Original Paper
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Repeated-sprints exercise in daylight fasting: carbohydrate mouth rinsing does not affect sprint and reaction time performance

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ABSTRACT: To determine the effect of carbohydrate mouth rinsing (CHO-MR) on physical and cognitive performance during repeated-sprints (RS) after 3 days of intermittent fasting (abstaining from food and fluid 14 h per day). In a randomized and counter-balanced manner 15 active healthy males in a fasted state performed a RS-protocol [RSP; 2 sets (SET1 and SET2) of 5×5 s maximal sprints, with each sprint interspersed with 25 s rest and 3 min of recovery between SET1 and SET2] on an instrumented non-motorized treadmill with embedded force sensors under three conditions: i) Control (CON; no-MR), ii) Placebo-MR (PLA-MR; 0% maltodextrin) and iii) CHO-MR (10% maltodextrin). Participants rinsed their mouth with either 10 mL of PLA-MR or CHO-MR solution for 5 s before each sprint. Sprint kinetics were measured for each sprint and reaction time (RTI) tasks (simple and complex) were assessed pre-, during- and post-RSP. There was no statistical main effect of CHO-MR on mean power, mean speed, and vertical stiffness during the sprints between the PLA-MR and CON condition. Additionally, no statistical main effect for CHO-MR on accuracy, movement time and reaction time during the RTI tasks was seen. CHO-MR did not affect physical (RSP) or cognitive (RTI) performance in participants who had observed 3 days of intermittent fasting (abstaining from food and fluid 14 h per day).

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INTRODUCTION

Muslims commonly fast during the holy month of Ramadan, or on occasions three days of the month throughout the year (intermittent fasting; 3d-IF). During Islamic religious fasting, Muslims abstain from consuming both food and fluid during daylight hours. Daylight fasting has been reported to reduce physical and cognitive performance [1], although the magnitude of this response has been debated [2, 3]. Despite these fasting-mediated challenges to cognitive processes and physical performance [1] many Muslim athletes, and those that voluntarily fast, may still need/wish to train and/or compete. Within fasted participants simple strategies such as mouth rinsing (MR) with either Carbohydrate (CHO) or water have shown efficacy to acquiesce some of the fasting induced endurance [4] and high-intensity [5] exercise performance decrements. Performance improvements through CHO-MR is dependent on several debated factors, including the fasted or fed state of participants [6], a dose response to the duration of MR (5 or 10 s [7]), concentration of the CHO rinse, the rinsing frequency [8] and the exercise mode [9]. Positive effects are principally attributed to orally-mediated activation

of brain regions associated with motivation [10, 11] and attentional processing [12]. Activation of higher centers in response to CHO-MR has been shown through electroencephalogram (EEG) analysis [10], specifically brain regions attributed to reward and motor control (primary and putative secondary taste cortices in the orbitofrontal cortex [12, 13]).

The ability to repeatedly express and recover from near maximal sprints [i.e. repeated-sprint-ability (RSA)] is a key physical attribute in many team-sports, with RSA central to many training programs [14]. Despite a growing number of publications regarding the effect of CHO-MR on performance during high-intensity exercise lasting ~1 h [6, 9, 15]. The effect of 10% CHO-MR on participants in a fasted state, relative to short intensive exercise (e.g. RSA) accompanied by assessment of cognitive performance (e.g. reaction time and mechanical/movement quality), has not been performed. Given the cognitive demands of both playing [16] and officiating [17] team sports match-play, whether recreationally or at an elite level, these latter cognitive considerations should also be of concern to the fasting athlete/official and their support network, in addition to physical performance related agendas.

Therefore, the present study examined the effects of CHO-MR after 3d-IF (consisting of ~14 h of total fasting per day, i.e., no ingestion of food and fluid) on RSA specific physical and cognitive performance in recreational participants. It was hypothesized that the use of CHO-MR would positively influence (i) RSA specific physical performance indices and (ii) the employed cognitive measures.

MATERIALS AND METHODS

Fifteen healthy, recreationally active males (30.7±4 yrs, 82.8±5 kg and 181.0±6 cm, frequency of exercise 4.6±1.0 times/week) volunteered for participation giving their written informed consent. The study was approved by the Anti-Doping-Lab of Qatar (ADLQ) Ethics Committee (F2015000116).

