



The ergogenic potency of carbohydrate mouth rinse on endurance running performance of dehydrated athletes

Harris Kamal Kamaruddin^{1,2} · Cheong Hwa Ooi² · Toby Mündel³ · Abdul Rashid Aziz⁴ · Ahmad Munir Che Muhamed²

Received: 2 October 2018 / Accepted: 9 May 2019 / Published online: 16 May 2019
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Abstract

Purpose To examine the effect of carbohydrate (CHO) mouth rinsing on endurance running responses and performance in dehydrated individuals.

Methods In a double blind, randomised crossover design, 12 well-trained male runners completed 4 running time to exhaustion (TTE) trials at a speed equivalent to 70% of VO_{2peak} in a thermoneutral condition. Throughout each run, participants mouth rinsed and expectorated every 15 min either 25 mL of 6% CHO or a placebo (PLA) solution for 10 s. The four TTEs consisted of two trials in the euhydrated (EU-CHO and EU-PLA) and two trials in the dehydrated (DY-CHO and DY-PLA) state. Prior to each TTE run, participants were dehydrated via exercise and allowed a passive rest period during which they were fed and either rehydrated equivalent to their body mass deficit (i.e., EU trials) or ingested only 50 mL of water (DY trials).

Results CHO mouth rinsing significantly improved TTE performance in the DY compared to the EU trials (78.2 ± 4.3 vs. 76.9 ± 3.8 min, $P = 0.02$). The arousal level of the runners was significantly higher in the DY compared to the EU trials ($P = 0.02$). There was no significant difference among trials in heart rate, plasma glucose and lactate, and psychological measures.

Conclusions CHO mouth rinsing enhanced running performance significantly more when participants were dehydrated vs. euhydrated due to the greater sensitivity of oral receptors related to thirst and central mediated activation. These results show that level of dehydration alters the effect of brain perception with presence of CHO.

Keywords Hypohydration · Exercise · Treadmill · Oral sensing

Abbreviations

CHO Carbohydrate
PLA Placebo
TTE Time to exhaustion
EU Euhydration

DY Dehydration
 VO_{2peak} Maximal aerobic power
 VO_2 Volume of oxygen
 VCO_2 Volume of carbon dioxide
HR Heart rate
EID Exercise induced dehydration
USG Urine specific gravity

Communicated by Narihiko Kondo.

✉ Ahmad Munir Che Muhamed
ahmadmunir@usm.my

Harris Kamal Kamaruddin
harris540@uitm.edu.my

Cheong Hwa Ooi
cheonghwa.ooi@usm.my

Toby Mündel
t.mundel@massey.ac.nz

Abdul Rashid Aziz
abdul_rashid_aziz@sport.gov.sg

¹ Faculty of Sports Science and Recreation, Universiti Teknologi MARA, Shah Alam, Malaysia

² Lifestyle Science Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Kepala Batas, Penang, Malaysia

³ School of Sport, Exercise and Nutrition, Massey University, Palmerston North, New Zealand

⁴ Sports Science and Medicine, Singapore Sport Institute, Singapore, Singapore

RH	Relative humidity
VAS	Visual analogue scale
FAS	Perceived activation scale
FS	Feeling scale
GI	Gastrointestinal
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion
T_{sk}	Skin temperature
T_{re}	Rectal temperature
fMRI	Functional magnetic resonance imagery

Introduction

Dehydration frequently occurs during prolonged exercise and can impair aerobic exercise performance (Sawka et al. 2007). Studies have shown that a decrease in bodily fluid equivalent to 2% in volume relative to pre-exercise body mass increases cardiovascular strain (Montain et al. 1995), decreases exercise performance (Maughan and Meyer 2013), impairs thermoregulation control (Jeukendrup 2011), and results in rapid depletion of muscle glycogen stores (Garber et al. 2011). Therefore, athletes are often advised to stay well hydrated and ingest sufficient amounts of fluid to avoid the detrimental effects of dehydration during exercise (Sawka et al. 2007). Indeed, ingesting fluids, especially carbohydrate (CHO) beverages, during exercise to maintain <2% of body mass loss can attenuate performance deterioration by attenuated hypoglycaemia, maintaining glycogen sparing (Tsintzas and Williams 1998), delaying fatigue (Coyle et al. 1983; Duckworth et al. 2013), enhancing evaporative cooling (Casa et al. 2010), and sustaining cardiac output (Montain and Coyle 1992).

However, the drinking behaviour of athletes during training and competitions generally is less than ideal, as many athletes commence their exercise in a dehydrated state and/or do not ingest sufficient fluids during exercise (Magee et al. 2017). Noakes (1995) reported that elite endurance runners seem to drink less during real-world competitive events and drink sparingly without developing specific medical complications. Voluntary fluid intake by endurance runners is reported to be 500 mL h⁻¹, which is far less than the recommended fluid intake during exercise (Noakes 2003, 2010; Berkulo et al. 2016). In addition, sub-optimal hydration among runners is also linked to the symptoms of gastrointestinal discomfort (van Nieuwenhoven et al. 2005). Van Nieuwenhoven et al. (2005) reported an increase in gastrointestinal discomfort among runners when they consumed 600 mL of CHO beverages during an 18 km race, with the average completion time of ~1 h. Numerous studies have reported a similar observation of gastrointestinal discomfort among runners during prolonged distance races (Peters et al. 2000; Sharwood et al. 2004; Dion et al. 2013). Indeed,

survey data showed that 30–50% of endurance athletes experienced some level of gastrointestinal issues during prolonged exercise (Beis et al. 2012).

It has also been shown in a competition setting that marathon runners were challenged with less opportunity to rehydrate due to the continuous running and distance between the water stations available (Racinais et al. 2015). Moreover, the palatability and consumption of fluid during prolonged endurance exercise are influenced by beverage temperature, resulting in athletes more likely to not ingest sufficient fluid (Burdon et al. 2013). While the aim of CHO ingestion during exercise is to maintain energy supply, numerous studies have reported that for 1-h running exercise, CHO availability is not a major limiting factor to exercise performance (Carter et al. 2004b; Jeukendrup et al. 2008). Ingesting fluid to match the amount of sweat loss is impractical for individuals with a high sweat rate; for example, Alberto Salazar was reported to have a sweat rate of 3.6 L h⁻¹ during exercise (Armstrong et al. 1986). An attempt to drink at a rate equal to sweat rate may develop symptoms of fullness/bloating and developed gastrointestinal discomfort (Costill et al. 1970). Indeed, the sweat rate is likely to differ between individuals and be influenced by environment and exercise intensity (Kenefick 2018). Moreover, body mass loss is not always associated with a negative effect on exercise; it may be advantageous at the end of prolonged exercise as theoretically this body mass loss will lower the energy cost of running at the same relative submaximal speed (Fudge et al. 2012). Therefore, the fluid ingestion within 1 h of running exercise may not be the major factor in enhancing performance.

