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The Effect of Blinded Hydration State on Thermoregulation and Performance in Male Cyclists

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Kinesiology

by

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Abstract

Purpose: The purpose of these studies was to observe the effect of dehydration on exercise performance while subjects were blinded to their hydration status. **Methods:** Study 1: Seven male cyclists (weight: 71 ± 8 kg, body fat: $14 \pm 6\%$, VO_{2peak} : 59.4 ± 6 ml·kg⁻¹·min⁻¹) exercised for 2 hours on a cycle ergometer at 55% VO_{2peak} , in a hot-dry environment (35°C, 30% rh), with a nasogastric (NG) tube under euhydrated (EUH-NT) and hypohydrated (DEH-NT) conditions. In both trials, thirst was matched by drinking 25 mL every 5 min. In the EUH-NT trial sweat losses were fully replaced via the NG tube. Following the 2 hours of steady state, the cyclists completed a 5-kilometer cycling time trial at 4% grade. Study 2: Eleven male cyclists (weight 75.8 ± 6.4 kg, VO_{2peak} : 64.9 ± 5.6 mL·kg·min⁻¹, body fat: $12.0 \pm 5.8\%$) performed three sets of criterium-like cycling, consisting of 20 min of steady state cycling at 50% peak power output, each followed by a 5-km time-trial at 3% grade. Subjects completed the protocol on two separate occasions either hypohydrated (HYP) or euhydrated (EUH). In both trials, subjects ingested 25 mL every 5 min during the steady-state and 25 mL every 1-km during the 5-km time-trials. In the EUH trial, sweat losses were fully replaced via intravenous infusion of isotonic saline while in the DEH trial, a sham IV was instrumented. **Results:** In Study 1, cyclists completed the 5-km time trial faster in the EUH-NT trial compared to the DEH-NT trial (23.2 ± 0.2 vs. 22.3 ± 0.3 km·h⁻¹, $P < 0.05$), while producing higher power output (295 ± 29 vs. 270 ± 26 W, $P < 0.05$). In Study 2, during the second and third time-trials, subjects displayed faster speed in the EUH trial (27.5 ± 3.0 and 27.2 ± 3.1 km·h⁻¹) compared to the HYP trial (26.2 ± 2.9 and 25.5 ± 3.3 km·h⁻¹; both $P < 0.05$). Core temperature (T_{re}) was also higher in the HYP trial throughout the third steady-state ($P < 0.05$) and continued to be higher throughout the third 5-km time-trial ($P < 0.05$). **Conclusions:** These data suggest that full fluid replacement, even in a blinded manner, provided a performance advantage by maintaining better hydration state. This benefit seems to be associated with the lower thermoregulatory strain, due to lower core temperatures.

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I. Introduction

The negative impact of dehydration on aerobic and endurance exercise performance is well documented (Cheuvront, Carter, & Sawka, 2003; Sawka, Cheuvront, & Kenefick, 2015). Physiologic factors that contribute to dehydration-mediated performance decrements include 1) increased cardiovascular strain, 2) increased heat strain, 3) altered central nervous system (CNS) function, and 4) altered metabolic function. Though each factor is unique, evidence suggests that they interact to contribute in concert, rather than in isolation, to degrading endurance performance. However, heat strain (hyperthermia) probably acts to accentuate the performance decrement the heaviest (González-Alonso et al., 1999).

The effects of dehydration on physiologic function and exercise performance have been studied using several different approaches for reducing body water. Fluid losses are achieved either before the exercise task (hypohydration; i.e., water deprivation, diuretics, sauna, exercise) or can develop during exercise (dehydration; i.e., water deprivation, exercise) (American College of Sports Medicine et al., 2007; Barr, 1999). Unfortunately, none of these methods allows for a blinding of the treatment, with participants clearly aware of the hydration status under which they are performing. It is possible, therefore, that a placebo effect could partially contribute to the reported performance outcomes shown in several laboratory-based studies (McClung & Collins, 2007).

One difficult symptom of dehydration that is difficult to mask is the simple aspect of thirst. The physical act of drinking can modify an individual's perception of thirst (Figaro & Mack, 1997) and conversely, increased thirst can lead to psychological and physiological fatigue (Brunstrom, Tribbeck, & MacRae, 2000; Cheung et al., 2015). Thirst plays an integral role in the body's homeostatic mechanism for fluid levels by acting as one of the key psychologic indicators to replenish lost fluid (McKinley & Johnson, 2004) and can potentially influence the motivation and cessation of exercise. It has been shown that when athletes drink only to satisfy thirst, they replace ~60% of the fluid loss (Greenleaf, 1992; Greenleaf & Sargent, 1965). However, some argue that exercise performance is not impaired by dehydration and that drinking to thirst is encouraged (Goulet, 2013; Hoffman, Cotter, Goulet, & Laursen, 2016).

Recently, several studies have attempted to investigate the effect of thirst and dehydration on exercise performance in a blinded manner (Cheung et al., 2015; Wall et al., 2015). Although both studies reported no differences in cycling performance when subjects were dehydrated to 3% body weight, higher thermoregulatory and cardiovascular strain was observed. Further, both studies did not allow the ingestion of water during exercise, with only one allowing mouth rinsing with water. Previous experiments have suggested that the act of swallowing reduces thirst, increases performance, and inhibits vasopressin release, via oropharyngeal stimulation (Figaro & Mack, 1997; S A Kavouras et al., 2016; Takamata, Mack, Gillen, Jozsi, & Nadel, 1995).

Lastly, since fluid ingestion was not provided during any of the hydration conditions in the previously mention studies, it remains possible that the similar performance outcomes were driven by a consistent impairment from a strong psychologic state of thirst rather than a lack of effect from hydration status. In order to test the hypothesis that dehydration impairs exercise performance even under blinded conditions, subjects completed an exercise protocol while being progressively dehydrated or while maintaining euhydration via sweat loss replacement. Further, subjects were allowed a structured drinking volume periodically to control for thirst while not affecting hydration state. This study design allowed for thorough investigation of how endurance exercise is affected by dehydration even when subjects were unaware of their hydration state.

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II. Study 1:

Dehydration Impairs Cycling Performance in The Heat in The Absence of Thirst: A Blinded Study

Dehydration Impairs Cycling Performance in The Heat in The Absence of Thirst: A Blinded Study

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ABSTRACT

Purpose: The aim of the present study was to examine the effect of dehydration on exercise performance in the absence of thirst with subjects blinded to their hydration status. **Methods:** Seven male cyclists (weight: 71 ± 8 kg, body fat: $14 \pm 6\%$, $VO_{2\text{peak}}$: 59.4 ± 6 ml·kg⁻¹·min⁻¹) exercised for 2 hours on a cycle ergometer at 55% $VO_{2\text{peak}}$, in a hot-dry environment (35°C, 30% rh), with a nasogastric (NG) tube under euhydrated (EUH-NT) and hypohydrated (DEH-NT) conditions. In both trials, thirst was matched by drinking 25 mL every 5 min (300 mL·h⁻¹). In the EUH-NT trial sweat losses were fully replaced via the NG tube (calculated from the familiarization visit). Following the 2 hours of steady state, the cyclists completed a 5-kilometer cycling time trial at 4% grade. **Results:** Following 2 hours of steady state cycling, post-exercise body mass loss for EUH-NT trial was -0.1% compared to the DEH-NT trial which was -2.2±0.4%. Thirst (28±11 vs. 42±12 mm) and stomach fullness (41±8 vs. 38±8 mm) did not differ between EUH-NT and DEH-NT trials ($P > 0.05$). Cyclists completed the 5-km time trial faster in the EUH-NT trial compared to the DEH-NT trial (23.2±0.2 vs. 22.3±0.3 km·h⁻¹, $P < 0.05$), while producing higher power output (295±29 vs. 270±26 W, $P < 0.05$). During the 5-km time trial, core temperature was higher in the DEH-NT trial (39.2±0.3°C) compared to the EUH-NT trial (38.8±0.2°C; $P > 0.05$). **Conclusion:** These data indicated that hypohydration decreased cycling performance and impaired thermoregulation in the absence of thirst, while the subjects were unaware of their hydration status.

Key words: core temperature, cycling, thirst, hydration

INTRODUCTION

During endurance exercise, especially in the heat, maintaining adequate hydration is recommended for optimal performance (American College of Sports Medicine et al., 2007; Cheuvront et al., 2003; Sawka et al., 2015). Proper fluid replacement reduces physiological strain (González-Alonso, Mora-Rodríguez, Below, & Coyle, 1995) and diminishes thirst (McKinley & Johnson, 2004). While thirst plays an integral role in water homeostasis by acting as a key psychologic indicator to fluid replenishment (McKinley & Johnson, 2004), it is suppressed by the act of drinking (Figaro & Mack, 1997). Some scientist have argued that thirst alone acting as part of an anticipatory regulatory system (Sawka & Noakes, 2007) could impair exercise performance in dehydrated subjects (Cheung et al., 2015).

Regardless of thirst, previous studies showing that dehydration impairs exercise performance have failed to blind their subjects to their fluid intake and hydration state. This absence of blinding could induce a bias that might affect the results based on subjects' perceptions and/or expectations. Studies that manipulate hydration status through fluid ingestion are limited by the nature of drinking and its effect on thirst.

Recently, two studies (Cheung et al., 2015; Wall et al., 2015) investigated the effect of hypohydration on exercise performance in a blinded manner via intravenous infusion of saline during exercise, while thirst was controlled via mouth rinsing with water. Although both studies reported no differences in cycling performance when subjects were dehydrated to 3% body weight, higher thermoregulatory and cardiovascular strain was observed. Further, both studies did not allow the ingestion of water during exercise. Previous experiments have suggested that the act of swallowing reduces thirst, increases performance, and inhibits vasopressin release, via oropharyngeal stimulation (Arnaoutis, Kavouras, Christaki, & Sidossis, 2012; Figaro & Mack, 1997; Takamata et al., 1995), as opposed to mouth rinsing. Interestingly, even though ingestion of small volumes of water alleviates thirst (Guest et al., 2006), drinking to thirst during exercise might impair performance (Armstrong et al., 2014; Armstrong, Johnson, & Bergeron, 2016; Greenleaf, 1992) via involuntary dehydration. Therefore, the aim of the present study was to investigate the effect of dehydration on cycling time-trial performance in the absence of thirst with subjects blinded to their hydration status.

METHODS

Participants

Twenty-nine cyclists signed informed consent to participate in the study. Twenty-one withdrew due to the discomfort associated with the nasogastric tube insertion, while one subject dropped out after the first trial. Seven male cyclists (weight: 71 ± 8 kg, body fat: $14 \pm 6\%$, VO_{2peak} : 59.4 ± 6 ml·kg⁻¹·min⁻¹) that completed the entire protocol were included in the analysis. All cyclists had extensive racing experience and competed regularly at USA Cycling category 3 or higher races. Eligibility criteria for participation included other than competitive cycling status, absence of any metabolic, cardiovascular, renal disease, and history of heat stroke. The study was approved by the University's Institutional Review Board and participants gave their written consent prior to enrolment.

