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Perceived dehydration impairs endurance cycling performance in the heat in active males

Mark P. Funnell^{a,1}, Jodie Moss^{a,1}, Daniel R. Brown^{a,b}, Stephen A. Mears^a, Lewis J. James^{a,*}

^a School of Sport, Exercise and Health Sciences, Loughborough University, Leicestershire, LE11 3TU, UK

^b Centre for Human Performance, Carnegie School of Sport, Leeds Beckett University, Leeds, LS6 3QT, UK

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ABSTRACT

Dehydration of >3 % body mass impairs endurance performance irrespective of the individual's knowledge of their hydration status, but whether knowledge of hydration status influences performance at lower levels of dehydration is unknown. This study examined whether perception of hydration status influenced endurance performance. After familiarisation, nine active males (age 25 ± 2 y, VO_{2peak} 52.5 ± 9.1 mL kg min^{-1}) completed two randomised trials at 34 °C. Trials involved an intermittent exercise preload (8 × 10 min cycling/5 min rest), followed by a 15 min all-out cycling performance test. During the preload in both trials, water was ingested orally every 10 min (0.3 mL kg body mass⁻¹), with additional water infused into the stomach via gastric feeding tube to produce dehydration of ~1.5 % body mass pre-performance test. Participants were told intra-gastric infusion was manipulated to produce euhydration (0 % dehydration; Perceived-EUH) or dehydration (2 % dehydration; Perceived-DEH) pre-performance test, which was told to them pre-preload and confirmed after body mass measurement pre-performance test. Body mass loss during the preload (Perceived-EUH 1.6 ± 0.2 %, Perceived-DEH 1.7 ± 0.2 %; $P = 0.459$), heart rate, gastrointestinal temperature and RPE ($P \geq 0.110$) were not different between trials. Thirst was greater at the end of the preload and performance test in Perceived-DEH ($P \leq 0.040$). Work completed during the performance test was 5.6 ± 6.1 % lower in Perceived-DEH (187.4 ± 37.0 kJ vs. 176.9 ± 36.0 kJ; $P = 0.038$). These results suggest that at lower levels of dehydration (<2 % body mass), an individual's perception of their hydration status could impair their performance, as well as their thirst perception.

1. Introduction

The effect of hydration status on endurance performance has been well-studied, with most studies reporting that dehydration impairs endurance performance, particularly when exercise takes place in warm/hot environmental conditions [1,2]. The impairment in performance likely derives from an array of both physiological (i.e., increased cardiovascular and thermal strain [3], reduced muscle [4] and cerebral bloodflow [5], and increased muscle glycogenolysis [6]) and perceptual mechanisms (i.e., increased thirst [7] and perception of effort [8] and compromised mood state and pain sensation [9]). Despite the wealth of research reporting that dehydration impairs endurance performance, debate continues around whether dehydration actually influences endurance performance [2,10]. Some of this debate has centered around the typical methods used in studies examining hydration status and

endurance performance [2,10–12].

One potential methodological issue with previous hydration research is the overtness of the intervention used, with all but a handful of recent studies [8,13–17] performing unblinded trials. Methods used to induce dehydration include severe/complete restriction of fluid in the days leading up to exercise [18] or during a preceding bout of exercise [19], or the administration of a diuretic drug to increase urine output [20]. In all cases, the order of the treatments/trials will be obvious to the participants and therefore placebo, nocebo or expectation effects might influence the results reported. Many nutritional interventions including carbohydrate [21], caffeine [22,23], sodium bicarbonate [24,25] and pre-exercise feeding [26,27] have been shown to have placebo effects on performance and it is possible that hydration status/water intake might also exert a similar effect.

Some recent studies using intra-gastric delivery of water to

* Corresponding author at: National Centre for Sport and Exercise Medicine, School of Sport, Exercise and Health Sciences, Loughborough University, UK.

E-mail address: L.James@lboro.ac.uk (L.J. James).

¹ Joint first authors.

manipulate hydration status have reported that dehydration impairs endurance cycling performance in the heat (at least in males) even when participants were blinded to their hydration status [8,14,16]. In these studies, dehydration of $>2\%$ was induced, suggesting that, consistent with the current scientific consensus [28], dehydration above this level likely negatively impacts performance. However, many factors should be considered when interpreting research findings, such as training status, environmental conditions, facing airflow, the performance criteria investigated, and mode of dehydration utilised [2].

