Original Research

Carbohydrate Mouth Rinse Improves 1.5 h Run Performance: Is There A Dose-Effect?

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ABSTRACT

International Journal of Exercise Science 6(4) : 328-340, 2013. There is a substantial body of recent evidence showing ergogenic effects of carbohydrate (CHO) mouth rinsing on endurance performance. However, there is a lack of research on the dose-effect and the aim of this study was to investigate the effect of two different concentrations (6% and 12% weight/volume, w/v) on 90 minute treadmill running performance. Seven active males took part in one familiarization trial and three experimental trials (90-minute self-paced performance trials). Solutions (placebo, 6% or 12% CHO-electrolyte solution, CHO-E) were rinsed in the mouth at the beginning, and at 15, 30 and 45 minutes during the run. The total distance covered was greater during the CHO-E trials (6%, 14.6 ± 1.7 km; 12%, 14.9 ± 1.6 km) compared to the placebo trial (13.9 ± 1.7 km, $P < 0.05$). There was no significant difference between the 6% and 12% trials ($P > 0.05$). There were no between trial differences ($P > 0.05$) in ratings of perceived exertion (RPE) and feeling or arousal ratings suggesting that the same subjective ratings were associated with higher speeds in the CHO-E trials. Enhanced performance in the CHO-E trials was due to higher speeds in the last 30 minutes even though rinses were not provided during the final 45 minutes, suggesting the effects persist for at least 20-45 minutes after rinsing. In conclusion, mouth rinsing with a CHO-E solution enhanced endurance running performance but there does not appear to be a dose-response effect with the higher concentration (12%) compared to a standard 6% solution.

KEY WORDS: Mouth wash, treadmill running, nutrition, endurance

INTRODUCTION

There is now considerable evidence for a 'non-metabolic' or 'central' effect of carbohydrate (CHO) on endurance performance (4, 6, 7, 12, 13, 16). This idea was first postulated when it was discovered that CHO ingestion, during activity that is not limited by CHO availability or oxidation rate, such as high intensity (e.g. > 70% VO_2max) relatively short duration (up to 1 h) exercise, is associated with enhanced performance (1, 10). This notion was further strengthened by the observations of Carter et al. (5) that the intravenous infusion of glucose during a 1 h cycling time-trial did not improve performance, despite the previous work showing ingestion to

improve performance. Following this, Carter et al. (4) were the first to provide evidence that CHO (maltodextrin) mouth rinses improved performance compared to that of a control rinse of water. This led to the suggestion that CHO sensing occurs in the mouth resulting in an ergogenic effect on performance via a central action, possibly by enhancing motor drive or motivation (or blunting their perturbation) during fatiguing exercise. A considerable body of research now exists showing that simply rinsing the mouth with a CHO-containing solution can have an ergogenic effect on endurance exercise (4, 6, 7, 11–13), although not all studies have observed benefits (2, 21). The work of Chambers et al. (6) is particularly important as they have demonstrated that CHO sensing in the mouth is associated with activation of reward centers in the brain and that this is independent of sweetness. Furthermore, Gant et al. (8) have provided evidence that the presence of a non-sweet carbohydrate (maltodextrin) in the mouth may enhance muscle function and facilitate corticomotor output. Together, these findings provide mechanistic evidence that CHO does have central, non-metabolic, ergogenic effects that can be induced simply by the presence of CHO in the mouth, although there is a lack of evidence on the effect of different doses.

The CHO concentrations used in all of the previous mouth rinse studies are ~ 6% weight/volume (w/v), which seems to be somewhat arbitrarily based on the composition of commercially available sports drinks and previous work on CHO ingestion. However, as the mechanisms for performance benefit with rinse are very different to those with ingestion there could be greater benefit with higher concentrations, but this has not yet been determined. Evidence suggests that the mechanisms responsible for the ergogenic effects of CHO mouth rinsing are related to CHO-sensing in the oral cavity (6). However, it is unknown whether these oral receptors are sensitive to the concentration of CHO in the solution and no dose-response studies have been conducted with CHO mouth rinsing. In rodents allowed free access to different solutions, it has been demonstrated that, for glucose as well as CHO polymer solutions, there is a concentration-dependent effect on affective behavior response. Although animals ingested the solutions, knockout of the T1R2 and T1R3 proteins demonstrated that these behaviors were attributable, at least in part, to oral CHO receptors (20). Interestingly, Treesukosol et al. (20) showed a dose-response effect with 9% w/v being the optimal concentration for glucose in their wild-type mice. There was little difference, compared to water, for solutions with a concentration of 4.5% and lower, whereas there was a plateau at concentrations above 9%. Equivalent evidence is lacking in humans and there are no dose-response studies with mouth rinsing rather than ingestion. However, Smeets et al. (17) conducted an fMRI study (to measure hypothalamic responses) with glucose ingestion at a variety of solution concentrations (0%, 8.3% and 25% w/v) and observed significant effects of the CHO within minutes of ingestion. Since these effects were observed immediately after ingestion (i.e. before any absorption or 'metabolic' effects would manifest) this does suggest similar 'non-metabolic' effects to those observed by Chambers et al. (6). In this study, these observed effects were more marked with the higher concentration glucose solution (17). Taken together, the