Preliminary Screening

During the preliminary screening, anthropometric characteristics were recorded during the first visit at the laboratory. Weight (Health O Meter Professional, 349 KLX, McCook, IL) and height (Seca, Model 700, Hamburg, Germany) were measured without shoes and with minimal clothing to the nearest 0.1 kg and 0.005 m, respectively. Body composition was determined via dual-energy X-ray absorptiometry (DXA; General Electric, Lunar Prodigy Promo, Chicago, IL). Peak oxygen uptake (VO_{2peak}) test was performed on an electronically braked cycle ergometer (Velotron, Racermate, Seattle, WA). Following standardized warm-up at 100 W power increased by 40 W every two minutes until volitional exhaustion. During the test, expiratory gases were analyzed via an online gas analyzer (Parvo Medics TrueOne 2400, Sandy, UT). At least three of the four following criteria were used to verify attainment of VO_{2peak} : 1) Oxygen uptake plateau with increased workload, 2) Respiratory exchange ratio greater than 1.1, 3) HR greater than 90% of age-predicted maximal value ($220 - \text{age}$) and 4) Perceived exertion based on the 6-20 Borg scale greater than 17 (Borg, 1982).

Experimental Protocol

All subjects completed the experimental protocol on three separate visits, first for familiarization and then, the two experimental trials in a counterbalanced manner. The protocol was consisted of 2 h steady state exercise ($55\% VO_{2peak}$) followed by a 5-km time-trial at 4% grade (Dantas, Pereira, & Nakamura, 2015). During the familiarization trial, the sweat rate of the participants was estimated while

drinking water ad libitum. The cyclist performed the two experimental trials without being thirsty while maintaining euhydration (euhydrated not-thirsty, EUH-NT) or while becoming progressively dehydrated (dehydrated not-thirsty, DEH-NT). To clamp thirst at low levels in both trials cyclists were drinking 25 mL of water every 5 minutes during the 2-h steady state phase of the protocol and every 1-km during the 5-km time-trial. During the EUH-NT trial, water was infused via the nasogastric tube at a rate to match sweat losses based on the subject's sweating rate assessed on the familiarization trial. The amount of water infused was corrected for water ingested to clamp thirst (25 mL every 5 min). The water infused was warmed to body temperature (37 °C) to prevent the subject from sensing the cooler water getting into the stomach during the infusion, as well to avoid any cooling effect. The experimental trials were performed in the morning, at the same time of the day, to avoid diurnal variations (Atkinson, Todd, Reilly, & Waterhouse, 2005).

Familiarization session

Prior to the two experimental trials, subjects completed the cycling session to get familiarized with the experimental protocol. During this familiarization, subjects were instructed to bring their own water bottles and drink as much as they wanted from the water provided. Sweating rate was estimated based on the changes of body weight corrected for water intake and urine output. The protocol of this session was identical with the two experimental trials, apart from blood draws and nasogastric tube placement.

Experimental Trials

Upon arrival to the laboratory, a urine sample was collected to assess pre-trial hydration state and proceeded to testing only when urine specific gravity was below 1.020 (American College of Sports Medicine et al., 2007). Subjects then self-inserted a rectal thermistor and a nasogastric tube (NG; 10-F, Corflo, Corpak Medsystems; Buffalo Grove, IL) was inserted at a depth equal to the distance between the tip of the nose, behind one ear, to the tip of the sternum. Placement was confirmed by analyzing gastric fluid for pH testing (pH <5). After securing NG tube on the nose with tape, the external portion of the nasogastric tube was connected to an extension tube running over the ear and toward the shoulder.

The subject then entered the environmental chamber (35±0.3 °C, 30±0.2% relative humidity) and sat on the ergometer for 20 min before a baseline blood sample was taken. Following baseline measurements, the subjects cycled for 2 h at 55% VO_{2peak} . Subjects performed both trials in a

randomized, counter-balanced fashion separated by at least 1 week. A fan producing an air speed of $4.5 \text{ m}\cdot\text{s}^{-1}$ was directed at the subjects throughout exercise, and subjects wore the same clothing for each trial.

Physiological and Perceptual measurements

Wireless skin temperature sensors (Maxim Integrated Products, Sunnyvale, CA) were attached on the arm, chest, thigh, and leg. Mean weighted skin temperature (T_{sk}) was calculated using the Ramanathan equation (Ramanathan, 1964). To record rectal temperature (T_{re}) rectal thermistors (Physiotemp Instruments Inc., Clifton, NJ) were inserted 10 cm past the anal sphincter. T_{re} and T_{sk} were recorded throughout exercise every 5 min. Heart rate (HR) was recorded every 5 min via wireless heart rate monitor (Polar Electro T31; Kempele, Finland). During the time-trial, all thermoregulatory and cardiovascular measurements were recorded every kilometer. Cycling power output (W) and finishing time (sec) of the 5-km time-trial were recorded in real time by the cycling computrainer software (RacerMate Inc., Seattle, WA). Subjects could view the screen profile of the course, but could not view their time, cadence, or power output. During the steady state, cyclists provided their rate of thirst (“how thirsty are you now”), and stomach fullness (“how full is your stomach now”) every 10 min with visual analog scales (33). The visual analog scales used were consisted of a 180-mm line with an anchor on the left side (0 mm, “not at all”) and a second anchor on the 125-mm mark with the label “extremely”. Since 25 mL of water was provided every 5 min the assessment of thirst was done prior to drinking water to provide fair and objective indication.

Blood and urine analyses

Blood samples were obtained via venipuncture without stasis at baseline, following steady state, and immediately after the time-trial. Urine was obtained at baseline as well as post-trial. Urine specific gravity (USG) and total plasma proteins (TTP) were measured using a hand-held refractometer (Atago SUR-NE, Tokyo, Japan). Hematocrit (Hct) was determined in triplicate from whole blood using the microcapillary technique, following centrifugation for 5 min at 10,000 rpm. Hemoglobin (Hb) was also measured in triplicate from whole blood via the cyanmethemoglobin technique, using a commercially available kit (Drabkin’s reagent, Sigma, Saint Louis, Missouri, USA). Percent change in plasma volume ($\%\Delta\text{PV}$) was calculated using the Dill and Costill equation (D.B. & D.L., 1974). Plasma (POsm) and urine

osmolality (UOsm) were measured in duplicate via freezing-point depression from fresh samples (3250 Osmometer; Advanced Instruments Inc, Norwood, MA).

Statistical Analysis

All variables are presented as mean \pm standard error, since they were normally distributed. Differences in the mean values or the distributions of parameters between EUH-NT and DEH-NT were assessed using Students paired t-tests. Two-way (treatment x time) repeated measures of ANOVA were used to analyze differences in variables across time points between treatments. Post-hoc analysis for comparing mean values between trials across time points, as well as different time points, was applied by using the sequential Bonferroni correction rule. All statistical analysis was performed using JMP Pro (version 12.1.0, SAS Inc., Gary, NC, USA). A value of $P < 0.05$ was regarded as statistically significant.

RESULTS

Familiarization Visit

During the 2-hour steady state of the familiarization visit, subject sweat loss was 2.3 ± 0.1 L with average sweating rates of 1.2 ± 0.0 L \cdot h $^{-1}$. Despite favorable conditions to drink ad libitum during the 2-hour steady state, subjects reached a mild level of hypohydration of $-1.2 \pm 0.3\%$. During the 5-km time-trial, subject sweat loss was -0.4 ± 0.1 L with average sweating rates of 1.7 ± 0.3 L \cdot h $^{-1}$. Final percent dehydration for the whole familiarization visit was $-1.8 \pm 0.4\%$ body weight. Time-trial average power output for the familiarization visit was 281 ± 15 W and average speed was 22.4 ± 0.1 km \cdot h $^{-1}$.

Fluid Balance Results

Pre-exercise body mass, USG, UOsm, and plasma osmolality did not differ between EUH-NT and DEH-NT (Table 1, $P > 0.05$). During the steady state protocol, both EUH-NT and DEH-NT trials subjects ingested of 0.6 L of water. However, in the EUH-NT trial, 1.7 ± 0.1 L of water was also infused into the subjects' stomach via the nasogastric tube, bringing the total fluid replacement to 2.3 ± 0.1 L. Following 2 hours of steady state exercise body mass loss for the EUH-NT and DEH-NT trials were -0.2 ± 0.2 ($0.2 \pm 0.6\%$) and -1.6 ± 0.1 kg ($2.2 \pm 0.4\%$), respectively ($P < 0.05$). No significant differences in changes of plasma volume were observed between trials (EUH-NT $-4.9 \pm 0.7\%$ and DEH-NT $-4.9 \pm 1.2\%$; $P > 0.05$).

During the 5-km time-trial, the cyclists ingested 0.1 L of water (25 ml at every one km) during both EUH-NT and DEH-NT. However, in the EUH-NT trial, gastric infusion of water was 0.3 ± 0.1 L, bringing the total fluid replacement to 0.4 ± 0.1 L. Following the 5-km time-trial, final weight loss for the EUH-NT and DEH-NT time-trial was 0.3 ± 0.1 vs. 0.5 ± 0.1 kg (-0.5 ± 0.4 vs. $-0.7\pm 0.3\%$; $P<0.05$). No statistically significant differences were observed on ΔPV (-4.4 ± 3.7 vs. $-7.6\pm 5.4\%$; $P>0.05$).

Physiological, Perceptual, and Performance Responses

During 2 hours of steady state cycling, there were no differences between EUH-NT and DEH-NT in T_{re} ($P>0.05$) with both trials reaching a 38.5 ± 0.1 °C at end of exercise. There were, however, significant differences in HR between EUH-NT and DEH-NT ($P<0.05$) from 55 min until the end of the 2-hour steady state bout. Final HR recording from 2 hours of steady state was 144 ± 4 for EUH-NT and 153 ± 4 for DEH-NT. Final mean T_{sk} recordings were 35.4 ± 0.3 vs. 35.3 ± 0.2 °C for EUH-NT and DEH-NT, respectively. The T_{re} data during the 5-km time-trial are presented in Figure 1. DEH-NT resulted in significantly higher rectal temperatures compared to the EUH-NT ($P<0.05$; Figure 1). HR during both EUH-NT and DEH-NT time-trials reached $85\pm 14\%$ of max HR but did not differ between the two trials ($P>0.05$; Figure 1). Thirst perception and stomach fullness did not change during the 2 h of steady state cycling and no differences were observed between trials (Figure 2). Mean power output was significantly greater during the performance test in the EUH-NT (294 ± 11 W; $P<0.05$; Figure 3) compared to DEH-NT (269 ± 10 W). Similarly, cycling speed was higher in the EUH-NT (23.2 ± 0.2 km·h⁻¹; Figure 3) compared to the DEH-NT (22.3 ± 0.3 km·h⁻¹). Further, EUH-NT had lower completion time (777 ± 18 s) compared to DEH-NT (822 ± 21 s; $P<0.05$). Five out of the seven cyclists performed better in the 5-km time-trial during the EUH-NT than the DEH-NT trial (Figure 4).

DISCUSSION

The aim of the present study was to investigate the effect of dehydration on cycling time-trial performance in the absence of thirst with subjects blinded to their hydration status. The main finding of this study was that euhydration (EUH-NT) lead to better exercise performance in the 5-km time-trial compared to the dehydration (DEH-NT). Further, these performance results occurred with similar, albeit

low, thirst perceptual responses. To our knowledge, this is first study to induce a blind hydration protocol while drinking water, thus stimulating the oropharyngeal receptors.