Participants reported to the laboratory on four separate occasions: Visit 1 (familiarization): Participants were familiarized to experimental MR solutions and their procedures prior to anthropometric data, body mass (precision 0.1 kg) and height (Seca-769-digital medical) being measured. Participants were also familiarized with the cognitive testing protocol [reaction time tasks (RTI)] [18].

Given the treadmill (ADAL3D-WR, Medical Development—HEF Tecmachine, Andrézieux-Bouthéon, France) employed was operated in a motorized (first part of warm-up) and non-motorized 'constant motor torque mode' (last part of warm-up and repeated sprint protocol (RSP); see below and [19]) manner, specific familiarization to each mode was performed. The RSP familiarization also provided determination of a participants maximal power output during sprinting [20, 21]. This value was used to determine whether participants were expressing maximal efforts in the first sprint of each completion of the RSP (i.e. criterion value) to ensure that adoption of a prospective pacing strategy was not seen (all observed experimental values were within ± 5% of this criterion). Importantly, participants were provided with standardized strong verbal encouragement when performing sprints.

Visits 2-4 (experimental): Three conditions were employed: control (CON; no-MR), placebo-MR (PLA-MR; 0% maltodextrin), and CHO-MR (10% maltodextrin). Administered in a randomized and counter-balanced manner, separated by at least 2 weeks (see Figure 1 for schematic of experimental design). Participants reported to the laboratory in the afternoon following total fasting of 14 h per day, for the 3 prior consecutive days. All exercise trials were performed at the same time of the day (between 14:00-16:00) to avoid circadian variation [12], the first laboratory arrival time was used across each further experimental visit. Participants performed the reaction time tests (RTI; simple and complex) and repeated sprint protocol (RSP) on each visit. All experimental procedures occurred in a standardized chronological order within standardized environmental conditions (22.7±1.1ºC and 46.03±3.5% relative humidity).

Experimental Day Procedures: Anthropometrics were assessed as previously described. Urine samples were collected upon arrival to assess urine-specific gravity (USG; Digital Pocket Refractometer, Atago 4410 PAL-10S, West Sussex, UK). If the USG value was outside of 1.005 – 1.030 [22], a participant was excluded (n = 1). Seated finger capillary blood samples were collected to determine resting pre-RSP blood lactate concentration (Lactate Scout+, Barleben, Germany). Hooper's [23] index questionnaires (i.e., summation of the subjective ratings of sleep quality, fatigue, stress and muscle soreness) were administered to assess participant's wellness. RTI was administered pre-RSP.

RSP and related procedures: Prior to the RSP, participants underwent a standardized warm-up: (i) 1 min at 8 km.h^{-1}, 1 min at 9 km.h^{-1} and finally 8 min at 10 km.h^{-1} (10 min total running time) on the treadmill (motorized mode), followed by (ii) 5 to 6 min of sprint-specific dynamic stretching and skipping muscular warm-up exercises off the treadmill, and (iii) 10 to 13 min of interval treadmill-sprints (non-motorized mode) [24]. Warm-up duration was consistent within participants for the 3 conditions (total time range: 25 – 28 min). The RSP was composed of two sets (SET1 and SET2) of repeated-sprints, separated by 3 min recovery. Each SET consisted of five sprints (maximal 5 s sprints), separated by 25 s passive recovery (figure 1). Heart rate (HR; Polar S-810, Kempele, Oy, Finland) was continuously monitored, with mean peak cardiac frequency (HRpeak) calculated for each set using commercially available software (Polar Pro Trainer 5, Bethpage, NY, USA).

Treadmill: The validated instrumented treadmill ergometer allowed the participants to sprint against the resistance of a tethered belt, in the non-motorized mode [19]. The motor torque was set between

140% and 160% of the default torque necessary to overcome the friction on the belt due to the participant's body weight. This allowed participants to sprint in a maximal manner whilst minimizing participant risk of losing balance. A belt attached to a stiff rope (1 cm in diameter, ~3 m in length) was used to tether participants to an anchor point on the wall behind them. An additional overhead safety harness with sufficient slack not to impede natural running mechanics was also fastened to the participants. When correctly attached, participants could lean forward in a crouched sprint-start position with their preferred foot forward. This starting position was standardized and consistently used in all sprint efforts. For each sprint, following a 5 s verbal and visual countdown, the treadmill was released and the belt began to accelerate as a result of participants applying a positive (i.e., propulsive) horizontal force, i.e. the start of the sprint.