Importantly, [16] tested two groups of trained cyclists, one blinded to the intervention and one unblinded, reporting that dehydration of $\sim 3\%$ body mass at the start of a time trial lasting ~ 15 min impaired performance to the same extent in both groups ($\sim 10\text{--}11\%$). This suggests that placebo/nocebo effects do not influence performance responses when dehydration is moderate in nature (i.e., the equivalent of $\sim 3\%$ body mass). However, studies have reported performance impairments at levels of dehydration less than 2% body mass [29–31] and sometimes when there are no obvious physiological differences between conditions. Whether any of these reported results are due to the participants knowing the treatment they are undertaking in each trial is not clear.

Therefore, this study aimed to examine whether a participant's belief of the trial they were completing (i.e., verbally informed euhydrated or dehydrated) influenced their performance during an endurance cycling performance test in the heat, whilst controlling their hydration status at a similar level in both trials. It was hypothesised that performance would be impaired when participants believed they were dehydrated.

2. Methods

2.1. Participants

Nine active healthy males (mean \pm SD: age 25 ± 2 y, height 1.77 ± 0.09 m, body mass 75.3 ± 9.9 kg, VO_{2peak} 52.5 ± 9.1 mL kg $^{-1}$ min $^{-1}$, W_{peak} 288 ± 38 W) completed a medical screening questionnaire and gave written and verbal consent before completing the study. The study received ethical approval from the Loughborough University Ethics Approvals (Human Participants) Sub Committee (Reference: R15-P045). Participants were physically active and were either trained cyclists or experienced with stationary cycling, but none were heat acclimated at the time of the study. Using the data from a previous experiment involving the same performance test [8], an α of 0.05 and a statistical power of 0.8, it was estimated that at least eight participants would be required to detect an 8% difference in endurance performance between trials.

2.2. Experimental overview, study blinding & cover story

Each participant completed three preliminary trials, followed by two experimental trials, each separated by ≥ 7 days (Fig. 1). To provide an adequate cover story and blind participants from the true aim of the study, participants were told there were three experimental trials (i.e., the final preliminary trial and the two actual experimental trials). These three trials in the heat (34°C , 50% relative humidity) involved a 120 min intermittent preload (8 blocks of 10 min cycling, 5 min rest),

followed by a 15 min performance test.

A gastric feeding tube (8 Fr, Sonde Gastro-duodenal Type Levin, Vygon Ltd, Cirencester, UK) was positioned orally or nasally (depending on participant preference) before the preload and used to deliver water to the stomach without the participant's knowledge. Participants were told the three trials involved one dehydrated trial where dehydration equivalent to $\sim 2\%$ body mass was induced during the preload, and two rehydrated trials with a different rehydration drink used in each trial to maintain euhydration ($\sim 0\%$ body mass loss). Participants were told the gastric tube was used to allow blinding of the rehydration drinks and they would not find out the details of the drinks until the end of the study, but that we would tell them whether they were undertaking a dehydrated or rehydrated trial. Really, the first trial was used to fully familiarise participants to the protocol and to allow precise manipulation of hydration status during the second and third trials, but participants were told this was a rehydrated trial. In the second and third trials, participants were, in a randomised order, told trials were dehydrated or euhydrated immediately before the preload, but fluid provided both orally and through the gastric tube was identical and at a volume estimated to produce a body mass loss of $\sim 1.5\%$ by the end of the preload (i.e., mild dehydration). This meant we produced two experimental trials where fluid intake was identical, but in which participants believed they were dehydrated (Perceived-DEH) or believed they were euhydrated (Perceived-EUH) at the start of the performance test. To further assist with the cover story, during pre-performance test body mass measurement and whilst participants were behind a screen, they were told they were 'dehydrated by $\sim 2\%$ of their body mass' in Perceived-DEH or they had 'maintained body mass and hydration status', in the Perceived-EUH trial.

2.3. Preliminary trials

During the first preliminary trial, body mass (AFW-120 K, Adam Equipment Co., Milton Keynes, UK) and height were measured. Cycling peak oxygen uptake (VO_{2peak}) and power output (W_{peak}) were determined (Lode Corival, Groningen, The Netherlands) using a progressive exercise test commencing at 95 W and increasing by 35 W every 3 min until volitional exhaustion. After completion of the maximal exercise test, participants were familiarised with the insertion of an 8 Fr gastric feeding tube which was inserted either orally or nasally (depending on individual preference) to a depth estimated to place the tube at the base of their stomach. A practice of the performance test was then completed. At the second preliminary visit, participants completed two of the eight preload stages used in experimental trials (2×10 min cycling, 5 min rest) in a controlled environment, followed by the 15 min performance test. Body mass change during the second stage was used to prescribe fluid intake for the third preliminary trial. The third preliminary trial was used to familiarise participants with the entire protocol and was identical to the two experimental trials with the exception that water was provided through the gastric tube to maintain euhydration. This preliminary trial was used as part of the cover story and participants were told it was a rehydrated trial where euhydration was maintained.