evidence discussed above provides support for the notions that the optimal CHO concentration to induce positive performance effects in humans could also be greater than the typical ~6% used in previous mouth rinse studies. Furthermore, no studies have yet determined the effects of CHO mouth rinsing on exercise of longer than 1 h in duration. Therefore, the aims of this study were 1) to determine whether a carbohydrate mouth rinse enhances performance in a 90 minute treadmill performance trial; and 2) to determine whether a higher concentration (12%) has a greater effect than a 6% solution.

METHODS

This study was conducted according to the guidelines laid down in the Declaration of Helsinki (2004). All procedures were approved by Aberystwyth University Research Ethics Committee for research involving human participants. Written informed consent was obtained from all subjects. Subjects also completed a pre-exercise screening questionnaire (Physical Activity Readiness Questionnaire) before participating in each test.

Participants

Seven male university students (age 21 ± 1 years; body mass 78 ± 7 kg; stature 1.81 ± 0.12 m; means ± standard deviation) participated in this study. All subjects were physically active and represented the university in a competitive sports team (e.g. football [soccer], rugby, field hockey) but were not specifically endurance trained.

All subjects took part in a familiarization trial and three main (experimental) trials: placebo (PLA, 0% CHO-electrolyte solution, CHO-E, rinse solution), 6% CHO-E rinse solution, and 12% CHO-E rinse solution. All trials took place at the same time of day (start time within 1 hour) for each subject, and were separated by at least five days. Participants first completed the familiarization trial, which was identical to the main trials except plain water was used as the mouth rinse solution. In this trial subjects were accustomed with the mouth rinse procedure. The main trials were conducted in randomized order and solutions were administered double-blind. Participants were required to be fasted for at least six hours before each trial. In addition they were required to keep a record of food and activity during the 24 hours before the first main trial and replicate this before any subsequent trials.

Protocol

All trials were conducted on a motorized treadmill (PPS 55med, Woodway GmbH, Weil am Rhein, Germany). Subjects were first asked to perform a 5 minute warm up at 6 km/h before beginning the performance trial. The test began with a rolling start (at a treadmill speed of 8 km/h) and subjects were allowed to freely control the treadmill speed using the manual controls located on the handrail. They were instructed to cover as much distance as possible during the 90 min test. Subjects were not able to see the treadmill speed or distance covered, or heart rate, on the display panel but they were allowed to see the clock showing time elapsed. No encouragement was provided to the subjects during all of the tests.

Carbohydrate-containing solutions were made with a commercially available CHO-electrolyte product (H5 Ltd., Derby, UK) supplied as a powder. The powder was

mixed with concentrated, artificially sweetened (saccharin), cordial drink and plain water (1:3 ratio concentrate:water) to give final CHO concentrations of 6% and 12% w/v, with approximately 418 mg and 836 mg of sodium per liter in the 6% and 12% solutions, respectively. The CHO was comprised of maltodextrin (95%), dextrose (3%) and maltose (2%) and the PLA solution did not have any of this powder added but contained additional sweeteners (saccharin) to help with blinding, in accordance with the methods of Chambers et al. (6).

For the rinse procedure subjects were given a plastic cup containing 25 ml of solution. They were required to rinse the solution in their mouth for 5 seconds before expectorating back into the cup. The cups were marked with a graduation at 25 ml so that the volume of expectorate could be inspected to ensure that none of the liquid was swallowed. The first mouth rinse procedures occurred after the warm up and then at 15, 30 and 45 minutes of the performance trial.

