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Sports drink intake pattern affects exogenous carbohydrate oxidation during running

PLEASE CITE THE PUBLISHED VERSION

<https://doi.org/10.1249/MSS.0000000000002334>

PUBLISHER

Lippincott, Williams & Wilkins

VERSION

AM (Accepted Manuscript)

PUBLISHER STATEMENT

This is a non-final version of an article published in final form in MEARS, S. ... et al, 2020. Sports drink intake pattern affects exogenous carbohydrate oxidation during running. *Medicine and Science in Sports and Exercise*, 52 (9), pp.1976-1982, doi: 10.1249/MSS.0000000000002334.

LICENCE

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REPOSITORY RECORD

Mears, Stephen, Benjamin Boxer, David Sheldon, Hannah Wardley, Caroline A Tarnowski, Lewis James, and Carl Hulston. 2020. "Sports Drink Intake Pattern Affects Exogenous Carbohydrate Oxidation During Running". Loughborough University. <https://hdl.handle.net/2134/12046290.v1>.

1 **Title Page**

2 **Sports drink intake pattern affects exogenous carbohydrate oxidation during running**

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Abstract

PURPOSE: To determine whether the pattern of carbohydrate sports drink ingestion during prolonged sub-maximal running affects exogenous carbohydrate oxidation rates and gastrointestinal (GI) comfort.

METHODS: Twelve well-trained male runners (27 ± 7 y, 67.9 ± 6.7 kg, $\dot{V}O_{2\text{peak}}$: 68 ± 7 mL·kg⁻¹·min⁻¹) completed two exercise trials of 100 min steady state running at 70% $\dot{V}O_{2\text{peak}}$. In each of the trials, 1 L of a 10% dextrose solution, enriched with [U-¹³C] glucose, was consumed as either 200 mL every 20 min (CHO-20) or 50 mL every 5 min (CHO-5). Expired breath and venous blood samples were collected at rest and every 20 min during exercise. Subjective scales of GI comfort were recorded at regular intervals.

RESULTS: Average exogenous carbohydrate oxidation rates were 23% higher during exercise in CHO-20 (0.38 ± 0.11 vs. 0.31 ± 0.11 g·min⁻¹; $P=0.017$). Peak exogenous carbohydrate oxidation was also higher in CHO-20 (0.68 ± 0.14 g·min⁻¹ vs. 0.61 ± 0.14 g·min⁻¹; $P=0.004$). During exercise, total carbohydrate oxidation (CHO-20: 2.15 ± 0.47 ; CHO-5: 2.23 ± 0.45 g·min⁻¹, $P=0.412$) and endogenous carbohydrate oxidation (CHO-20: 1.78 ± 0.45 ; CHO-5: 1.92 ± 0.40 g·min⁻¹; $P=0.148$) were not different between trials. Average serum glucose ($P=0.952$) and insulin ($P=0.373$) concentrations were not different between trials. There were no differences in reported symptoms of GI comfort and stomach bloatedness ($P>0.05$), with only 3% of reported scores classed as severe (≥ 5 out of 10).

CONCLUSION: Ingestion of a larger volume of carbohydrate solution at less frequent intervals during prolonged submaximal running increased exogenous carbohydrate oxidation rates. Neither drinking pattern resulted in increased markers of GI discomfort to a severe level.

Key Words: metabolism, submaximal, exercise, fluid

38 **Introduction**

39 It is well established that carbohydrate ingestion during prolonged sub-maximal exercise
40 increases plasma glucose availability during exercise, contributing to delayed fatigue (1) and
41 enhanced performance (reviewed by (2)). Specific recommendations for carbohydrate
42 ingestion will depend on the duration and intensity of the event. Current recommendations are
43 to consume 30-60 g·h⁻¹ carbohydrate for events lasting 1-2.5 h and up to 90 g·h⁻¹ of multiple
44 sources of carbohydrate for events over 2.5 h (3). For a rapidly absorbed single source of
45 carbohydrate such as glucose, the maximal rate of exogenous carbohydrate oxidation is
46 approximately 1.0-1.2 g·min⁻¹ (4). Utilization of exogenous carbohydrate is primarily limited
47 by intestinal absorption (5,6,7) but other limiting factors may include gastric emptying, hepatic
48 glucose extraction, and/ or uptake at the muscle (2).

