1 2 13C-glucose-fructose labelling reveals comparable exogenous CHO oxidation during 3 exercise when consuming 120 g/h in fluid, gel, jelly chew or co-ingestion 4 5 Mark A Hearris¹, Jamie N Pugh¹, Carl Langan-Evans¹, Stephen J Mann², Louise Burke³, Trent Stellingwerff^{4,5}, Javier T Gonzalez^{6,7}, James P Morton¹ 6 7 8 9 10 ¹Research Institute for Sport and Exercise Sciences 11 Liverpool John Moores University 12 Liverpool, UK 13 14 ²Science in Sport PLC 15 London, UK 16 17 ³Exercise and Nutrition Research Program 18 Mary MacKillop Institute for Health Research 19 Australian Catholic University 20 Melbourne, Australia 21 ⁴Exercise Science, Physical and Health Education 22 23 University of Victoria 24 Victoria, Canada 25 26 ⁵Pacific Institute for Sport Excellence 27 Canadian Sport Institute-Pacific 28 Victoria, Canada 29 30 ⁶Centre for Nutrition, Exercise and Metabolism 31 University of Bath 32 Bath, UK 33 34 ⁷Department for Health, 35 University of Bath, 36 Bath, UK 37 38 Running head: CHO oxidation from fluid, semi-solid and solid 39 40 Address for correspondence: 41 Prof James Morton 42 Research Institute for Sport and Exercise Sciences 43 Liverpool John Moores University 44 Tom Reilly Building 45 Liverpool, L3 3AF 46 United Kingdom 47 Email: J.P.Morton@ljmu.ac.uk 48 Tel: +44 151 904 6233

Abstract

We examined the effects of carbohydrate (CHO) delivery form on exogenous CHO
oxidation, gastrointestinal discomfort, and exercise capacity. In a randomised repeated
measures design (after 24 h of high CHO intake (8 g·kg ⁻¹) and pre-exercise meal (2 g·kg ⁻¹)),
nine trained males ingested 120 g CHO·h ⁻¹ from fluid (DRINK), semi-solid gel (GEL), solid
jelly chew (CHEW), or a co-ingestion approach (MIX). Participants cycled for 180 min at
95% lactate threshold followed by an exercise capacity test (150% lactate threshold). Peak
rates of exogenous CHO oxidation (DRINK, 1.56 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.16 ; GEL, 1.59 ± 0
0.08; MIX, 1.66 \pm 0.02 g·min ⁻¹) and oxidation efficiency (DRINK, 72 \pm 8; GEL, 72 \pm 5;
CHEW, 75 \pm 5; MIX, 75 \pm 6%) were not different between trials (all $P > 0.05$). Despite
ingesting 120 g·h ⁻¹ , participants reported minimal symptoms of gastrointestinal distress
across all trials. Exercise capacity was also not significantly different (all $P < 0.05$) between
conditions (DRINK, 446 ± 350 ; GEL, 529 ± 396 ; CHEW, 596 ± 416 ; MIX, 469 ± 395 sec).
Data represent the first time that rates of exogenous CHO oxidation (via stable isotope
methodology) have been simultaneously assessed using feeding strategies (i.e., pre-exercise
CHO feeding and the different forms and combinations of CHO during exercise) commonly
adopted by elite endurance athletes. We conclude 120 g·h ⁻¹ CHO (in a 1:0.8 ratio of
maltodextrin or glucose:fructose) is a practically tolerable strategy to promote high CHO
availability and oxidation during exercise.

Keywords

72 Stable isotopes, fructose, maltodextrin, metabolism

New & Noteworthy We demonstrate comparable rates of exogenous CHO oxidation from fluid, semi-solid, solid or a combination of sources. Considering the sustained high rates of total and exogenous carbohydrate oxidation, and relative lack of gastrointestinal symptoms, consuming 120 g CHO·h⁻¹ appears a well-tolerated strategy to promote high CHO availability during exercise. Additionally, this is the first time that rates of exogenous CHO oxidation have been assessed using feeding strategies (e.g., co-ingestion of multiple CHO forms) typically reported by endurance athletes.