Room temperature and atmospheric pressure were monitored and recorded, prior to each trial, with a temperature probe (Rotronic Hygromer Pt100, Grant Instruments, Cambridge, UK) connected to an electronic data logger (Squirrel SQ2020, Grant Instruments, Cambridge, UK) and a mercury column direct reading barometer (Cranlea, Birmingham, UK), respectively. The distance and speed were recorded every 10 minutes during each trial and total distance was recorded at the completion of the 90-minute period. The distance covered at each 10 min split was used to calculate average speed over each segment. Heart rate was measured using a telemetric device (Polar S610i, Kempele, Finland). Rating of perceived exertion (RPE), and subjective ratings of Feeling and Arousal were expressed using the Borg scale (3), Feeling scale (9), and Arousal scale (19), respectively. These measures were recorded after the warm up and every 15 minutes during each trial. Heart rate was also recorded at rest before the warm up.

Expired respiratory gas was collected at 15, 30 and 45 minutes during the trials using 150 L Douglas bags. Oxygen and carbon dioxide concentrations were determined using paramagnetic oxygen and infrared carbon dioxide analyzers (Servomex 4100, Crowborough, UK) and gas volume was measured with a dry gas meter (Harvard Apparatus Ltd., Edenbridge, UK) in order to determine oxygen consumption and carbon dioxide output. These values were used to calculate respiratory exchange ratio (RER). Capillary blood samples were obtained from a fingertip pre- (5 min before warm-up) and post-exercise (immediately on completion of the 90 min run) using an automatic lancet device (Soft clix pro, Accu-check, Mannheim, Germany) and collected into Lithium-Heparin treated microtubes (Microvette cb300, Sarstedt, Nümbrecht, Germany) for the determination of blood glucose and lactate concentrations using an automated analyzer (YSI 2300 Stat Plus, Yellow Springs, OH, USA).

Statistical Analysis

Data analyses were carried out using the software package SPSS (v17.00; SPSS Inc., Chicago, IL, USA). All data were normally distributed as determined by Z-scores for skewness and kurtosis (within ± 2), with the exception of Feeling scale data. One-way repeated measures ANOVA tests (with Holm-Bonferroni corrected post hoc paired

t-tests, where necessary) were used to compare performance (distance covered), and ambient conditions between trials. For normally distributed data, 2-way (trial × time) repeated measures ANOVA was used to compare variables with multiple measurement points during the trials (distance, speed, heart rate, RPE, Arousal, blood [glucose], blood [lactate], and respiratory variables) between trials. Mauchley's test was used to determine if the assumption of sphericity was met. If the sphericity assumption was violated the Greenhouse-Geisser correction was applied to ANOVA P values (indicated by subscript $_{GH}$ after P values in the text), otherwise no correction was applied. For the Feeling scale data non-parametric tests were used. Overall comparisons were made between trials and within trials (across time) with the Friedman test. Also, the discrepancy between the first and last times was compared between trials for equivalence with the trial × time interaction comparisons in a 2-way ANOVA. These data were normally distributed so a 1-way ANOVA was used. Statistical significance was accepted when $P < 0.05$. All results are presented as mean ± standard deviation unless otherwise stated.

RESULTS

Room temperature (ANOVA, P = 0.725) and barometric pressure (ANOVA, P = 0.282) were relatively stable and similar between trials. Mean temperature was 19.5 ± 1.0 °C, 19.7 ± 1.2 °C, and 19.2 ± 1.6 °C for the PLA, 6% CHO-E and 12% CHO-E trials, respectively. Mean barometric pressure was 743 ± 6 mmHg, 752 ± 11 mmHg, and 755 ± 21 mmHg for the PLA, 6% CHO-E and 12% CHO-E trials, respectively.

There was a significant difference between trials in distance covered during the 90 minute performance run (ANOVA, P = 0.001, see Table 1a and Table 1b). Post Hoc analyses revealed that there was a significant difference between the PLA and 6% CHO-E trials (P = 0.035) and between the PLA and 12% CHO-E trials (P = 0.003). There was no difference between the 6% CHO-E and 12% CHO-E trials (P = 0.196).

Table 1a. Distance covered during 90 min performance run on each trial.

	PLA	6% CHO-E	12 %CHO-E
Distance (km)	13.9 ± 1.7	14.6 ± 1.7*	14.9 ± 1.6**

Values are mean ± SD. Significantly different from PLA trial (*P < 0.05; **P < 0.01).

Table 1b. Individual subject improvement in each CHO-E trial compared to PLA.

	6% CHO-E	12 %CHO-E
Subject 1	5.6	6.0
Subject 2	0.7	4.8
Subject 3	6.4	4.5
Subject 4	2.8	4.3
Subject 5	0.3	6.9
Subject 6	11.3	8.7
Subject 7	13.5	18.6

Values show percentage improvement compared to the PLA trial.