49 Exogenous carbohydrate oxidation studies are often conducted using cycling as the mode of
50 exercise. Many of these studies have adopted aggressive ingestion strategies to maximise
51 carbohydrate delivery to the intestines through stimulation of gastric emptying, (5,6,7,8).
52 Typically, a large volume (400-600 mL) of fluid is provided immediately prior to exercise,
53 with additional large boluses (100-200 mL) ingested at regular intervals (every 10-15 min)
54 during exercise. This has resulted in maximal exogenous carbohydrate oxidation rates >1.0
55 g·min⁻¹. The frequent ingestion of large volumes of carbohydrate-containing beverages has
56 been well tolerated in laboratory-based cycling experiments. However, such ingestion
57 strategies may not be feasible in the real-world, especially in sports such as running where it is
58 difficult to carry as much fluid as on the bike and where increased risk of gastrointestinal (GI)
59 disturbances may be a consideration for fluid/ carbohydrate intake.

60 In distance running, the mechanical motion has been linked to increased GI disturbances (9).
61 As such, runners are unlikely to ingest a large pre-race bolus and will typically consume a

maximum of ~150-200 mL at drinks stations (positioned every 5 km: ~15-20 min). Recently, elite athletes in major marathon races and record attempts have experimented with ingesting drinks more frequently (every 1-2 km; ~2.5-6.0 min), allowing total delivery of a large amount of carbohydrate in relatively small volumes (~50-100 mL). However, the effect of this revised drinking strategy on exogenous carbohydrate oxidation has not been assessed in runners (or cyclists). Smaller, more frequent volumes of carbohydrate may minimise GI issues, but they also may reduce gastric emptying and limit exogenous carbohydrate oxidation, particularly in situations where a large 'priming' bolus of drink is not ingested immediately before exercise. There are also practical challenges of collecting and then consuming fluid whilst running at high speeds. A large volume may be more difficult to ingest, but frequent consumption of smaller volumes may be inconvenient and make it more difficult to maintain race pace. Therefore, there remains a question surrounding the balance between optimal drink volume and frequency to maximise carbohydrate delivery and utilization, whilst minimizing gastrointestinal disturbances.

The aim of this study was to investigate the effect of drink pattern (volume and frequency) on exogenous carbohydrate oxidation and markers of GI comfort. It was hypothesised that ingestion of less frequent, larger volumes of carbohydrate would increase exogenous carbohydrate oxidation but also increase the prevalence of GI discomfort.

Methods

Subjects

Twelve well trained male runners and triathletes (27 ± 7 y, 67.9 ± 6.7 kg, $\dot{V}O_{2\text{peak}}$: 68 ± 7 mL·kg⁻¹·min⁻¹) participated in this study. Subjects were provided with verbal and written information regarding the study before providing written informed consent and completing a health screen questionnaire. The study was approved by the Loughborough University Ethical Approvals (Human Participants) Sub-Committee. It was estimated that 12 subjects were required to detect a 10% difference in exogenous carbohydrate oxidation between trials based on a statistical power of 0.8 and an alpha of 0.05 (10).

Subjects visited the laboratory on 4 occasions: a preliminary peak oxygen uptake ($\dot{V}O_{2\text{peak}}$) test, a familiarization trial, in which water was ingested at a rate of 200 mL every 20 min (this trial also provided a background correction for ¹³CO₂ breath enrichment), and two experimental trials in which a 10% dextrose solution was ingested as either 200 mL every 20 min (CHO-20) or 50 mL every 5 min (CHO-5). The two carbohydrate trials were performed in a counter-balanced cross-over design, separated by ≥ 7 d.

