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ABSTRACT

PURPOSE: Beneficial effects of carbohydrate (CHO) ingestion on exogenous CHO oxidation and endurance performance require a well-functioning gastrointestinal (GI) tract. However, GI complaints are common during endurance running. This study investigated the effect of a CHO solution-containing sodium alginate and pectin (hydrogel) on endurance running performance, exogenous and endogenous CHO oxidation and GI symptoms. **METHODS:** Eleven trained male runners, using a randomised, double-blind design, completed three 120-minute steady state runs at 68% $\dot{V}O_2$max, followed by a 5-km time-trial. Participants ingested 90 $g \cdot h^{-1}$ of 2:1 glucose:fructose (^{13}C enriched) either as a CHO hydrogel, a standard CHO solution (non-hydrogel), or a CHO-free placebo during the 120 minutes. Fat oxidation, total and exogenous CHO oxidation, plasma glucose oxidation and endogenous glucose oxidation from liver and muscle glycogen were calculated using indirect calorimetry and isotope ratio mass spectrometry. GI symptoms were recorded throughout the trial. **RESULTS:** Time-trial performance was 7.6% and 5.6% faster after hydrogel ([minutes:seconds]19:29±2:24; $p<0.001$) and non-hydrogel (19:54±2:23, $p=0.002$), respectively, versus placebo (21:05±2:34). Time-trial performance after hydrogel was 2.1% faster ($p=0.033$) than non-hydrogel. Absolute and relative exogenous CHO oxidation was greater with hydrogel (68.6±10.8g, 31.9±2.7%; $p=0.01$) versus non-hydrogel (63.4±8.1g, 29.3±2.0%; $p=0.003$). Absolute and relative endogenous CHO oxidation were lower in both CHO conditions compared with placebo ($p<0.001$), with no difference between CHO conditions. Absolute and relative liver glucose and muscle glycogen oxidation were not different between CHO conditions. Total GI symptoms were not different between hydrogel and placebo, but GI symptoms was higher in non-hydrogel compared with placebo and hydrogel ($p<0.001$).

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CONCLUSION: Ingestion of glucose and fructose in hydrogel form during running benefited endurance performance, exogenous CHO oxidation and GI symptoms, compared with a standard CHO solution. **Keywords:** ^{13}C tracer; Time-trial; Encapsulation; Metabolism; Endurance

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INTRODUCTION

It is well established that carbohydrate (CHO) ingestion during prolonged exercise can enhance endurance performance (1). This is associated with the maintenance of plasma glucose concentration and CHO oxidation during the latter stages of prolonged exercise (2). CHO ingestion can also prevent the depletion or attenuate the use of liver glycogen (3, 4) and in some instances, muscle glycogen (5, 6). American College of Sports Medicine (ACSM) guidelines recommend consuming up to 90 $g \cdot h^{-1}$ of CHO during exercise lasting >2.5 hours or where endogenous CHO stores will be depleted (7). However, these guidelines are largely based on the accumulated evidence from studies that used cycle ergometer protocols, and so may not be suitable for adoption by individuals during distance running. In fact, the mean rate of CHO ingestion during marathon running (8, 9) is far below current recommendations (7). This supports anecdotal evidence from practitioners and athletes (10) that the recommendation to consume up to 90 $g \cdot h^{-1}$ (7) is not always practical or tolerable for runners.

The ergogenic effects of CHO ingestion require a well-functioning gastrointestinal (GI) tract (10), yet surveys suggest individuals experience upper and lower GI symptoms during distance running (11, 12). Moreover, GI symptoms are perceived to negatively affect running performance (13). Symptoms may include nausea, vomiting, stomach cramps, urge for bowel movement, reflux, fullness, bloating and diarrhoea (12). Evidence suggests over a one-month period 78-84% of runners have reported experiencing at least one GI symptom whilst running, and 14% of males, and 22% of females, have encountered moderate-to-severe GI symptoms (14).

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The aetiology of GI symptoms during distance running is likely multifactoral, influenced by exercise intensity and fluid osmolality amongst others (15). The upper range of the exercise intensities (60-75% maximal oxygen consumption; $\dot{V}O_2max$) that are typically achieved by (non)elite distance runners during marathon running events (16) are associated with delayed gastric emptying (17, 18), and the latter is thought to be a main cause of GI symptoms (19). Higher CHO concentrations (>6%) are associated with delayed absorption of CHO (20) resulting in increased residual CHO and water retention in the intestines (21), likely causing elevated GI symptoms. For example, consuming hypertonic CHO beverages in large quantities have been reported to cause a greater prevalence of GI symptoms whilst running (22). Ingestion of multiple transportable CHO (glucose and fructose) can reduce the prevalence of GI symptoms (23), whilst also increasing CHO oxidation rates (24). However, some individuals are still susceptible to GI symptoms (25). Therefore, strategies or methods to increase CHO intake without causing GI symptoms are of significant interest to runners and nutrition practitioners.

Hydrogel food technology has recently become commercially available in sports nutrition products (26), and may provide a novel means of delivering 90 $g \cdot h^{-1}$ CHO during running whilst potentially reducing the severity of GI symptoms. The addition of sodium alginate and pectin to CHO and water creates a pH-sensitive solution that forms a hydrogel that swells when exposed to the low pH environment in the stomach (27). The hydrogel stays complexed in acid, at this lower pH. Once in the small intestine, the higher alkaline pH causes the breakdown of the gel and the release of the CHO (27).

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Emerging research suggests that the ingestion of a CHO, sodium alginate and pectin solution can enhance the rate of gastric emptying compared to a standard non-hydrogel CHO solution (28). However, no study to date (29) including running (30, 31), has reported a benefit to exercise performance, total whole-body substrate metabolism, exogenous CHO oxidation, or GI symptoms when CHO was consumed as a hydrogel during endurance exercise. These findings could be related to the mode of exercise, and the exercise intensities (45-60% $\dot{V}O_2$max) studied. As a result, previous CHO hydrogel research using such exercise intensities may not have sufficiently depleted the endogenous CHO stores, or impaired the GI tract to elicit sufficient alterations in CHO absorption or GI symptoms. It is unclear whether the metabolic and GI responses to CHO hydrogel ingestion are different from a non-hydrogel when running at a higher exercise intensity.

This study aimed to investigate the effects of a multiple transportable CHO hydrogel, against a non-hydrogel solution and placebo, on endurance running performance, substrate utilisation and markers of GI symptoms. Using indirect calorimetry and ^{13}C tracer techniques, this study aimed to assess exogenous and endogenous (liver and muscle) substrate utilisation in runners. It was hypothesised that the addition of sodium alginate and pectin with CHO would improve endurance performance, exogenous CHO oxidation and GI symptoms.

METHODS

Participants

Eleven trained, healthy male runners volunteered to participate in this study (mean ± SD, age 29 ± 6 years, body mass 68.7 ± 2.6 kg, body mass index 21.0 ± 1.3 kg/m^2, and $\dot{V}O_2$max 62.6 ± 4.2

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$mL \cdot kg^{-1} \cdot min^{-1}$). Inclusion criteria required participants to have trained for >4 times per week in running-specific training for at least the last 3 years, completed a marathon within the last 18 months with a time under 2 hour 40 minutes or achieved a $\dot{V}O_2max$ >60 $mL \cdot kg^{-1} \cdot min^{-1}$. Procedures and potential risks were explained before the study and all participants provided written informed consent. Before commencing, this study gained institutional ethical approval from Leeds Beckett University (Ref No. 57552) and was conducted in accordance with the Declaration of Helsinki.

Experimental design

Preliminary testing, conducted 7 days before the first experimental trial, consisted of a submaximal incremental test and maximal exercise test to volitional exhaustion (32) to determine the specific submaximal running speed at 68% $\dot{V}O_2max$ for the experimental trial. This was followed by familiarisation of the GI questionnaire and 5-km time-trial. Following preliminary testing, participants completed three experimental trials (each separated by 7 days) consisting of a 120-minute steady state run at 68% $\dot{V}O_2max$, followed by a 5-km time-trial. During each trial, participants ingested one of three taste-matched solutions (CHO hydrogel, non-hydrogel CHO solution, or placebo) in a randomised, double-blind order. Participants ingested a 200 mL bolus immediately before exercise and then 100 mL of a 18% CHO solution every 15 minutes throughout the steady state run delivering CHO at a rate of 90 $g \cdot h^{-1}$.

Diet and physical activity before testing

Physical activity and food intake during the 48 hours before the first experimental trial were recorded and participants were instructed to repeat the same diet and activity pattern before

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subsequent trials. Before each experimental trial, participants were required to not undertake any strenuous physical activity and avoid alcohol and caffeine consumption for 24 hours. Before and for the duration of the study, participants were instructed to refrain from ingesting foods with a high natural ^{13}C abundance (i.e. plants with a C4 photosynthetic cycle, or animals fed with such plants) (33). This precaution ensured that background $^{13}CO_2$ abundance was less likely to be perturbed from oxidation of endogenous and dietary substrate stores from naturally "enriched" C4 origin. Prior to each trial, participants consumed a standardised evening meal consisting of a total of ~1196 kcal; 58% CHO, 14% fat, 28% protein (fibre: ~12 g) 10-12 hours before arriving at the laboratory.