These data agree with previous literature concluding that mild hypohydration impairs endurance exercise performance. Logan-Springer et al. (Logan-Sprenger, Heigenhauser, Killian, & Spriet, 2012) found that progressive dehydration significantly increased core temperature, heart rate, whole body carbohydrate oxidation, and muscle glycogenolysis, and these changes were apparent in the first hour of exercise when body mass losses were <1%. Further, Bardis et al. (Bardis et al., 2017) found that hypohydration of -1.8% body mass as a response to ad libitum drinking resulted in increased core temperature, lower cycling power output and slower time-trial time.

In the present study, although no differences in core temperature were observed during the steady state cycling, the DEH-NT trial induced higher core temperature in the time-trial. This core temperature difference between the two trial was almost 0.5 °C when the subjects finished the 5-km. It should also be noted that the DEH-NT trial had higher core temperature despite cycling at a slower speed, by cycling at a lower power output. Since the metabolic heat production was lower in the DEH-NT due to the lower power output, the higher core temperatures were due to the inability to dissipate heat, via sweating and skin blood flow. The fact that sweating responds were similar between the trials even though the thermoregulatory strain was greater in the DEH-NT trial could indicate that sweat sensitivity deteriorates due to water deficit.

Kenefick et al. (R. W. Kenefick, Chevront, Palombo, Ely, & Sawka, 2010) found that increasing skin temperature proportionally accentuated plasma volume shrinkage and any additional plasma volume loss likely results from increased net filtration rate at the capillaries. Despite several other studies concluding that skin temperature modifies the impact of hydration state on endurance performance (González-Alonso et al., 1999; Robert W Kenefick, Sollanek, Charkoudian, & Sawka, 2014), no differences in skin temperature were found in the present study following 2-hours of steady state exercise.

Numerous studies have shown that dehydration impairs cardiovascular function during exercise (González-Alonso, Mora-Rodríguez, Below, & Coyle, 1997; R. W. Kenefick et al., 2010; Montain & Coyle, 1992) via elevated heart rate to compensate for the decreased stroke volume (González-Alonso et al.,

1999). In the present study, heart rate was higher during the 2-hour steady state cycling in the DEH-NT trial from 55 to 120 min of the steady-state exercise component. However, despite the gastric infusion of water, no differences were found in PV between trials. Lastly, no differences were observed in maximal heart rate during the time-trial as seen elsewhere (R. W. Kenefick et al., 2010).

Despite the large body of literature that consistently shows hypohydration decreases exercise performance (Bardis, Kavouras, Arnaoutis, Panagiotakos, & Sidossis, 2013; Bardis, Kavouras, Kosti, Markousi, & Sidossis, 2013; Cheuvront & Kenefick, 2014; R. W. Kenefick et al., 2010; Sawka et al., 2015), the vast majority of these studies are confounded by the lack of experimental blinding on the hydration state. In an experimental setting, hydration has been manipulated via a number of methods such as: exercise-induced hypohydration (Armstrong et al., 1997; Barr, 1999), overnight fluid restriction (Arnaoutis et al., 2017), diuretics (Gebruers, Hall, O'Brien, O'Leary, & Plant, 1985), and intravenous fluid administration (Cheung et al., 2015; Wall et al., 2015). With two exceptions (Cheung et al., 2015; Wall et al., 2015), the previous methods have been conducted in a non-blind manner, thus, the subjects were aware of whether they were in the hypohydrated or euhydrated trial. Despite the majority of the data showing that fluid restriction impairs exercise performance (David Cotter, Thornton, Lee, & Laursen, 2014), there is a possibility that knowing that you are dehydrated and expecting a decline in performance could work as placebo. However, in this present study, hydration status was manipulated during exercise in a blinded manner injecting water to the stomach, via a nasogastric tube. Therefore, the impairment in performance seen in the present study is in not the result of subjects' expectation.

Recently, two separate studies have attempted to eliminate the bias of thirst on exercise performance and blind subjects to their fluid balance. In both of the previous studies (Cheung et al., 2015; Wall et al., 2015), Wall et al. (Wall et al., 2015) found that when cyclists were dehydrated by 3% of body mass, they exhibited higher core temperatures during their 25-km time-trial, despite having similar ratings for thirst perception. In this study, intravenous infusion of isotonic saline was used to rehydrate subjects following exercise-induced dehydration of -3%. Similarly, Cheung et al. (Cheung et al., 2015) found that when cyclists were hypohydrated by 3% body mass they experienced higher core temperatures and heart rates during the last half of their 20-km time-trial. Further, the cyclists were also provided mouth rinse to control for thirst, as both previous studies did not allow for drinking. The process of drinking water has

been shown to reduce thirst, increase performance, and inhibit vasopressin release, via oropharyngeal stimulation (Arnaoutis et al., 2012; Figaro & Mack, 1997). In the present study, subjects were provided a small amount of drinking throughout the protocol. This technique did not prevent dehydration, but could keep thirst perception low and similar between the two conditions (Fig. 2).

Interestingly, even though a significant amount of water was infused in the stomach of the cyclist during exercise, no differences in stomach fullness was observed between treatments. Probably, the slow, continuous gastric infusion did not cause a significant distention that would be perceived by the subjects. It would be interesting to note the role of stomach fullness in relation to thirst. A classic animal-model study by Adolph et al. (Adolph & Northrop, 1950) discussed the relationship between stomach fullness and thirst when drinking and infusing fluid directly into the stomach. The authors of this study conclude that the factor of gastric filling concerns only the drinking that occurs immediately after a large volume is placed into the stomach. If time is allowed for the passage of the large amount of fluid administered into the intestine and for its absorption, then thirst is inhibited. In the present study, the gastric infusion of water was at a rate matching sweating rate and sweating amount, meaning the EUH-NT received about 0.85 L per hour. This amount is maximal gastric emptying capacity and readily absorbed as water does not need to pass through the alimentary tract to be absorbed.

Lastly, in the present study all subjects performed a familiarization trial. Although the purpose of the familiarization was to assess sweat losses, one cannot ignore the observation that despite favorable conditions for rehydration during exercise, subjects finished the 2-hour steady state in a mild level of hypohydration (-1.2%) while drinking ad-libitum and reached -1.8% following the 5-km time trial. The subjects average speed during the time-trial was slower than the EUH-NT trial coupled with a lower power output. This observation might suggest that exercise induced dehydration associated with drinking to thirst impaired performance, even though the subject did not wear the nasogastric tube that could create more discomfort.

In conclusion, dehydration impaired cycling performance during a 5-km time trial in the heat, even in the absence of thirst, when subjects were unaware of their hydration state. Further research is needed to evaluate the effect of blinded rehydration in different sports of varying intensities and environments.

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Table 1. Pre-exercise fluid balance results for both DEH-NT and EUH-NT

	Body mass (kg)	UOsm (mmol·kg⁻¹)	USG	POsm (mmol·kg⁻¹)
DEH-NT	72.2±3.0	515±89	1.012±0.002	291±1
EUH-NT	72.4±2.9	368±63	1.010±0.001	292±1

Table 2. Fluid balance measurements during and following 2-hour steady state protocol.

	Water Ingestion (L)	Gastric Infusion (L)	Total Fluid (L)	% BM Change	ΔPV (%)
DEH-NT	0.6	0.0 \pm 0.0	0.6 \pm 0.0	-2.2 \pm 0.1*	-4.9 \pm 3.4
EUH-NT	0.6	1.7 \pm 0.1	2.3 \pm 0.1	-0.2 \pm 0.2	-4.9 \pm 1.8

*Denotes statistically significant different ($P < 0.05$) between DEH-NT and EUH-NT trials.

FIGURE LEGENDS

Figure 1. Core temperature and heart rate during 5km cycling time-trial between DEH-NT and EUH-NT. *denotes statistically significant differences, $P < 0.05$ between trials at same time point.

Figure 2. Thirst and stomach fullness visual analog scale responses during 2-hour steady state between DEH-NT and EUH-NT.

Figure 3. Mean cycling speed and mean power output during the 5km cycling time-trial between DEH-NT and EUH-NT. *denotes statistically significant differences, $P < 0.05$ between trials.

Figure 4. Individual performance data during the 5km time-trial in DEH-NT and EUH-NT trials plotted with a line of identity. Each point represents a different individual participant.

Figure 1.

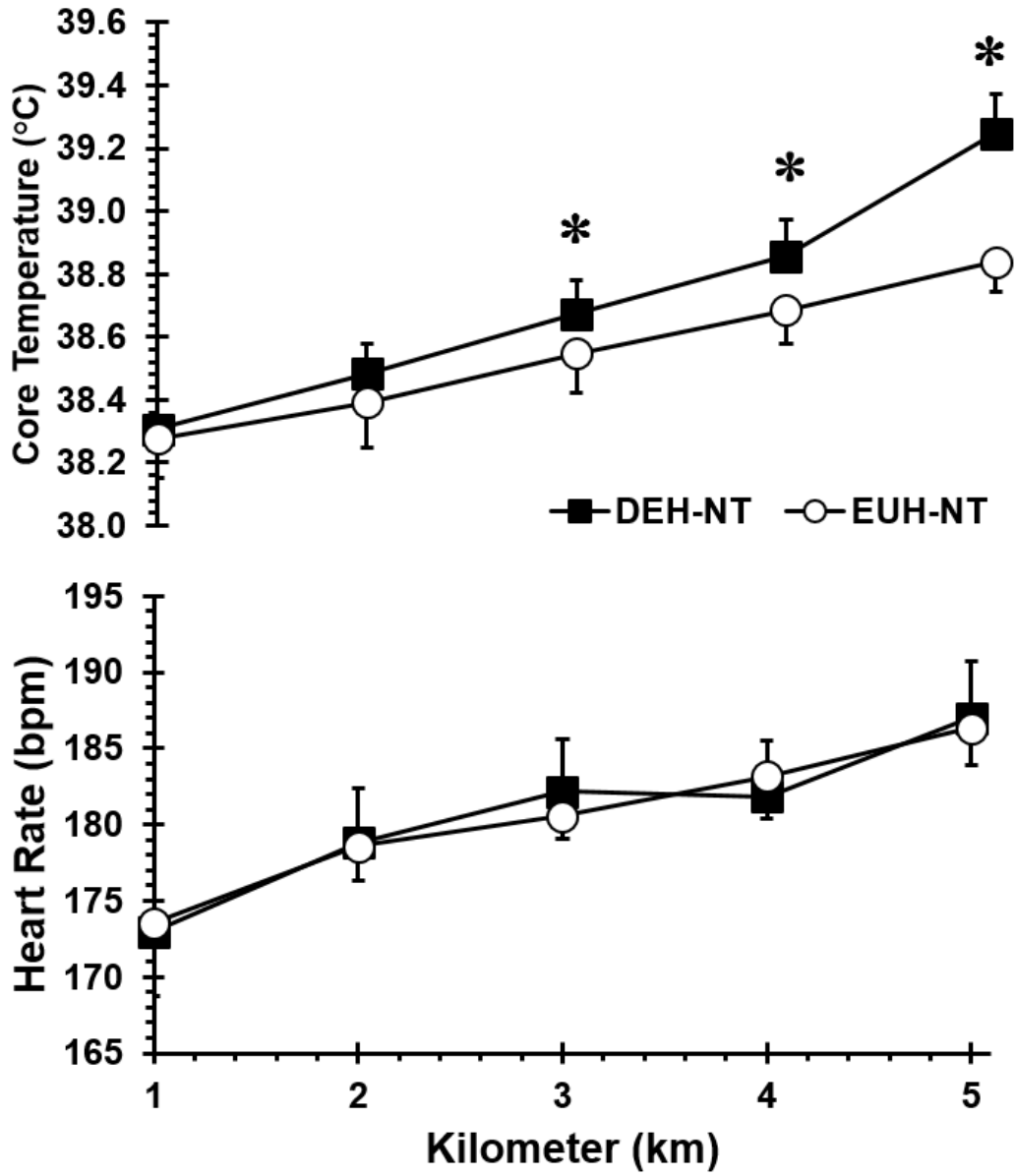


Figure 2.