Within the last decade, repeated CHO mouth rinsing had been demonstrated to provide ergogenic benefits to athletes engaged in prolonged endurance exercise (Carter et al. 2004a; Rollo et al. 2008; Fraga et al. 2015). It has been suggested that the oral receptors within the mouth sense the availability of CHO and directly stimulate the corresponding brain regions that are associated with motor control, reward, and motivation (Chambers et al. 2009) and enhance the neural activation networks involved in sensory perception (Turner et al. 2014). The premise is that CHO mouth rinsing may exert a central effect that results in the athlete experiencing a feeling of pleasure, thus lowering the rating of perceived exertion (RPE) during prolonged exercise (Backhouse et al. 2005, 2007). Thus, the brain's sensory activation may be linked to improved exercise performance with CHO mouth rinsing.

The ergogenic benefits of CHO mouth rinse during prolonged running performance have been previously reported.

Rollo et al. (2008) reported that runners who practiced CHO mouth rinsing were able to increase their self-selected running speed during a 30 min run with enhanced sensation of pleasure compared a placebo (PLA) solution. This was later supported by other several studies that running exercise

performance were enhanced with CHO mouth rinsing and were believed due to positive effect to the subject's feeling during the exercise (Wright and Davison 2013; Fraga et al. 2015; Rollo et al. 2015). Hence, the practise of CHO mouth rinsing may provide an ergogenic benefit and be a useful practical strategy for individuals who have gastrointestinal problems when ingesting liquid CHO during exercise.

Several factors influence the effectiveness of CHO mouth rinsing, such as training status, feeding status (Lane et al. 2013), duration of rinsing (Sinclair et al. 2014), and percentage of CHO in the solution (Hawkins et al. 2017; James et al. 2017). However, it remains unclear whether an individual's hydration status impacts prolonged running exercise performance. Few studies have examined the impact of mouth rinsing among dehydrated individuals. Che Muhamed et al. (2014) showed that mouth rinsing with CHO and placebo (artificial sweetener) shortened the time to complete 10 km of cycling after 30 min of cycle exercise at 65% peak rate of oxygen consumption when compared with no-fluid ingestion in a restricted fluid condition. However, general applicability of this finding is limited because the individuals tested were in the Ramadan-fasted state (i.e., they were in both dehydrated and fasted states). In the only published study conducted to examine the effectiveness of CHO mouth rinsing among dehydrated subjects, Arnautis et al. (2012) concluded that fluid mouth rinsing did not enhance performance when compared with no fluid ingestion during a ~1 h cycle time to exhaustion (TTE) trial in the dehydrated state. The major limitation in this study was that the mouth rinse solution used was plain water instead of a sweet and caloric solution containing CHO (Arnautis et al. 2012) that was previously shown to stimulate brain regions that are associated with reward and motor control (Chambers et al. 2009). Thus, the use of plain water instead of CHO solution did not allow the study to elucidate the true ergogenic benefit to central activation during exercise in the dehydrated state.

While numerous studies have been reported the ergogenic benefits of CHO mouth rinsing, little is known about its effect during dehydrated state and required further clarification. Therefore, it would be beneficial to examine the impact of CHO mouth rinsing during prolonged exercise in the sub-optimal dehydrated state. The purpose of this study was to examine the impact of CHO mouth rinsing on the responses and performance of athletes during prolonged treadmill running in both hydrated and dehydrated states. Use of the placebo mouth rinse in the hydrated and dehydrated states served as the corresponding control conditions.

Materials and methods

Participants

Twelve male endurance-trained runners (age 23 ± 2 years; stature 171 ± 5 cm; body mass 58.5 ± 3.5 kg; maximal aerobic power (or VO_{2peak}) 58.3 ± 3.3 mL kg^{-1} min^{-1}) volunteered for the study. Participants were runners who trained 5 ± 1 day $week^{-1}$ and logged training distances of 79.2 ± 18.9 km $week^{-1}$. All participants were briefed on all measuring procedures, but they were not fully informed about the true purpose of the study. The pre-participation interview revealed that none of the participants had previously practised CHO mouth rinsing while exercising. Further, none of them were aware of the postulated ergogenic benefits of CHO mouth rinsing during endurance exercise. All participants underwent a medical check-up and health screening procedure and provided written informed consent before participation. The study was approved by the University Sains Malaysia Human Ethics Committee (USM/JEPeM/15070254) and conformed to the latest version of the Declaration of Helsinki.

Experimental design

Participants made a total of five visits to the laboratory. The first visit included assessment of the participant's VO_{2peak} , determination of his individualised submaximal speed for the TTE exercise, and familiarisation with the equipment and mouth-rinsing procedures. The next four visits were experimental trials that consisted of two dehydrated mouth-rinsing TTE runs with and without CHO (i.e., DY-CHO and DY-PLA, respectively) and two euhydrated mouth-rinsing TTE runs with and without CHO (i.e., EU-CHO and EU-PLA, respectively). All four experimental trials were completed in a counterbalanced, randomised, double-blind design and were conducted at the same time of the day with an inter-trial interval of 7 days. Each experimental session consisted of an exercise-induced dehydration (EID) phase, a rest phase (in which rehydration or no rehydration took place), and a time to exhaustion exercise phase during which mouth rinsing intervention was given (Fig. 1). Participants arrived at the laboratory at 08:00 after an overnight fast. To ensure that starting glycogen concentrations were similar before each experimental trial, participants were instructed to record dietary intake and physical activity during the day before the familiarisation trial and to replicate them in the day prior to the subsequent experimental trials. No strenuous physical activity or alcohol and caffeine consumption were allowed within the 24 h before each trial.

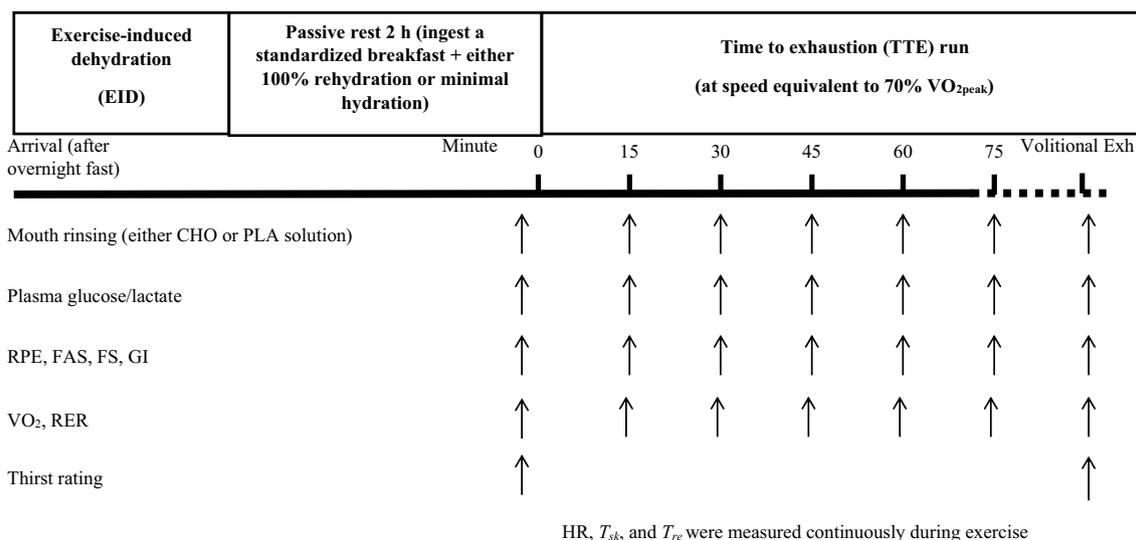


Fig. 1 Schematic diagram of the experimental session. Exh, exhaustion; CHO, carbohydrate; PLA, placebo; RPE, rating of perceived exertion; FAS, perceived arousal scale; FS, feeling scale; GI, gas-

trointestinal comfort; VO_2 , oxygen consumption; RER, respiratory exchange ratio; T_{sk} , skin temperature; T_{re} , rectal temperature; HR, heart rate