Experimental trials

After an overnight fast, participants reported to the laboratory at the same time in the morning to avoid any influence of circadian variance. Upon arrival at the laboratory, an in-dwelling catheter (18-gauge Introcan Safety®, B. Braun Medical Ltd, Sheffield, UK) was inserted into an antecubital forearm vein. Resting blood samples were drawn for plasma glucose, plasma lactate, serum free fatty acid (FFA) and serum insulin. Subsequently, a 10-minute resting $\dot{V}O_2$ and $\dot{V}CO_2$ measurement was made using an online gas analysis system (Metalyser, Cortex, Germany), calibrated to the manufacturer's instructions. For the measurement of $^{13}CO_2$:$^{12}CO_2$ in expired air at rest, 12 ml Exetainers (SerCon Ltd, Crewe, UK) of expired gas were collected in duplicate via a mixing chamber (Jaeger, Germany). Participants then consumed a 200 mL bolus of the experimental drink solution immediately before starting the 120 minutes of running at 68% $\dot{V}O_2$max on a treadmill (Woodway, USA). Additional boluses (100 mL) of each solution were provided every 15 minutes throughout the 120-minute exercise period. Expired gas breath

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samples were collected, and measurements of $\dot{V}O_2$, $\dot{V}CO_2$, were measured every 15 minutes during the steady state run. Samples of expired gas for $^{13}CO_2$ analysis were collected during the final 60 seconds of each collection period at 60, 90 and 120 minutes. Venous blood samples for the analysis of plasma glucose, plasma lactate, serum FFA and serum insulin were drawn every 15 minutes, and for ^{13}C plasma glucose enrichment at 60 minutes and every 30 minutes thereafter. Heart rate measurements were taken every 15 minutes during steady state exercise. In addition, every 30 minutes during the 120 minutes of running, participants completed a GI questionnaire (34) covering three sections (upper, lower & systemic GI symptoms), with a 10-point scale ranging from 1 (no problem at all) to 10 (the worst it has ever been), and a score of ≥ 5 was classified as having severe GI symptoms. Participants were familiarised with the GI questionnaire during preliminary testing. After the steady state run, participants completed a self-paced 5-km time-trial, with a rolling start of a running speed at 68% $\dot{V}O_2$max. Only feedback on distance completed was given at 1, 2, 2.5, 3, 4, 4.5, 4.6, 4.7, 4.8, 4.9 and 5-km. Participants were blinded of their finishing times until all three experimental trials were completed.

Experimental drinks

The 180 grams of CHO used within the hydrogel and non-hydrogel experimental drinks were a 2:1 ratio of glucose (120 g) (D-glucose; Thornton and Ross Ltd, Huddersfield, UK) and fructose (60 g) (Danisco, Kettering, UK). The natural $\delta^{13}C$ abundance of the stock glucose and fructose were measured by isotope ratio mass spectrometry (IRMS, Isoprime, Cheadle, UK), using L-fucose as an isotopic internal standard as previously described (35) and determined to be -25.68 ‰ and -12.27 ‰ respectively. All ^{13}C measurements are quoted with reference to the internationally accepted standard for carbon isotope measurements, Vienna Pee Dee Belemnite

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(VPDB). Both CHO solutions were enriched with 150 mg per 75 g CHO of universally labelled (U-$^{13}C_6$) glucose and (U-$^{13}C_6$) fructose tracer (2:1 ratio) (Sigma Aldrich, St Louis, MO). The final isotopic enrichment of each ingested CHO solution were 143.81 ± 5.18 ‰ for the hydrogel solution and 142.32 ± 5.32 ‰ for the non-hydrogel solution. The hydrogel solution contained high methoxy pectin and sodium alginate at 0.45 wt% with a ratio of 1.25:1. All formulations contained 2.55 mmol·L^{-1} of NaCl (Saxa, Herts, UK), with the placebo drink containing artificial sweetener (aspartame, Morrisons' plc, Bradford, UK) to blind the participants to each condition. On completion of all three trials participants were asked to stipulate the order of their conditions, only 3 of the 11 determining the order correctly.

Analyses

Blood samples were centrifuged and aliquots of plasma and serum were stored at -80^{O}C until analysis. Plasma glucose (glucose oxidase kit; Instrumentation Laboratory, Monza, Italy, Inter-assay CV: 4.9%, Intra assay CV: 2.3%) and plasma lactate (Lactate kit, Randox, County Antrim, UK, Inter CV: 4.5%, Intra CV: 2.7%) concentrations were analysed by spectrophotometry (iLab 300 plus, iLab, UK). Serum insulin was analysed using a chemoilumino-metric immunoassay (ADIVA Centaur, Bayer diagnostics, Berkshire, UK, Inter CV: 3.2–4.6%, Intra CV: 2.6–5.9%). Serum FFA concentration was analysed by an acyl-CoA synthetase and oxidase assay (NEFA-HR2, Wako Chemicals GmbH, Germany, Inter assay CV: 1.5%). Isotope ratio mass spectrometry (IRMS; AP2003, GVI Instruments Ltd, Manchester, UK) were used to determine the $^{13}CO_2$:$^{12}CO_2$ in expired air as described previously (36). The ^{13}C:^{12}C in plasma glucose was determined using liquid chromatography linked-isotope ratio mass spectrometry (LC-IRMS), as previously described (35). Briefly, plasma samples were spiked with an internal standard (L-

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fucose, Sigma Aldrich, Poole, UK) and prepared by ultrafiltration (30000 MWCO, Amicon Ultra 4, Millipore, Watford, UK) for LC-IRMS analysis of ^{13}C-glucose enrichment (Δ^{13}C-glucose).

Calculations

Total CHO and fat oxidation ($g \cdot min^{-1}$) were calculated using the stoichiometric equations (equation 1 and 2) proposed by Jeukendrup & Wallis (37), with protein oxidation during running assumed to be negligible.

$$\text{CHO oxidation} = 4.210\ \dot{V}CO_2 - 2.962\ \dot{V}O_2 \qquad (1)$$

$$\text{fat oxidation} = 1.695\ \dot{V}O_2 - 1.701\ \dot{V}CO_2 \qquad (2)$$

To calculate absolute (g) whole body CHO, exogenous and endogenous (liver and muscle) CHO and fat oxidation the area under the curve technique was applied to the respective rates ($g \cdot min^{-1}$). Energy expenditure contributions from CHO and fat were calculated from absolute values, by applying their respective energy potentials (4.07 kcal and 9.75 kcal (37)). Total ingested glucose and fructose isotopic enrichment, (R_{exo}), and expired air (R_{exp}) were expressed in standard δ^{13}C units (‰) relative to VPDB (38). The rate of exogenous CHO oxidation derived from the combined ingestion of glucose and fructose (CHO_{EX}) were computed using equation 4 (39).

$$CHO_{EX}\ (g \cdot min^{-1}) = \dot{V}CO_2\ [(R_{exp} - R_{ref}) / (R_{exo} - R_{ref})] / k \qquad (4)$$

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Where $\dot{V}CO_2$ is in liters per minute, R_{exp} is the isotopic composition of expired CO_2 and R_{ref} is the isotopic composition of expired CO_2 at the same time point with ingestion of placebo. The isotopic composition of the ingested solution computed as R_{exo} and the k (0.747 $L \cdot g^{-1}$) is the volume of CO_2 provided by the complete oxidation of glucose. The oxidation efficiency, percentage of the ingested CHO utilised was calculated (40).

Computations were made on the assumption that, in response to exercise, ^{13}C is not irreversibly lost in pools of tricarboxylic acid cycle intermediates and/or bicarbonate, and that lactate produced from either glucose or fructose is either oxidized in muscle or recycled through gluconeogenesis to be used subsequently by complete oxidation. Essentially exogenous CHO oxidation is calculated irrespective of the pathway that finally produces $^{13}CO_2$ that can be measured. The calculations assume that $^{13}CO_2$ recovery in expired gases were complete or almost complete during exercise. Such computation has been shown to underestimate exogenous oxidation rates at the beginning of exercise because of the delay between $^{13}CO_2$ production in tissues and expired $^{13}CO_2$ at the mouth (41). Therefore, exogenous CHO oxidation data are presented for the final 60 minutes of the 120-minute running period, where it is expected that there would be isotopic equilibrium in the tissues and at the mouth (42).

The oxidation rate of plasma glucose was calculated based on the ^{13}C isotopic composition of plasma glucose (R_{glu}) (equation 5, 43):

$$\text{plasma CHO } (g \cdot min^{-1}) = \dot{V}CO_2 \left[(R_{exp} - R_{ref}) / (R_{glu} - R_{ref})\right] / k \qquad (5)$$

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Endogenous CHO oxidation is presented as the difference between total CHO oxidation and exogenous CHO oxidation. The oxidation rate of muscle glycogen ($g \cdot min^{-1}$), either directly or through the lactate shuttle (44), were calculated by subtracting plasma glucose oxidation from total CHO oxidation. Finally, glucose oxidation derived from the liver was estimated as the difference between plasma glucose oxidation and exogenous CHO oxidation (43).

Statistical analyses

Data evaluation was performed using Prism (8.3.1) (GraphPad Software, La Jolla California USA). Eleven trained male runners were recruited for this study, providing 92% power to detect differences in performance, with an expected mean difference of 1.6% between CHO hydrogel and non-hydrogel (29), assuming a standard deviation of 1.43% at an alpha of 0.05.

In addition, eleven male runners would provide 90% power to detect differences in the rate of exogenous CHO oxidation, with an expected mean difference of 0.11 $g \cdot min^{-1}$ between CHO hydrogel and non-hydrogel, assuming a standard deviation of 0.10 $g \cdot min^{-1}$ at an alpha of 0.05. Variables were checked for normality prior to performing statistical tests (Kolmogorov–Smirnov test). Differences in $\dot{V}O_2$, $\dot{V}CO_2$, RER, HR, rate of substrate utilisation, plasma and serum metabolites were analysed using a two-factor (time x treatment) analysis of variance (ANOVA) for repeated measures. Time-trial completion time, total energy expenditure, endogenous CHO oxidation and relative substrate data for all three conditions was analysed using a one-way ANOVA. Post hoc analysis was performed for any significant time and condition main effects or interactions using paired sample *t*-tests with Bonferroni adjustment. Only CHO conditions were compared when variables considered $\delta^{13}CO_2$ of expired gas and $\delta^{13}C$ in plasma glucose. A paired sample *t*-test was used to analyse absolute and relative exogenous CHO oxidation and the

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oxidation of muscle, liver and plasma glucose, as well as the percentage of the exogenous source of CHO utilised. GI symptoms were analysed using Wilcoxon matched-pairs signed rank test. Relationship between time-trial performance and GI symptoms was assessed using a Spearman's rho correlations. Data are presented as mean ± SD and statistical significance was set at $p < 0.05$.