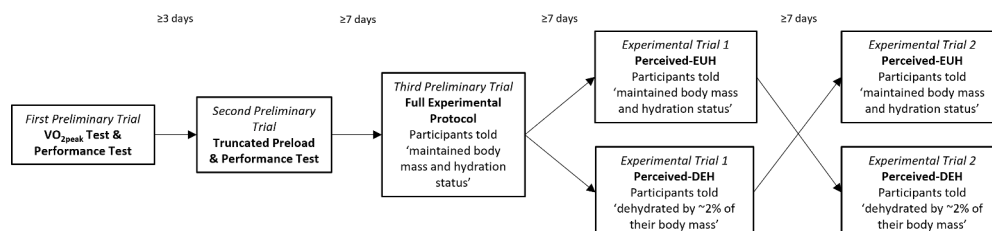


Fig. 1. Schematic of the study design. Participants completed the two experimental trials in a randomised, counter-balanced, cross-over design.

2.4. Pre-trial standardisation

For the 24 h preceding the third visit (i.e., final preliminary trial), participants recorded their dietary intake and physical activity, and replicated these patterns before the two experimental trials, with adherence verbally checked upon arrival for trials. Strenuous exercise and alcohol intake were not permitted during this period. To ensure adequate fluid intake before experimental trials, participants were instructed to consume a minimum of 40 mL kg body mass⁻¹ fluid the day before trials. This was distributed as 8 mL kg body mass⁻¹ fluid during the morning, 16 mL kg body mass⁻¹ fluid during the afternoon, and 16 mL kg body mass⁻¹ fluid during the evening. Participants consumed a standardised breakfast consisting of carbohydrate sports drink and cereal bars (providing 1.5 g carbohydrate•kg body mass⁻¹ and 8 mL kg body mass⁻¹) 1.5 h before arriving at the laboratory. Participants also ingested a disposable temperature sensor capsule (CorTemp sensor, HQInc, Palmetto, USA) at 22:00 the night before each trial for measurement of gastrointestinal temperature during trials.

2.5. Experimental trials

Trials began in the morning at a time standardised within participants, between 08:00–09:00. Upon arrival, participants voided their bladder into a plastic container, which was used to immediately determine osmolality (Osmocheck Digital Refractometer, Vitech Scientific Ltd, Partidge Green, UK) with a urine osmolality of <900 mOsm kg H₂O⁻¹ required for trial participation [32]. No participant produced a urine sample of >700 mOsm kg H₂O⁻¹. Apart from this urine sample collected upon arrival, no other urine samples were collected to avoid alerting participants to the true purpose of the study. Nude body mass was then measured behind a private curtain/screen to the nearest 0.01 kg using a digital scale (AFW-120K, Adam Equipment Co., Milton Keynes, UK). Participants then inserted an 8 Fr gastric feeding tube (orally or nasally; standardised within participants) to the base of their stomach, before a heart rate monitor (Polar Beat, Kempele, Finland) was attached. The gastric tube was placed behind the ear and taped (Transpore, 3 M Health Care, St Paul, MN) onto the upper back, so water was infused outside the participants field of vision. Thereafter, participants entered a climatic chamber maintained at 34.8 ± 0.4 °C and 56.5 ± 3.0 % relative humidity. After 15 min rest on the cycle ergometer, resting measures of heart rate, gastrointestinal temperature, and thirst (100 mm visual analogue scale; 0 mm = “not at all thirsty”, 100 mm = “extremely thirsty”; [8]) were recorded.

Participants then completed an intermittent exercise preload consisting of eight blocks of 10 min cycling at 50 % W_{peak}, each separated by 5 min rest in the environmental chamber (i.e., preload). Facing airflow (~2 m s⁻¹) was provided by two fans placed in front of the cycle ergometer, one aimed at the upper body, and one at the lower body. During both trials, participants orally ingested 0.3 mL kg body mass⁻¹ water every 10 min of the preload, this water was located outside of the environmental chamber and was 18.7 ± 1.1 °C. Additional water was infused directly into the stomach through the gastric feeding tube every 5 min during the preload to produce body mass loss of ~1.5 %, based on the final preliminary trial. The infusion process was identical in each trial, with an investigator connecting a syringe to the gastric feeding tube and delivering the water over ~1 min. The infused water was maintained (in a temperature-controlled water bath) at 35.2 ± 0.6 °C to remove any sensation of cold water passing through the tube and prevent cooling.