Two-way repeated measures ANOVA revealed a significant main effect of trial (P = 0.001) for average speed over each 10-minute segment of the run (Figure 1). There was also a trend for an effect of time (P = 0.053) but no significant trial × time interaction (P = 0.436). Due to the main effect of trial, the average speed for each 10 min segments were compared between

trials with 1-way ANOVA and post hoc paired t-tests (Holm-Bonferroni corrected) where necessary (see Figure 1). There were no significant differences between trials in the first 60 min (1-way ANOVA, P = 0.506, 0.213, 0.823, 0.359$_{GH}$, 0.933, 0.373 for the first 6 segments, respectively). For the 7th and 8th segments there were significant differences (P = 0.001 and 0.010, respectively) but there were no differences for final segment (P = 0.141). Post hoc comparisons revealed that the average segment speed, in the 7th segment, was significantly lower in the PLA trial compared to the 6% CHO-E (P = 0.014) and 12% CHO-E (P = 0.003) trials with no difference between the 6% and 12% CHO-E trials (P = 0.156). In the 8th segment average speed was lower in the PLA trial compared to the 6% CHO-E (P = 0.038) and 12% CHO-E (P = 0.021) trials with no difference between the 6% and 12% CHO-E trials (P = 0.889).

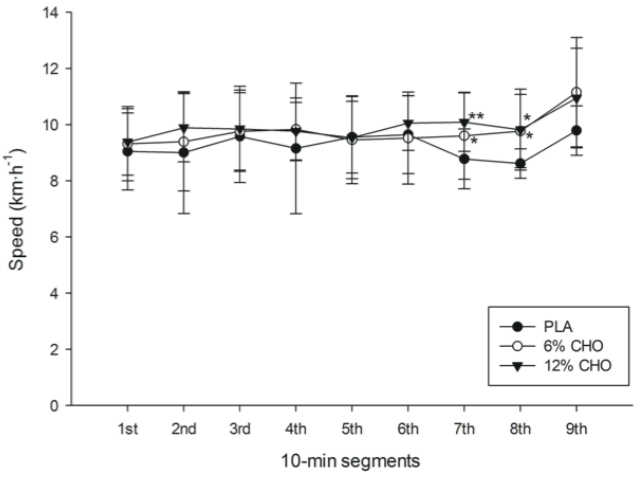

Figure 1. Average running speed in each 10-minute segment of the performance trials. Significantly different from PLA trial (*P < 0.05; **P < 0.01). PLA = placebo solution, 6% CHO = CHO-E solution containing 6% w/v carbohydrate, 12% CHO = CHO-E solution containing 12% w/v carbohydrate.

For heart rate (Table 2), 2-way repeated measures ANOVA showed no significant main effect of trial (P = 0.131) or trial × time interaction (p = 0.097). There was a significant effect of time (P < 0.001). Heart rate increased progressively during the trial with each point significantly higher than the previous one (all P < 0.05) with one exception, in that the heart rate at 75 minutes was not significantly different from 60 minutes (P = 0.573). For blood glucose concentration (Table 2) the 2-way repeated measures ANOVA showed no significant main effect of trial (P = 0.246) and trial × time interaction (P = 0.511). There was a significant main effect of time (P = 0.018) with higher concentrations post-exercise. For blood lactate concentration (Table 2) the 2-way repeated measures ANOVA showed no significant main effect of trial (P = 0.761) and trial × time interaction (P = 0.938). There was a significant main effect of time (P = 0.018) with higher concentrations post-exercise. For Rating of Perceived Exertion 2-way repeated measures ANOVA showed no significant main effect of trial (P = 0.258) and trial × time interaction (P = 0.657). There was a significant main effect of time (P < 0.001$_{GH}$). RPE increased progressively during the trial with each point significantly higher than the previous one (all P < 0.05, see Table 2).

For Feeling scale ratings (Figure 2), a Friedman test revealed a significant effect of time (P < 0.001) in all trials (PLA, 6% CHO-E and 12% CHO-E). There were no between trial differences at any of the time points although there was a trend at 90 min (Friedman, P = 0.084). A 1-way ANOVA on the discrepancy data (which were normally distributed) revealed a significant difference between trials (P = 0.030). Post hoc analysis for the discrepancy data revealed no difference between the PLA

Table 2. Physiological and subjective responses.