RESULTS

Time-Trial Performance

A one-way ANOVA determined that mean 5-km time-trial performance [minutes:seconds] was significantly different between conditions ($p = < 0.0001$). Post hoc analysis revealed significant improvements in time-trial performance with the ingestion of hydrogel (19:29 ± 2:24, 7.6%, $p < 0.001$) and non-hydrogel (19:54 ± 2:23, 5.6%, $p = 0.002$) compared with placebo (21:05 ± 2:34, Figure 1). Time-trial performance for hydrogel was also significantly faster (2.1%) compared with non-hydrogel ($p = 0.033$).

$\dot{V}O_2$, $\dot{V}CO_2$, RER and Heart Rate

A two-way ANOVA showed that there was a significant condition and time interaction for $\dot{V}O_2$ ($p = 0.001$), $\dot{V}CO_2$ ($p = 0.003$) and RER ($p = 0.001$) during the 120 minutes of running. Post hoc analysis at each time period during the steady state run revealed $\dot{V}O_2$ was not significantly different between hydrogel and non-hydrogel ($p = 0.33$ to 0.99), but $\dot{V}O_2$ was significantly lower in both CHO conditions compared with placebo ($p = 0.001$ to 0.03; Table 1). $\dot{V}CO_2$ was not significantly different between conditions in the first 60 minutes of the steady state run ($p = 0.70$ to 0.99). In the final 60 minutes of the steady state run, $\dot{V}CO_2$ was not significantly different between hydrogel and non-hydrogel ($p = 0.43$ to 0.99), but $\dot{V}CO_2$ was significantly greater in

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both CHO conditions compared with placebo (p = 0.001 to 0.03). Throughout the steady state run, RER was significantly higher in both CHO conditions relative to placebo (p = 0.001 to 0.02), with no significant difference in RER between hydrogel and non-hydrogel conditions (p = 0.09 to 0.5). In the placebo condition, RER was significantly lower during the final 60 minutes compared with the initial 60 minutes of the steady state run (p = 0.01). HR was not significantly different across time (p = 0.43) or between conditions (p = 0.87) during the steady state run.

Energy Expenditure, Total Carbohydrate and Fat Oxidation

A one-way ANOVA showed that there was no main effect of condition for total energy expenditure during the steady state run (hydrogel, 1746 ± 135 kcal; non-hydrogel, 1760 ± 137 kcal; placebo, 1818 ± 167 kcal; p = 0.83). A two-way ANOVA showed that there was a significant condition and time interaction for both absolute whole-body CHO (p = 0.001*)* and fat oxidation (p = 0.001). Post hoc analysis indicated that absolute whole-body CHO oxidation in hydrogel (324.2 ± 17.6 g) and non-hydrogel (318.3 ± 20.7 g) were not significantly different during the 120-minute steady state run (p = 0.09), but the absolute CHO oxidation in both CHO conditions was significantly higher than placebo (260.9 ± 16.5 g; p < 0.00001 & p < 0.001). In addition, absolute whole-body CHO oxidation in hydrogel and non-hydrogel were not significantly different during the first (p = 0.99) and second (p = 0.99) hour of steady state running, however, both CHO conditions were significantly higher compared with placebo (p < 0.001; Table 1). Conversely, absolute fat oxidation was significantly lower throughout the 120-minute steady state run for both CHO conditions (hydrogel = 43.5 ± 8.3 g; non-hydrogel, 47.6 ± 7.9 g) compared with placebo (77.8 ± 15.2 g; p < 0.0001 & p < 0.0001) as well as during the first (hydrogel: p = 0.0003; non-hydrogel: p = 0.0003) and second hour (hydrogel: p < 0.0001; non-

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hydrogel: $p < 0.0001$) (Table 1). Fat oxidation was also significantly lower in hydrogel compared with non-hydrogel during the first ($p = 0.002$) and second hour ($p = 0.001$) of exercise and over the 120-minute steady state run ($p = 0.002$). A one-way ANOVA showed that there was a main effect of condition ($p < 0.0001$) for the relative contribution of CHO and fat to energy expenditure during the 120-minute steady state run (Figure 2). Post hoc analysis showed that the relative contribution of CHO to energy expenditure was significantly greater in both CHO conditions (hydrogel, 76.1 ± 4.4%; non-hydrogel, 74.1 ± 5.2%), compared with placebo (58.2 ± 6.5%; $p = 0.001$ and $p = 0.001$), with no significant difference between CHO conditions ($p = 0.12$). The relative contribution of fat to energy expenditure was significantly lower for the CHO conditions (hydrogel, 23.9 ± 2.1%; non-hydrogel, 25.9 ± 1.8%), compared with placebo (41.8 ± 4.9%, $p < 0.0001$ & $p < 0.0001$), with the hydrogel being significantly lower compared with the non-hydrogel ($p = 0.002$).

$\delta^{13}CO_2$ in expired gas and $\delta^{13}C$ in plasma glucose

A two-way ANOVA showed that there was a significant main effect of time and condition and time interactions for $\delta^{13}CO_2$ in expired gas ($p < 0.0001$ and $p < 0.0001$). Post hoc analysis for $\delta^{13}CO_2$ in expired gas showed that there was no significant difference between conditions at rest ($p = 0.45$ to 0.99, Figure 3A). In placebo, the $\delta^{13}CO_2$ in expired gas significantly increased over time by 1.28‰ from the start to the end of the steady state run ($p < 0.0001$). These data were then used as the background correction for the calculation of exogenous CHO and plasma glucose oxidation for each CHO condition. The $\delta^{13}CO_2$ in expired gas significantly increased over time from the start of exercise following the ingestion of the ^{13}C enriched CHO solutions ($p = 0.001$) and peak values were reached at 120 minutes. As shown in figure 3A, post hoc analysis

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indicated that the hydrogel was significantly higher compared with the non-hydrogel at 60 (p = 0.005), 90 (p = 0.009), and 120 minutes (p = 0.012). The isotopic composition of plasma glucose ($\delta^{13}C$) significantly increased by 1.28‰ from 60 to 120 minutes during the steady state run with ingestion of placebo ($p < 0.0001$, Figure 3B). In both CHO conditions, there was a significant main effect of time (p = 0.001) for plasma glucose $\delta^{13}C$, with post hoc analysis revealing a significant rise between 60 and 120 minutes (p = 0.004). However, there was no significant condition or time interaction for plasma glucose $\delta^{13}CO_2$ between CHO conditions (p = 0.14).

Exogenous and Endogenous Carbohydrate Oxidation

A two-way ANOVA showed a significant main effect of time for the rate of exogenous CHO oxidation ($p < 0.0001$). Post hoc analysis revealed that the rate of exogenous CHO oxidation increased significantly (p = 0.001) in both CHO conditions during the final 60 minutes of the steady state run, peaking at 120 minutes during each condition (hydrogel: 1.27 ± 0.17 $g \cdot min^{-1}$; non-hydrogel: 1.18 ± 0.13 $g \cdot min^{-1}$, Figure 4A). There was also a condition and time interaction (p = 0.005), with post hoc analysis showing that exogenous CHO oxidation was significantly greater in hydrogel compared with non-hydrogel at 60 (1.03 ± 0.19 vs 0.93 ± 0.14 $g \cdot min^{-1}$, p = 0.001), 90 (1.14 ± 0.19 vs 1.06 ± 0.14 $g \cdot min^{-1}$, p = 0.009) and 120 minutes (1.27 ± 0.17 vs 1.18 ± 0.13 $g \cdot min^{-1}$, p = 0.001). A paired sample t-test showed that absolute exogenous CHO oxidation during the final 60 minutes of the steady state run (Table 2) was significantly greater with hydrogel compared with non-hydrogel (p = 0.003). In addition, relative exogenous CHO (Figure 2) was also significantly greater with hydrogel (31.9 ± 2.69%) compared with the non-hydrogel (29.3 ± 1.96%, p = 0.003). The percentage of the exogenous source of CHO utilised was also significantly greater in the hydrogel (76.2%) compared with the non-hydrogel condition (70.6%,

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p = 0.003). A two-way ANOVA showed that there was a significant main effect of condition for the rate of endogenous CHO oxidation during the final 60 minutes of the steady state run. Post hoc analysis showed that rate of endogenous CHO oxidation was lower for both CHO conditions (hydrogel 1.64 ± 0.15 g·min^{-1}; non-hydrogel 1.68 ± 0.14 g·min^{-1}) compared with placebo (2.03 ± 0.19 g·min^{-1}, p = 0.001), but reporting no significant condition and time interaction (p = 0.07). A one-way ANOVA showed that the absolute endogenous CHO oxidation, during the final 60 minutes of the steady state run was not significantly different between the hydrogel and non-hydrogel conditions (Table 2, p = 0.58). There was also no significant difference in the relative contribution of endogenous CHO oxidation to energy expenditure between the hydrogel (46.0 ± 2.3%) and non-hydrogel conditions (46.8 ± 1.4%, p = 0.46).