Introduction

The introduction of the muscle biopsy technique in the late 1960s (1) has allowed robust documentation of the importance of muscle glycogen in determining exercise capacity and performance in endurance events (2). In addition to high endogenous carbohydrate (CHO) availability, consumption of CHO during exercise can also enhance exercise performance (3-5), an effect likely mediated by liver glycogen sparing (6), the maintenance of plasma glucose concentrations and CHO oxidation rates (7) and/or via direct effects on the central nervous system (8). Indeed, the provision of exogenous CHO during exercise can address the finite capacity of muscle glycogen stores, particularly during prolonged strenuous events (> 2.5 h). In such scenarios, current consensus guidelines recommend a CHO intake of 90 g·h⁻¹ (9) while other contemporary reviews recommend CHO intakes rates of 100+ g·h⁻¹, if gastrointestinal (GI) outcomes are individually tolerated (10). However, the latter recommendations are based more on practitioner experience and field data, and have not yet been tested using multiple forms of CHO sources, in ecologically valid conditions.

The oxidation of ingested CHO by skeletal muscle during exercise is thought to be limited by CHO absorption through the intestinal membrane (11). In this regard, it is well established that a mixture of multiple-source CHO blends (e.g. glucose polymers, glucose and fructose etc.) are oxidised at 20-50% higher rates when compared to single source formulations (12-14). Indeed, whereas the exogenous oxidation of single-transportable CHO plateaus at \sim 60 g·h⁻¹, exogenous oxidation of multiple-transportable CHO continues to rise with CHO ingestion up to 144 g·h⁻¹ (15). When ingesting CHO at a rate \geq 90 g·h⁻¹, the ratio at which sources of CHO are co-ingested also influences their subsequent oxidation, whereby a 1:0.8 ratio of maltodextrin to fructose yields higher rates of oxidation when compared with an isocaloric 2:1 ratio (16, 17). It is noteworthy, however, that for an individual to achieve high

125 oxidation efficiency of less than 100% (15). Taken together, these data suggest that to 126 maximise CHO availability and oxidation, athletes should ingest multiple-transportable CHO's, co-ingested in ratios closer to unity (18) and at absolute intakes above 90 g·h⁻¹. 127 128 In practice, athletes typically utilise a variety of CHO forms to meet these targets, including 129 liquids (i.e. sports drinks), semi-solids (i.e. energy gels) and solids (i.e. energy bars) (19). 130 Previous comparisons between liquid, semi-solid and solid carbohydrates have demonstrated these forms are oxidised at similar rates (albeit ingested at 93-108 g·h⁻¹ using a 2:1 ratio of 131 glucose and fructose) during prolonged endurance exercise (20, 21), thus suggesting that 132 133 athletes can tailor their chosen feeding strategy to meet their personalised CHO intake targets. 134 However, given that elite endurance athletes (e.g., Grand Tour cyclists, triathletes) often ingest a mix of such forms during prolonged exercise, at rates of up to at least 120 g·h⁻¹ (19), 135 136 an examination of oxidation rates when multiple forms of CHO are co-ingested at these high 137 ingestion rates is highly warranted. Furthermore, recent food innovations have led to the 138 development of commercially available "jelly chews", providing a "solid" food form with the 139 absence of the protein, fat, and fibre content of energy bars, which are often associated with 140 gastrointestinal complaints during exercise (22). Despite the popular use of jelly chews 141 among athletic populations, it is currently unknown if this delivery form achieves similar 142 peak rates of CHO oxidation to CHO fluids (i.e. sports drinks) and semi-solid forms (i.e. 143 gels). 144 With this in mind, the primary aim of the present study was to quantify rates of exogenous 145 CHO oxidation from the individual ingestion of liquid, gel and jelly chew forms of CHO as 146 well as the combination of the three forms. To this end, trained male cyclists consumed CHO at a rate of 120 g·h⁻¹ (using a 1:0.8 ratio of maltodextrin or glucose to fructose) during three 147

rates of oxidation (e.g. $\geq 1.5 \text{ g} \cdot \text{min}^{-1}$), they should likely ingest 90-120 g·h⁻¹ to account for an