The following procedures were administered post SET1 and SET2 of the RSP, whilst participants were stood on the braked treadmill. Rating of perceived exertion (RPE; 1 – 10 scale [25]) and subjective ratings for pleasantness (feeling scale; FS) using a 'feeling scale' (+2= very pleasant, 1= pleasant, 0= neutral, -1= unpleasant and -2= very unpleasant [26]), immediately followed by a standing capillary blood sample, after which the harness was removed. Participants then stepped off the treadmill and completed the RTI procedures in a standardized standing position, taking between 60 – 90 s. A final body mass measure was taken after the final RTI post SET2 procedure was completed. The intervention procedures (CHO-M or PLA-MR) were administered prior to each sprint (see below).

Mouth rinse solutions and protocol: A single blind design was employed, whereby all but the principal investigator were blinded to MR solution. Solutions were prepared daily by the principal investigator and kept at room temperature. The 10% CHO-MR solution (10% w/v) contained 12 g of commercially available maltodextrin powder (SIS company, Nelson, UK) dissolved in a final volume 120-ml of distilled water. The PLA-MR solution was obtained from a commercially available non-caloric effervescent tablet (GO Hydro-SIS company, Nelson, UK), one tablet per 1000 ml of distilled water. Both solutions were indistinguishable in regard to color, odor, and taste. MR consisted of rinsing with 10 mL of solution for ~5 s and then expectoration of the solution into an empty plastic cup. Participants were instructed

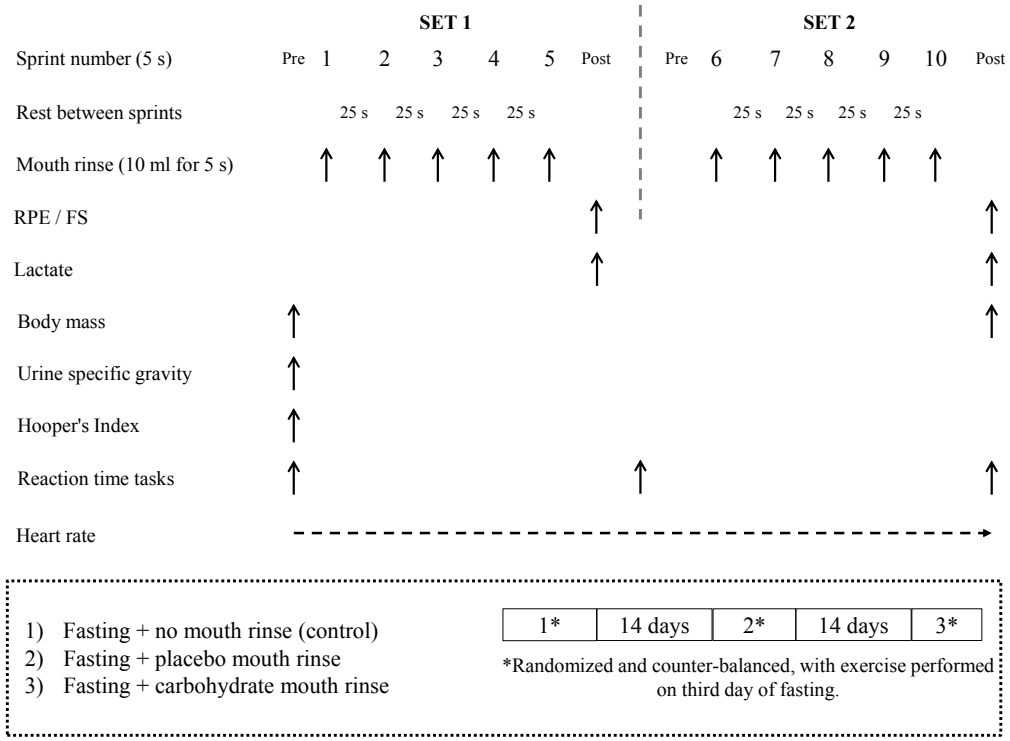

FIG. 1. Study design. Repeated-sprints protocol: [2sets: (5 x 5 s maximal sprints (25 s passive recovery between sprints) with 3 min recovery in-between sets]. A Heart rate (HR) monitor continuously recorded HR throughout the experiment. In the 2 mouth rinse trials (carbohydrate and placebo), each participant mouth rinsed the solution before each sprint. At Pre SET1: Urine and blood capillary samples (lactate) collection; body mass, Hooper's index and height measurement; Reaction time tasks (RTI) were assessed before starting the warm up. At Post SET1 and during the 3 min of recovery: Rating of Perceived Exertion (RPE), feeling scales (FS) and lactate were collected before starting the RTI test. At Post SET2: RPE, FS and lactate were collected before starting the RTI test and body mass measurement. The three experimental trials were separated by 15 days each and conducted in fasted state in a randomized and counter-balanced.