A two-way ANOVA showed a significant main effect of time for plasma glucose oxidation (p < 0.001) during the final 60 minutes of steady state running, reaching peak rates at 120 minutes during each CHO condition (hydrogel: 1.58 ± 0.24 g·min^{-1}; non-hydrogel: 1.49 ± 0.20 g·min^{-1}, Figure 4B). However, there was no significant condition and time interaction between the hydrogel and non-hydrogel trials (p = 0.86). In contrast, a paired sample t-test showed that plasma glucose oxidation was significantly higher in hydrogel compared with non-hydrogel when expressed in absolute (p = 0.001, Table 2) and relative terms (41.0 ± 2.1% vs 38.1 ± 1.8%, p = 0.001).

The rate of glucose oxidation derived from the liver remained stable in both CHO conditions during the final 60 minutes of steady state run (Figure 4C), with a two-way ANOVA showing that there was no significant condition and time interaction between the hydrogel and non-

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hydrogel conditions (p = 0.78). A paired sample t-test showed that liver glucose oxidation was also not significantly different between the hydrogel and non-hydrogel conditions when expressed in absolute (p = 0.83, Table 2) or relative terms (9.1 ± 1.5% vs 8.7 ± 0.9%, p = 0.83, Figure 2). Muscle glycogen oxidation rates (Figure 4D) remained stable over time, with a two-way ANOVA showing that there was no significant condition and time interaction between CHO conditions (p = 0.46). In addition, a paired sample t-test showed that muscle glycogen oxidation was not significantly different between the hydrogel and non-hydrogel when expressed in absolute (p = 0.26, Table 2) or relative terms (36.9 ± 3.4% vs. 38.1 ± 1.8%, p = 0.26).

Circulatory Metabolites and Insulin

A two-way ANOVA showed that there were significant condition and time interactions for plasma glucose (p = 0.04), plasma lactate (p = 0.03), serum FFA (p < 0.0001) and serum insulin (p < 0.0001). Post hoc analysis showed that plasma glucose concentrations (Figure 5A) during the 120-minute run were significantly greater in both CHO conditions compared with placebo at each time point (p < 0.001). Plasma glucose concentrations were not significantly different between hydrogel and non-hydrogel at any time point (p = 0.74 to 0.99). Plasma lactate concentration were significantly higher in the CHO conditions compared with placebo (p = 0.001 to 0.01) throughout the steady state run (Figure 5B). However, plasma lactate was not significantly different between hydrogel and non-hydrogel conditions (p = 0.66 to 0.94). Serum FFA concentration (Figure 5C) increased significantly over time throughout the steady state run in the placebo condition (p < 0.001). Serum FFA was significantly lower in the CHO conditions at each time point compared with placebo (p = 0.002 to 0.01) and was not significantly different between CHO conditions at any time points (p = 0.34 to 0.99). Serum insulin concentration

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(Figure 5D) was significantly higher in both CHO conditions throughout the steady state run compared with placebo ($p < 0.001$) and was not significantly different between CHO conditions ($p = 0.53$ to 0.99).

Gastrointestinal Response

Mean GI symptom scores, the minimum and maximum scores, and percentage of participants who reported GI symptom scores ≥5 are presented in Table 3. A Wilcoxon signed rank test showed there was no significant differences in total scores of GI symptoms between hydrogel and placebo conditions ($p = 0.19$). However, the total GI symptom scores for the hydrogel and placebo were both significantly lower compared with non-hydrogel ($p = 0.001$ & $p = 0.001$ respectively). The prevalence of upper and lower GI symptoms ranged from 0-18% in the placebo, 18-36% in the hydrogel, and 18-64% in the non-hydrogel condition. Upper GI symptom scores were significantly greater during the non-hydrogel compared with hydrogel (Table 3, $p = 0.025$) and placebo ($p = 0.001$) conditions, and was significantly greater in hydrogel compared with the placebo condition ($p = 0.011$). Lower GI symptom scores were significantly greater during the non-hydrogel compared with hydrogel (Table 3, $p = 0.006$) and placebo ($p < 0.001$) conditions, and was significantly greater in hydrogel compared with the placebo condition ($p = 0.007$). Systemic symptom score was significantly greater during the non-hydrogel compared with hydrogel (Table 3, $p = 0.039$) and placebo ($p = 0.002$) conditions and was not significantly different in hydrogel compared with placebo ($p = 0.98$). A Spearman's rank-order correlation showed a strong positive correlation between combined total GI symptom scores and time-trial performance for all three conditions ($r_s = .693$, $p = 0.021$). In the non-hydrogel condition, there was a strong positive correlation between GI symptom scores and time-trial performance ($r_s =$

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.746, p = 0.011). No correlation was shown in the hydrogel and placebo conditions (r_s = .536, p = 0.094 and r_s = .560, p = 0.067, respectively).

DISCUSSION

This is the first study, to our knowledge, to demonstrate that ingestion of 90 g·h^{-1} of glucose and fructose in hydrogel form whilst running at 68% $\dot{V}O_2$max for 120 minutes improves 5-km time-trial performance in trained runners compared with a non-hydrogel CHO. This improved performance could be attributed to the increase exogenous CHO oxidation, decreased fat oxidation and reduced GI symptoms following hydrogel ingestion, as liver and muscle glycogen oxidation during the last hour of the 120 minutes of running were not different between hydrogel and non-hydrogel. Thus, the CHO hydrogel may allow athletes to consume more adequate amounts of CHO during prolonged running, subsequently improving performance.

The novel performance effects observed in the present study when ingesting CHO hydrogel whilst running conflict with previous evidence that have failed to detect a performance benefit across cycling, cross-country skiing and running (29). The reasons for the discrepancy are unclear, but it may relate to differences in exercise intensity, duration, CHO type and dose, training status, exercise mode, or the performance test employed. To the authors' knowledge, only one study has investigated the effect of consuming a commercially available CHO hydrogel (90 g·h^{-1}) against a non-hydrogel CHO (90 g·h^{-1}) solution on running performance (30). However, the incremental time to exhaustion treadmill test following a 180-minute run at 60% $\dot{V}O_2$max is maximal in nature and may not have been appropriate to detect an effect of CHO hydrogel on running performance.

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In the present study, total CHO oxidation was higher, and fat oxidation was lower when CHO was ingested in either hydrogel or non-hydrogel conditions relative to placebo, which is consistent with the known effects of CHO ingestion on whole body substrate metabolism (45). In addition, lower fat oxidation was observed with hydrogel compared with non-hydrogel during the 120-minute run, which cannot be attributed to between-condition differences in serum free fatty acid and serum insulin concentration. However, there was no difference in whole-body CHO oxidation, liver glucose oxidation and muscle glycogen oxidation between the hydrogel and non-hydrogel conditions during 60-120 minutes of the steady state run. Thus, the improvement in 5-km time-trial performance following the ingestion of hydrogel compared with the non-hydrogel CHO solution is not related to sparing of either liver or muscle glycogen during the final hour of the 120-minute steady state run.

Exogenous CHO oxidation rates during the final 60 minutes for the non-hydrogel CHO solution (1.06 ± 0.13 g·min^{-1}) are consistent with existing running literature that also administered 90 g·h^{-1} of a 2:1 ratio of glucose and fructose (46). For the first time we show that significantly higher (8.2%) exogenous CHO oxidation rates can be achieved with the ingestion of hydrogel compared with non-hydrogel. The higher exogenous CHO oxidation resulted in a greater utilisation with the hydrogel (76.2%), compared with non-hydrogel condition (70.6%). The elevations in plasma glucose oxidation due to exogenous CHO oxidation may therefore have contributed, at least partially, to the improvement in 5-km time-trial performance following the hydrogel compared with non-hydrogel CHO solution. Of the three studies that previously measured exogenous CHO oxidation following CHO hydrogel ingestion (31, 47, 48), only Barber *et al.,* (31) included a comparative CHO condition. In contrast to the present study, Barber *et al.* (31) found no

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difference in exogenous CHO oxidation between the hydrogel (maltodextrin-fructose) and CHO-matched non-hydrogel conditions in trained runners during 120 minutes of running at 60% $\dot{V}O_2$max. The higher exercise intensity used in our study compared with Barber *et al.*, (31) may be a potential explanation for the disparity, as exogenous CHO oxidation is well accepted to increase with exercise intensity. In addition, a strength of the present study design is that both CHO solutions were enriched with a high dose of universally labelled ^{13}C tracers which enhances the signal to noise-ratio and the ability to definitively detect oxidative differences in ^{13}C labelled substrate metabolism.

To our knowledge, we are the first to report the effect of CHO hydrogel ingestion during running at exercise intensities that align with (non)elite marathon running (16), and delayed gastric emptying (17, 18). The potential for a lower rate of gastric emptying during running (18, 49) may have been tempered in the present study by the hydrogel solution, since a faster rate of gastric emptying can be achieved when CHO is ingested as a hydrogel compared to a standard CHO solution (28). This may result in a more effective intestinal absorption of CHO (49), and would be consistent with the greater oxidation of exogenous CHO observed in the present study, relative to the non-hydrogel CHO solution. This interpretation is supported by a glucose infusion study which suggested exogenous CHO oxidation to be limited by intestinal absorption (50).