		Rest	0	15	30	45	60	75	90	Post
Heart Rate	PLA	83 ± 11	129 ± 17	157 ± 12	161 ± 14	168 ± 15	171 ± 15	173 ± 14	179 ± 14	–
(bpm)	6% CHO-E	81 ± 8	122 ± 23	152 ± 10	158 ± 10	165 ± 16	169 ± 15	168 ± 16	180 ± 14	–
	12% CHO-E	80 ± 8	113 ± 27	154 ± 10	158 ± 8	159 ± 12	167 ± 9	169 ± 7	180 ± 12	–
Post hoc (time)			#	#	#	#	#		##	
RPE	PLA	–	8 ± 2	11 ± 2	13 ± 2	14 ± 2	16 ± 2	17 ± 1	17 ± 1	–
	6% CHO-E	–	8 ± 2	10 ± 3	13 ± 2	15 ± 1	16 ± 1	17 ± 1	18 ± 1	–
	12% CHO-E	–	8 ± 2	11 ± 2	12 ± 1	14 ± 2	15 ± 2	16 ± 2	18 ± 1	–
Post hoc (time)				#	#	##	##	##	#	
Arousal	PLA	–	2 ± 1	3 ± 1	3 ± 2	3 ± 1	4 ± 2	4 ± 2	4 ± 2	–
	6% CHO-E	–	3 ± 2	3 ± 1	4 ± 1	4 ± 2	4 ± 2	4 ± 2	5 ± 1	–
	12% CHO-E	–	2 ± 1	3 ± 1	3 ± 2	3 ± 2	4 ± 2	4 ± 2	5 ± 2	–
Post hoc (time)										
Blood glucose	PLA	4.4 ± 0.1	–	–	–	–	–	–	–	4.9 ± 0.5
(mmol/L)	6% CHO-E	4.3 ± 0.7	–	–	–	–	–	–	–	4.3 ± 0.4
	12% CHO-E	4.4 ± 0.5	–	–	–	–	–	–	–	4.7 ± 0.7
Post hoc (time)										#
Blood lactate	PLA	0.8 ± 0.3	–	–	–	–	–	–	–	1.9 ± 0.9
(mmol/L)	6% CHO-E	1.0 ± 0.3	–	–	–	–	–	–	–	2.0 ± 1.1
	12% CHO-E	0.8 ± 0.4	–	–	–	–	–	–	–	1.9 ± 1.0
Post hoc (time)										#

Values are mean ± SD. Time point 0 is post-warm up. Time effect: Significantly different from previous point (#P < 0.05; ##P < 0.01). PLA = placebo solution, 6% CHO = CHO-E solution containing 6% w/v carbohydrate, 12% CHO = CHO-E solution containing 12% w/v carbohydrate.

and 6% CHO-E trials (P = 0.173), a significant difference between the PLA and 12% CHO-E trials (P = 0.030) and no difference between the 6% and 12% CHO-E trials (P = 0.386). When analyzed in 30-minute segments Feeling data were normally distributed and 2-way repeated measures ANOVA revealed a significant main effect of time ($P < 0.001_{GH}$). There was no significant main effect of trial (P = 0.593) and a trend for a trial × time interaction (P = 0.071). Post hoc analysis for the time effect showed that Feeling ratings were significantly lower in the last 30-minute segment compared to the first 30-minute (P = 0.002) and second 30-minute (P = 0.003) segments. Ratings were also significantly lower in the second compared to first 30-minute segment (P < 0.001). For Arousal ratings (Table 2), 2-way repeated measures ANOVA showed no significant main effect of trial (P = 0.328), time ($P = 0.125_{GH}$) and trial × time interaction (P = 0.377).

For oxygen consumption (Table 3), 2-way repeated measures ANOVA showed no significant effect of trial (P = 0.247), time (P = 0.082) or trial × time interaction (P = 0.244). For carbon dioxide output (Table 3), 2-way repeated measures ANOVA showed no significant effect of trial (P = 0.066), time ($P = 0.476_{GH}$), or trial × time interaction ($P = 0.151_{GH}$). For Respiratory Exchange Ratio (Table 3), 2-way repeated measures ANOVA showed no significant main effect of trial (P = 0.886), time (P = 0.533) and trial × time interaction (P = 0.477).

DISCUSSION

The main findings of the present study are that rinsing the mouth with a Carbohydrate-electrolyte (CHO-E) solution,

Table 3. Respiratory measures.