VO_{2peak} test and familiarisation with the study design

Each participant performed a running economy test followed by a maximal exercise test on a treadmill (HP Cosmos, Nussdorf, Germany). First, the participants ran at four submaximal velocities of 7.0, 9.0, 11.0, and 13.0 km h⁻¹ for 4 min at each speed. After the submaximal running, participants engaged in an active recovery in which they walked for 5 min at the speed of 5.0 km h⁻¹. This was followed by a graded exercise test to determine the VO_{2peak} . The test required the participants to run at a constant speed of 12.0 km h⁻¹ with the treadmill gradient being elevated by 2% every 2 min until volitional exhaustion. Respiratory gases (oxygen uptake (VO_2), and carbon dioxide (V_{CO_2})) were sampled using a calibrated respiratory gas system (TrueOne 2400, Parvomedics, Sandy, Utah, USA) and heart rate (HR; Polar Electro, Kempele, Finland) was continuously monitored throughout the submaximal and maximal tests. Post-test, a linear regression line was plotted between submaximal VO_2 and treadmill velocity to determine each participant's running speed at the intensity of 60% and 70% VO_{2peak} . These values were used for the individual's EID and TTE runs, respectively. After the VO_{2peak} test, participants were familiarized with the other procedures of the study. During this visit, participants also practiced the mouth rinse procedure while running on the treadmill.

EID phase

Upon their arrival to the laboratory at 08:00, participants emptied their bladder and a urine sample was collected to determine urine specific gravity (USG) using a refractometer (Atago Co. Ltd, PAL-10 s, Tokyo, Japan). Their pre-exercise nude body weight then was measured (Mettler Toledo, Columbus, OH, USA). The dehydration protocol consisted of 30 min of running on the treadmill at a speed equivalent to the individual's 60% VO_{2peak} followed by a 30 min of passive rest in a hot humid climatic chamber (35 °C and 70% relative humidity (RH)). This protocol has been shown to induce a mean body deficit of ~2% (based on our own unpublished data from six individuals who were not part of the present study). At the end of the 30 min rest duration, participants were towelled dry, their post-exercise nude body mass was measured, and another urine sample was collected.

Resting phase

Immediately after the EID phase, participants rested in a room (22 °C and 40% RH) for 120 min. During the first 15 min of this resting period, they consumed a standardized breakfast that contained calories equivalent to 2.5 g kg⁻¹ of their body mass (Lane et al. 2013). For the EU trials, participants ingested a volume of plain water (with no calories) equivalent to their body mass deficit that resulted from the EID phase; this helped to ensure that the participants were

well hydrated before the TTE run. Participants were not informed of the volume of fluid given to minimize the possibility of any psychological bias. For the DY trials, participants consumed the same amount of food, but were provided with only 50 mL of plain water so as to remain dehydrated for the subsequent TTE runs.

TTE running performance test phase

Following the resting phase, participants were instrumented before being transferred into the climatic chamber where they remained seated for 5 min. Participants performed their TTE run at a speed equivalent to 70% of their VO_{2peak} until volitional exhaustion in a thermoneutral condition (22 °C and 40% RH). TTE was determined as the time at which participants reached volition of fatigue and inability to maintain the run with the present intensity (Fraga et al. 2015). The participants had no knowledge of their performance time or any other measures such as HR, lactate level, and core temperature during the TTE so as to not influence their subsequent experimental trials. Respiratory gases were sampled for 3 min at pre-exercise and at every 15 min during exercise, and HR was continuously monitored using previously mentioned equipment throughout the TTE run. Rectal temperature (T_{re}) was recorded using a disposable thermistor probe (YSI 400 series, Mallinckrodt Medical, Kansas City, MO, USA) inserted 12 cm beyond the anal sphincter. A data logger (Cole Parmer, Vernon Hills, IL, USA) was used to record the T_{re} . Skin temperature (T_{sk}) was measured at four different sites (left shoulder, left chest, right mid-thigh, and right mid-shin) using iButton temperature sensors (Maxim Integrated Products, Sunnyvale, CA, USA), and mean T_{sk} was calculated from all four sites (Ramanathan 1964). T_{re} and T_{sk} were sampled continuously throughout the TTE exercise. Participants were asked to state their subjective RPE (category scale 6–20) before exercise and at 15 min intervals during exercise (Borg 1982). At the end of the TTE run, participants dismounted and removed all clothing and instrumentation; they were towelled dry before they were weighed (in the nude) and a urine sample was taken.

Mouth rinse solutions and procedures

An external researcher who was not part of the study prepared and administered the mouth rinse solution. The CHO solution consisted of 6% glucose (Sim Company, Penang, Malaysia) and the PLA consisted of artificial sweeteners (Sucralose, Diabetasol, Jakarta, Indonesia). All solutions were freshly prepared before each trial and were kept in a chiller at a temperature of 15 ± 0.5 °C. The CHO and PLA solutions were indifferent and matched to the same colour and appearance. During the four experimental trials, participants received 25 mL of each respective mouth

rinse solution. Participants swirled the fluid around their mouth for 10 s before expectorating it into a beaker. The beaker was weighed (Tanita KD-160, Tokyo, Japan) before and after each rinse to determine the volume of rinsed and expectorated solution and to confirm that no fluid had been ingested. Mouth rinsing was administered at the start and every 15 min throughout the TTE run (Fig. 1). The participants were informed of the purpose of the study and the solutions they had received at the completion of the study.

Blood collection

A total of 4 mL of whole blood was collected prior and intermittently during TTE run via a radial vein using a 22G IV catheter insertion (Surflo IV Catheter, Terumo Med Corporation, Elkton, MD, USA). After each sample collection, the catheter was flushed with 2–3 mL of 0.9% sterile saline to ensure patency of the vein. All blood samples were kept in tubes containing sodium fluoride/potassium oxalate (BD Vacutainer, Lakes USA) and centrifuged at 3500 rpm for 5 min. The plasma was stored at -80 °C for later analysis. Plasma lactate concentration was measured using the electro-enzymatic method (YSI 1500 Sport, Yellow Spring, OH, USA), and plasma glucose level was measured using an ultraviolet spectrophotometer (Optima SP-3000 Plus, Tokyo, Japan) and commercially available kits (Randox, Daytona, UK).