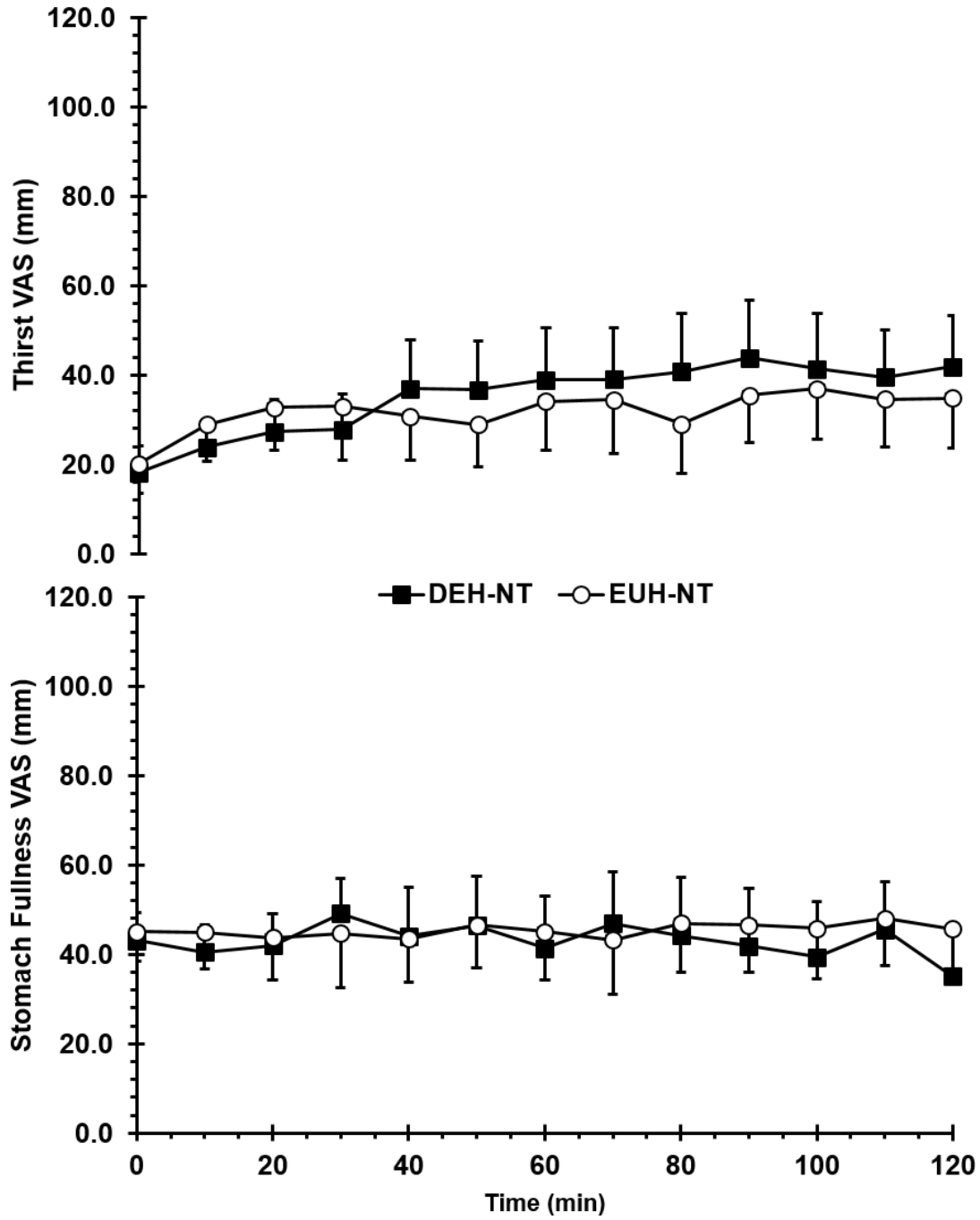


Figure 3.

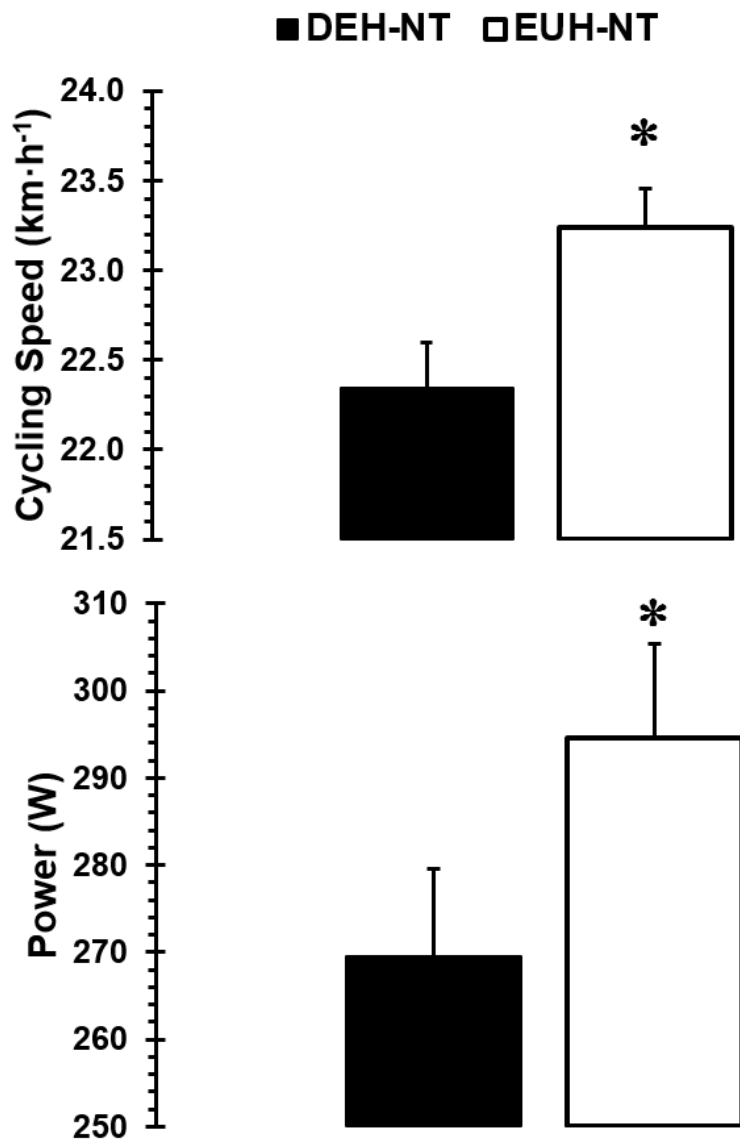
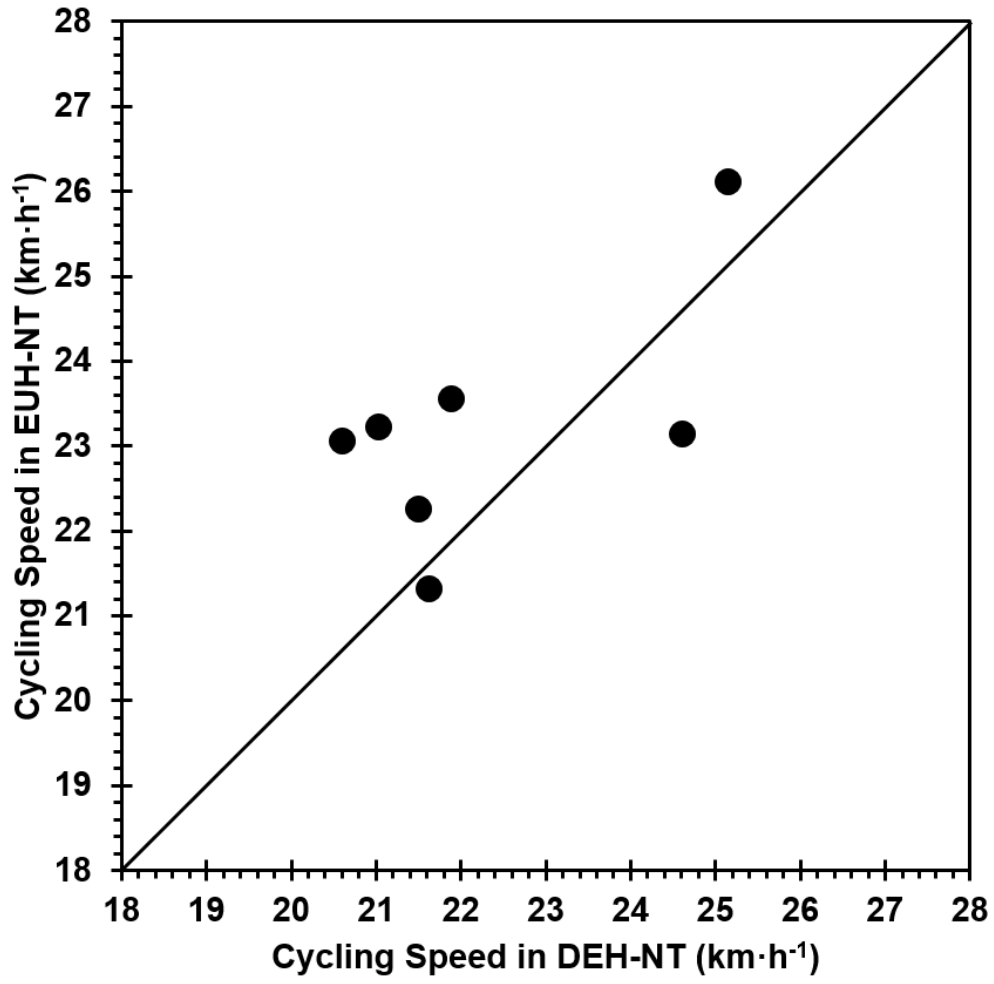


Figure 4.