Heart rate and gastrointestinal temperature were measured during the last min of each 10 min exercise block. Rating of perceived exertion (RPE; 6–20 scale; [33]), thermal sensation (-10 to +10 scale; [34]), and stomach fullness and bloating (0–12 scale; [8]) were recorded during the final min of exercise in the 1st, 4th and 8th blocks (i.e., 10, 55 and 115 min). Expired gas was collected during the final min of exercise in the 4th (54–55 min) and 8th (114–115 min) blocks using the Douglas bag

method, with O₂ and CO₂ content (Servomex 1400 Gas Analyzer, Servomex), volume (Harvard Dry Gas Meter, Harvard Apparatus) and temperature determined. Ambient air was collected simultaneously with expires gas samples to correct VO₂ and VCO₂ values [35]. Carbohydrate and fat oxidation rates were determined using the method of [36]. Ambient temperature and relative humidity were recorded during the final min of exercise in the 1st, 4th and 8th blocks (i.e., 10, 55 and 115 min) (Kestrel 4400, Nielsen–Kellerman Co.). At the end of the preload, the gastric feeding tube was removed, nude body mass was measured, and thirst sensation recorded. Participants then completed a 15 min cycling performance test.

2.6. Performance test

Participants were given a standard set of verbal instructions before each performance test and instructed to complete as much work as possible in 15 min. The workload was initially set to 90 % W_{peak} and participants could increase or decrease the workload by pressing up or down on the ergometer’s console. No encouragement was given to the participants and the only feedback provided was the time remaining. A screen separated the participant from the researcher to minimise distractions. Work completed, heart rate and gastrointestinal temperature were recorded every 5 min without disturbing the participant. This performance test has been shown to be a reliable performance measure in recreationally active individuals with a mean coefficient of variation of 1.0 % (range: 0.2–1.8 %) after two familiarisation trials [37].

2.7. Post-study interview

All participants were interviewed upon completion of the final experimental trial to determine the success of the blinding, and their views/knowledge of dehydration and exercise performance. Participants were asked six questions: (1) dehydration impairs athletic performance (agree/undecided/disagree); (2) what is the smallest percentage of body mass loss/dehydration when performance is impaired?; (3) 2 % body mass loss/dehydration has what effect on performance, -6 to +6 [-6 = “extreme decrease in performance”, -4 = “very decreased performance”, -2 = “slight decreased performance”, 0–2 = “no effect”, +2 = “slightly increased performance”, +4 = “very increased performance”, +6 = “extreme increase in performance”]; (4) could you tell the difference between the two rehydration drinks?; (5) what do you think the two rehydration drinks were?; (6) the study was not investigating rehydration drinks, what do you think the purpose of the study was?. Upon completion of the post-study interview, participants were informed of the true purpose of the study.

2.8. Statistical analysis

Data were analysed using SPSS (version 27, IBM SPSS Inc., Illinois, USA) and were initially checked for normality of distribution using a Shapiro-Wilk test. Data containing one factor (i.e., trial) were analysed using paired *t*-tests for normally distributed data, and Wilcoxon signed rank tests for non-normally distributed data. Data containing two factors (i.e., time and trial) were analysed using a two-way repeated measures ANOVA. Where the assumption of sphericity was violated, the degrees of freedom were corrected using the Greenhouse-Geisser estimate. Significant interaction effects were followed-up by *post-hoc* paired *t*-tests or Wilcoxon signed rank tests, as appropriate. The familywise error rate was controlled using the Holm-Bonferroni correction. Relationships between variables were assessed by Pearson’s correlation coefficient. Gastrointestinal temperature data includes seven participants as the CorTemp capsule did not work during one experimental trial for two participants. Expired gas analyses and substrate oxidation data include eight participants due to collection error during one experimental trial for one participant. Data sets were accepted as being significantly different when *P* < 0.05. All data are presented as mean ± SD.

3. Results

3.1. Trial conditions

No differences were present for ambient temperature (Perceived-EUH 34.8 ± 0.4 °C, Perceived-DEH 34.8 ± 0.3 °C; $P = 0.561$) or relative humidity (Perceived-EUH 56.4 ± 3.1 %, Perceived-DEH 56.7 ± 3.0 %; $P = 0.280$) between trials. There were no differences between trials for pre-trial body mass (Perceived-EUH 75.8 ± 9.2 kg, Perceived-DEH 75.9 ± 9.5 kg; $P = 0.644$) or thirst sensation ($P = 0.419$; Fig. 2).