Perceived thirst and psychological scales

Each participant's perceived thirst was recorded pre- and post-TTE using a straight line 100 mm visual analogue scale (VAS) on which the anchor point was labelled “not thirsty” at the 0 mm mark and “very thirsty” at the 100 mm mark. The feeling activated scale (FAS) ranged from “1” indicating low arousal to “6” indicating high arousal (Svebak and Murgatroyd 1985). The feeling scale (FS) was an 11-point scale ranging from -5 (“feeling very bad”) to 0 (“feeling neutral”) to $+5$ (“feeling very good”) (Hardy and Rejeski 1989). A 12-point gastrointestinal (GI) comfort scale was used to determine the participant's gastrointestinal condition; the scale had anchors at 0 (“neutral”), 4 (“uncomfortable”), 8 (“very uncomfortable”), and 12 (“painful”) (Rollo et al. 2010). The FAS, FS, and GI scales were administered at the start and at 15-min intervals during the TTE (Fig. 1).

Statistical analysis

All data are presented as mean \pm standard deviation (SD) and were analysed using SPSS (ver. 22.0, Chicago, IL, USA). To examine the main effects of treatments (EU-CHO, EU-PLA, DY-CHO, DY-PLA) on TTE performance, the data were analysed using two-way

analysis of variance (ANOVA) for repeated measures. Other physiological (e.g., body mass, USG, VO_2 , respiratory exchange ratio (RER), HR, T_{re} , T_{sk}) and psychological (e.g., perceived thirst, RPE, FAS, FS, GI) data were analysed using a two-way (treatment \times times) ANOVA for repeated measures. Mauchly's test of sphericity was applied, and if sphericity was violated the Huynh–Feldt estimate was used to correct the data. When significant differences between treatments were identified, post hoc Student's *t* tests using the Holm–Bonferroni adjustment were performed. Where any differences were identified, 95% confidence intervals (95% CI) were used to display the likely range of the true value in the sample population. Sample size calculation was performed using PS: Power and Sample software (version 3.1.2; Dupont and Plummer 1990) for repeated measures ANOVA; to detect a small effect size, α as 0.05, and a $1 - \beta$ error probability of 0.8, a sample size of 12 participants were required. Furthermore, effect size using partial eta squared (η_p^2) and Cohen's *d* were calculated, which were defined as trivial (0–0.19), small (0.20–0.49), moderate (0.50–0.79), or large (> 0.80) (Cohen 1992).

Results

Pre-TTE exercise hydration status

In the dehydration trials, participants started the TTE runs with a body mass deficit of $-2.06 \pm 0.68\%$ for DY-CHO and $-2.05 \pm 0.15\%$ for DY-PLA when compared to pre-EID stage ($P < 0.001$, respectively). For the euhydration trials, participants started the TTE with minimum changes in body mass (i.e., $0.11 \pm 0.23\%$ for EU-CHO and $-0.08 \pm 0.24\%$ for EU-PLA) (Table 1). These conditions were supported by the USG data, which showed significant dehydration in the DY-CHO trial (1.025 ± 0.003 ; 95% CI 1.023, 1.027) compared with the EU-CHO trial (1.006 ± 0.00 ; 95% CI 1.004, 1.008) (mean difference: 0.018; $P < 0.001$) and in the DY-PLA trial (1.026 ± 0.002 ; 95% CI 1.024, 1.027) compared with the EU-PLA trial (1.007 ± 0.003 ; 95% CI 1.004, 1.008) (mean difference: 0.019; $P < 0.001$) (Table 1). Similarly, pre-TTE perceived thirst was significantly greater in the DY-CHO trial (61 ± 7 mm; 95% CI 57, 66) than in the EU-CHO trial (22 ± 8 mm; 95% CI 13, 25) (mean difference: 39 mm; $P < 0.001$) and in the DY-PLA trial (63 ± 12 mm; 95% CI 55, 70) compared to the EU-PLA trial (20 ± 7 mm; 95% CI 15, 24) (mean difference: 43 mm; $P < 0.001$) (Table 1).

Table 1 Body mass, percent change in body mass, urine specific gravity, and perceived thirst (based on visual analogue scale) measured at various phases of the experiment

Variable	Trial	Pre EID	Post EID	Pre TTE	Post TTE
Body mass (kg)	DY-CHO	58.53 ± 3.47	$57.32 \pm 3.42^*$	57.33 ± 3.42	$55.89 \pm 3.45^{*\#}$
	EU-CHO	58.58 ± 3.37	$57.37 \pm 3.33^*$	58.52 ± 3.35	$57.47 \pm 3.34^{*\#}$
	DY-PLA	58.52 ± 3.70	$57.33 \pm 3.66^*$	57.31 ± 3.55	$55.95 \pm 3.62^{*\#}$
	EU-PLA	58.58 ± 3.60	$57.39 \pm 3.55^*$	58.54 ± 3.67	$57.58 \pm 3.66^{*\#}$
Changes in body mass (%)	DY-CHO	–	-2.07 ± 0.11	-2.06 ± 0.65	-4.52 ± 0.74
	EU-CHO	–	-2.07 ± 0.13	-0.11 ± 0.23	-1.91 ± 0.32
	DY-PLA	–	-2.04 ± 0.15	-2.05 ± 0.17	-4.38 ± 0.32
	EU-PLA	–	-2.03 ± 0.14	-0.08 ± 0.24	-1.71 ± 0.34
Urine specific gravity (USG)	DY-CHO	1.016 ± 0.008	1.017 ± 0.006	$1.025 \pm 0.003^{\dagger*}$	$1.029 \pm 0.002^{\dagger*\#}$
	EU-CHO	1.011 ± 0.005	1.011 ± 0.005	1.006 ± 0.003	$1.015 \pm 0.002^{*\#}$
	DY-PLA	1.015 ± 0.007	1.015 ± 0.008	$1.026 \pm 0.002^{\ddagger*}$	$1.029 \pm 0.002^{\ddagger*\#}$
	EU-PLA	1.012 ± 0.007	1.013 ± 0.006	1.007 ± 0.003	$1.014 \pm 0.002^{*\#}$
Perceived thirst scale (mm)	DY-CHO	30 ± 13	$70 \pm 12^*$	$61 \pm 7^{\dagger}$	$86 \pm 7^{\dagger*\#}$
	EU-CHO	28 ± 14	$64 \pm 13^*$	$22 \pm 8^*$	$59 \pm 16^{*\#}$
	DY-PLA	28 ± 11	$73 \pm 9^*$	$63 \pm 12^{\ddagger}$	$86 \pm 8^{\ddagger*\#}$
	EU-PLA	22 ± 12	$62 \pm 17^*$	$20 \pm 7^*$	$61 \pm 16^{*\#}$

Values are presented \pm as the mean SD

EID, exercise-induced dehydration; TTE, time to exhaustion; min, minutes; EU, euhydration; DY, dehydration; CHO, carbohydrate; PLA, placebo

† Significant difference between DY-CHO and EU-CHO ($P < 0.001$); ‡ significant difference between DY-PLA and EU-PLA ($P < 0.001$); $*$ significant difference between previous phase ($P < 0.001$); $\#$ significant effect of time ($P < 0.001$)