Preliminary testing

After measuring height and nude body mass (AFW-120K, Adam Equipment Co., UK), subjects performed a $\dot{V}O_{2\text{peak}}$ test on a motorised treadmill (mercury h/p/cosmos, Nussdorf, Germany). Subjects completed 4 min stages at progressive speeds until a heart rate >160 b·min⁻¹ was elicited (measured using Polar M400, Polar, Kemple, Finland). Expired gas was collected into Douglas bags during the final minute of each stage and analysed for oxygen and carbon dioxide concentrations (Servomex 1400 Oxygen and Carbon Dioxide Gas Analyser, Servomex, Crowborough, UK). Gas volume (Harvard dry gas meter, Harvard Apparatus Ltd, Edenbridge, UK) and temperature (RS Pro digital thermometer, RS Components, Corby, UK) were

measured and corrected to standard temperature and pressure, dry. To correct $\dot{V}O_2$ and $\dot{V}CO_2$ values, ambient air was collected simultaneously with expired gas samples (11). Following a short period of rest (~10-15 min), subjects ran to volitional exhaustion using a ramped protocol. Set at the speed of the final sub-maximal stage, incline was increased by 1% each min until volitional exhaustion. An expired gas sample was collected during the final minute of exercise.

Pre-trial standardization

Subjects were asked to record physical activity and dietary intake in the 24 h before the water trial and were then asked to replicate this before each of the carbohydrate trials. They were also asked to refrain from strenuous exercise in the 24 h before all trials. In addition, subjects were asked to follow a specific exercise and diet regime designed to reduce the background shift in $^{13}CO_2$ (12). At least 2-4 d prior to each experimental trial, subjects completed an exhaustive bout of exercise (replicated at the same time prior to subsequent trials). This was to oxidise ^{13}C enriched glycogen. Following this, subjects were asked to refrain from eating foods that had a naturally high abundance of ^{13}C including commercially available sports drinks and carbohydrate derived from C4 plants (e.g. cane sugar and maize). A detailed list of foods to avoid was provided to each subject. Subjects confirmed adherence to all pre-trial standardization requirements before commencing each trial.

Experimental trials

For each trial, subjects arrived at the laboratory at the same time of day (standardized for each subject between 0600-1000 h) following an overnight fast and 90 min after ingesting 500 mL water.

On arrival, subjects provided a urine sample and had nude body mass measured. A 20-gauge Teflon cannula was then inserted into an antecubital vein of one arm to allow repeated blood sampling during the trial. After 20 min sitting, expired breath samples were collected into 12 mL evacuated glass tubes (Exetainers, Labco, Ceredigion, UK) for determination of $^{13}\text{CO}_2$ enrichment, followed by a 5-min collection of expired gas (analysed for oxygen and carbon dioxide content and gas volume and temperature as described for the preliminary testing). A 5.5 mL venous blood sample was then collected and heart rate was recorded. Subjects were asked to rate feelings of thirst, GI comfort and stomach bloatedness using scales (0= no feeling, 10=extreme feeling). As used in similar studies (13), responses ≥ 5 were classed as severe, with values < 5 classed as non-severe.

Immediately prior to the start of exercise, subjects consumed the first drink. They then completed 100 min running at 70% $\dot{V}\text{O}_{2\text{peak}}$ ($13.1 \pm 1.0 \text{ km}\cdot\text{h}^{-1}$). A sample period occurred every 20 min: at 18 min, expired breath samples were collected into exetainers, a 60 s expired gas collection occurred at 19 min, with heart rate and rating of perceived exertion (RPE) recorded during this collection. At 20 min, subjects straddled the treadmill for 90 s and a 5.5 mL blood sample was taken. Once running had recommenced, the next drink was ingested. This was repeated every 20 min. Additional drinks were ingested in CHO-5 at 5 min intervals, with final drinks consumed at 80 min (water and CHO-20) and 95 min (CHO-5). Heart rate, RPE and subjective scales of thirst, GI comfort and stomach bloatedness were measured every 10 min, with all scales measured on completion of exercise. Air flow provided by a 0.5 m diameter fan was placed on the floor and angled at the chest to provide similar air flow in both trials ($\sim 3.0\text{-}3.5 \text{ m}\cdot\text{s}^{-1}$). After the completion of exercise, subjects towel dried to remove unevaporated sweat and had nude body mass measured.