hours of steady-state cycling at 95% of lactate threshold. To assess rates of exogenous CHO oxidation, all forms were uniformly enriched with both ¹³C-glucose and ¹³C-fructose during the manufacturing process, thus representing the first study to incorporate dual stable isotope tracers at high enrichment into both solid and semi-solid CHO sources ingested during exercise. To assess the effects of CHO on exercise capacity, a time to exhaustion test (at 150% of lactate threshold) was also performed after the completion of the 3 h steady-state protocol within each of the trials. We hypothesised that: 1) peak rates of exogenous CHO oxidation would be comparable between all fuelling approaches, 2) consumption of 120 g·h⁻¹ CHO would not cause negative gastrointestinal symptoms and 3) exercise capacity would not differ between the various feeding forms and formats.

Methodology

Ethical approval

All participants gave written informed consent prior to participation after all experimental procedures and potential risks had been fully explained. The study was approved by the Ethics Committee of Liverpool John Moores University and conformed to the standards set by the latest revision of the *Declaration of Helsinki* (except for registration in a database).

Participants

Nine endurance trained, amateur male cyclists (mean \pm SD: age, 25 \pm 8 years; body mass, 75.6 \pm 7.0 kg; height, 179.1 \pm 4.7 cm) volunteered to participate in the study. Mean $\dot{V}O_{2max}$, peak power output (PPO) and power output at lactate threshold were 64.9 \pm 6.8 ml·kg⁻¹·min⁻¹, 438 \pm 79 W and 226 \pm 37 W, respectively. Subjects were defined as either highly-trained (Tier 3) or trained (Tier 2) in accordance with the criteria specified by McKay et al. (23).

Sample size was determined according to our primary outcome variable (i.e. exogenous CHO oxidation) assuming an effect of feeding form (liquid vs. solid) of 0.18 g.min⁻¹ (0.96 ± 0.13 g.min⁻¹ with solid vs 1.14 ± 0.16 g.min⁻¹ with liquid), as reported by Pfeiffer et al. (20) between 60 and 180 minutes of exercise. These data give an effect size of dz = 1.22, where a sample size of 8 would provide an α -value of 0.05 and a power of 0.80 (G*Power, version 3.1.9.6). None of the subjects had any history of musculoskeletal or neurological disease, nor were they under any pharmacological treatments during the testing period.

Experimental overview

In a repeated measures (> 6 days, but < 15 days apart), randomised, cross-over design, with each experimental trial separated by a minimum of 7 days, participants completed a prolonged endurance-based cycling exercise protocol, consisting of 180 min of submaximal exercise (undertaken at 95% of lactate threshold) followed by an exercise capacity test to exhaustion (undertaken at 150% of lactate threshold) on five separate occasions. The initial trial (WATER; where water only was consumed during exercise) was performed to provide a full familiarisation to the exercise protocol and to examine any background shifts in breath 13 CO₂ appearance. In the following four randomised trials, subjects ingested CHO at a rate of 120 g·h⁻¹ from fluid (DRINK), gels (GEL), jelly chews (CHEW) or a combination of all three delivery forms (MIX). Each experimental trial was commenced following 24-h of high CHO intake (8 g·kg⁻¹) and 3 h after the consumption of a CHO rich pre-exercise meal (2 g·kg⁻¹). An overview of the experimental design and nutritional protocols is displayed in Figure 1.

Preliminary testing

At least 7 days prior to experimental trials, subjects performed a two-part incremental cycle test (Lode Excalibur Sport, Groningen, Netherlands) to determine lactate threshold, maximal

oxygen consumption ($\dot{V}O_{2max}$) and PPO as previously described (24). Briefly, the first part of the test was commenced at 100 W and increased by 25 W at the end of each 4-minute stage. A fingertip blood sample was collected during the final 30 seconds of each stage for the determination of blood lactate concentrations (Biosen C-Line; EKF Diagnostics, Cardiff, UK) and the lactate threshold (LT) was determined using the D_{max} method (25). Subjects commenced the second part of the test at an intensity corresponding to that of the penultimate stage completed in the previous part, whereby exercise intensity increased by 25 W every minute until volitional exhaustion. The end time and power output at the point of exhaustion were used to calculate PPO using the following equation (26):