3.2. Fluid balance measures

There were no differences in body mass loss during the preload (Perceived-EUH 1.2 ± 0.2 kg, Perceived-DEH 1.3 ± 0.2 kg; $P = 0.342$) or performance test (Perceived-EUH 0.5 ± 0.1 kg, Perceived-DEH 0.6 ± 0.2 kg; $P = 0.519$) between trials. Percentage change in body mass during the preload (Perceived-EUH 1.6 ± 0.2 %, Perceived-DEH 1.7 ± 0.2 %; $P = 0.459$) and entire trial was not different between trials (Perceived-EUH 2.4 ± 0.3 %, Perceived-DEH 2.4 ± 0.4 %; $P = 0.438$).

There were time ($P < 0.001$), trial ($P = 0.009$), and interaction ($P = 0.034$) effects for thirst sensation. Thirst sensation increased from pre-exercise to the end of the preload and end of the performance test in both trials, but was greater at the end of the preload ($P = 0.040$) and end of the performance test ($P = 0.033$) in Perceived-DEH (Fig. 2).

3.3. Preload responses

There were main effects of time for heart rate, gastrointestinal temperature, and RPE (all $P < 0.001$), with all increasing progressively throughout the preload. There were no differences in heart rate ($P = 0.110$; Fig. 3A), gastrointestinal temperature ($P = 0.182$; Fig. 3B), RPE ($P = 0.110$; Fig. 4A), or thermal sensation ($P = 0.647$; Fig. 4B) between trials. There were no differences in ratings of stomach fullness ($P = 0.138$; Fig. 4C) or bloating ($P = 0.098$; Fig. 4D) between trials.

There were no time ($P = 0.512$) or interaction ($P = 0.620$) effects for VO_2 , but there was a main effect of trial ($P = 0.014$). Mean VO_2 during the preload was higher in Perceived-DEH (Perceived-EUH 2.12 ± 0.31 L min^{-1} , Perceived-DEH 2.23 ± 0.34 L min^{-1} ; $P = 0.014$). There were no time, trial, or interaction effects for VCO_2 between trials ($P \geq 0.279$; data not displayed). There were no time ($P = 0.353$), trial ($P = 0.065$), or interaction effects ($P = 0.792$) for carbohydrate oxidation between trials (mean carbohydrate oxidation: Perceived-EUH 2.33 ± 0.48 g min^{-1} , Perceived-DEH 2.16 ± 0.54 g min^{-1}). There were no time ($P = 0.111$) or interaction ($P = 0.925$) effects for fat oxidation, but there was a main effect of trial ($P = 0.004$). Mean fat oxidation during the preload was higher in Perceived-DEH (Perceived-EUH 0.20 ± 0.22 g min^{-1} ,

Perceived-DEH 0.30 ± 0.19 g min^{-1} ; $P = 0.012$).

3.4. Performance test

Total work completed during the performance test was lower in Perceived-DEH ($P = 0.038$; Fig. 5A). Seven of the nine participants completed less work during the performance test in Perceived-DEH (range: -17.3 % to $+1.7$ %; Fig. 5A), with an average decrement of 5.6 ± 6.1 %. To determine the pacing strategy, the performance test was separated into 5 min segments. The amount of work completed during Perceived-EUH compared to Perceived-DEH between 0 and 5 min ($P = 0.176$), 5–10 min ($P = 0.088$), and 10–15 min ($P = 0.206$) was not different between trials (Fig. 5B). Heart rate and gastrointestinal temperature increased throughout the performance test but were not different between trials (heart rate $P = 0.327$, Fig. 3A; gastrointestinal temperature $P = 0.609$, Fig. 3B). There was a strong, non-significant, correlation (Pearson's $r = -0.618$ [strong]; $P = 0.076$) between the percentage change in performance and the difference in thirst sensation between trials at the end of the preload.

3.5. Post-study interview

Responses from individual participants to the post-study questions are displayed in Table 1. Based on the knowledge questions (Q1–3), participants had an understanding of the potential implications of dehydration on exercise performance, and had preconceived thoughts that dehydration impairs exercise performance. From the responses of Q4–6, the blinding of the study was successful, with no participant correctly describing what the rehydration drinks were, eight participants incorrectly describing the purpose of the study, and one participant answering “level of dehydration” to Q6 once they were informed the true purpose was not rehydration drinks.

4. Discussion

This study examined whether participants' perception of the trial they were completing (i.e., euhydrated or dehydrated) influenced their endurance cycling performance in the heat, whilst controlling their hydration status to a similar level in both trials (~ 1.6 % body mass loss at the beginning of the performance test). The main finding was that when participants believed they were ‘dehydrated by ~ 2 % of their body mass’, cycling endurance performance was impaired by ~ 6 %, despite no difference in their hydration status. The decrement in performance occurred despite no differences in heart rate, rating of perceived exertion, thermal sensation, or gastrointestinal temperature between trials. The decrement in performance likely derived from predispositions/knowledge that dehydration impairs performance and/or increased thirst sensation.