Drink composition

For each carbohydrate trial, 137.5 g of glucose (D-glucose monohydrate, MyProtein, Northwich, UK) was measured and then made up to a volume of 1250 mL to produce a 10% carbohydrate solution. To account for the different molecular masses of glucose and dextrose monohydrate, the amount of glucose was multiplied by 1.1. The glucose had a low natural abundance of ^{13}C (-27.22‰ vs. Pee Dee Belemnite (PDB)), therefore drinks were enriched with uniformly labelled ^{13}C glucose ($\sim 0.100\text{ g [U-}^{13}\text{C] glucose}\cdot\text{L}^{-1}$, Cambridge Isotopes Laboratory Inc., Andover, MA, USA). A total of 1.0 L of each drink, with ^{13}C mean glucose enrichment of $62.5 \pm 3.7\text{‰}$ vs. PDB, was ingested during each trial to provide an average ingestion rate of $1.0\text{ g}\cdot\text{min}^{-1}$.

Analysis

For each 5.5 mL of venous blood, 1.0 mL was aliquoted into a tube containing anticoagulant for analysis of hemoglobin (Cyanmethemoglobin method: Sigma, St Louis, MO, USA) and hematocrit (micro-centrifugation: Hawksley, Worthing, UK). Plasma volume changes were calculated from hemoglobin and hematocrit values (14). The remaining blood was allowed to clot at room temperature before centrifugation at $2200 \times g$ for 15 min at 4°C . The serum was removed and frozen at -20°C until later analysis of glucose (spectrophotometry, ABX Pentra 400; Horiba Medical, Northampton, UK) and insulin concentration (enzyme immunoassay, DRG Instruments GmbH, Marburg, Germany). Urine was immediately analysed for osmolality (refractometry, Osmocheck; Vitech Scientific, Horsham, UK).

Breath (gas chromatography isotope ratio mass spectrometry, Hydra 20-20 IRMS; Europa Scientific, Crewe, UK) and drink (elemental analyser isotope ratio mass spectrometry, 20-20 IRMS; Europa Scientific, Crewe, UK) samples were analysed for $^{13}\text{C}/^{12}\text{C}$ ratio (both Iso-Analytical Ltd., Crewe, UK).

Calculations

173 Stoichiometric equations were used to calculate rates of total carbohydrate and fat oxidation
174 (15), with the assumption that oxidation of protein was negligible:

175 Carbohydrate oxidation ($\text{g} \cdot \text{min}^{-1}$) = $4.210 \dot{V}\text{CO}_2 - 2.962 \dot{V}\text{O}_2$

176 Fat oxidation ($\text{g} \cdot \text{min}^{-1}$) = $1.695 \dot{V}\text{O}_2 - 1.701 \dot{V}\text{CO}_2$

177 For each expired breath sample, the isotopic enrichment was expressed as δ per mL difference
178 between $^{13}\text{C}/^{12}\text{C}$ ratio of the sample and a known laboratory reference standard. The formula
179 (16) used was:

180
$$\delta^{13}\text{C} = \left[\left(\frac{^{13}\text{C}/^{12}\text{C sample}}{^{13}\text{C}/^{12}\text{C standard}} \right) - 1 \right] \times 10^3 \text{ per mil}$$

181 Following this, $\delta^{13}\text{C}$ was then related to the international standard Vienna PDB.

182 Exogenous carbohydrate oxidation rates were calculated by:

183
$$\text{exogenous carbohydrate oxidation (g} \cdot \text{min}^{-1}\text{)} = \dot{V}\text{CO}_2 \left(\frac{\delta\text{Exp} - \text{Exp}_{\text{bkg}}}{\delta\text{Ing} - \text{Exp}_{\text{bkg}}} \right) \left(\frac{1}{k} \right)$$

184 where δExp is the ^{13}C expired breath enrichment, δIng is the ^{13}C enrichment of the ingested
185 beverage, $\delta\text{Exp}_{\text{bkg}}$ is the ^{13}C expired breath enrichment of the water trial (background), k is the
186 CO_2 volume that is produced by oxidation of 1 g of glucose ($k = 0.7467 \text{ L}$).

187 When calculating exogenous carbohydrate oxidation rates using $^{13}\text{CO}_2$ from expired gas, a
188 methodological consideration is the $^{13}\text{CO}_2$ trapped in the bicarbonate pool. During the onset of
189 exercise, some CO_2 arising from the oxidation of glucose will be retained (17), however $\dot{V}\text{CO}_2$
190 will increase during exercise until a condition of physiological steady-state occurs resulting in
191 equilibration between the $^{13}\text{CO}_2$ in expired gas and the CO_2/HCO_3 pool. Recovery of $^{13}\text{CO}_2$
192 from oxidation of enriched glucose will approach 100% after approximately 60 min (18).