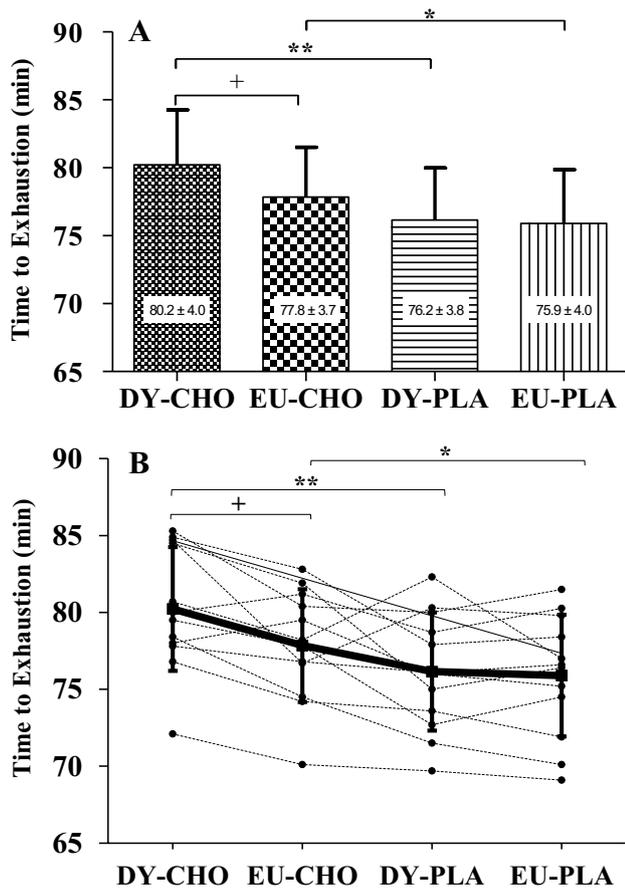


Fig. 2 Exercise performance: **a** mean TTE performance and **b** individual TTE performance during TTE for the EU-CHO, EU-PLA, DY-CHO, and DY-PLA trials. DY, dehydration; EU, euhydration; CHO, carbohydrate; PLA, placebo. *Significant difference between EU-CHO and EU-PLA ($P=0.024$); **significant difference between DY-CHO and DY-PLA ($P=0.001$); +significant difference between DY-CHO and EU-CHO ($P=0.008$). Data are expressed as the mean \pm SD

Exercise performance

There was no trial order effect for the four trials during the TTE exercise performance analysis ($P=0.68$). For the TTE, there was a small significant main effect of hydration status ($F=7.29$, $P=0.02$, $\eta^2_p=0.45$), a moderate effect for solution used ($F=18.4$, $P=0.001$, $\eta^2_p=0.63$), and a small hydration–solution interaction effect ($F=5.88$, $P=0.03$, $\eta^2_p=0.35$) (Fig. 2). Post hoc analysis revealed that TTE exercise performance in the DY-CHO (80.2 ± 4.0 min) was significantly higher than both DY-PLA (76.1 ± 3.8 min; $P<0.001$, $d=1.30$; mean change 95% CI = $5.0 \pm 3.9\%$) and EU-CHO trial (77.8 ± 3.7 min; $P=0.008$, $d=0.94$; mean change 95% CI = $3.0 \pm 2.9\%$), respectively (Fig. 2a). Similarly, there was a significantly higher time performance in EU-CHO (77.8 ± 3.7 min) than EU-PLA trial (75.9 ± 4.0 min; $P=0.024$, $d=0.76$; mean change 95%

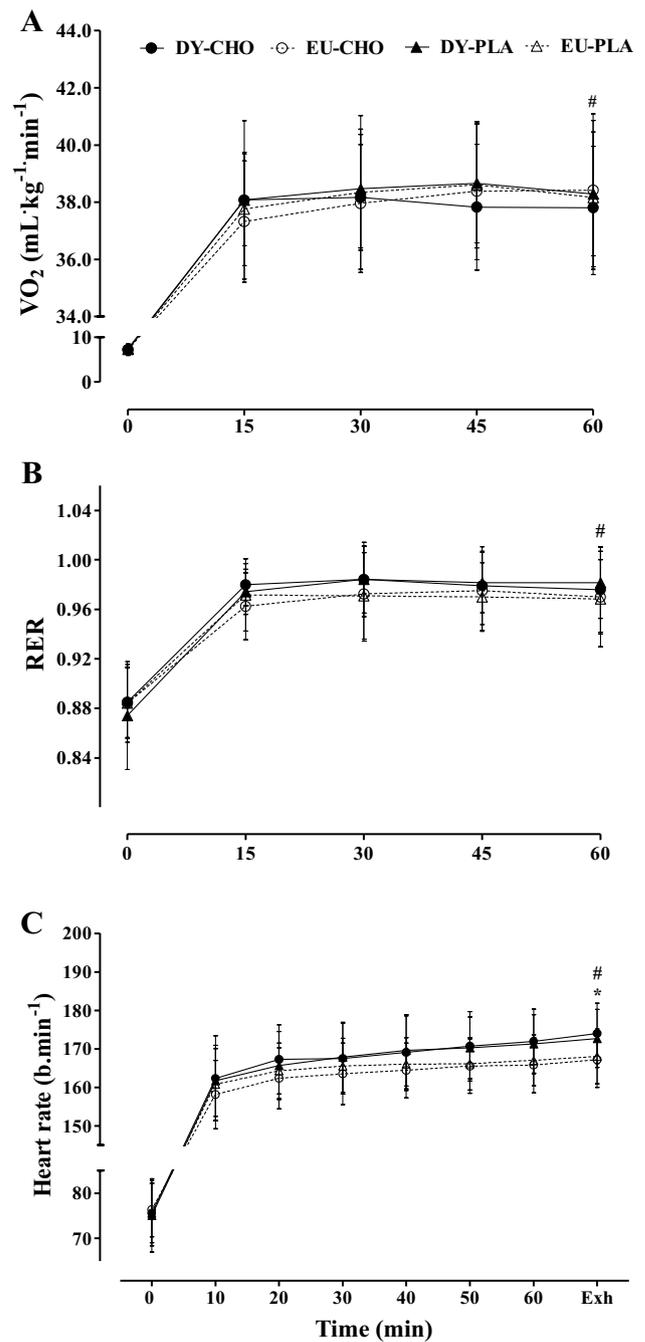


Fig. 3 Physiological responses during TTE for the EU-CHO, EU-PLA, DY-CHO, and DY-PLA trials. **a** Oxygen uptake (VO_2), **b** respiratory exchange ratio (RER), and **c** heart rate (HR). *Significant difference between DY-CHO and EU-PLA ($P<0.05$); #significant effect of time ($P<0.001$). Data are expressed as the mean \pm SD

CI = $2.4 \pm 3.1\%$). Specifically, 11 of 12 participants ran longer during the DY-CHO compared to DY-PLA trial and 10 of 12 participants when compared to EU-CHO. Similarly, 10 of 12 participants ran longer during the DY-CHO compared to EU-CHO trial (Fig. 2b).