Eight out of nine participants stated that they ‘agree’ that dehydration impairs athletic performance, and eight out of nine believed that 1 or 2 % body mass loss was the threshold for which performance was impaired. These responses indicate that the participants recruited had an understanding of the potential implications of dehydration on exercise performance [28], and the predispositions/knowledge that dehydration impairs performance may have resulted in a nocebo/placebo effect that led to the lesser performance in the perceived dehydration condition (i.e., when participants were told they were ‘dehydrated by ~ 2 % of their body mass’) compared to the perceived euhydrated condition. Similar nocebo/placebo effects have been evidenced in other exercise nutrition research, including carbohydrate [21], caffeine [22, 23], sodium bicarbonate [24,25] and pre-exercise feeding [26,27].

Thirst sensation was greater at both the end of the preload and end of the performance test when participants believed they were ‘dehydrated by ~ 2 % of their body mass’. Participants reported greater thirst despite no differences in body mass loss, or fluid intake. The alteration in thirst sensation likely derived from predispositions/previous experience(s) of

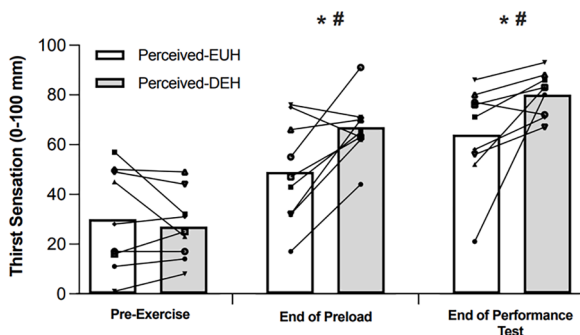


Fig. 2. Thirst sensation pre-exercise, at the end of the preload, and end of the performance test during Perceived-EUH and Perceived-DEH trials. Lines represent individual participants. * denotes Perceived-DEH significantly different from Perceived-EUH. # denotes significantly different from Pre-Exercise within trial.

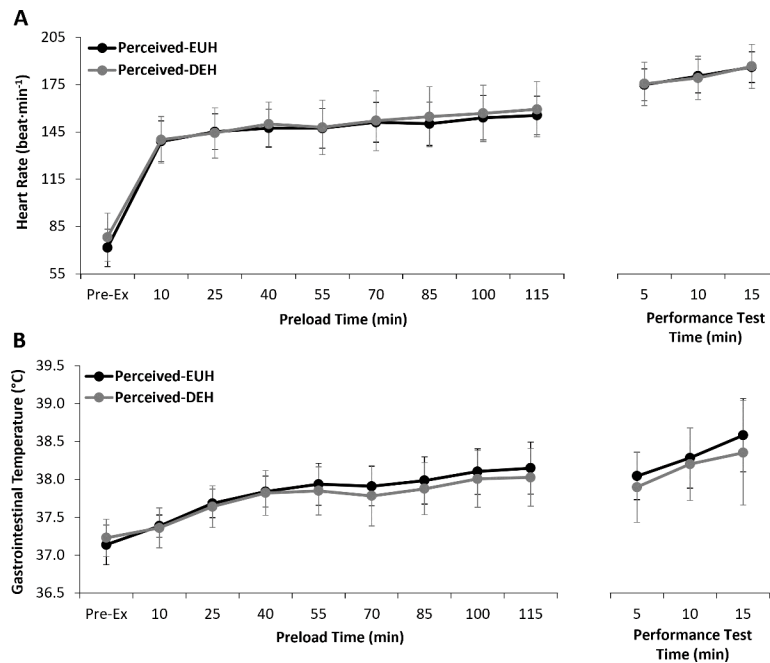


Fig. 3. (A) heart rate and (B) gastrointestinal temperature ($n = 7$) during the preload and performance test for Perceived-EUH and Perceived-DEH trials.