III. Study 2:

The Effect of Blinded Hydration State on Thermoregulation and Performance in Male Cyclists

The Effect of Blinded Hydration State on Thermoregulation and Performance in Male Cyclists

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ABSTRACT

Purpose: The aim of the present study was to observe the effect of dehydration on exercise performance with subjects blinded to their hydration status via intravenous infusion. **Methods:** Eleven male cyclists (weight 75.8 ± 6.4 kg, $\text{VO}_{2\text{peak}}$: 64.9 ± 5.6 mL·kg·min⁻¹, body fat: $12.0 \pm 5.8\%$, $\text{Power}_{\text{max}}$: 409 ± 40 W) performed three sets of criterium-like cycling, consisting of 20 min of steady state cycling at 50% peak power output, each followed by a 5-km time-trial at 3% grade. Subjects completed the protocol, in counter-balanced fashion, on two separate occasions in dry heat (30 °C, 30% rh) either hypohydrated (HYP) or euhydrated (EUH). In both trials, subjects ingested 25 mL every 5 min during the steady-state and 25 mL every 1-km during the 5-km time-trials. In the EUH trial, sweat losses were fully replaced via intravenous infusion of isotonic saline while in the DEH trial, a sham IV was instrumented. **Results:** Following the exercise protocol, the subjects dehydrated by $-0.1 \pm 0.1\%$ and $-1.8 \pm 0.2\%$ of their body weight for the EUH and HYP trial, respectively. During the second and third time-trials, subjects displayed faster speed in the EUH trial (27.5 ± 3.0 and 27.2 ± 3.1 km·h⁻¹) compared to the HYP trial (26.2 ± 2.9 and 25.5 ± 3.3 km·h⁻¹; both $P < 0.05$). Core temperature (T_{re}) was also higher in the HYP trial throughout the third steady-state ($P < 0.05$) and continued to be higher throughout the third 5-km time-trial ($P < 0.05$). Final T_{re} of the third time-trial for HYP and EUH was 39.1 ± 0.3 vs. 38.6 ± 0.6 °C. **Conclusion:** These data suggest that full fluid replacement, even in a blinded manner, provided a performance advantage by maintaining better hydration state. This benefit seems to be associated with the lower thermoregulatory strain, due to lower core temperatures.

INTRODUCTION

Ad-libitum water intake during endurance exercise is rarely sufficient to replace water loss, and consequently dehydration is common at the end of prolonged exercise (Sawka et al., 2015). The American College of Sports Medicine position statement on exercise and fluid replacement states that hypohydration greater than 2% body mass impairs endurance exercise performance in temperate, warm and hot conditions (1). However, studies have shown that even a mild degree of hypohydration can impair exercise performance via thermoregulatory and cardiovascular strain (Bardis et al., 2017; Below, Mora-Rodríguez, González-Alonso, & Coyle, 1995; Logan-Sprenger et al., 2012).

Sufficient fluid replacement during exercise reduces physiological strain (González-Alonso et al., 1995) and thirst (McKinley & Johnson, 2004). Thirst, however, is a poor indicator for fluid replacement (Greenleaf, 1992) as it is suppressed by the act of drinking (Figaro & Mack, 1997; Stavros A. Kavouras, 2013). A classic study by Greenleaf et al. (Greenleaf & Sargent, 1965) showed that when drinking to satisfy thirst, subjects replace ~60% of fluid requirements. Further, some have postulated that thirst alone could impair exercise performance in dehydrated subjects (Cheung et al., 2015; Sawka & Noakes, 2007). Regardless of thirst, the majority of previous studies showing that dehydration impairs exercise performance have failed to blind their subjects to their fluid intake and/or hydration state. This absence of blinding could induce a bias that might affect the results based on subjects' perceptions and/or expectations. Studies that manipulate hydration status through fluid ingestion are limited by the nature of drinking and its effect on thirst (Armstrong et al., 2016; Arnaoutis et al., 2012).

Currently, only two studies have implemented intravenous saline infusion during exercise to blind fluid replacement and overall hydration state (Cheung et al., 2015; Wall et al., 2015). Although both studies reported no differences in cycling performance when subjects were dehydrated to 3% body weight, higher thermoregulatory and cardiovascular strain was observed. Further, both studies did not allow water ingestion during exercise, while only one allowed mouth rinsing with water. Previous experiments have suggested that the act of swallowing reduces thirst, increases performance, and inhibits vasopressin release, via oropharyngeal stimulation (Arnaoutis et al., 2012; Figaro & Mack, 1997; Takamata et al., 1995), as opposed to mouth rinsing. Interestingly, even though a small volume of water

could alleviate thirst (Guest et al., 2006), drinking to thirst during exercise might impair exercise performance (Armstrong et al., 2014, 2016; Greenleaf & Sargent, 1965).

Further, since fluid ingestion was not provided during any of the hydration conditions in the previously mentioned studies, it remains a possibility that performance outcomes could be due to dehydration coupled with the activation of oropharyngeal receptors. Therefore, the purpose of this study was to investigate the effect of hypohydration on cycling performance in a blinded manner, while also attempting to control for thirst with small volume of water ingestion.

METHODS

Participants

Eleven male cyclists (28 ± 7 y; 75.9 ± 6.4 kg; VO_{2peak} : 64.9 ± 5.6 mL·kg·min⁻¹; $12.0 \pm 5.8\%$ body fat, $Power_{max}$: 409 ± 40 W) were recruited to participate in the study. All cyclists had extensive racing experience and competed regularly at USA Cycling category 3 or higher races. Eligibility criteria for participation included other than competitive cycling status, absence of any metabolic, cardiovascular, renal disease, and history of heat stroke. The study was approved by the university's institutional review board and participants gave their written consent prior to enrolment.

Preliminary Screening

During the preliminary screening, anthropometric characteristics were recorded. Weight (Health O Meter Professional, 349 KLX, McCook, IL) and height (Seca, Model 700, Hamburg, Germany) were measured without shoes and with minimal clothing to the nearest 0.1 kg and 0.005 m, respectively. Body composition was determined via dual-energy X-ray absorptiometry (DXA; General Electric, Lunar Prodigy Promo, Chicago, IL). Peak oxygen uptake (VO_{2peak}) test was performed on an electronically braked cycle ergometer (Velotron, Racermate, Seattle, WA). Following standardized warm-up at 100 W power increased by 40 W every two minutes until volitional exhaustion. During the test, expiratory gases were analyzed via an online gas analyzer (Parvo Medics TrueOne 2400, Sandy, UT). At least three of the four following criteria were used to verify attainment of VO_{2peak} : 1) Oxygen uptake plateau with increased workload, 2) Respiratory exchange ratio greater than 1.1, 3) HR greater than 90% of age-predicted

maximal value (220 - age) and 4) Perceived exertion based on the 6-20 Borg scale greater than 17 (Borg, 1982).

Experimental Protocol

All subjects performed in a counterbalanced manner two experimental criterium-like cycling tests in the laboratory under hot, dry conditions with moderate wind speed (30 °C, 30% rh; 4.5 m·s⁻¹). The protocol consisted of three 20-min steady-state cycling (SS) sets at 50% peak power output, each followed by a 5-km time-trial at 3% grade (TT). During the protocol cyclists ingested 25-mL of water every 5 minutes of each steady state, and every 1-km of each time-trial. The two experimental trials were identical with the exception of the hydration protocols. During the euhydrated trial (EUH), cyclists were continually infused isotonic saline with flow rate and amount controlled by an electronic infusion pump set to match the rate of sweat loss measured during the familiarization trial. During the hypohydrated trial (HYP), cyclists were given the same drinking protocol, however, the cyclists were sham-instrumented with an identical intravenous (IV) infusion configuration, and the technician performed similar movements and manipulations (e.g., checking the pump, massaging, and changing the saline bag). In all conditions, saline bags were pre-warmed to 37 °C, with a small amount infused following IV-line insertion to avoid conscious awareness of hydration condition. The IV pump was positioned behind the participant to prevent visual feedback of the infusion state. All subjects were given a commercially available frozen meal (600 kcal, 44 g carbohydrates, 8 g fat, and 12 g protein) to consume the night before. Prior to the protocol subjects ingested a carbohydrate-rich shake (240 kcal, 41 g carbohydrates, 4 g fat, and 10 g protein) 20-min before the protocol. To minimize differences in starting muscle glycogen concentrations in between trials, participants recorded their diet 24 h before their first visit. Diet records were copied and returned to the participants with instructions to follow the same diet before the next subsequent visit. Participants were also given platform scales to measure body weight 3 days prior to each experimental protocol to ensure no changes in body weight occurred over the course of the experiment. All subjects were advised to treat the protocol as an interval workout and to refrain from heavy training and competition while participating in the study.

Familiarization Trial

Prior to the experimental trials, participants completed the entire protocol in the same ambient conditions to get familiarized with the experimental protocol. All subjects were instructed to drink as much as they wanted from their own water bottles. During this trial, sweating rate was estimated based on changes in body weight corrected for water intake and urine output. To record body weight changes during the trial without changing body posture, the cycle ergometer was mounted on the top of a large platform scale (Model VS-2501, WeighSouth Inc, Asheville, NC). The volume and rate of fluid infused during the EUH trial was estimated based on the familiarization trial to replace 100% of fluid losses. The protocol of this session was identical with the two experimental trials, with the exception of blood draws and perceptual measures.

Experimental Trials

Upon arrival to the laboratory, a urine sample was collected to assess pre-trial hydration state and proceeded to testing only when urine specific gravity was below 1.020 (American College of Sports Medicine et al., 2007). A venous catheter was then placed in an antecubital vein which was coupled with an extension set and a 3-way stopcock for blood sampling and IV saline infusion. Following instrumentation, participants entered the environmental chamber and sat quietly on the cycle ergometer for 20 min before the first blood sample to normalize posture. A fan producing an air speed of $4.5 \text{ m}\cdot\text{s}^{-1}$ was directed at the subjects throughout exercise, and subjects wore the same clothing for each trial.

Thermoregulatory Measurements

Wireless skin temperature sensors (Maxim Integrated Products, Sunnyvale, CA) were attached on the arm, chest, thigh, and leg. Mean weighted skin temperature (T_{sk}) was calculated using the Ramanathan equation (Ramanathan, 1964). To record rectal temperature (T_{re}), a rectal thermistor (Physiotemp Instruments Inc., Clifton, NJ) was inserted 10 cm past the anal sphincter. T_{re} and T_{sk} were recorded throughout exercise every 5-min. During the time-trials, all thermoregulatory measurements were recorded every kilometer.

Cardiovascular Measurements

Heart rate (HR) was recorded every 5 min via wireless heart rate monitor (Polar Electro T31; Kempele, Finland). Cardiac output (Q) was measured using the indirect CO_2 -rebreathing method, as described by Jones et al. (Inman, Hughson, & Jones, 1985), using the Parvo Medics metabolic system

and software. This involved measuring the $\dot{V}CO_2$, end-tidal CO_2 concentration, and the equilibrium CO_2 concentration after rebreathing in succession. HR was measured every 5-min during the 20-min of steady state and every kilometer during the time-trials. Q was measured the last 5 min of the steady state sessions.

Performance and Perceptual Measurements

Average power output and time were recorded by the online computrainer software (Computrainer, RacerMate Inc., Seattle, WA). At baseline and every 10 minutes, subjects were asked to rate their perceived exertion their perceived thirst (“how thirsty are you now”), and their stomach fullness (“how full is your stomach now”). Thirst and stomach fullness was assessed via a visual analog scale used consisted of a 180-mm line with an anchor on the left side (0 mm, “not at all”) and a second anchor on the 125-mm mark with the label “extremely”.