to thoroughly swill the fluid throughout their oral cavity for the MR duration. The MR fluid was weighed before and after each rinse using an electronic balance (Ohaus, New Jersey, USA) to assess the potential quantity of solution that could have remained in the oral cavity. The fluid was expressed by mass with the assumption that 1 g of the beverage equals 1 mL [7, 13]. Pilot testing with various volumes (5, 10, 20 and 25 ml) demonstrated that the 25 ml MR volume, although used elsewhere in resting participants [10], was not compatible with the vigorous exercise associated with the RSP.

RTI: The Cambridge Battery of Tests from the Cantab software (CANTABeclipse, Cambridge, UK) and hardware suite [27] were used to assess RTI, through a touch screen computer and a button box. Participants completed these RTI tasks in a standardized standing position, within an environmentally and noise-controlled room, with standardized verbal instructions provided to participants. Specifically, the RTI test measures *movement time*, *reaction time* and *response accuracy*. The overall task was divided into five stages, which require increasingly complex chains of responses. The RTI test outcome measures were divided into *simple* and *complex reaction scores*. Each measure was obtained by averaging the score over four trials. In the *simple-RTI*, a yellow dot appeared on the black screen in one pre-determined location whilst in the *complex-RTI*, the yellow dot appeared in one of five pre-determined different locations. At the sight of the dot appearing on the screen, the participant had to release the button and immediately hit the dot on the screen in the fastest time possible. In line with manufacturer's instructions, the computer software defined *reaction time* (as the time taken between the dot appearance on the screen and button being released), *movement time* (time between button release and screen touch) and *response accuracy* (hitting or missing the target area) as previously described [28].

Treadmill biomechanical parameters: The three-dimensional ground reaction force (GRF) and treadmill belt velocity were sampled at 1,000 Hz over the 5 s sprints, and digitally filtered with a zero phase-lag fourth-order Butterworth low-pass filter with a 30 Hz cut-off. Sprint onset was defined as the time when the belt speed exceeded 0.2 $m \cdot s^{-1}$. For each step, power in the anterior-posterior direction was computed as $P = F_H V$, where F_H is the horizontal GRF, and V is the treadmill belt velocity [21]. Vertical stiffness (K_{vert}) was calculated as $K_{vert} = F_{max}/\Delta COM$, where F_{max} is the vertical GRF peak, and ΔCOM is the maximum vertical displacement of the center of mass, which was calculated by integrating the vertical acceleration twice with respect to time [20]. All calculations were processed using a custom written Matlab (MathWorks, Version 8.4) program.

Statistical analysis

Statistical analysis was performed using SPSS version 21.0. All values are presented as means±SD. The data was screened for outliers

TABLE 1. Physiological parameters, Hooper's index, urine samples and sleep reported duration during the 24 h preceding the experimental testing collected at pre-RSP; Blood capillary samples (Lactate), RPE and HR taken at pre-, mid and post-RSP (mean ± SD; statistical outcome and P-value) of participants (n=15) in Control, CHO-MR and PLA-MR conditions.