Cardiorespiratory responses

A large increase in VO_2 (Fig. 3a) was observed during the exercise trials ($F=3983.1$, $P<0.001$, $\eta^2_p=0.99$), but there was no interaction effect ($F=0.513$, $P=0.66$, $\eta^2_p=0.14$) and no main effect of hydration or solution ($P>0.05$). A large increase in RER (Fig. 3b) was observed throughout the exercise periods ($F=103.4$, $P<0.001$, $\eta^2_p=0.74$), but only a small significant interaction effect ($F=0.11$, $P=0.39$, $\eta^2_p=0.28$) and no main effect of hydration or solution ($P>0.05$) were detected. Statistical analysis of HR data (Fig. 3c) revealed a large increase during exercise ($F=3982.9$, $P<0.001$, $\eta^2_p=0.99$), but the interaction effects of solution ($F=0.71$, $P=0.49$, $\eta^2_p=0.17$) and hydration condition was trivial ($F=3.84$, $P=0.027$, $\eta^2_p=0.48$) were not significant. Post hoc analysis revealed a greater increase in HR in the DY-CHO trial (174 ± 8 b min^{-1} ; 95% CI 169, 179) compared to the EU-CHO trial (167 ± 7 b min^{-1} ; 95% CI 163, 172) (mean difference: 7 b min^{-1} ; $P=0.04$, $d=2.16$) than between the DY-PLA trial (172 ± 8 b min^{-1} ; 95% CI 168, 178) and the EU-PLA trial (168 ± 7 b min^{-1} ; 95% CI 163, 176) (mean difference: 5 b min^{-1} ; $P<0.001$, $d=2.09$) at the end of the TTE exercise.

Thermoregulatory responses

A moderate increase in T_{re} (Fig. 4a) was detected during exercise ($F=1100.9$, $P<0.001$, $\eta^2_p=0.96$), although only a trivial interaction effect ($F=0.50$, $P=0.74$, $\eta^2_p=0.11$) and a small effect of hydration condition ($F=15.6$, $P<0.001$, $\eta^2_p=0.28$) were found. Post-hoc analysis showed a higher T_{re} in the DY-CHO trial compared to the EU-CHO trial and in the DY-PLA trial compared to the EU-PLA trial after ~40 min into the TTE run ($P<0.05$; Fig. 4a). The mean T_{sk} gradually decreased throughout the exercise period, but there was only a trivial significant interaction effect ($F=1.15$, $P=0.32$, $\eta^2_p=0.03$), however, there was no significant main effects of hydration or solutions ($P>0.05$).

Ratings of perceived exertion, psychological measures, and blood measures

A large increase in RPE (Fig. 5a) was observed during exercise, but no significant interaction ($F=2.00$, $P=0.99$, $\eta^2_p=0.43$) or main effects were found ($P>0.05$). FAS (Fig. 5b) increased throughout the exercise period ($F=10.9$, $P<0.001$, $\eta^2_p=0.20$), and no significant interaction effect was found ($F=0.64$, $P=0.99$, $\eta^2_p=0.01$). However, a moderate effect of hydration status was detected ($F=3.88$, $P=0.004$, $\eta^2_p=0.51$). Post hoc analysis showed higher FAS from 60 min to the end of the TTE in the DY versus the EU trials ($P<0.05$) (Fig. 5b). Specifically, higher FAS was observed at the end of exercise in

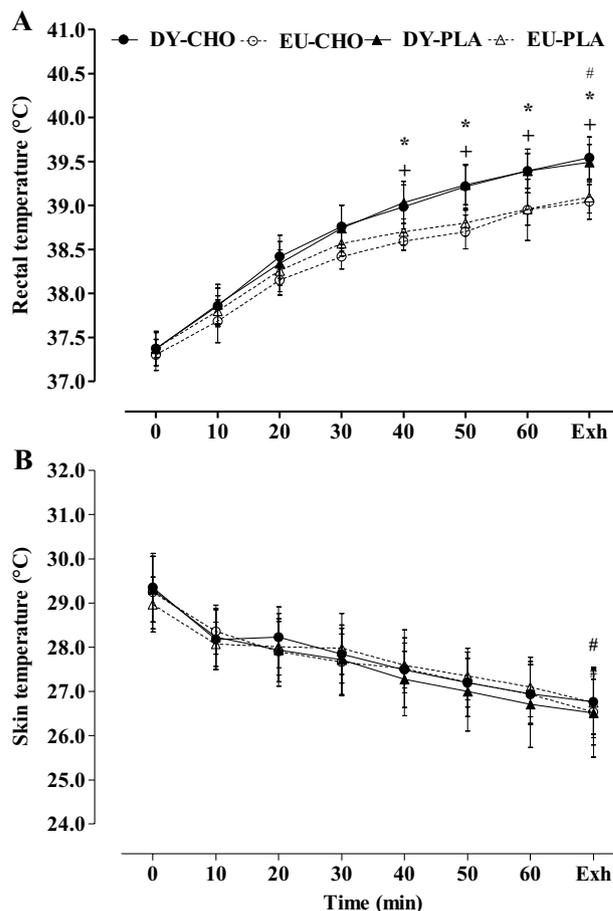
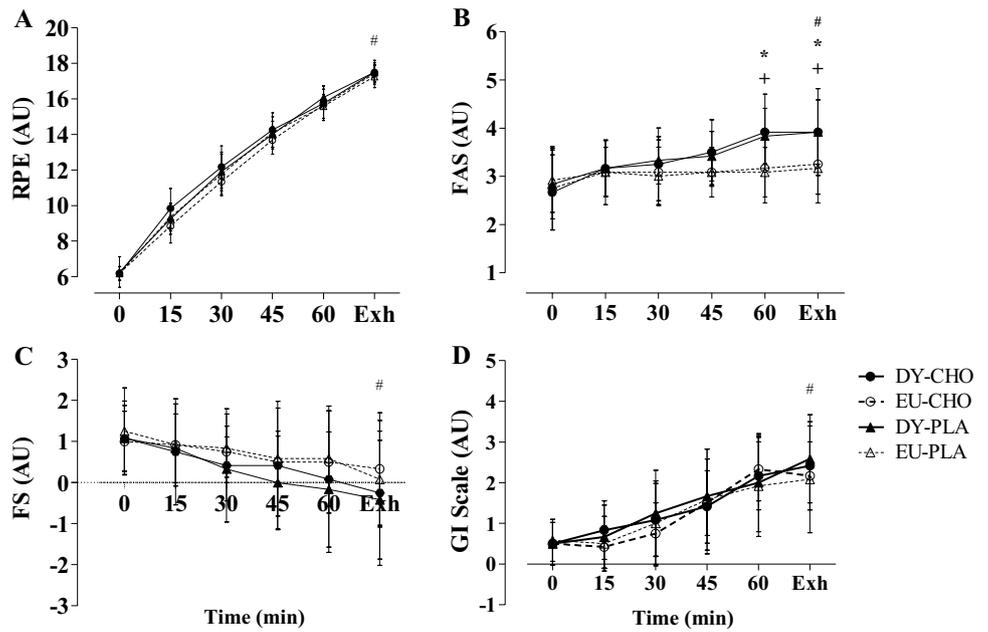