Blood and urine analysis

Urine specific gravity (USG) and total plasma proteins (TTP) were measured using a hand-held refractometer (Atago SUR-NE, Tokyo, Japan). Hematocrit (Hct) was determined in triplicate from whole blood using the microcapillary technique, following centrifugation for 5-min at 10,000 rpm. Hemoglobin (Hb) was also measured in triplicate from whole blood via the cyanmethemoglobin technique, using a commercially available kit (Drabkin’s reagent, Sigma, Saint Louis, Missouri, USA). Percent change in plasma volume ($\% \Delta PV$) was calculated using the Dill and Costill equation (D.B. & D.L., 1974). Plasma (POsm) and urine osmolality (UOsm) were measured in duplicate via freezing-point depression from fresh samples (3250 Osmometer; Advanced Instruments Inc, Norwood, MA). Whole blood lactate was also measured at the end of each 5-km climb with a lactate analyzer (Accutrend Lactate, Roche Diagnostics, Mannheim, Germany). Post-exercise blood glucose was measured using the HemoCue 201+ Glucose analyzer (HemoCue Ltd, Dronfield, UK).

Statistical Analysis

All variables are presented as mean \pm standard deviation, unless stated otherwise. Differences in the mean values or the distributions of parameters between EUH and HYP were assessed using Students paired t-tests. Two-way (treatment x time) repeated measures of ANOVA were used to analyze differences in variables across time points between treatments. Post-hoc analysis for comparing mean

values between trials across time points, as well as different time points, was applied by using the sequential Bonferroni correction rule. Using data from a similar study (Bardis et al., 2017), an α of 0.05 and a statistical power of 0.8, it was estimated that 10 subjects would be required to reject the null hypothesis. All statistical analysis was performed using JMP Pro (version 13.0, SAS Inc., Gary, NC, USA). A value of $P < 0.05$ was regarded as statistically significant.

RESULTS

Familiarization Visit

During the three 20-min steady-state bouts, subject sweat loss was 0.3 ± 0.1 , 0.5 ± 0.1 , and 0.4 ± 0.2 L. During the 5-km time-trials, subject sweating rates were 1.3 ± 0.5 , 1.7 ± 1.0 , and 1.5 ± 0.6 L·h⁻¹. While drinking ad-libitum with favorable conditions for rehydration, subjects reached a mild level of hypohydration of $-0.8 \pm 0.8\%$ prior to the last time-trial and completed the familiarization visit at $-0.8 \pm 0.8\%$ body weight. Average power output for the first, second, and third 5-km time-trials were 298 ± 52 , 280 ± 47 , and 277 ± 62 W while average speeds during the time-trials were 26.7 ± 3.0 , 25.8 ± 2.9 , 25.6 ± 4.0 km·h⁻¹.

Blood and Body weight parameters

Pre-exercise body mass, USG, and POsm did not differ between EUH and HYP (Table 1, $P > 0.05$). Subjects finished the first, second, and third 5-km time-trials in the HYP trial with a cumulative water deficit of -0.4 ± 0.2 , -0.9 ± 0.2 , and -1.5 ± 1.0 kg, respectively. However, during the EUH trial, subjects maintained euhydration (-0.1 ± 0.2 , -0.1 ± 0.3 , -0.1 ± 0.4 kg). At the start of the second 5-km time-trial, subjects in the HYP trial were dehydrated by almost 1% of their body weight ($-0.9 \pm 0.2\%$), while subjects in the EUH trial remained euhydrated ($-0.1 \pm 0.1\%$). Prior to the last 5-km time-trial, subjects in the HYP trial were at a mild level of hypohydration at $-1.5 \pm 0.3\%$ body weight, and finished the entire protocol at $-1.8 \pm 0.2\%$ body weight. In the EUH trial, subjects were euhydrated approaching the last 5-km time-trial ($-0.1 \pm 0.4\%$ body weight) and maintained that euhydration through the last time-trial ($-0.1 \pm 0.5\%$ body weight). Subjects in both trials ingested 25 mL of tap water every 5-min (300 mL total) of the steady-state exercise, and another 25 mL every 1-km of the time-trials (375 mL total). However, in the EUH trial, subjects were also infused intravenously a total of 1.3 ± 0.4 L bringing the total fluid replacement for the EUH trial to 2.0 ± 0.4 L. Both the water ingested and the saline infused were warmed up to 37 °C to avoid

cooling effect. Despite constant infusion of 0.9% NaCl, POsm following the third time-trial in the EUH trial reached 303 ± 5 mmol·kg⁻¹ which did not differ from the HYP trial 302 ± 7 mmol·kg⁻¹ ($P>0.05$; Figure 1)

Thermoregulatory results

T_{re} was higher in the HYP trial than the EUH trial during the second steady-state from 15 min to 20 min ($P<0.05$; Figure 2) and maintained that higher core temperature during the second 5-km time-trial up to the last kilometer ($P<0.05$). Final T_{re} of the second time-trial for HYP and EUH was 38.9 ± 0.3 vs. 38.7 ± 0.5 °C ($P=0.06$). T_{re} was also higher in the HYP trial throughout the third steady-state ($P<0.05$) and continued to be higher throughout the third 5-km time-trial ($P<0.05$). Final T_{re} of the third time-trial for HYP and EUH was 39.1 ± 0.3 vs. 38.6 ± 0.6 °C. Mean T_{sk} was not different between HYP and EUH for the first steady-state (33.4 ± 0.9 vs. 33.5 ± 0.6 °C), first time-trial (both 33.5 ± 0.7 °C), second steady-state (33.1 ± 0.9 vs. 33.3 ± 0.6 °C), and second time-trial (33.3 ± 1.0 vs. 33.6 ± 0.8 °C; all $P>0.05$). Mean T_{sk} was higher, however, in the third steady-state from 10 minutes on (33.1 ± 1.1 vs. 33.5 ± 0.8 °C; $P<0.05$), but not during the third time-trial with final mean T_{sk} for HYP and EUH being 33.2 ± 1.2 vs. 33.6 ± 1.0 °C. Sweating rates for the HYP and EUH trials were not different for the first (1.5 ± 0.4 vs. 1.5 ± 0.9 L·h⁻¹) second (1.5 ± 0.6 vs. 2.0 ± 1.0 L·h⁻¹) and third time-trials (1.9 ± 1.0 vs. 1.7 ± 0.5 L·h⁻¹; all $P>0.05$).

Cardiovascular results

HR during the first (135 ± 13 vs. 134 ± 13 beats·min⁻¹), second (143 ± 16 vs. 140 ± 14 beats·min⁻¹), and third steady-states (146 ± 17 vs. 139 ± 14 beats·min⁻¹; all $P>0.05$) were not significantly different. Similarly, HR during the 5-km time-trials was near maximal and did not differ significantly between the two trials (HYP: 170 ± 16 , 171 ± 16 , and 174 ± 13 beats·min⁻¹; EUH: 173 ± 13 , 173 ± 14 , 174 ± 14 beats·min⁻¹, $P>0.05$). We were able to collect complete Q data only from four subjects, since several subjects were not willing to perform the measurement. Q during the third steady-state showed no differences between HYP (21.0 ± 1.0 L·min⁻¹) and EUH (21.3 ± 1.0 L·min⁻¹). Oxygen uptake data did not differ between EUH (2.7 ± 0.2 , 2.7 ± 0.3 , and 2.70 ± 0.3 L·min) and HYP trials (2.6 ± 0.2 , 2.8 ± 0.2 , and 2.7 ± 0.3 L·min; $P>0.05$)

Perceptual Results

After the first steady-state, no differences were observed in thirst perception (Figure 3) between HYP (40 ± 20 mm) and EUH trials (39 ± 24 mm; $P>0.05$) and this continued until the end of the second steady state (64 ± 26 mm vs. 50 ± 26 mm; $P>0.05$). However, during the third steady state, thirst perception

ratings were higher in the HYP trial at all timepoints (72 ± 29 , 72 ± 24 , and 79 ± 29 mm) compared to the EUH trial (46 ± 26 , 60 ± 32 , and 60 ± 32 mm; $P<0.05$). No differences in stomach fullness were observed between HYP and EUH trials following the first (46 ± 28 vs. 46 ± 26 mm), second (43 ± 27 vs. 37 ± 19 mm), and third steady states (35 ± 19 vs. 37 ± 22 mm; all $P>0.05$). Similarly, RPE responses (Figure 3) were similar throughout the protocol between HYP and EUH following the first (9 ± 2 vs. 9 ± 2), second (11 ± 2 vs. 11 ± 2), and third steady states (12 ± 2 vs. 11 ± 1 ; all $P>0.05$).

Performance Results

Average cycling speed during the first 5-km time-trial was not significantly different between EUH and HYP (27.3 ± 2.9 vs. 26.8 ± 3.0 km·h⁻¹, Figure 4). During the second and third time-trials, subjects displayed faster speed in the EUH trial (27.5 ± 3.0 and 27.2 ± 3.1 km·h⁻¹) compared to the HYP trial (26.2 ± 2.9 and 25.5 ± 3.3 km·h⁻¹; both $P<0.05$). Individual data for each time-trial is plotted in Figure 5. Interestingly, nine out of the eleven subjects performed the last time-trial better in the EUH trial than in the HYP trial, with one subject performing the same. Mean power output during the first time-trial was not different between the two experiments (EUH: 306 ± 52 vs. HYP: 297 ± 53 W; $P>0.05$). During the second and third time-trials, subjects produced higher power outputs in the EUH trial (309 ± 52 and 306 ± 55 W) compared to the HYP trial (287 ± 49 and 276 ± 54 W; both $P<0.05$; Figure 4).

DISCUSSION

This study investigated the effects of mild progressive dehydration on cycling performance while subjects were blinded to their hydration state. Our data indicated that subjects during the EUH trial performed better in two out of the three 5-km time-trials, as indicated by both speed and power output. We found that by the end of the protocol, core temperature in the HYP trial was greater than in the EUH trial, indicating that even a small degree of hypohydration could induce greater thermoregulatory strain. These findings occurred in a blinded manner, meaning the subject was blind to which trial they were in and their current hydration state. To our knowledge, this is the first study to use intravenous infusion to blind subjects while also implementing a drinking protocol to attempt to control for thirst responses.

Maintaining hydration status to avoid losing greater than 2% body weight has been well established in the literature (American College of Sports Medicine et al., 2007). However, several recent

studies have concluded that even mild levels of dehydration (<2% body weight) can negatively impact performance. Bardis et al. (Bardis et al., 2017) found that hypohydration of -1.8% body mass as a response to ad libitum drinking resulted in increased core temperature, lower cycling power output and slower time-trial performance. Below et al. (Below et al., 1995) showed that when cyclists were hypohydrated by less than -2%, their exercise performance declined by 6.5%. Walsh et al. (Walsh, Noakes, Hawley, & Dennis, 1994) examined cyclists' performance in 32 °C and found that the time to exhaustion at 90% VO_{2max} was decreased by 31% when participants started cycling in a mild hypohydrated state of -1.8%. In our study, subjects in the HYP trial had lower power output and cycling speed in the second and third time-trial when hypohydrated to -0.9 and -1.5%, respectively. Although not significant, the HYP trial had lower power output and slower cycling speeds in the first time-trial, meaning a dismal level of hypohydration of -0.5% was almost enough to significantly impair performance.