	Control	CHO-MR	Placebo-MR	P-value
Night (h)	7.03±3.03	6.57±1.94	6.87±2.22	0.74
Total Sleep (h)	7.53±2.60	7.24±1.68	7.33±2.09	0.91
Hooper's Index (au)	8.93±4.06	9.20±3.61	9.27±3.22	0.96
Body mass loss (kg)	0.46±0.14	0.55±0.16	0.50±0.12	0.20
USG pre (au)	1.028±0.015	1.023±0.002	1.023±0.048	0.96
Pre-Lactate (mmol/L)	0.93±0.44	1.00±0.60	0.89±0.34	0.92
Mid-Lactate (mmol/L)	9.51±2.82	8.51±3.22	10.03±3.13	0.31
Post-Lactate (mmol/L)	12.06±3.41	11.22±2.44	11.37±2.89	0.51
Mid-RPE (au)	5.00±1.56	5.15±2.13	4.80±1.21	0.77
Post-RPE (au)	5.87±1.64	5.53±1.51	5.50±1.40	0.68
HR-Pre (bpm)	81.3±15.3	76.0±10.1	74.5±9.8	0.29
HR-Mid (bpm)	140.5±15.5	136.7±16.7	130.7±21.3	0.34
HR-Post (bpm)	136.1±14.5	135.1±14.9	136.4±17.4	0.97

RPE – Rating of Perceived Exertion; HR- Heart rate; Hooper's Index: summation of self-ratings of fatigue, stress, delayed onset muscle soreness and sleep; au: arbitrary units; CHO-MR: Carbohydrate mouth rinse solution; USG: Urine-specific gravity.
Pre: resting pre RSP, Mid: during the 3-minutes of recovery in-between 2 sets and Post: at post RSP (repeated-sprint protocol).

and deviation from normality using the Shapiro-Wilk test. USG and resting pre-RSP blood lactate values were not normally distributed and were thus log transformed with associated p-values based on a non-parametric equivalent (Friedman's ANOVA). A two way repeated measures ANOVA was used to determine: the effect of condition (PLA-MR, CON, and CHO-MR) and time (sprint 1 to 10) on mean power, mean speed and vertical stiffness, and the effect of condition (PLA-MR, CON, and CHO-MR) and time (pre-, mid- and post-RSP) on cognitive parameters (RTI simple and complex). A Linear Mixed Model was performed to analyze the effect of condition on RTI parameters at mid- and post-RSP after adjusting for pre-RSP RTI parameters. If a primary significant difference was observed, the post-hoc Bonferroni correction for multiple comparisons was used to detect where the differences occurred. The level of two tailed statistical significance was set at $p<0.05$ with effect sizes (ES) calculated from Partial Eta-squared. A large effect size was determined as ≥ 0.25, medium as >0.09 and small as ≤ 0.09 [29].

RESULTS

No effect of condition on all physiological measures were seen (table 1). No effect of condition (PLA-MR, CON, and CHO-MR) and time (sprint 1 to 10) on mean power (P=0.630, ES=0.032), mean speed (P=0.835, ES=0.014) and vertical stiffness (P=0.670, ES=0.030) (table 2), and on cognitive parameters (RTI simple and complex) (table 3), were observed. Specifically, no effect of condition on RTI tests of accuracy (simple P=0.357, ES=0.071; complex P=0.259, ES=0.092), movement time (simple P=0.546, ES=0.042; complex P=0.902, ES=0.007), and reaction time (simple P=0.514, ES=0.046; complex P=0.758, ES=0.020) were seen. No significant differences were present for USG, mean HR,

TABLE 2. Average maximal power (w/kg), maximal speed (m/s) and vertical stiffness (kN/m) of the participants during the three experimental conditions (CON, CHO-MR and PLA-MR) for 10 repeated sprints (S1 to S10) of 15 participants. Values are presented as means ± SD and range (minimum - maximum).