Fig. 4 Thermoregulatory responses during TTE for the EU-CHO, EU-PLA, DY-CHO, and DY-PLA trials. **a** Rectal temperature (T_{re}) and **b** mean skin temperature (T_{sk}). *Significant difference between DY-CHO and EU-CHO ($P<0.05$); +significant difference between DY-PLA and EU-PLA ($P<0.05$); #significant effect of time ($P<0.001$). Data are expressed as the mean \pm SD

the DY-CHO trial (3.9 ± 0.9 AU; 95% CI 3.3, 4.5) compared to the EU-CHO trial (3.3 ± 0.6 AU; 95% CI 2.9, 3.6) (mean difference: 0.67 AU; $P=0.013$, $d=0.86$) and in the DY-PLA trial (3.9 ± 0.7 AU; 95% CI 3.5, 4.3) compared to the EU-PLA trial (3.2 ± 0.72 AU; 95% CI 2.7, 3.6) (mean difference: 0.70 AU; $P=0.03$, $d=0.71$). No significant interaction ($F=0.24$, $P=0.79$, $\eta^2_p=0.01$) or main effects ($P>0.05$) were found for perceived mood (Fig. 5c). GI discomfort ratings also indicated a trivial interaction ($F=0.71$, $P=0.56$, $\eta^2_p=0.02$) and no significant main effects ($P>0.05$) (Fig. 5d). Plasma glucose and plasma lactate concentrations increased (Fig. 6a, b) during exercise ($P<0.001$), but showed no significant interaction ($P>0.05$) or main effects ($P>0.05$). Furthermore, there were no significant differences among all four trials at any time point for both plasma glucose and plasma lactate levels ($P>0.05$).

Fig. 5 Mean psychological responses during TTE for the EU-CHO, EU-PLA, DY-CHO, and DY-PLA trials. Arbitrary unit (AU). **a** Rating of perceived exertion (RPE), **b** perceived activation scale (FAS), **c** feeling scale (FS), **d** gastrointestinal scale (GI). *Significant difference between DY-CHO and EU-CHO ($P < 0.05$); +significant difference between DY-PLA and EU-PLA ($P < 0.05$); #significant effect of time ($P < 0.001$). Data are expressed as the mean \pm SD



Discussion

The aim of the present study was to examine the effects of CHO mouth rinsing on endurance running performance in dehydrated athletes as a practical strategy that may potentially enhance exercise performance. The main finding was that CHO mouth rinsing significantly improved endurance running performance (~1 h) among well trained subjects whilst experiencing dehydration. These findings corroborated the results of previous studies that reported that TTE performance was extended by repeated CHO mouth rinsing (Fraga et al. 2015; Bastos-Silva et al. 2016). Our findings are at odds with the common belief that dehydration (> 2% of body mass) could degrade aerobic exercise performance and the greater the dehydration, the more severe the physiological strain and aerobic exercise performance (Montain and Coyle 1992). However, our data revealed that CHO mouth rinsing was an effective ergogenic aid whilst subjects began exercise dehydrated (2% of body mass loss) and continued exercising to exhaustion in endurance-trained athletes. It should be taken into consideration that the exercise improvement in this study was significant up to dehydration of ~4.5% body mass loss and among well-trained runners that have a high tolerance to dehydration (Beis et al. 2012). The enhanced exercise performance with CHO mouth rinsing may be associated with the increased perceived arousal level without any variation in metabolic and cardiovascular processes.

The improved exercise performance that accompanies CHO mouth rinsing previously was attributed to a central effect from the stimulation of oral receptors, which would lead to activation of the supraspinal pathway to the brain

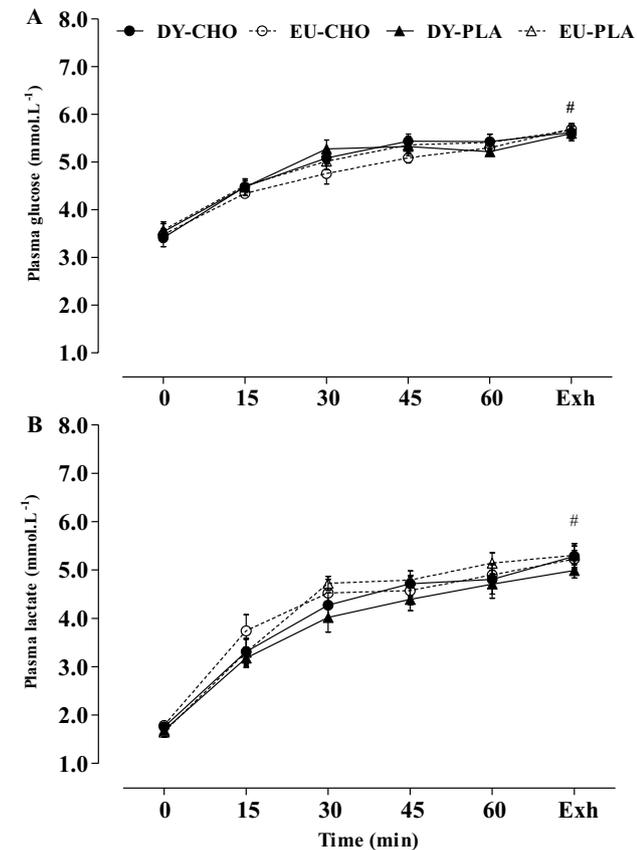


Fig. 6 Mean metabolic responses during TTE for the EU-CHO, EU-PLA, DY-CHO, and DY-PLA trials. Arbitrary unit (AU). **a** Plasma glucose and **b** plasma lactate concentrations. #Significant effect of time ($P < 0.001$). Data are expressed as the mean \pm SD

(Chambers et al. 2009; Turner et al. 2014). Specifically, the presence of CHO in the mouth is thought to trigger the dorso-lateral prefrontal cortex and striatum, which are related to motor control behaviour and motivational responses (Chambers et al. 2009). Given the longer TTE in both of the CHO mouth rinse solution trials compared to both of the PLA (which contained artificial sweetener) mouth rinsing trials, the observed outcome seems to indicate that it is the “caloric” or energy content within the CHO mouth rinse solution that is the potent component, and that it activated the individual’s brain reward or motivation centre to persist in exercising longer. This view of the potency of the nutritive value of CHO is supported by the findings of Hawkins et al. (2017). They compared the effects of mouth-rinsing with water, a sweetened nutritive (taste sweet + calories) solution, and a sweetened non-nutritive (taste sweet + no calories) solution on ~1 h running performance (Hawkins et al. 2017). The sweetened nutritive solution improved endurance performance compared to water, and there was no difference in performance between the sweetened non-nutritive and water groups. These findings implied that the “energy” content in the rinsing solution was key to eliciting the observed performance enhancement and that the sweet taste alone was not sufficient to impact performance (Hawkins et al. 2017). There may be different receptors for nutritive fluid that stimulate the reward-related brain areas differently or to varying degrees as compared to the sweet but non-caloric fluid (Frank et al. 2008). Indeed, the oropharynx contains many receptors, such as those for taste, texture, and temperature (Rolls 2007), and it is likely that one of these specialized receptors senses the presence of “energy” in the CHO rinsing solution independent of taste (i.e., sweetness).