An improved rate of gastric emptying during running in the hydrogel condition may have also contributed to the lower severity and incidence of GI symptoms reported by our cohort, given that delayed gastric emptying is thought to be one of the main contributors to GI symptoms during exercise (19). However, the lower GI symptoms with hydrogel contrasts with the

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literature (29). The reasons for the discrepancy between others (29) and the present study may be related to our robust familiarisation of participants to the GI questionnaire, a higher exercise intensity and a different CHO type and dose. In the present study, both CHO conditions used a high concentration of CHO (18%) in the form of glucose:fructose (2:1 ratio) as opposed to maltodextrin:fructose (7.8-15.8%: 1:0.7 ratio) (29). As glucose is monomeric and maltodextrin is polymeric the potential for osmotic differences exists which could also account for the increased prevalence of GI symptoms in the non-hydrogel condition in this study compared with previous literature (29). The high rate of hydrogel ingestion (90 $g \cdot h^{-1}$) used in the present study did not completely nullify GI symptoms for some individuals, and future research should look to see whether moderate rates of non-hydrogel ingestion (50-60 $g \cdot h^{-1}$), which are associated with lower GI symptoms (51) are equally efficacious. A limitation of the present study and the literature, is the reporting of how accustomed participants are to consuming CHO, as gut training may alleviate some GI symptoms, such a stomach comfort following CHO ingestion (52). Thus, further research is required to establish whether gut training diminishes the positive effect of hydrogel on GI symptoms seen in this study. In addition, further research is required in measuring the rate of gastric emptying when ingesting CHO in hydrogel form, as the viscosity of the ingested liquid (i.e. CHO hydrogel) could be a limitation of the double sampling gastric aspiration technique previously administered (28). Nevertheless, our data suggest that when men running at exercise intensities consistent with (non)elite marathon running (16) and delayed gastric emptying (17, 18), hydrogel ingestion may be an effective means to increase the rate of CHO ingestion during marathon running (8, 9), in line with ACSM guidelines (7). This would subsequently decrease the incidence and severity of GI symptoms, and improve running

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performance. However due to females also reporting GI symptoms when running (14), further research is required to establish if CHO hydrogel ingestion is equally efficacious.

Conclusion

Ingestion of glucose and fructose (90 $g \cdot h^{-1}$) in hydrogel form whilst running at 68% $\dot{V}O_2$max for 120 minutes improved subsequent 5-km time-trial performance relative to a CHO-matched non-hydrogel solution and placebo. This occurred alongside increased exogenous CHO oxidation, decreased fat oxidation, and a reduction in symptoms of GI when ingesting the hydrogel solution. If individuals choose to ingest a high rate of glucose and fructose (90 $g \cdot h^{-1}$) during prolonged running, they may benefit from ingesting monomeric CHO in hydrogel form as compared to a standard non-hydrogel CHO solution.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest. The results of the present study do not constitute endorsement by the American College of Sports Medicine. The results of the study are presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation.

AUTHOR CONTRIBUTIONS

JTR, RK, AK, OJW and JOH designed the research. JTR, DJM and TP conducted the research. JTR analysed the data and performed the statistical analysis. JTR, RK, OJW and JOH interpreted the data. JTR wrote the paper, and all authors edited the manuscript. All authors read and approved the final version of the manuscript.

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REFERENCES

1. Stellingwerff T, Cox GR. Systematic review: Carbohydrate supplementation on exercise performance or capacity of varying durations. *Appl Physiol Nutr Metab*. 2014;39(9):998-1011.
2. Coyle EF, Coggan AR, Hemmert MK, Ivy JL. Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J Appl Physiol* (1985). 1986;61(1):165-72.
3. Jeukendrup AE, Raben A, Gijsen A, et al. Glucose kinetics during prolonged exercise in highly trained human subjects: effect of glucose ingestion. *J Physiol*. 1999;515 (Pt 2):579-89.
4. Gonzalez JT, Fuchs CJ, Smith FE, et al. Ingestion of glucose or sucrose prevents liver but not muscle glycogen depletion during prolonged endurance-type exercise in trained cyclists. *Am J Physiol Endocrinol Metab*. 2015;309(12):E1032-9.
5. Tsintzas OK, Williams C, Boobis L, Greenhaff P. Carbohydrate ingestion and single muscle fiber glycogen metabolism during prolonged running in men. *J Appl Physiol* (1985). 1996;81(2):801-9.
6. Stellingwerff T, Boon H, Gijsen AP, Stegen JH, Kuipers H, van Loon LJ. Carbohydrate supplementation during prolonged cycling exercise spares muscle glycogen but does not affect intramyocellular lipid use. *Pflugers Arch*. 2007;454(4):635-47.
7. Thomas DT, Erdman KA, Burke LM. American College of Sports Medicine Joint Position Statement. Nutrition and Athletic Performance. *Med Sci Sports Exerc*. 2016;48(3):543-68.

ACCEPTED

Copyright © 2021 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

8. Pfeiffer B, Stellingwerff T, Hodgson AB, et al. Nutritional intake and gastrointestinal problems during competitive endurance events. *Med Sci Sports Exerc*. 2012;44(2):344-51.

9. Pugh JN, Kirk B, Fearn R, Morton JP, Close GL. Prevalence, Severity and Potential Nutritional Causes of Gastrointestinal Symptoms during a Marathon in Recreational Runners. *Nutrients*. 2018;10(7):811.

10. Costa RJS, Snipe RMJ, Kitic CM, Gibson PR. Systematic review: exercise-induced gastrointestinal syndrome-implications for health and intestinal disease. *Aliment Pharmacol Ther*. 2017;46(3):246-65.

11. Keeffe EB, Lowe DK, Goss JR, Wayne R. Gastrointestinal symptoms of marathon runners. *West J Med*. 1984;141(4):481-4.

12. Riddoch C, Trinick T. Gastrointestinal disturbances in marathon runners. Br J Sports Med. 1988;22(2):71-4.

13. Halvorsen FA, Lyng J, Glomsaker T, Ritland S. Gastrointestinal disturbances in marathon runners. Br J Sports Med. 1990;24(4):266-8.

14. Wilson PB. Frequency of Chronic Gastrointestinal Distress in Runners: Validity and Reliability of a Retrospective Questionnaire. *Int J Sport Nutr Exerc Metab*. 2017;27(4):370-376.

15. Brouns F, Beckers E. Is the gut an athletic organ? Digestion, absorption and exercise. *Sports Med*. 1993;15(4):242-57.

16. Maughan RJ, Leiper JB. Aerobic capacity and fractional utilisation of aerobic capacity in elite and non-elite male and female marathon runners. Eur J Appl Physiol Occup Physiol. 1983;52(1):80-7.

ACCEPTED

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17. Costill DL, Saltin B. Factors limiting gastric emptying during rest and exercise. *J Appl Physiol*. 1974;37(5):679-83.

18. Neufer PD, Young AJ, Sawka MN. Gastric emptying during walking and running: effects of varied exercise intensity. *Eur J Appl Physiol Occup Physiol*. 1989;58(4):440-5.

19. de Oliveira EP, Burini RC. Food-dependent, exercise-induced gastrointestinal distress. *J Int Soc Sports Nutr*. 2011;8:12

20. Rehrer NJ, Wagenmakers AJ, Beckers EJ, et al. Gastric emptying, absorption, and carbohydrate oxidation during prolonged exercise. *J Appl Physiol*. 1985. 1992;72(2):468-75.

21. Shi X, Horn MK, Osterberg KL, et al. Gastrointestinal discomfort during intermittent high-intensity exercise: effect of carbohydrate-electrolyte beverage. *Int J Sport Nutr Exerc Metab*. 2004;14(6):673-83.

22. Morton DP, Aragón-Vargas LF, Callister R. Effect of Ingested Fluid Composition on Exercise-Related Transient Abdominal Pain. *Int J Sport Nutr Exerc Metab*. 2004;14(2), 197-208.

23. Wilson PB, Ingraham SJ. Glucose-fructose likely improves gastrointestinal comfort and endurance running performance relative to glucose-only. *Scand J Med Sci Sports*. 2015;25(6):613-20.

24. Jentjens R, Moseley L, Waring RH, Harding LK, Jeukendrup AE. Oxidation of combined ingestion of glucose and fructose during exercise. *J Appl Physiol.* 2004;96(4):1277-1284.

25. Rowlands DS, Swift M, Ros M, Green JG. Composite versus single transportable carbohydrate solution enhances race and laboratory cycling performance. *Appl Physiol Nutr Metab*. 2012;37(3):425-36

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26. Sutehall S, Muniz-Pardos B, Bosch AN, Di Gianfrancesco A, Pitsiladis YP. Sports Drinks on the Edge of a New Era. *Curr Sports Med Rep*. 2018;17(4):112-6.

27. Ahmed EM. Hydrogel: Preparation, characterization, and applications: A review. *J Adv Res*. 2015;6(2):105-21.

28. Sutehall S, Galloway SDR, Bosch A, Pitsiladis Y. Addition of an Alginate Hydrogel to a Carbohydrate Beverage Enhances Gastric Emptying. *Med Sci Sports Exerc*. 2020;52(8):1785-92.

29. King AJ, Rowe JT, Burke LM. Carbohydrate Hydrogel Products Do Not Improve Performance or Gastrointestinal Distress During Moderate-Intensity Endurance Exercise. *Int J Sport Nutr Exerc Metab*. 2020:1-10.

30. McCubbin AJ, Zhu A, Gaskell SK, Costa RJS. Hydrogel Carbohydrate-Electrolyte Beverage Does Not Improve Glucose Availability, Substrate Oxidation, Gastrointestinal Symptoms or Exercise Performance, Compared With a Concentration and Nutrient-Matched Placebo. *Int J Sport Nutr Exerc Metab*. 2019:1-9.

31. Barber JFP, Thomas J, Narang B, et al. Pectin-Alginate Does Not Further Enhance Exogenous Carbohydrate Oxidation in Running. *Med Sci Sports Exerc*. 2020;52(6):1376-84.

32. Winter E, Jones A, Davidson RC, Bromley P, Mercer T. *Sport and Exercise Physiology Testing Guidelines: Volume I - Sport Testing: The British Association of Sport and Exercise Sciences Guide*. 1st ed. Routledge. 2007. p. 147-154.

33. Morrison DJ, Dodson B, Slater C, Preston T. (13)C natural abundance in the British diet: implications for (13)C breath tests. *Rapid Commun Mass Spectrom*. 2000;14(15):1321-4.