	Maximal Power (w/kg)			Maximal Speed (m/s)			Vertical Stiffness (kN/m)		
Sprints	CON	CHO-MR	PLA-MR	CON	CHO-MR	PLA-MR	CON	CHO-MR	PLA-MR
S1	15.4±4.2	14.1±3.8	13.7±3.6	4.7±0.9	4.5±0.9	4.5±0.8	95.8±18.7	92.7±18.7	92.6±15.3
	15.1 (6.9-22.0)	13.3 (6.3-19.6)	14.9 (6.0-18.7)	3.1 (2.9-6.0)	3.1 (2.6-5.7)	3.2 (2.9-6.1)	59.2 (70.0-129.2)	61.1 (66.8-127.9)	49.1 (63.6-112.7)
S2	13.1±3.3	12.8±3.5	13.3±3.9	4.4±0.8	4.3±0.8	4.3±0.9	87.8±18.1	89.0±17.5	87.9±15.7
	13.3 (5.8-19.1)	12.7 (5.9-18.6)	13.2 (5.5-18.7)	3.0 (2.5-5.5)	2.9 (2.5-5.4)	3.0 (2.5-5.5)	63.6 (69.3-132.8)	70.9 (55.5-126.4)	45.2 (64.8-110.0)
S3	12.8±4.3	12.6±3.9	12.2±3.8	4.2±1.0	4.3±0.9	4.3±1.0	85.9±18.8	85.5±16.9	84.8±13.2
	14.3 (4.3-18.6)	11.8 (6.0-17.8)	14.6 (4.1-18.7)	3.3 (2.3-5.6)	2.9 (2.5-5.4)	3.3 (2.3-5.7)	49.0 (68.3-117.3)	62.0 (52.6-114.6)	61.1 (54.5-115.6)
S4	11.6±3.8	15.1±3.7	13.9±3.7	4.1±0.9	4.7±0.9	4.5±1.0	84.4±18.3	83.7±16.5	84.2±13.2
	13.5 (5.2-18.9)	12.2 (5.5-17.8)	13.9 (4.0-17.9)	3.0 (2.6-5.6)	2.9 (2.5-5.4)	3.0 (2.6-5.6)	53.4 (66.8-120.2)	54.7 (66.0-120.7)	63.4 (62.4-126.1)
S5	13.6±3.9	13.3±3.7	13.0±4.1	4.4±1.0	4.4±0.9	4.3±1.0	82.6±19.2	83.6±18.1	83.0±12.1
	14.3 (4.3-18.6)	13.1 (5.6-18.7)	14.9 (3.2-18.1)	3.3 (2.2-5.5)	3.0 (2.4-5.4)	3.3 (2.3-5.5)	54.5 (70.2-124.7)	66.5 (54.3-120.8)	38.5 (65.3-103.8)
S6	13.6±4.44	12.1±5.0	13.1±4.1	4.4±1.0	4.4±1.0	4.4±1.0	88.7±19.0	84.7±19.1	91.4±16.7
	14.8 (4.2-18.9)	13.3 (5.9-19.2)	14.9 (4.9-19.9)	3.6 (2.1-5.7)	3.0 (2.5-5.5)	3.6 (2.1-5.7)	64.7 (63.4-128.1)	55.4 (54.9-110.3)	63.5 (53.6-117.1)
S7	12.6±3.5	12.5±3.9	15.0±3.4	4.3±0.9	4.3±1.0	4.6±0.7	87.3±16.5	83.5±22.0	87.4±17.2
	16.2 (2.8-19.0)	13.5 (5.4-18.9)	18.1 (0.0-18.1)	4.2 (1.5-5.6)	3.2 (2.4-5.6)	4.1 (1.5-5.6)	57.7 (60.1-118.4)	48.7 (61.8-110.4)	56.9 (55.3-112.2)
S8	14.0±3.5	13.8±3.3	13.5±3.5	4.5±0.8	4.4±0.8	4.4±0.8	83.6±20.2	80.6±17.2	84.8±15.7
	16.6 (3.3-18.9)	14.1 (4.5-18.6)	14.9 (3.7-18.6)	3.7 (1.9-5.6)	3.4 (2.1-5.5)	3.5 (1.9-5.6)	52.0 (65.5-117.5)	71.3 (55.5-126.8)	60.0 (70.3-130.3)
S9	13.2±3.5	14.1±3.8	13.4±4.1	4.4±0.8	4.5±0.8	4.4±1.0	80.3±19.5	79.3±17.2	82.8±13.9
	15.5 (3.5-19.0)	13.7 (4.9-18.6)	11.9 (5.4-17.3)	3.7 (1.9-5.6)	3.3 (2.3-5.5)	3.7 (1.9-5.6)	47.7 (64.6-112.3)	67.7 (54.2-121.9)	53.8 (55.8-126.8)
S10	13.1±3.7	12.6±3.7	12.9±3.6	4.3±0.9	4.2±0.9	4.3±0.9	82.9±21.4	81.3±18.8	86.4±17.1
	15.1 (3.6-19.0)	12.1 (6.9-19.1)	14.6 (2.8-17.4)	3.4 (2.1-5.5)	3.0 (2.7-5.7)	3.4 (2.1-5.5)	49.9 (61.6-111.5)	60.1 (66.5-126.6)	71.3 (55.5-126.8)

No significant difference was observed between trials ($P > 0.05$).