$$PPO = W_{final} + (t/60) * PI)$$

where W_{final} is the power output of the final completed stage, t is the time spent in the final uncompleted stage (seconds), 60 is the duration of each stage (seconds) and PI is the increase in power output between stages. During the test, gas exchange measurements were made using an online gas analysis system (Moxus Modular Metabolic System; AEI Technologies, IL, USA) and $\dot{V}O_{2max}$ was determined as the highest $\dot{V}O_2$ captured over a 30 second period. The same gas analyser was used during all subsequent trials.

Pre-experimental controls

For 2-days prior to all experimental trials, subjects were asked to minimise the consumption of foods with a high natural abundance of ¹³C to minimise the background shift from glycogen stores during exercise. Foods with a high natural abundance of ¹³C (e.g. corn and sugar cane) were also avoided in the standardised diet and pre-exercise breakfast. Twenty-four hours prior to experimental trials, subjects were provided with a pre-packaged high CHO

diet containing precisely 8 g·kg⁻¹ CHO, 2 g·kg⁻¹ protein and 1 g·kg⁻¹ fat to standardise dietary intake between trials. During this period, subjects also refrained from any form of exercise as well as caffeine and alcohol consumption. Subjects were also provided with a pre-packaged high CHO breakfast containing 2 g·kg⁻¹ CHO, ~20 g protein and ~5 g fat, which was consumed 3 hours prior to the commencement of exercise in accordance with contemporary sports nutrition guidelines for endurance exercise (9).

227

228

229

230

231

232

233

234

235

236

237

238

239

240

241

242

243

244

245

221

222

223

224

225

226

180-min steady state cycling

On the morning of the main experimental trials, subjects reported to the laboratory at ~ 10.00 h having consumed the pre-packaged, standardised breakfast provided (see above). Upon arrival, an indwelling cannula (Safety Lock 22G; BD Biosciences, West Sussex, UK) was inserted into the antecubital vein in the anterior crease of the forearm and a resting blood sample drawn and subsequently flushed with ~5 ml of sterile saline (Kays Medical, Liverpool, UK). Resting expired breath samples were collected in duplicate into evacuated 10 mL Exetainer tubes (Labco, High Wycombe, UK), sampled directly from the mixing chamber, to determine the ¹³C/¹²C ratio in CO₂ at rest. Following the collection of resting measures, subjects completed a 10-min warm-up at 100 W and began the 180 min cycling protocol at 95% LT (215 \pm 35 W). This relative exercise intensity was chosen as it has been suggested as an appropriate method of matching metabolic stress between subjects when compared with exercising at a percentage of $\dot{V}O_{2max}$ (27). Heart rate (Polar H10; Polar, Kempele, Finland), ratings of perceived exertion (RPE) (28) and cycling cadence were obtained at 30 min intervals throughout. Expired gas was collected for a 5 min period at 30 min intervals to calculate whole body substrate utilisation. The final minute of this period was used to collect expired gas into the evacuated Exetainer tubes to determine the ¹³C/¹²C ratio in CO₂. Gastrointestinal (GI) symptoms (nausea, regurgitation, fullness, cramps, gas,

and urge to defecate) were recorded at 30-min intervals during exercise using a 0-10 visual analogue scale (0 = no discomfort, and 10 = very severe discomfort) (29). Subjects were instructed that a score > 4 should be regarded as a moderate symptom that was detrimental to their ability to exercise. The sum of scores at each time point were collated for each gastrointestinal symptom, resulting in maximum scores of 60 for each symptom. Immediately following the 180 min submaximal cycle, subjects began the exercise capacity test, where they cycled at 150% LT (339 \pm 55 W) until task failure, defined as an inability to maintain a cadence > 60 rpm. During the capacity test, the only information available to the subjects was the fixed power output and pedal cadence and no performance results were provided to subjects until they had completed all experimental trials. All exercise tests were performed at the same time of day under normal laboratory conditions (20-22°C and 50-60% humidity) using the same electrically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) and automated gas analyser (Moxus Modular Metabolic System; AEI Technologies, IL, USA). During all exercise trials, subjects were cooled with a floor fan to minimise thermal stress. Participants were not provided with any prior information from the researchers that would influence their bias on which form of CHO would be superior for exercise performance.