Therefore, there is likely to be some underestimation of exogenous carbohydrate oxidation rates during the first 60 min. Data should be interpreted as the minimum estimates.

Statistical Analysis

Data were checked for normality of distribution using Shapiro-Wilks tests. All data with one factor (urine osmolality, sweat losses, body mass) were normally distributed and analysed using a one-way ANOVA. All data with two factors except thirst and GI measures were normally distributed. Data with two factors (variables measured over time in each trial) were measured using a two-way repeated measures ANOVA. When a significant two-way ANOVA was observed, a paired samples t-test with Holm-Bonferroni correction was performed to identify where the difference occurred. Statistical significance was accepted when $P \leq 0.05$. Data were presented as mean $\pm$ SD. All statistical analysis was conducted using Statistical Package for the Social Sciences for Windows (version 23.0; SPSS, Chicago, IL, USA).

Results

Baseline measures and pre-trial standardization

Body mass (CHO-20: 67.5 ± 6.5 , CHO-5: 67.4 ± 6.5 kg; $P=0.384$) and urine osmolality (CHO-20: 319 ± 193 , CHO-5: 398 ± 265 mOsmol $\cdot$ kg $^{-1}$ H $_2$ O; $P=0.076$) were not different between trials, providing a good indication that subjects arrived at the laboratory in a similar state of hydration.

Resting values of ^{13}C breath enrichment were not different between water and CHO trials (WAT: -27.14 ± 0.32 ‰ vs. PDB, CHO-20: -27.27 ± 0.38 ‰ vs. PDB, CHO-5: -27.18 ± 0.34 ‰ vs. PDB; $P=0.635$).

Exercise measures

Mean $\dot{V}\text{O}_2$, $\dot{V}\text{CO}_2$ and RER during exercise were not different between trials (table 1). Exogenous carbohydrate oxidation rates were greater in CHO-20 than CHO-5. This effect was observed both early (20-60 min) and late (60-100 min) in exercise, with average exogenous carbohydrate oxidation rates across the whole exercise period being 23% higher in CHO-20 than CHO-5 (table 1). There was also a time x trial interaction effect for exogenous carbohydrate oxidation and post-hoc analysis detected this at the 100 min timepoint (CHO-20: 0.68 ± 0.14 g $\cdot$ min $^{-1}$, CHO-5: 0.61 ± 0.14 g $\cdot$ min $^{-1}$; figure 1a). These values represent the peak exogenous carbohydrate oxidation rates for each trial. Exogenous carbohydrate oxidation efficiency was a minimum estimate of 38% in CHO-20 compared to 31% in CHO-5. Total carbohydrate oxidation and average endogenous carbohydrate oxidation rates during exercise were not different between trials (table 1). There was a significant time x trial interaction effect ($P=0.041$) for endogenous carbohydrate oxidation rates, but after correction for multiple comparisons, no post-hoc differences were detected. During exercise, mean fat oxidation was not different between trials (table 1).

Venous blood data was collected in 11 out of 12 subjects due to sampling problems in one trial and therefore presented accordingly. In both carbohydrate trials, serum glucose concentrations increased from baseline following drink ingestion (time effect: $P<0.0001$, figure 2a). There was no trial ($P=0.938$) or time x trial interaction effect ($P=0.095$). Serum insulin concentrations (figure 2b) were not different between trials (mean value during exercise, CHO-20: 42.9 ± 21.5 pmol·L⁻¹, CHO-5: 38.2 ± 16.2 pmol·L⁻¹, $P=0.373$).

Following the onset of exercise, plasma volume decreased from resting values in both trials (approximately -10.0 to -12.5% decrease from baseline; time effect, $P=0.020$, figure 3). The pattern of drink ingestion did not influence changes in plasma volume (time x trial interaction effect, $P=0.425$).