TABLE 3. Response times during cognitive testing. Mean values (± SD) (n=15) and range (minimum - maximum) of the accuracy (%), movement time (ms) and reaction time (ms) in simple and complex reaction time test (RTI), respectively at pre, mid and post-RSP in Control, PLA-MR and CHO-MR.

		RTI Accuracy score (%)		RTI Movement time (ms)		RTI Reaction time (ms)	
Condition	**Time**	**Simple**	**Complex**	**Simple**	**Complex**	**Simple**	**Complex**
CON	Pre	8.6±0.6 2 (7 – 9)	7.4±0.8 3 (5 – 8)	302.5±196.9 715 (123-837)	198.0±69.2 272 (134-406)	294.9±36.8 136 (234-370)	317.3±38.8 146 (272-418)
	Mid	8.5±0.5 2 (7 – 9)	7.5±0.7 2 (6 – 8)	188.3±75.1 283 (112-395)	180.1±37.5 133 (117-249)	311.7±36.5 132 (252-384)	316.2±35.9 111 (267-378)
	Post	8.4±0.7 2 (7 – 9)	7.6±0.6 2 (6 – 8)	223.2±86.3 284 (112-395)	199.9±46.8 146 (120-265)	305.4±55.5 175 (236-411)	312.1±36.7 135 (252-387)
PLA-MR	Pre	8.2±0.8 5 (7 – 12)	7.8±0.4 1 (8 – 7)	269.7±136.8 484 (128-612)	180.1±27.4 99 (138-237)	279.0±52.1 220 (226-446)	294.4±36.9 143 (248-391)
	Mid	8.4±0.8 2 (7 – 9)	7.9±0.4 1 (8 – 7)	183.6±68.4 281 (121-402)	188.6±46.6 168 (126-295)	313.9±66.7 228 (239-467)	310.5±39.6 148 (247-395)
	Post	8.7±1.1 2 (8 – 10)	7.5±0.6 2 (8 – 6)	231.9±84.9 331 (131-463)	210.6±61.6 228 (150-378)	296.0±46.2 164 (228-392)	325.6±70.4 265 (233-498)
CHO-MR	Pre	8.8±1.2 2 (7 – 9)	7.6±0.6 2 (6 – 8)	373.6±286.7 1007 (122-1129)	209.1±80.2 277 (118-395)	299.9±45.2 164 (217-381)	340.2±132.4 546 (263-809)
	Mid	8.3±0.7 2 (7 – 9)	7.6±0.7 2 (6 – 8)	197.1±85.9 355 (112-467)	184.4±62.7 242 (118-360)	303.1±43.4 140 (236-375)	308.8±34.3 130 (241-370)
	Post	8.8±0.6 5 (7 – 12)	7.7±0.9 4 (6 – 10)	207.8±86.0 330 (108-438)	172.5±42.5 167 (117-284)	280.4±35.9 132 (217-349)	308.3±41.5 132 (264-396)

ms – milliseconds. RSP: Repeated-sprints protocol. Pre: resting pre RSP, Mid: during the 3-minutes of recovery in-between 2 sets and Post: at post RSP. No significant difference was observed between trials ($P > 0.05$).

blood lactate and RPE throughout the exercise (all $p>0.05$) between conditions. The mean expectorated volume per MR did not statistically differ ($P=0.871$) between conditions (CHO-MR: 9.97±0.15 ml; PLA-MR 9.98±0.13 ml) inclusive of saliva volume. Participants (CHO-MR 75%; PLA-MR 67%) generally reported MR solutions to be of a pleasant taste.

DISCUSSION

The present study demonstrated that 10% CHO-MR for 5 s in a fasted state (third day of 3d-IF, composed ~14 h of fasting per day) during the RSP, had no significant effect on physical performance or cognitive function; thus the stated experimental hypothesis is rejected. This running based RSA based data contributes to previous specific observations that CHO-MR (6.4% CHO-MR for 10 to 15 s) following overnight fasting elicits no performance effect during anaerobically biased short duration exercises, including maximal strength and strength endurance exercise [30], and 30 s cycle sprint cycling (6.4% CHO-MR for 5 s [31]). However, such conclusions must be tempered by evidence that CHO-MR may improve high-intensity interval running capacity (1 min at 80% VO_2 max interspersed with 1 min walking at 6 $km.h^{-1}$) in CHO restricted, not fasted, participants [32].