It is known that exercise increases body temperature (R J Maughan, Leiper, & Thompson, 1985), especially in warm environments. Further, hypohydration can play a detrimental role in exercise-induced hyperthermia (Montain & Coyle, 1992). We found that by the end of the second steady-state, core temperature was higher in the HYP trial than in the EUH trial, indicating that even a small degree of hypohydration induced greater thermoregulatory strain. The fact that sweating responses were similar between trials even though the core temperature was greater in the HYP trial indicated that sweat sensitivity deteriorates due to water deficit. It should be noted that the steady-states were meant to be a "cool-down" from the previous time-trial as well as functioning as a "warm-up" for the next time-trial. Despite this, core temperature continued to stay elevated in the HYP trial throughout the end of protocol.

Kenefick et al. (Robert W Kenefick et al., 2014) found that increasing skin temperature proportionally accentuated plasma volume shrinkage and any additional plasma volume loss likely results from increased net filtration rate at the capillaries. Despite several other studies concluding that skin temperature modifies the impact of hydration state on endurance performance (González-Alonso et al., 1999; R. W. Kenefick et al., 2010), no differences in skin temperature were found until the last steady-state in which the EUH trial had higher skin temperatures than the HYP trial.

Numerous studies have shown that dehydration impairs cardiovascular function during exercise (González-Alonso et al., 1997; Montain & Coyle, 1992; Wingo, Lafrenz, Ganio, Edwards, & Cureton,

2005) via elevated heart rate to compensate for the reduction in stroke volume (González-Alonso et al., 1999). In the present study, no differences were observed in heart rate during the steady-states or the time-trials. Further, no differences were observed in cardiac output either, which could mean that the small degree of hypohydration in the HYP trial was not enough to negatively impact the cardiovascular system, despite differences in percent changes in plasma volume. Therefore, the performance differences observed stem from more mechanistic actions such as skin blood flow or rather baroreceptor responses due to volume changes.

Recently, two separate studies have attempted to eliminate the bias of thirst on exercise performance and blind subjects to their fluid balance. Wall et al. (Wall et al., 2015) found that when cyclists were dehydrated by 3% of body mass, they exhibited higher core temperatures during their 25-km time-trial, despite having similar thirst perception ratings. Similar to our study, intravenous infusion of 0.9% NaCl was used to rehydrate subjects following exercise-induced dehydration of -3%, coupled with a sham IV infusion in another trial to ensure blinding. In the other study, Cheung et al. (Cheung et al., 2015) concluded that when cyclists were hypohydrated by 3% body mass they experienced higher core temperatures and heart rates during the last half of their 20-km time-trial. Further, the cyclists were provided mouth rinse of water whenever they desired to control for thirst, as both previous studies did not allow for drinking. The process of drinking water has been shown to reduce thirst, increase performance, and inhibit vasopressin release, via oropharyngeal stimulation (Arnaoutis et al., 2012; Figaro & Mack, 1997). In our study, subjects were provided a standardized amount of water to drink at regular intervals. This was designed to keep thirst perception low, albeit similar between trials. However, this technique of small volume ingestion was not able to control thirst during the high intensity nature of the protocol as seen elsewhere (Mears & Shirreffs, 2013; Mears, Watson, & Shirreffs, 2016).

In contrast to other performance based experiments, a unique aspect of our study was the implementation of a familiarization trial. In many published studies, however, there is no mention of adequate familiarization trials having been performed, nor is there any mention of whether the results were tested for the presence of an effect of trial order (Ronald J. Maughan, 2012). Further, an interesting note from the familiarization trial is the hydration habits of the subjects. During the familiarization trial, subjects were instructed to bring their own water bottles and drink ad libitum throughout the protocol.

Despite the low level of hypohydration in favorable conditions for drinking, the time-trial speeds were comparable to those in the HYP trial. In conclusion, the data showed that mild dehydration, even in a blinded manner, lead to performance impairment probably due to greater thermoregulatory strain.

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Table 1.

Variables	HYP		EUH	
	Pre	Post	Pre	Post
BW, kg	77.2±6.1	75.7±6.0*	77.4±6.2	77.4±6.2
ΔBW, %		-1.8±0.2*		-0.1±0.5
USG	1.012±0.008		1.013±0.008	
POsm, mmol·kg⁻¹	291±2	302±7	292±2	303±5
TPP, g·L⁻¹	7.4±0.4	8.0±0.7	7.4±.4	7.5±0.5
Glucose		93±18		100±25
ΔPV, %		-9.5±5.1*		2.8±5.9
Lactate		5.2±3.2		6.5±2.1

BW: Body Weight, ΔBW: Change in Body Weight, UOsm: Urine Osmolality, USG: Urine Specific Gravity, POsm: Plasma Osmolality, TPP: Total Plasma Protein, ΔPV: Changes in Plasma Volume. Values are presented as mean±SD.

* denotes statistically significant difference ($P<0.05$) between EUH and HYP trials at corresponding time.

FIGURE LEGENDS

Figure 1. Plasma osmolality during steady-states and time-trials between HYP and EUH.

Figure 2. Core temperature during steady-states and time-trials between EUH and HYP. *denotes statistically significant differences, $P < 0.05$ between trials at same time point. Values are presented as mean \pm SE

Figure 3. Thirst visual analog scale response during steady-states between EUH and HYP. *denotes statistically significant differences, $P < 0.05$ between trials at same time point. Values are presented as mean \pm SE

Figure 4. Mean cycling speed and mean power output during the 5-km cycling time-trials between HYP and EUH. *denotes statistically significant differences, $P < 0.05$ between trials. Values are presented as mean \pm SE

Figure 5. Individual performance data during the 5km time-trials in HYP and EUH trials plotted with a line of identity. Each point represents a different individual participant's average speed for that trial.

Figure 1.

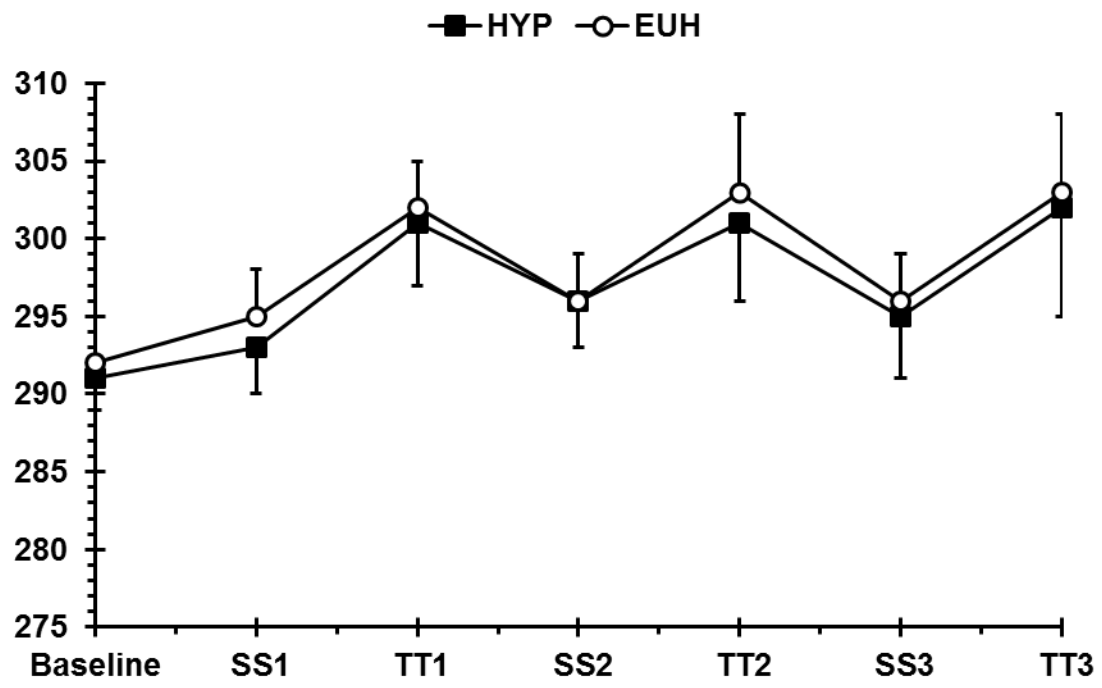


Figure 2.

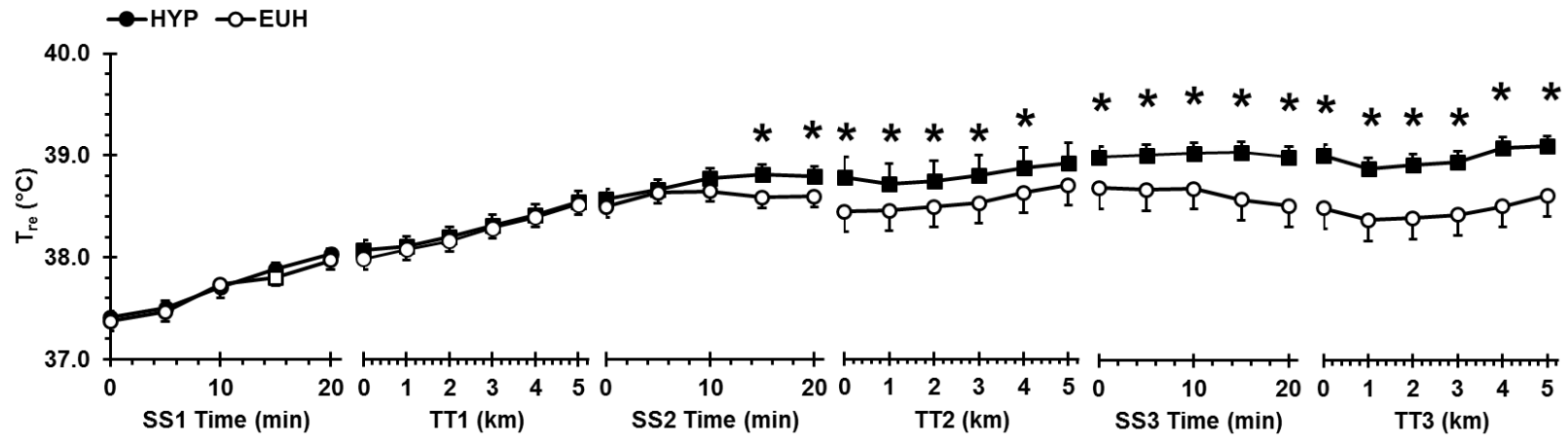


Figure 3.