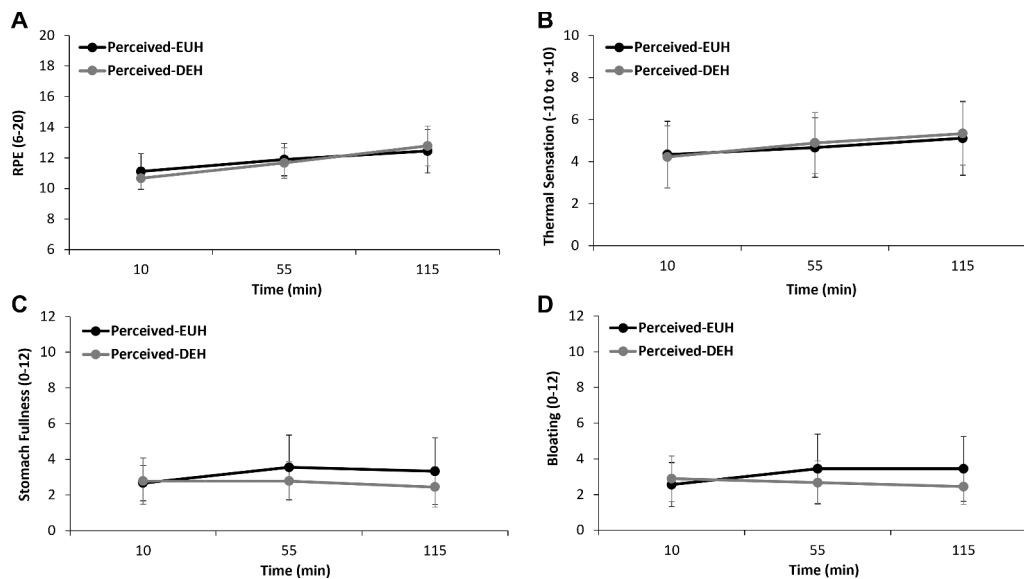


Fig. 4. (A) rating of perceived exertion, (B) thermal sensation, (C) stomach fullness, and (D) bloating for Perceived-EUH and Perceived-DEH trials.

increased thirst associated with dehydration, rather than altered physiological stimulus between trials, for example, increased plasma osmolality, however, this cannot be confirmed. Thirst perception is a key signal in water balance regulation as it prompts drinking or fluid seeking behaviour if no drink is available [38]. Exercise induced dehydration leads to increased thirst sensation [1,8,16], which is likely at least one of several factors that might explain why dehydration impairs endurance performance [2]. Indeed, some researchers suggest that thirst, and not fluid loss, may explain the impaired performance with dehydration [10, 39]. However, to date, no study has isolated the effect of thirst on performance.

On the contrary, [14] used intragastric rehydration to produce blinded euhydration or dehydration (~ 2.2 % body mass loss) at the end of 120 min of exercise. Water (25 mL) was ingested every 5 min of the 120 min to suppress thirst. Thirst was similar between trials, but 5 km

time trial performances was ~ 6 % slower with dehydration, suggesting dehydration impaired performance independent of thirst. These results should not be interpreted that thirst does not contribute to dehydration-induced impairments in performance, but rather that the negative effects of dehydration are not completely mediated by thirst. In the present study, there was a strong, albeit non-significant, correlation (Pearson's $r = -0.618$ [strong]; $P = 0.076$) between the percentage change in performance and the difference in thirst sensation between trials at the end of the preload. Therefore, the present study suggests that the increase in thirst sensation likely contributed in some degree to the decrement in performance in the perceived dehydrated trial [2,11,39]. Nevertheless, the increase in thirst sensation in the perceived dehydrated trial further reinforces that the study blinding was successful.

Mean VO_2 and fat oxidation were greater during the preload when participants perceived they were dehydrated. Alterations in substrate