Heart rate increased progressively throughout exercise and was not different between trials, with mean exercise values of 155 ± 13 b·min⁻¹ and 157 ± 13 b·min⁻¹ for CHO-20 and CHO-5 trials respectively ($P=0.310$). Mean RPE values were not different between trials (CHO-20: 11 ± 2 , CHO-5: 11 ± 2 ; $P=0.723$). Sweat losses during (CHO-20: 2.04 ± 0.34 L, CHO-5: 2.09 ± 0.40 L, $P=0.416$) and percentage body mass loss at the end (CHO-20: $1.52 \pm 0.40\%$, CHO-5: $1.59 \pm 0.44\%$, $P=0.385$) of exercise were not different between trials. Mean subjective feelings for thirst (CHO-20: 1 ± 1 , CHO-5: 1 ± 1 ; trial effect: $P=0.836$), GI comfort (CHO-20: 2 ± 1 , CHO-5: 2 ± 1 ; trial effect: $P=0.830$) and stomach bloatedness (CHO-20: 2 ± 1 , CHO-5: 2 ± 1 ; trial effect: $P=0.880$) were during both trials. There were no time x trial interaction effects (thirst: $P=0.682$, GI comfort: $P=0.313$, stomach bloatedness: $P=0.126$). Subjective feelings of GI discomfort and stomach bloatedness gradually increased over the course of the exercise period (time effect: $P<0.0001$, figure 4). For GI comfort, four subjects reported a score of 5 in both trials, with a further subject reporting a score of 5 in the CHO-20 trial. Two subjects reported a score of 6 at single timepoints in CHO-5. For reported measures of stomach

253 bloatedness, 3 subjects reported a score of 5 in both trials, with a further subject reporting a
254 score of 5 in CHO-5.

Discussion

The aim of the study was to examine the effect of different drinking patterns (frequency and volume) on exogenous carbohydrate oxidation and gastrointestinal comfort during prolonged submaximal running. The main finding was that consuming larger volumes at less frequent intervals (200 mL every 20 min) increased exogenous carbohydrate oxidation compared to consuming smaller volumes at more frequent intervals (50 mL every 5 min). In addition, both drinking strategies were well-tolerated and there was no difference in the reported level of GI comfort between trials.

Average exogenous carbohydrate oxidation during exercise was 23% higher in CHO-20 than in CHO-5, which, given the same amount of carbohydrate was ingested, is a possibly important result, that may have beneficial implications in a performance situation. Over the course of the trials, mean fluid delivery was the same (200 mL per 20 min), but the larger boluses provided in CHO-20 at the start and throughout exercise likely increased gastric pressure, resulting in increased gastric emptying (19,20). As fluid enters the stomach, there is increased pressure on the antral region, which will contribute to an increased emptying rate (21). Increased gastric contents and pressure will stimulate stretch receptors in the gastric mucosa, therefore increasing the rate of gastric emptying (22,23). Costill and Saltin (20) examined progressively larger volumes of fluid than the initial boluses in the present study and found increased gastric emptying up to 600 mL. It was therefore likely that neither of the initial boluses in the present study were large enough to maximise carbohydrate delivery to the intestines, providing a partial explanation for the relatively low exogenous oxidation rates as well as the failure to observe a plateau. The findings by Costill and Saltin (20) were conducted at rest, and whilst they provide an indication of the role of drink volume in gastric emptying there must also be consideration for other factors, such as exercise intensity, carbohydrate concentration and dehydration.

Peak rates of exogenous carbohydrate oxidation were comparable with studies providing similar amounts of carbohydrate during exercise that either included (24) or did not include (10,25,26,27) a bolus of carbohydrate prior to exercise. Whilst exogenous oxidation rates were similar in Hulston et al. (24), there was a marked difference in oxidation efficiency (63%) compared to the present study (minimum estimate of at least 38% in CHO-20 and 31% in CHO-5). Despite delivery of slightly less carbohydrate during exercise ($0.8 \text{ g} \cdot \text{min}^{-1}$) in Hulston et al. (24), this increased efficiency is likely driven by the provision of a large, 600 mL bolus of carbohydrate (6% glucose solution) prior to exercise. It also appears, from other studies, that in order to achieve maximal exogenous oxidation rates of $1.0\text{-}1.2 \text{ g} \cdot \text{min}^{-1}$, both a pre-exercise bolus of carbohydrate is required as well as a much larger amount of carbohydrate ingestion during exercise (5,6,7,8), however, particularly in runners, this combination may exacerbate GI distress. As exogenous oxidation rates were still increasing at 100 min, there are potential improvements to both strategies used in this study, but this must be balanced against the practicalities of the exercise. Whilst mode of exercise has shown similar exogenous carbohydrate oxidation rates between running and cycling (28), exogenous carbohydrate oxidation rates in the present study may have been affected by using runners with limited experience of carbohydrate ingestion during exercise (four subjects had completed at least one marathon and had used carbohydrate in the build-up and during the race but, along with the other subjects were not regular users of carbohydrate during typical training). Running has been shown to decrease gastric emptying (19), but training with carbohydrate ingestion (i.e. increased exposure to carbohydrate ingestion) has been shown to enhance exogenous oxidation rates (29).