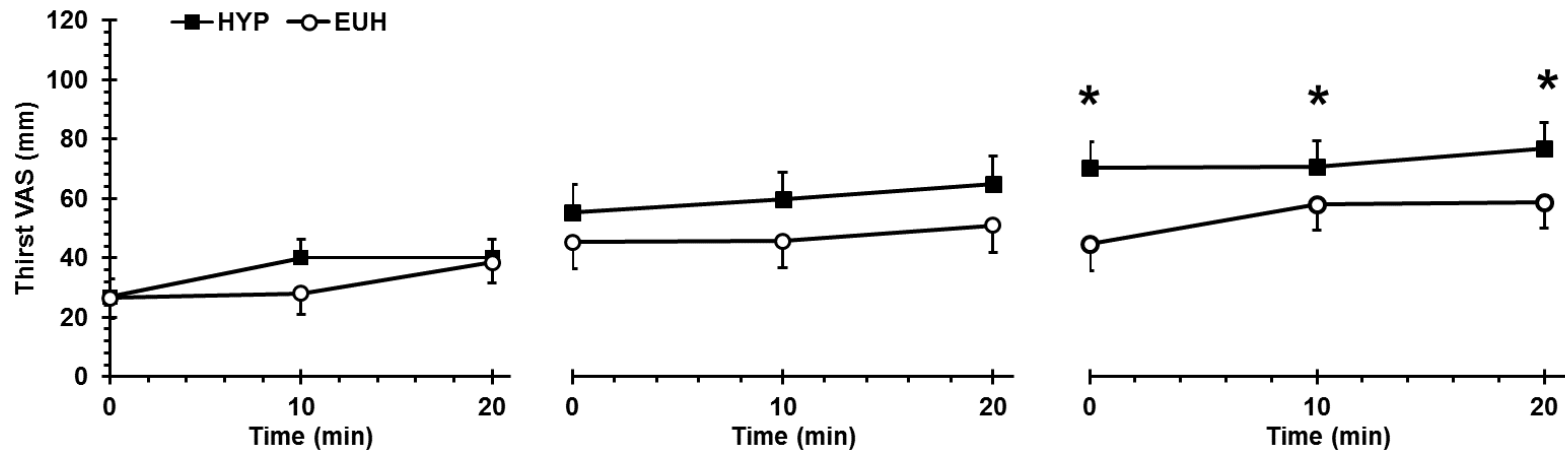


Figure 4.

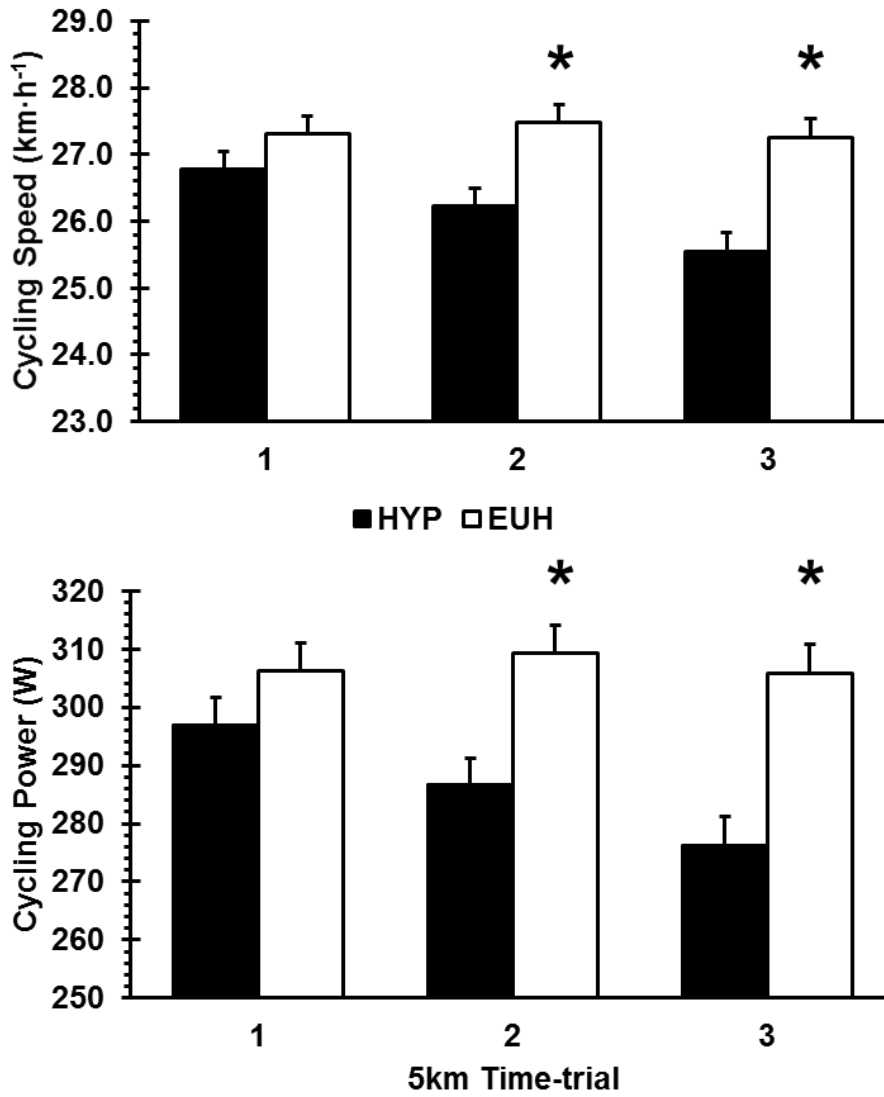
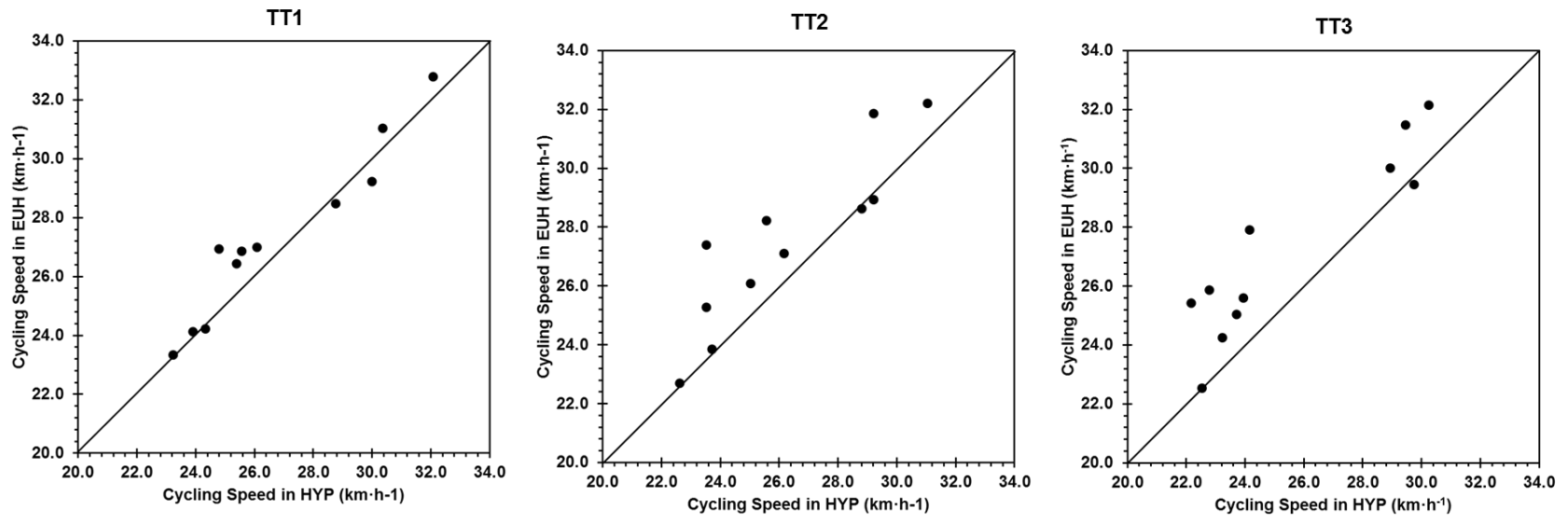


Figure 5.



IV. **Conclusions**

These two studies sought to determine if dehydration impairs exercise performance in male cyclists even in a blinded manner. To investigate this, we used multiple blinding methods in the subjects to manipulate hydration status (i.e., nasogastric tubes, intravenous infusion). Further, we utilized these to maintain euhydration or to blind a subject to their progressive dehydration. Lastly, we observed these methods under warm-dry conditions to stress the system and to detect the mechanisms as to which the performance impairment is coming from (i.e., thermoregulatory strain, cardiovascular strain, etc.).

In Study 1, subjects had higher power output and faster cycling speeds when they were euhydrated compared to being dehydrated. These performance differences were observed despite no differences in thirst and stomach fullness, which ensured the investigators that the subjects were blinded to their protocol. Further, the subjects experienced higher heart rates during their steady-state as well as higher core temperatures during the time-trial, ensuing that the impairment stemmed from some sort of physiological strain brought on by the elevated cardiovascular and thermoregulatory responses, rather than the perceptual responses.

In Study 2, subjects had faster cycling speeds, higher power outputs, and faster completion times when they were euhydrated compared to being dehydrated. In this study, the differences in performance were paralleled with differences in core temperature as the subjects in the dehydrated trial had higher core temperature throughout the last half of the protocol compared to the euhydrated trial. However, the performance impairment observed was unlikely caused by cardiovascular strain, as there were no differences in either heart rate or cardiac output during the protocol.

These data suggest that during exercise-heat stress, dehydration impairs exercise performance, even in a blinded manner. Moreover, the impairment appears to stem from thermoregulatory strain rather than cardiovascular strain, in the milder settings of dehydration (<2%). In addition, these studies were conducted without the knowledge of one's hydration status which takes away the perceptual bias experiences in previous studies using other methods of hydration manipulation. The exact mechanisms of the impaired exercise performance are not clear, but further research is needed utilizing more in-depth techniques for both cardiovascular and thermoregulatory responses (skin blood flow, blood pressure, etc.).

Appendix A



Office of Research Compliance
Institutional Review Board

October 14, 2015

MEMORANDUM

TO: J.D. Adams Lisa Jansen
Alison Schroeder Jillian Fry
Tracie Kirkland Jordan Smith
Yasuki Sekiguchi Cameron Nichols
Stavros Kavouras

FROM: Ro Windwalker
IRB Coordinator

RE: New Protocol Approval

IRB Protocol #: 15-02-507

Protocol Title: *Effect of Mode of Fluid Ingestion on Cycling Performance*

Review Type: EXEMPT EXPEDITED FULL IRB

Approved Project Period: Start Date: 10/14/2015 Expiration Date: 09/13/2016

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form *Continuing Review for IRB Approved Projects*, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (<https://vpred.uark.edu/units/rscp/index.php>). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

This protocol has been approved for 30 participants. If you wish to make *any* modifications in the approved protocol, including enrolling more than this number, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 109 MLKG Building, 5-2208, or irb@uark.edu.

Appendix B



UNIVERSITY OF ARKANSAS

Office of Research Compliance
Institutional Review Board

October 27, 2016

MEMORANDUM

TO: J.D. Adams Lisa Jansen
Adam Seal Hyun-Gyu Sean Suh
Elika Hamer Charlayne Mitchell
Natasha Brand Cameron Sprong
Dylan Scott Cory Butts
Stavros Kavouras

FROM: Ro Windwalker
IRB Coordinator

RE: New Protocol Approval

IRB Protocol #: 16-10-135

Protocol Title: *The Role of Hydration on Exercise Performance - ODIN*

Review Type: EXEMPT EXPEDITED FULL IRB

Approved Project Period: Start Date: 10/27/2016 Expiration Date: 10/13/2017

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form *Continuing Review for IRB Approved Projects*, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (<https://vpred.uark.edu/units/rscp/index.php>). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

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If you have questions or need any assistance from the IRB, please contact me at 109 MLKG Building, 5-2208, or irb@uark.edu.