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34. Jeukendrup A, Vet-Joop K, Sturk A, et al. Relationship between gastro-intestinal complaints and endotoxaemia, cytokine release and the acute-phase reaction during and after a long-distance triathlon in highly trained men. *Clinical Science* 2000;98:47-55.

35. Morrison DJ, O'Hara JP, King RF, Preston T. Quantitation of plasma 13C-galactose and 13C-glucose during exercise by liquid chromatography/isotope ratio mass spectrometry. *Rapid Commun Mass Spectrom*. 2011;25(17):2484-8.

36. O'Hara JP, Carroll S, Cooke CB, Morrison DJ, Preston T, King RF. Preexercise galactose and glucose ingestion on fuel use during exercise. *Med Sci Sports Exerc*. 2012;44(10):1958-67.

37. Jeukendrup AE, Wallis GA. Measurement of substrate oxidation during exercise by means of gas exchange measurements. *Int J Sports Med*. 2005;26 Suppl 1:S28-37.

38. Craig H. The geochemistry of the stable carbon isotopes. *Geochimica et Cosmochimica Acta*. 1953;3(2):53-92.

39. Péronnet F, Massicotte D, Brisson G, Hillaire-Marcel C. Use of 13C substrates for metabolic studies in exercise: methodological considerations. *J Appl Physiol* (1985). 1990;69(3):1047-52.

40. Jeukendrup AE, Jentjens R. Oxidation of carbohydrate feedings during prolonged exercise: current thoughts, guidelines and directions for future research. *Sports Med*. 2000;29(6):407-24.

41. Pallikarakis N, Sphiris N, Lefebvre P. Influence of the bicarbonate pool and on the occurrence of 13CO2 in exhaled air. *Eur J Appl Physiol Occup Physiol*. 1991;63(3-4):179-83.

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42. Robert JJ, Koziet J, Chauvet D, Darmaun D, Desjeux JF, Young VR. Use of 13C-labeled glucose for estimating glucose oxidation: some design considerations. *J Appl Physiol* (1985). 1987;63(5):1725-32.

43. Péronnet F, Rhéaume N, Lavoie C, Hillaire-Marcel C, Massicotte D. Oral [13C]glucose oxidation during prolonged exercise after high- and low-carbohydrate diets. *J Appl Physiol* (1985). 1998;85(2):723-30.

44. Brooks GA. Lactate production under fully aerobic conditions: the lactate shuttle during rest and exercise. *Fed Proc*. 1986;45(13):2924-9.

45. Pfeiffer B, Stellingwerff T, Zaltas E, Jeukendrup AE. CHO oxidation from a CHO gel compared with a drink during exercise. *Med Sci Sports Exerc*. 2010;42(11):2038-45.

46. Pfeiffer B, Stellingwerff T, Zaltas E, Hodgson AB, Jeukendrup AE. Carbohydrate Oxidation from a Drink during Running Compared with Cycling Exercise. *Med Sci Sports Exerc*. 2011; 43(2):327-34.

47. Pettersson S, Edin F, Bakkman L, McGawley K. Effects of supplementing with an 18% carbohydrate-hydrogel drink versus a placebo during whole-body exercise in -5 °C with elite cross-country ski athletes: a crossover study. *J Int Soc Sports Nutr*. 2019;16(1):46.

48. Pettersson S, Ahnoff M, Edin F, Lingström P, Simark Mattsson C, Andersson-Hall U. A Hydrogel Drink With High Fructose Content Generates Higher Exogenous Carbohydrate Oxidation and Lower Dental Biofilm pH Compared to Two Other, Commercially Available, Carbohydrate Sports Drinks. *Front Nutr*. 2020;7:88.

49. Lang JA, Gisolfi CV, Lambert GP. Effect of exercise intensity on active and passive glucose absorption. *Int J Sport Nutr Exerc Metab*. 2006;16(5):485-93.

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50. Hawley JA, Bosch AN, Weltan SM, Dennis SC, Noakes TD. Glucose kinetics during prolonged exercise in euglycaemic and hyperglycaemic subjects. *Pflugers Arch*. 1994;426(5):378-86.

51. Baur DA, Schroer AB, Luden ND, Womack CJ, Smyth SA, Saunders MJ. Glucose–Fructose Enhances Performance versus Isocaloric, but Not Moderate, Glucose. *Med Sci Sports Exerc.* 2014;46(9): 1778-86.

52. Lambert GP, Lang J, Bull A, Eckerson J, Lanspa S, O'Brien J. Fluid tolerance while running: effect of repeated trials. Int J Sports Med. 2008;29(11):878-82.