		15 min	30 min	45 min
Oxygen uptake (l/min)	PLA	2.91 ± 0.41	3.10 ± 0.38	2.96 ± 0.34
	6% CHO-E	2.95 ± 0.39	3.11 ± 0.54	3.15 ± 0.47
	12% CHO-E	2.86 ± 0.34	2.87 ± 0.34	2.98 ± 0.37
Carbon dioxide output (l/min)	PLA	2.40 ± 0.36	2.88 ± 0.33	2.48 ± 0.31
	6% CHO-E	2.42 ± 0.33	2.47 ± 0.29	2.61 ± 0.35
	12% CHO-E	2.35 ± 0.32	2.33 ± 0.32	2.26 ± 0.66
RER	PLA	0.82 ± 0.02	0.83 ± 0.02	0.83 ± 0.03
	6% CHO-E	0.82 ± 0.05	0.81 ± 0.10	0.83 ± 0.04
	12% CHO-E	0.83 ± 0.08	0.81 ± 0.08	0.81 ± 0.08

Values are mean ± SD. PLA = placebo solution, 6% CHO = CHO-E solution containing 6% w/v carbohydrate, 12% CHO = CHO-E solution containing 12% w/v carbohydrate.

compared to a CHO-E-free placebo, resulted in the accumulation of a greater distance in a 90-minute running performance trial on a motorized treadmill at a self-selected pace. However, a higher CHO concentration solution (12% w/v) did not result in additional performance benefit compared to a standard CHO concentration of 6% w/v. These findings agree with previous research showing enhanced endurance performance with CHO and CHO-E mouth rinses but this is the first study to show that there is no dose-response effect above concentrations of ~6%.

Significantly more distance was covered in the 6% CHO-E (P = 0.035) and 12% CHO-E (P = 0.003) trials compared to the placebo trial. However, there was no significant difference (P = 0.196) between the two CHO-E containing solutions (Table 1). It would appear that the performance differences were attributable to a better speed maintenance in the final 20 min of the CHO-E trials (Figure 1), despite the fact that the last solution was provided 45 min before the end of the trial. This suggests that the beneficial effects of CHO-E mouth rinsing during prolonged exercise may persist for at least 20 – 45 minutes, which may have practical relevance in situations in which free access to drinks/solutions is restricted by the nature of the sport or activity (e.g. drinks stations in endurance races or breaks in match play).

The speed profile in the present study suggests that the performance benefit is evident in the latter stages of the trial, at a time when fatigue becomes more apparent (i.e. speed or power output tends to decrease) rather than increasing speed in the earlier stages. This agrees with the findings of Chambers et al. (6), Pottier et al. (12) and Rollo et al. (13) but differs from

Carter et al. (4) who observed differences in the first 3 quarters of a cycling time-trial (although differences could be related to differences between studies in trial duration and exercise mode). However, in the present study, no rinses were provided after 45 minutes meaning that the effects either persist for more than 20 minutes post-rinse or are caused by other mechanistic pathway(s). Other potential mechanisms include: some CHO from the rinse remaining adhered to receptors in mouth (i.e. not rinsed away) after expectoration; or there is some cephalic phase hormonal response which exhibits a lag of effect duration, and/or has some effect on performance (or fatigue) in the latter stages. However, these mechanisms cannot be confirmed or refuted by the present data and further research is now needed to determine the mechanisms responsible for this apparent 'persistent' effect and the duration for which the effects remain after the final (or each) rinse. Interestingly, Smeets et al. (18) observed changes in fMRI signal that persisted for at least 30 minutes after the ingestion of glucose and energy-free, artificially sweetened beverages. These data lead the present authors to suggest that the effects observed in our study were due to central effects persisting for this time period (i.e. at least 30 minutes post-rinse). Although, the study of Smeets et al. (18) was an ingestion study the fact that these effects persisted for at least 30 minutes in the energy-free drink condition suggest that some taste receptors, albeit for sweetness in this instance, may be able to stimulate brain responses that persist for this time period. Although it is believed that the performance effects are due to different receptors (for CHO, not sweetness) this data seems to support the notion that receptor-mediated mechanisms of action for CHO rinsing (and hence oral detection) stimulates central effects that persist (or remain above control conditions, being beneficial) for at least 30 minutes, although this must be confirmed with similar studies on CHO rinsing before this theory can be accepted.

Overall, the present results agree with Rollo et al. (13) with a similar design and protocol to this study. Rollo et al. (13) used a 1 hour performance run, in which subjects were instructed to run as far as possible in the allowed time, and observed that greater distance was covered with a CHO-E compared to PLA mouth rinse. Mean running speed was relatively stable throughout most of the trial with the exception of the first 5 minutes when it was slower (presumably whilst subjects were adjusting and 'settling in' to their preferred pace), and the final 5 minutes when mean speed was increased significantly (the familiar 'sprint' finish that is commonly observed in such performance trials, (15)). Interestingly, mean running speed was significantly higher in the CHO-E condition at two points in the middle of the run (5-minute average sections 25-30 min and 35-40 min) as well as in the final 5 minutes, which combined to produce better overall performance in the CHO-E trial. A similar profile was evident in the present study in that mean running speed was relatively stable over the duration of the 90-minute run, with segmental analysis showing no differences between trials until the final 30 minutes, where mean running speed was significantly higher than PLA in both CHO-E trials. There were no significant differences in the present study between the two CHO-E solutions (6% and 12 % CHO). This suggests that CHO-E rinsing in the current study had no impact on the