This study was designed to explore whether dehydration had any influence on exercise performance when performing CHO mouth rinsing. The improvement was more pronounced in the dehydrated state, likely due to the oral senses becoming more sensitive to the presence of CHO. We postulated that the improved exercise performance in the dehydrated state was largely due to the individual’s oral senses becoming more responsive to the calorie-containing CHO solutions, as was previously reported (Chambers et al. 2009). Indeed, using fMRI, they observed that oral taste receptors appeared to be receptive to calorie-containing CHO but not to artificial sweeteners (non-caloric) and that the CHO activated various brain regions to induce motivational and motor control (Chambers et al. 2009). Dehydration may also cause the oral receptors to send stronger and/or faster signals to the brain motor-control area as described by de Araujo et al. (2003), thus resulting in the ability of the participants to maintain their submaximal effort for a longer duration. In another fMRI study, the level of brain activation was directly associated with the level of thirst among participants (de Araujo et al. 2003). The brain activation was specifically

detected within the primary taste cortex, which reflected the level of thirst or motivation states of the participants. Because the participants in the current study reported higher thirst sensation during dehydration trials than euhydration trials (Table 1), the presence of the CHO solution in the oral cavity may have triggered a greater magnitude of central response that may have led to longer running performance. It has also been reported that CHO mouth rinsing may contribute to increase oral-pharyngeal responses due to mouth wetting (Takamata et al. 1995; Figaro and Mack 1997). The act of drinking has a reflex effect on the oral-pharyngeal response that regulates sweating and on the secretion of arginine vasopressin among dehydrated participants (Takamata et al. 1995; Figaro and Mack 1997). The increased reflex of the oral-pharyngeal responses in turn may influence fluid balance, thermoregulation, and possibly exercise performance (Takamata et al. 1995; Figaro and Mack 1997; Arnaoutis et al. 2012). The inclusion of a ‘control’ condition, i.e., drinking PLA or CHO could have strengthened the current results.

In the present study, the better performance observed in the CHO compared to the PLA mouth rinse trials could additionally be due to the impact that the exercise protocol (duration) had on endogenous glycogen stores. The “fixed” velocity throughout the exercise in TTE implies that the ergogenic impact of CHO mouth rinsing on performance naturally refers to extending or prolonging the exercise time, whereby the positive effects of the mouth rinsing were observed towards the end-part of the prolonged TTE (i.e., beyond the ~70 min mark). At this point in time, it was possible that the individuals’ endogenous muscle glycogen levels may have been reduced to critically low levels (Torrens et al. 2016). As such, when continuing to exercise beyond this time point, the impact of caloric or energy content rather than the sweetness (or taste) of the CHO mouth rinse could be deemed by the brain to be far more critical and/or “useful” as future fuel for the working muscles relative to that of the need of the body for either fluid replacement or taste (or sweetness) (Rauch et al. 2005). This could have translated into a longer exercise duration in the two CHO mouth rinsing trials compared to that in the two PLA mouth rinsing trials.

Despite differences in exercise performance observed between the EU-CHO and DY-CHO trials, the physiological responses, such as heart rate, VO_2 , RER, and plasma glucose and lactate levels, did not show any concurrent differences between trials. These results indicate that there were no cardiorespiratory or metabolic factors that influenced the exercise performance observed, which was similarly observed in other studies (Rollo et al. 2010; Fraga et al. 2015). Since there were no substantial differences in physiological responses between CHO and PLA trials, we suggest that the central effect was likely the main mechanism for the exercise improvement in the CHO mouth

rinse trials. We speculate that the CHO solution may have produced an amplified sensation of reward (i.e., greater self-motivation) that altered the individual's subsequent exercise behaviour. This premise is partially supported by the significantly higher FAS values in the DY trials than the EU trials, which indicate a greater level of arousal in the DY trials; this result coupled with the higher exercise HR observed towards the latter part of the TTE exercise indicates additional physical effort was made by the participants. Indeed, the participants were able to run longer with CHO versus PLA mouth rinsing at the same level of RPE.

The present study had several limitations. The TTE protocol used in this study has been demonstrated to be less reliable than time trial tests (Jeukendrup et al. 1996; Hopkins et al. 2001). However, the small (3–5%) performance improvement evoked by mouth rinsing in the present study is in disagreement with previously reported margins of +12% (Fares and Kayser 2011) and +29% (Fraga et al. 2015) increase in TTE cycling and running performance, respectively. Thus, it appears that our findings are more similar to performance enhancement in time trials (+1.5 to 3%) that were attributed to CHO mouth rinsing (Carter et al. 2004a; Rollo and Williams 2011). The small performance change may be attributed to participants performing a familiarisation trial and being well-trained athletes and to careful control of the experimental protocol. Individual performance times also indicated a consistent trend of improvement with CHO mouth rinsing during EU and DY conditions (Fig. 2b). Although the significant effects were small (3–5%), they could be a meaningful effect that ultimately differentiates the winner from other athletes.

The TTE exercise model as an exercise performance indicator is not a replica of a real-world endurance race, and it can be influenced by subjective factors. However, the psychological and physiological variables, including VO_2 , RER, HR, RPE, and plasma glucose and lactate levels, measured at the time point of exhaustion suggested that participants exerted themselves approximately to the same level of fatigue in all four experimental trials when the exercise was terminated. The lack of a no mouth rinse condition or a fluid ingestion trial could be another limitation of the study. However, we did not inform the participants about the true purpose of the study and blinded them to their responses and exercise performance results until completion of the study. Moreover, the participants had no previous knowledge of the potential ergogenic benefits of CHO mouth rinsing. Finally, a no rinse or fluid ingestion trial is not a truly blinded trial, and, therefore, the inclusion of the result of such a trial would have had a bias placebo (or nocebo) effect that potentially may have confounded the overall outcome of the present study.

Conclusions

In this study, the level of dehydration had a positive influence on the ergogenic benefits of CHO mouth rinsing during exercise. The mechanism behind this benefit is likely to be related to the increasing sensitivity of the oral receptors relating to thirst and brain activation that are closely associated with greater self-arousal and motivational levels.

Acknowledgements Harris Kamal Kamaruddin is thankful to Universiti Teknologi MARA Malaysia and the Ministry of Higher Education of Malaysia for his Ph.D. scholarship. The authors thank all runners who took part in this study and all technical staff members of the Advanced Medical and Dental Institute, Universiti Sains Malaysia.

Author contributions HKK, OCH and AMCM conceived and designed research. HKK and AMCM conducted experiments. HKK and TM analyzed data. HKK and AMCM wrote the manuscript. All authors provided comments to the manuscript after proofreading and finally approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest. No financial support was received from any organization.

Ethical approval All procedures were performed in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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