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LIST OF FIGURES

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Figure 1

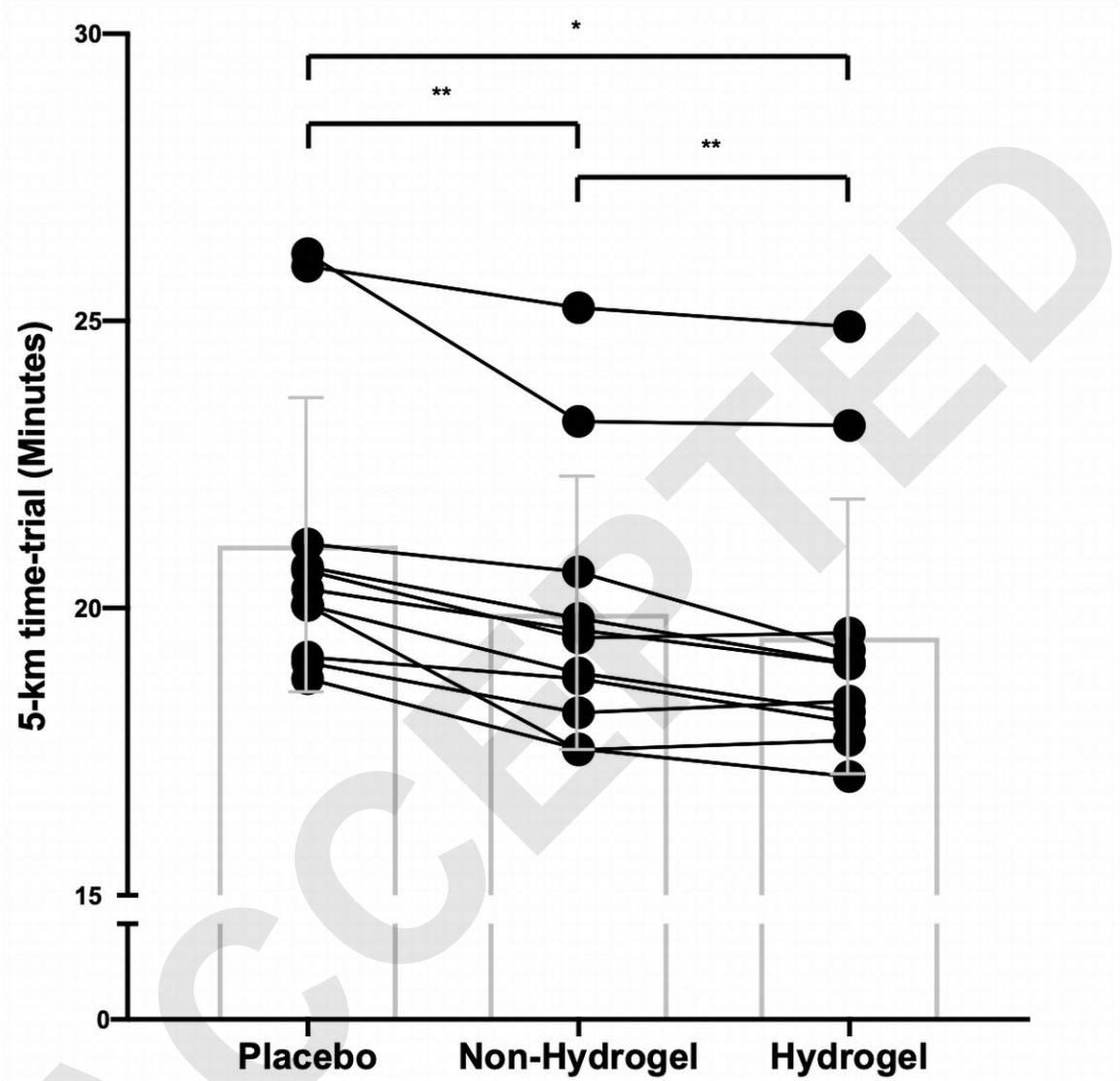

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Figure 2

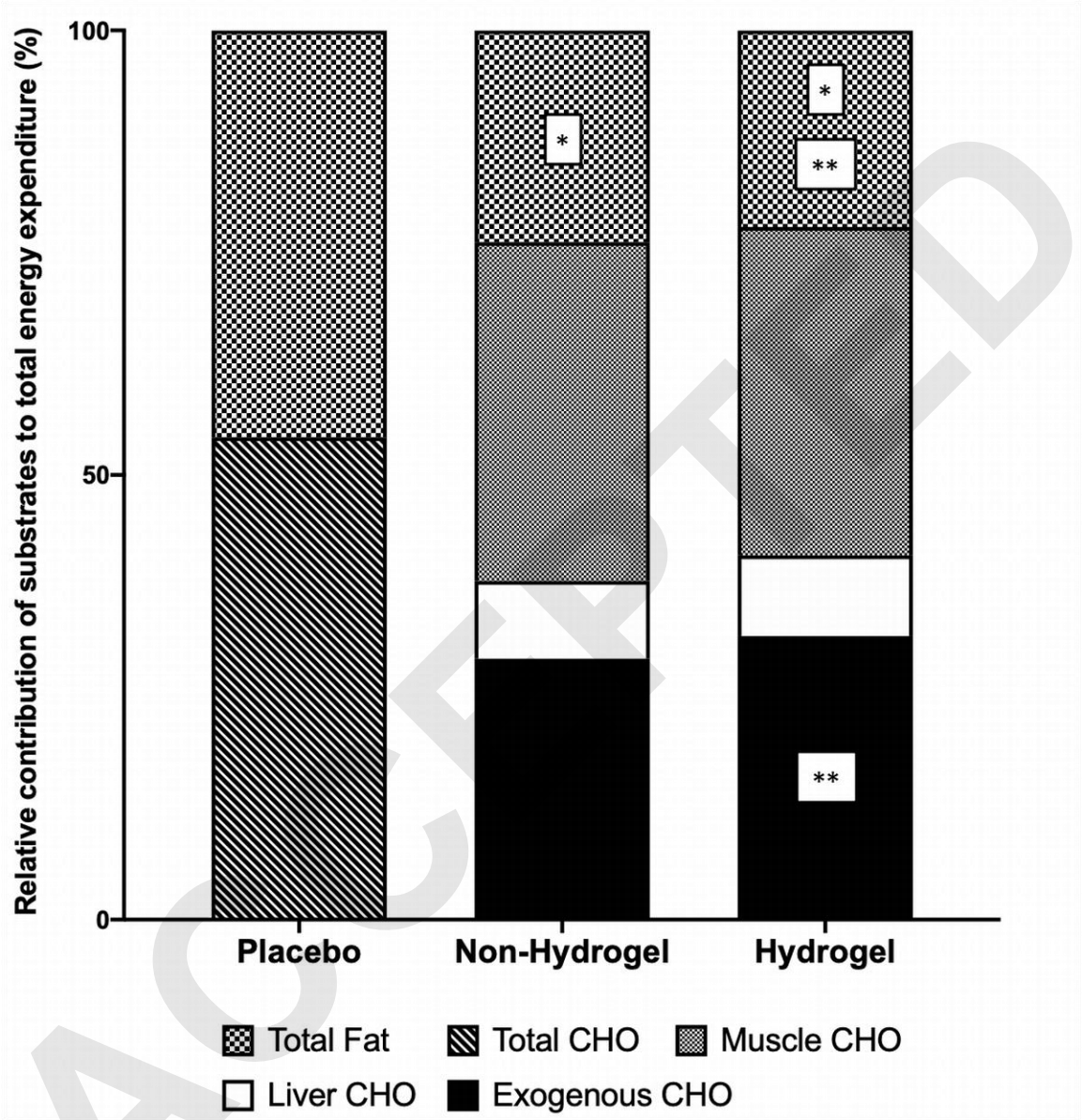

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Figure 3

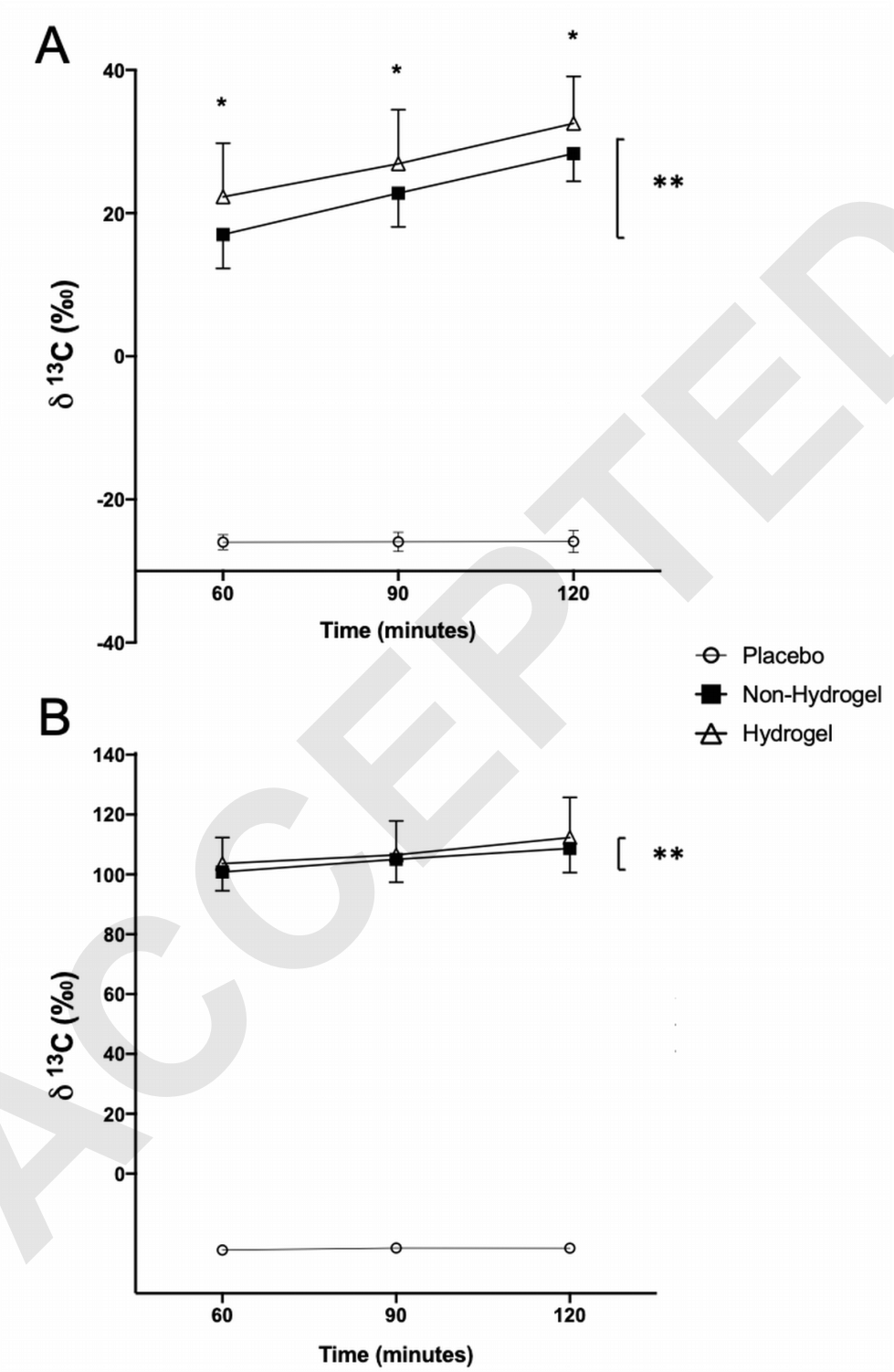

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Figure 4

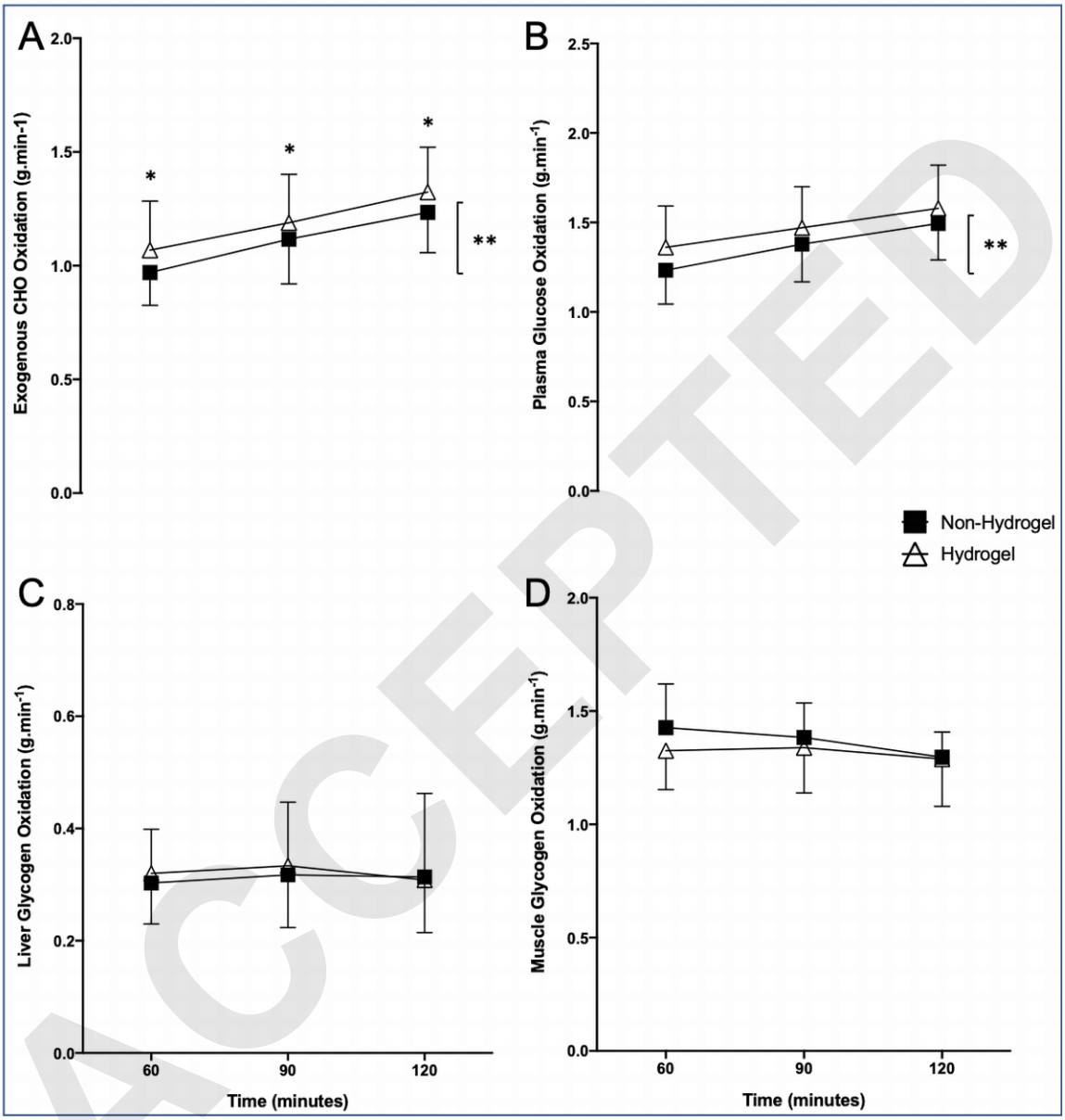

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Figure 5

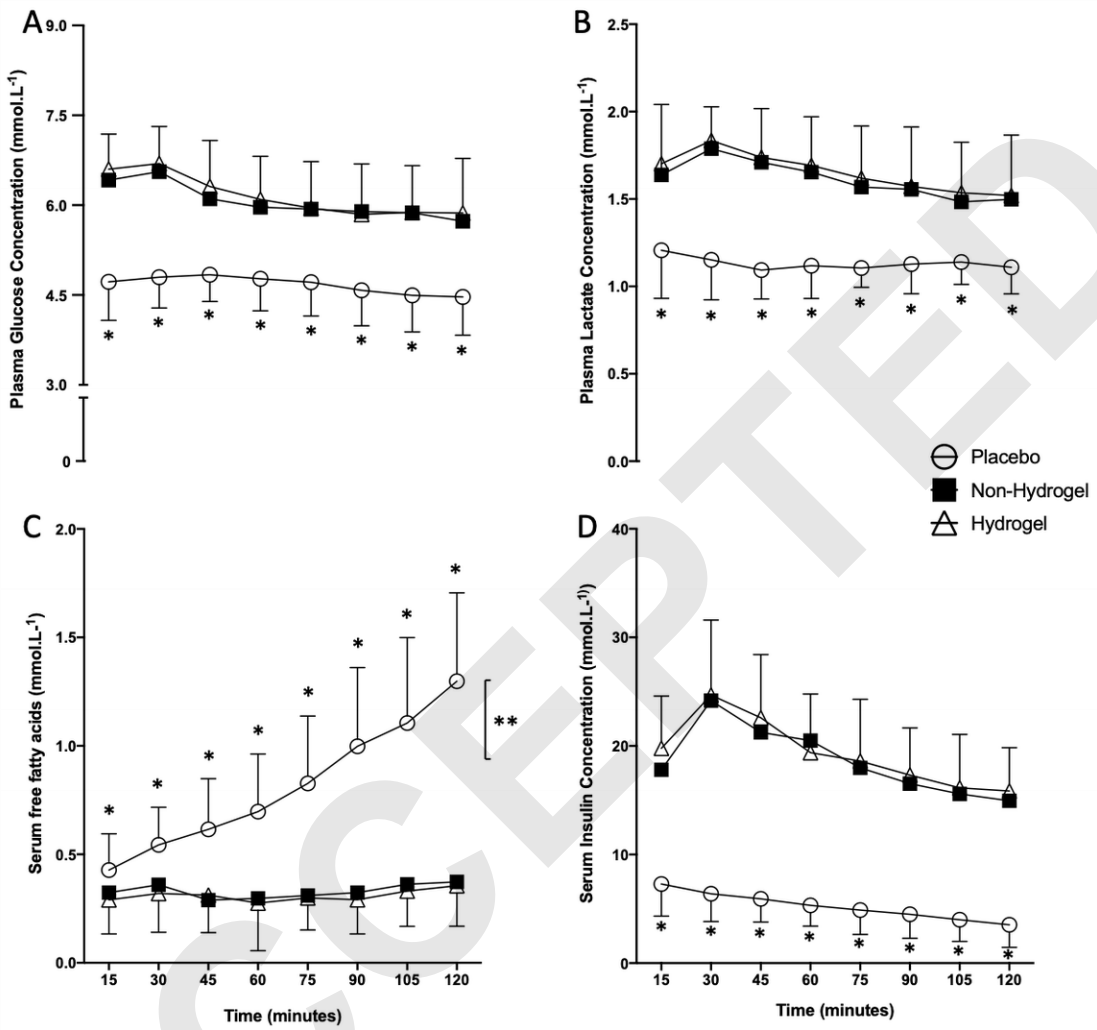

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Table 1. Comparisons of oxygen uptake, carbon dioxide production, total carbohydrate oxidation, total fat oxidation and heart rate over the first and second 60 minutes of the 120 minute steady state run.

		Condition		
	60-min period	Placebo	Non-Hydrogel	Hydrogel
VO_2 ($L \cdot min^{-1}$)	First	3.04 ± 0.31*	2.95 ± 0.26	2.91 ± 0.25
	Second	3.08 ± 0.27*	2.94 ± 0.21	2.93 ± 0.21
VCO_2 ($L \cdot min^{-1}$)	First	2.69 ± 0.24	2.69 ± 0.22	2.67 ± 0.21
	Second	2.64 ± 0.20*	2.73 ± 0.22	2.73 ± 0.18
RER	First	0.89 ± 0.02*	0.91 ± 0.01	0.92 ± 0.01
	Second	0.86 ± 0.02*†	0.93 ± 0.01	0.93 ± 0.01
CHO_{ox} (g)	First	138.8 ± 7.8*	153.9 ± 11.4	156.9 ± 9.5
	Second	122.1 ± 10.8*†	164.7 ± 9.8†	167.3 ± 8.3†
Fat_{ox} (g)	First	34.8 ± 7.7 *	26.1 ± 5.4	23.6 ± 4.8**
	Second	43.0 ± 8.2 *†	21.6 ± 3.3†	19.8 ± 3.8**†
HR ($b \cdot min^{-1}$)	First	152 ± 11	153 ± 12	152 ± 13
	Second	155 ± 12	154 ± 13	154 ± 13

VO_2: Oxygen consumption. VCO_2: Carbon dioxide production. RER: Respiratory Exchange Ratio. CHO_{ox}: CHO oxidation. Fat_{ox}: Fat oxidation. HR: Heart rate. All values are mean ± SD. * significantly different from hydrogel and non-hydrogel. ** significantly different from non-hydrogel. † significantly different to first 60 minute period.

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Table 2. Comparison of carbohydrate oxidation from various sources between non-hydrogel and hydrogel during the final 60 minutes of the 120-minute steady state run