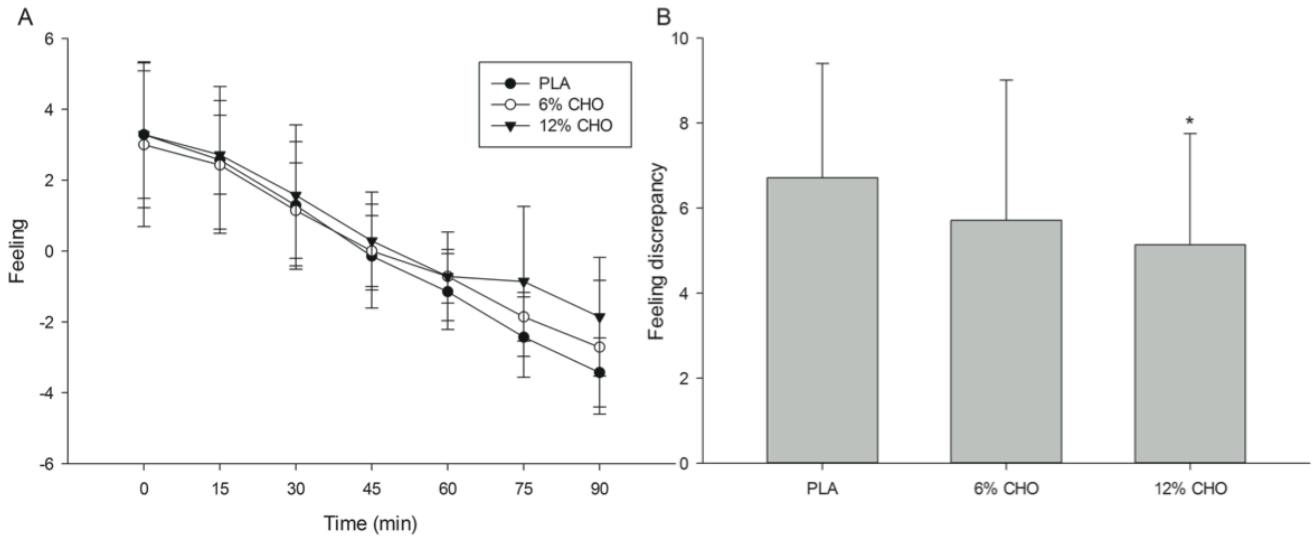

Figure 2. Feeling scale ratings during the performance trials (A) and Feeling scale discrepancy scores in each trial (B). Significantly different from PLA trial (*P < 0.05). PLA = placebo solution, 6% CHO = CHO-E solution containing 6% w/v carbohydrate, 12% CHO = CHO-E solution containing 12% w/v carbohydrate.

early and middle stages of the trials, which differs from the findings of Rollo *et al*. (13, 14). The present findings are in agreement, however, with Whitham and McKinney (21), although they reported no significant difference between CHO and PLA mouth rinses for a 45-minute running performance trial, as the benefits in our study only become evident after 60 minutes or more.

The RPE results showed significant differences across time (P < 0.001), which differs from the suggestions of Carter et al (4) in that subjects did not select speeds that maintained a constant RPE. Rather, average speed was relatively stable over the first two thirds of the trial whilst RPE progressively increased, culminating with near maximal ratings at the end (coinciding with the familiar 'sprint finish' as mentioned above). However, this appears to be more typical of running rather than cycling protocols (13). Nevertheless, the fact that RPE was not different between trials shows that more work was performed for the same relative subjective exertion, in agreement with previous studies in cycling (4), and running (13). A similar pattern was also evident for the Feeling scale ratings, in that there was a significant decrease in ratings as the trial progressed but there were no differences between trials (Figure 2A) showing that faster times and more work were achieved in the CHO-E trials for the same (or less) relative decrease in feeling ratings. It was suggested by Rollo et al. (13) and Chambers et al. (6) that enhanced feeling ratings contributed to the enhanced performance with CHO mouth rinsing. In the present study feelings ratings, when analyzed in 30 minute segments did show a trend (P = 0.071) for a trial × time interaction. Furthermore, analysis of the feeling rating discrepancy scores showed a smaller discrepancy with CHO-E, although this only reached statistical significance (compared to PLA) in the 12% CHO-E mouth rinse trial (P = 0.030). It would seem, therefore, that the higher concentration mouth rinse may

better limit the typical reduction in feeling ratings observed during prolonged exercise but this does not appear to be of sufficient magnitude to further enhance performance when compared to the 6% CHO-E rinse solution, although this requires further research.