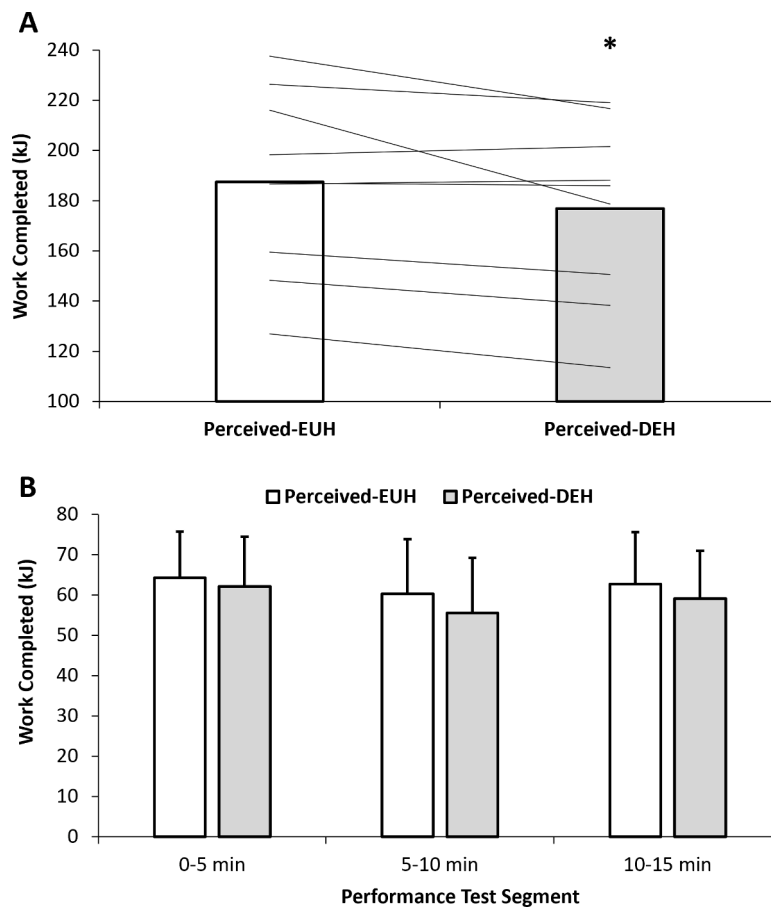


Fig. 5. (A) total work completed during the 15 min performance test, and (B) work completed during each 5 min segment of the performance test during Perceived-EUH and Perceived-DEH trials. Lines represent individual participants. * denotes Perceived-DEH significantly different from Perceived-EUH.

Table 1
Individual participant post-study interview responses.

Participant	Post-trial question					
	Dehydration impairs athletic performance (agree/undecided/disagree)	What is the smallest percentage of body mass loss/dehydration when performance is impaired?	2 % body mass loss/dehydration has what effect on performance, -6 to +6?	Could you tell the difference between the two rehydration drinks?	What do you think the two rehydration drinks were?	The study was not investigating rehydration drinks, what do you think the purpose of the study was?
1	Agree	2	-2	No	CHO vs CHO & Protein	Do not know
2	Agree	2	-2	“1st (Perceived-EUH) better than 2nd (Perceived-DEH)”	CHO vs Protein	Do not know
3	Agree	1	-3	No	Salt or Electrolyte/CHO	Gastric effects
4	Agree	3	0	No	Different electrolyte composition	Level of dehydration
5	Undecided	2	-1	No	Do not know	Do not know
6	Agree	2	-2	No	CHO	Do not know
7	Agree	2	-2	“More hydrated and full than 1st trial (Perceived-EUH)”	Do not know	Fullness
8	Agree	1	-4	No	Different electrolyte composition	Different fluid volumes
9	Agree	2	-3	“1st (Perceived-DEH) slightly more bloated, but nothing obvious”	Do not know	Do not know

CHO = carbohydrate.

oxidation, but not VO_2 , have been shown in other dehydration and exercise studies as early as 10 min into a steady-state cycling preload, when differences in hydration status would not be present/pronounced [16]. Dehydration also altered muscle glycogen use as early as 0–45 min into a 2 h exercise bout, when dehydration was very low [6]. The present study suggests some of these alterations in exercise metabolism may have manifested due to psychological mechanisms, however, the mechanisms surrounding these changes in VO_2 and possible changes in substrate oxidation are unclear, and certainly warrant reaffirmation and further investigation.

The conclusions of previous hydration research, where dehydration exceeds 3 % body mass, are unlikely to be misconstrued by a nocebo/placebo effect of hydration [2,16] examined the impact of blinding dehydration in two pair-matched groups of trained cyclists, with both groups completing 120 min of cycling before a ~15-minute time trial. Water intake was manipulated to either maintain euhydration or produce dehydration (~3 % body mass) at the end of 120 min. One group (blinded group) had water delivered through a nasogastric feeding tube, whilst in the other group (unblinded group) had all water provided orally. The decrement in performance with dehydration was not different between groups (~11 % blinded group, ~10 % unblinded group). These results suggest that when dehydration of ~3 % body mass is present, impairments in endurance performance are not caused or exaggerated by a lack of study blinding. The present study suggests that at lower levels of dehydration there may be a greater likelihood that any negative performance effects of dehydration are exaggerated or explained by placebo/nocebo effects, due to smaller changes in physiological and psychological mechanisms associated with dehydration [2].

5. Conclusions

In summary, this study demonstrated that when participants believed they were 'dehydrated by ~2 % of their body mass' cycling endurance performance in the heat was impaired by ~6 %. This decrement in performance likely derived from participants predispositions/knowledge that dehydration impairs performance and/or increased thirst sensation. The negative performance effects of previous unblinded hydration research, at least at lower levels of dehydration (<2 % body mass loss), may be exaggerated by a nocebo effect of dehydration.

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CRediT authorship contribution statement

Mark P. Funnell: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Jodie Moss:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Daniel R. Brown:** Investigation, Methodology, Writing – review & editing. **Stephen A. Mears:** Conceptualization, Investigation, Methodology, Writing – review & editing, Supervision. **Lewis J. James:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

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Data availability

Data generated or analysed during this study are available from the corresponding author upon request.

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