Markers of GI discomfort progressively increased throughout exercise in both conditions, however there was no difference between drinking pattern and only 3% of timepoints could be considered as severe (≥ 5 out of 10). GI issues can be increased by a greater volume and/ or an

increase in the carbohydrate concentration/ osmolality of the beverage (30). When large volumes of carbohydrate drinks have been ingested less frequently, an increase in GI discomfort has been reported (31), however, in the low frequency group, drink ingestion was every 60 min in a far greater volume (~690 mL) than the current study. The high frequency, small volume pattern used in the study by Stocks et al. (31) was more closely related to the CHO-20 condition with similar GI responses reported.

The intensity used in the present study was 70% $\dot{V}O_{2peak}$ which is comparable with several other studies (5,6,24,32,33,34,35). At this intensity there is a high reliance on carbohydrate oxidation (36) and, depending on the ingestion strategy, it is possible to achieve peak oxidation rates of ~0.9-1.0 g·min⁻¹ (5,32). It is also an intensity that can be sustained for exercise durations/ race distances over which carbohydrate ingestion is known to be beneficial to performance. Whilst elite marathon runners will race at even higher intensities (~75-85% $\dot{V}O_{2max}$; (37)), this may not be achievable for sub-elite and recreational runners, especially when performing on a treadmill. Therefore, we selected an exercise intensity and duration that was both achievable for our subjects and that could provide translational results for competitive running races. There does, however, need to be further investigation into the effect of drinking pattern at an increased intensity, as more severe GI symptoms may be reported. The potential increases in symptoms may be exacerbated by decreased gastric emptying rates above 75% $\dot{V}O_{2max}$ (38) and/ or the increased mechanical stress placed on the GI system when running at these increased velocities (39). Additionally, at an increased pace, the process of drinking a large volume may be hindered due to increased speed and breathing frequency.

Further work is needed to investigate the impact of drinking pattern on performance. Whilst this study was able to demonstrate a metabolic advantage with CHO-20, there is no guarantee that this will directly translate to improved performance, as there must also be consideration for factors such as the non-oxidative roles of carbohydrate. The effect of carbohydrate sensing

in the mouth on performance should be also be considered (3) as more frequent sensations of carbohydrate by the central nervous system may be more advantageous. In addition, from a practical perspective, there should be consideration of the opportunity to drink during a race setting as well as the time saved when drinks are consumed less frequently. Whilst a treadmill setting allows greater measurements to improve mechanistic understanding, there is reduced ecological validity in terms of recreating race practicalities. The design of the current study restricts comparison to one of two distinct drinking strategies, whereas in practice athletes are free to consume different volumes as they see fit. It may be that the preferential strategy is to combine both approaches by ingesting a larger volume at the start and during the early part of the race, thereby priming the system, before switching to a smaller volume as the race progresses.

A limitation of the study was that the glycogen depleting exercise was not supervised, whilst it is also preferential to provide a standardised dietary intake (40) rather than just providing guidance and ask subjects to repeat their intake. However, the use of well-motivated subjects, pre-trial interviews confirming diet and depletion exercise and the minimal shift in expired ^{13}C during the water trial were indicative of good adherence to pre-trial standardization procedures.

Conclusion

In conclusion, drinking a larger volume of a single source of carbohydrate (glucose) every 20 min increased exogenous carbohydrate oxidation compared to the same volume of fluid ingested in smaller volumes every 5 min. At this intensity there was no difference in reported GI issues and neither pattern induced prolonged severe symptoms. However, further work is warranted to investigate the effect of increasing intensity and also, how drinking pattern may affect performance.