It is possible that subjects could have ingested some of the solutions during the rinse procedure. However, clear instructions were provided to expectorate all of the solution and this was practiced in the familiarization trials. The expectorated solution was visually inspected to ensure a volume similar to that taken into the mouth was expelled (beakers were clearly marked to aid this). Whilst it is possible that this could be confounded by saliva output, this volume is negligible (saliva flow rate is usually less than 0.5 ml) in the time allowed for rinsing.

As the sample size was quite small it is conceivable that there was insufficient statistical power to detect differences between the 6% and 12% doses, which may be expected to be more subtle than the differences between PLA and CHO-containing solutions. However, post hoc power analysis on the present data revealed that a larger sample size would be unlikely to result in a finding of a significant difference between doses. Nevertheless, we cannot exclude the possibility that a much greater sample size (n = 30 or more) would have resulted in a significant difference between CHO doses (6% and 12%) but further research is required to determine whether this would actually be the case.

Another possible limitation to the study was the potential placebo effect. However, the solutions were taste matched and all drinks were flavored and strongly sweetened with artificial sweeteners. We believe that we were successful at blinding the subjects from trial order as when questioned after each trial (which were at least 1 week apart) subjects could not distinguish between the solutions. After all 3 trials had been completed subjects were also asked to reflect on all trails again and suggest which solution they received in each. Only one subject guessed all trials correctly and 4 guessed 1 trial correctly. However, all 7 participants covered a greater distance in both CHO-E trials compared to the PLA. Hence, whilst we cannot rule out the possibility of a placebo effect in some subjects, because of the fact that all subjects performed better with CHO-E (regardless of how they guessed) we are confident that the observed effects are due to CHO-sensing in the mouth as suggested previously (4, 6, 13). It should also be noted that metabolic data (e.g. gas exchange variables) were only collected in the first 45 minutes yet the differences observed in the performance tests did not occur until after 60 minutes. Whilst we are confident that the observed effects of CHO rinsing were indeed 'non-metabolic' (also supported by blood glucose and lactate measurements at the beginning and end of trials) it would be beneficial to also measure gas exchange throughout the whole exercise bout in future studies.

In the study by Rollo et al. (13) they used a customized automated treadmill to allow self-paced running whereas the current study used a traditional motorized treadmill with manual controls located on the handrail. According to Whitham and Mckinney, (21) studies in which runners manually change their running speed (e.g. using a traditional motorized treadmill)

might not have the same degree of sensitivity to nutritional interventions as is the case when using an automated treadmill. This does not seem to have been true in the present study however, possibly due to the longer duration of the performance trial. Therefore, it is possible that the use of longer duration running (e.g. 90 minutes) provides sufficient sensitivity to detect differences in self-paced treadmill running, even with a manual treadmill.

In summary, we have demonstrated that rinsing the mouth with a CHO-E solution, compared to placebo, enhances distance covered in a 90-minute running performance trial. This is the first study to show that a higher concentration solution (12% CHO w/v) does not offer any additional benefit compared to a standard concentration of 6% w/v, thus there is no dose-response effect with CHO concentration above ~6%. It is not known whether 6% is actually the optimal concentration for a CHO-containing mouth rinse solution or whether similar effects can be achieved with lower concentrations. Hence, the minimal concentration of CHO that is required to elicit these ergogenic effects has not been determined and this requires further research. The CHO-E mouth rinse seemed to have a positive effect on the subjects' feelings in the later stages of the 90-minute running performance trial and the speed of the athletes in the final 10-30 minutes were greater in the CHO-E trials compared to the PLA trials, despite the fact that the last rinse procedure occurred 45 minutes before the end of the trial. Furthermore, there was no difference in RPE despite greater speeds being obtained in the CHO-E trials. This supports previous work suggesting that CHO mouth rinsing acts via a central action related to motivation, perceptions of effort and/or motor drive but shows, for the first time, that this effect is also capable of having ergogenic effects in more prolonged exercise. Based on the current results it would seem that it is not the quantity of CHO in the mouth rinse that enhances performance, it is the fact there is a presence of CHO in the mouth. In addition, the benefits seem to last for at least 20-45 minutes after the final mouth rinse, which could have practical relevance in situations when access to drinks/rinsing is limited or not readily available at all times.

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