Whilst it appears that CHO-MR may have specific effects dependent upon the participants fed state, with enhanced performance effects during cycling exercise (subjects were asked to maintain their highest sustainable average power output within the 60 min) when athletes were in a fasted as opposed to fed state [6]. It is important to note that CHO-MR exercise performance evidence is skewed to non-fasted [9, 12, 33] rather than fasted [4, 34] endurance exercise experimental designs. Consequently, it is difficult to critically compare the present fasted RSP data (5 min total exercise duration; 2 x 5 x 5 s maximal effort sprints = 100 s sprinting) to fasted experimental designs involving ~60 min endurance exercise [4, 6, 9]. Therefore, whilst CHO-MR is mechanistically capable of stimulating reward and/or motivation centers in the brain [12], this stimulus was insufficient to influence the cognitive and physical performance outcome variables employed within the present design. It is plausible that there is a lag time between MR and the activation of the required brain regions ('mouth-to-muscle' hypothesis) which when observed elsewhere has been suggested to underpin enhanced mean power in an ~1 h high-intensity time trial [12, 33]. Such a lag time may underpin the null experimental findings within the present design, however specific deductive experimental designs are required to provide empirical evidence in this regard.

To our knowledge this experiment is the first to determine the effect of repeated 10% CHO-MR on cognitive performance in a fasted state during and after RS exercise. The present data demonstrates CHO-MR (5 s before each sprint) does not influence RTI tasks. This supports other data [35] whereby 5 s of 6% CHO-MR solution was administered following an overnight fast, which failed to elicit improvement in a 20 min continuous performance task (a cognitive test requiring sustained attention, working memory, response time, and error monitoring for 20 min) at rest. Whilst significant increases in the excitability of the corticomotor pathway have been shown in response to CHO-MR (10%) for 15 to 60 s where participants fasted for 12 h overnight [36,37]. Increased brain activity such as this (orbitofrontal) in response to 6.4% CHO-MR for 20 s failed to enhance reaction time within fed participants at rest [10]. Whilst the fed or fasted state of the participants [6], and the CHO rinse concentration [5] and duration [7] all have varying efficacy to activate several higher centers [12], this activation often does not elicit improvements in a range of cognitive processes [10]; with such effects complicated by the presence and proximity of various exercise modalities [9]. Deductive experimental designs are therefore required to precisely characterize the dose response of various CHO-MR strategies, in fed and fasted states, and subsequent mechanistically underpinned effects on physical and cognitive performance. The use of EEG may provide valuable insights into the precise oropharyngeal receptors and subsequent mechanisms of activation of brain regions implicated within CHO-MR. However, with specificity to the presented RSA based experimental design, EEG utilization is challenging relative to brief very intense exercise bouts.

In this study, the expectorated volume per MR was similar in both CHO-MR [range: 3.1 (8.33 – 11.43)] and PLA-MR [range: 1.98 (9.14 – 11.12)], as shown elsewhere [38]. This suggests a small amount of the MR solution was retained within the mouth. Whether this retained solution is exhaled during participant raised ventilation during exercise and/or swallowed cannot be determined by the present design. However, across the 20 MR procedures (CHO-MR and PLA-MR across SET1 and SET2) performed, 3 participants reported having been aware of inadvertently swallowing small quantities of solution. Although this represents only ~1.5% of the total MR procedures, it is an important consideration for participants fasting for religious reasons. An overt experimental limitation is that it is practically impossible to blind the participant to the fact that they were (i) fasting without mouth rinsing (MR) or (ii) fasting with MR. Therefore, although the presented data must be interpreted carefully relative to the above experimental limitations, the null experimental findings make it unlikely that that these limitations confounded the main findings of this research project.

CONCLUSIONS

CHO-MR (5 s, 10% CHO, 10 mL) in a fasted state (abstaining from food and fluid 14 h per day) did not affect physical (RSP) or cognitive (RTI) performance during the RSP. The lack of experimental effect may be due to insufficient stimulation (volume and duration of MR) of the implicated oral receptors.

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Conflicts of interest

All the authors declare that they have no conflicts of interest relevant to the content of this manuscript. Informed consent was obtained from all individual participants included in the study. The authors declare that they do not have anything to disclose regarding funding with respect to this study.

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