352 **Acknowledgements**

353 The authors acknowledge the work of Marianna Apicella and Kelsie Johnson in assisting with
354 data collection.

355 **Conflict of Interest**

356 No external funding was obtained for the current study. The results of the present study do not
357 constitute endorsement by the American College of Sports Medicine. The results of the study
358 are presented clearly, honestly, and without fabrication, falsification, or inappropriate data
359 manipulation. LJJ has previously received funding from PepsiCo Inc. and Volac International,
360 performed consultancy for PepsiCo Inc. and Lucozade, Ribena Suntory and received
361 conference fees from PepsiCo Inc. and Danone Nutricia. In all cases, monies have always been
362 paid to LJJ's institution and not directly to LJJ.

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464 **Figure Captions**

465 Figure 1. Exogenous (a), total (b) and endogenous (c) carbohydrate oxidation rates during
466 exercise in CHO-20 and CHO-5. *denotes difference between trials, #denotes increase from
467 baseline for both CHO-20 and CHO-5, all $P < 0.05$. (a) time x trial interaction effect: $P =$
468 0.045. (b) time effect: $P < 0.0001$, trial effect: $P = 0.461$, time x trial interaction effect: $P =$
469 0.081. (c) time x trial interaction effect: $P = 0.041$.

470 Figure 2. Serum glucose (a) and insulin (b) concentrations at rest and during exercise.
471 #denotes increase from baseline for both CHO-20 and CHO-5, $P < 0.05$. (a) time effect:
472 $P < 0.0001$, trial effect: $P = 0.938$, time x trial interaction effect: $P = 0.095$. (b) time effect: $P =$
473 0.002, trial effect: $P = 0.404$, time x trial interaction effect: $P = 0.361$.

474 Figure 3. Percentage plasma volume change from baseline #denotes increase/ decrease from
475 baseline for both CHO-20 and CHO-5, all $P < 0.05$. Time effect: $P = 0.020$, trial effect: $P =$
476 0.356, time x trial interaction effect: $P = 0.425$.

477 Figure 4. Reported subjective feelings for gastrointestinal comfort (a) and stomach
478 bloatedness (b). 0 = very comfortable, 10 = extreme discomfort. (a) time effect: $P < 0.0001$,
479 trial effect: $P = 0.830$, time x trial interaction effect: $P = 0.313$. (b) time effect: $P < 0.0001$,
480 trial effect: $P = 0.880$, time x trial interaction effect: $P = 0.126$.

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Table 1. Whole-body oxygen consumption, carbon dioxide production, respiratory exchange ratio (RER), total CHO and fat oxidation rates and exogenous and endogenous CHO oxidation rates during the steady-state exercise period (20-100 min). Exogenous CHO oxidation has also been shown for 20-60 and 60-100 min.

	CHO-20	CHO-5	<i>P</i>
$\dot{V}O_2$ (L·min ⁻¹)	3.16 ± 0.37	3.11 ± 0.36	0.188
$\dot{V}CO_2$ (L·min ⁻¹)	2.73 ± 0.34	2.72 ± 0.33	0.644
RER	0.87 ± 0.03	0.87 ± 0.03	0.350
Total CHO oxidation (g·min ⁻¹)	2.15 ± 0.47	2.23 ± 0.45	0.412
Fat oxidation (g·min ⁻¹)	0.70 ± 0.18	0.65 ± 0.15	0.234
Exogenous CHO oxidation (g·min ⁻¹)	0.38 ± 0.11	0.31 ± 0.11	0.017
Endogenous CHO oxidation (g·min ⁻¹)	1.78 ± 0.45	1.92 ± 0.40	0.148
Exogenous contribution to total CHO oxidation (%)	17 ± 5	14 ± 4	0.022
Endogenous contribution to total CHO oxidation (%)	83 ± 5	86 ± 4	0.022
<i>Early and late phase exogenous CHO oxidation rates</i>			
Exogenous CHO oxidation, 20-60 min (g·min ⁻¹)	0.21 ± 0.09	0.15 ± 0.08	0.047
Exogenous CHO oxidation, 60-100 min (g·min ⁻¹)	0.55 ± 0.14	0.47 ± 0.15	0.019

Values are presented as Mean ± SD

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