	Non-hydrogel (g)	Hydrogel (g)	*p-value*
Exogenous CHO oxidation	63.4 ± 8.1	68.6 ± 10.8	$p = 0.003$
Endogenous CHO oxidation	101.2 ± 6.5	98.9 ± 9.1	$p = 0.58$
Plasma glucose oxidation	82.3 ± 11.7	88.1 ± 13.1	$p = 0.001$
Glucose oxidation from liver	18.8 ± 4.6	19.5 ± 6.5	$p = 0.83$
Muscle glycogen oxidation	82.4 ± 7.5	79.4 ± 10.8	$p = 0.26$

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Table 3. Comparison of GI symptoms between placebo, non-hydrogel and hydrogel conditions

		Placebo			Non-Hydrogel			Hydrogel		
	Symptoms	Score	Range	%	Score	Range	%	Score	Range	%
Upper-gastrointestinal symptoms	Belching	2 ± 1	1 - 5	9	4 ± 1**	2 - 7	64	3 ± 1**	1 - 6	36
	Stomach Burn	1 ± 1	1 - 5	9	4 ± 1*	1 - 7	46	2 ± 1**	1 - 5	27
	Urge to Vomit	1 ± 0	1 - 4	0	2 ± 1**	1 - 5	36	2 ± 1**	1 - 5	27
	Bloatedness	3 ± 1	1 - 5	9	4 ± 1*	1 - 7	55	3 ± 1	1 - 5	36
	Nausea	3 ± 1	1 - 5	18	3 ± 1*	1 - 6	36	2 ± 1	1 - 5	27
Mean Score		**2 ± 1**			**4 ± 1***			**3 ± 1****		
Lower-gastrointestinal symptoms	Stomach Problems	2 ± 1	1 - 5	9	4 ± 1*	1 - 7	64	3 ± 1**	1 - 5	27
	Flatulence	1 ± 1	1 - 4	0	4 ± 1*	1 - 7	55	2 ± 1**	1 - 5	27
	Urge to Defecate	2 ± 1	1 - 5	9	3 ± 1**	1 - 6	36	2 ± 1	1 - 6	36
	Side Ache (Left)	1 ± 1	1 - 3	0	2 ± 1**	1 - 5	18	1 ± 0	1 - 5	18
	Side Ache (Right)	1 ± 1	1 - 4	0	4 ± 1*	1 - 6	64	1 ± 1	1 - 5	27
	Stomach Cramps	2 ± 1	1 - 4	0	4 ± 1*	1 - 7	64	1 ± 1	1 - 5	27
Mean Score		**1 ± 1**			**4 ± 1***			**2 ± 1****		
Systemic	Dizziness	2 ± 1	1 - 5	9	2 ± 1	1 - 5	34	2 ± 1	1 - 6	27
	Headache	2 ± 1	1 - 4	0	3 ± 1*	1 - 6	27	2 ± 1	1 - 5	18
	Urge to Urinate	3 ± 2	1 - 8	55	3 ± 2	1 - 7	55	3 ± 2	1 - 5	36
Mean Score		**2 ± 1**			**3 ± 1***			**2 ± 1**		
Total Mean Score		**2 ± 1**			**3 ± 1***			**2 ± 1**		

Score, mean ± SD; Range. min minimum and maximum score, %, percentage of participants who reported scores of ≥5. * significantly different from placebo and hydrogel. ** significantly different from placebo.

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