263

264

265

266

267

268

269

270

262

246

247

248

249

250

251

252

253

254

255

256

257

258

259

260

261

Carbohydrate feeding

During exercise, subjects consumed CHO at a rate of 120 g·h⁻¹ using multiple transportable carbohydrates in a 1:0.8 ratio of maltodextrin (or glucose for CHEW) to fructose. Carbohydrate drinks (Beta Fuel powder, Science in Sport, UK) and gels (Beta Fuel gels, Science in Sport, UK) were made from maltodextrin and fructose whilst the jelly chew (Beta Fuel chew, Science in Sport, UK) was made from glucose and fructose. This ratio was chosen as it has been previously shown to allow for improved rates of oxidation and gastrointestinal

comfort during exercise (16, 17, 30). Furthermore, maltodextrin and glucose can be considered as broadly interchangeable with respect to exogenous carbohydrate oxidation rates during exercise, since the rate of hydrolysis and exogenous oxidation between carbohydrate monomers and polymers are comparable (31, 32). Carbohydrate was ingested immediately prior to exercise and subsequently at 20 min intervals during exercise in equal amounts of 40 g CHO per serving. During production, all forms were equivalently enriched with 100 mg of U-13C-glucose (56 mg) and U-13C-fructose (44 mg; CK isotopes, Ibstock, UK) per 120 g CHO to ensure equivalent proportions in relation to their tracee and match the ratio of maltodextrin (glucose) and fructose in each form (Table 1). The MIX condition provided an equal mixture of all three forms and was consumed in the same order for all subjects (e.g., DRINK, CHEW, GEL) at 20 min intervals. Carbohydrate drinks were mixed with 800 ml water (per 120 g CHO), resulting in a 15% CHO solution and a fluid intake of 800 ml.h⁻¹. During all other trials, subjects consumed an equivalent amount of water (267 ml at each feeding point) to ensure total fluid intake and the pattern of ingestion was matched across all trials.

Blood sampling and analysis

Venous blood samples were collected into vacutainers containing K₂ EDTA, lithium heparin or serum separation tubes (BD Biosciences, UK) and stored on ice or at room temperature until centrifugation at 1500 g for 10 min at 4°C. Following centrifugation, plasma and serum was aliquoted and stored at -80°C for subsequent analysis. Samples were later analysed for plasma glucose, lactate non-esterified fatty acids (NEFA), and glycerol using commercially available enzymatic spectrophotometric assays (RX Daytona+ Analyser; Randox, UK) as per the manufacturer's instructions.

Estimates of whole-body substrate oxidation and energy expenditure

Rates of whole-body CHO and fat oxidation (g·min⁻¹) were calculated at 30 min intervals during the 180 min submaximal cycle using the equations of Jeukendrup and Wallis (33). Total energy expenditure was estimated for each trial assuming an energy yield of 17.57 kJ and 39.33 kJ for 1 g of CHO and fat, respectively. Substrate utilisation data during the MIX trial is missing for one participant due to a technical error and at the 180 min time point during the WATER trial for two participants due to volitational fatigue.

¹³C/¹²C analysis of breath CO₂

Isotopic enrichment of breath samples was determined using gas chromatography isotope ratio mass spectrometry (Iso-analytical, Crewe, UK). The 13 C enrichment from breath samples are expressed as δ^{13} C ‰ versus Pee Dee Belemnite (PDB). Exogenous carbohydrate oxidation was calculated using the following formula

Exogenous carbohydrate oxidation =
$$\dot{V}CO_2 \cdot \left(\frac{\delta Exp - \delta EXP_{bkg}}{\delta Ing - \delta Exp_{bkg}}\right) \left(\frac{1}{k}\right)$$

Where δExp is the ¹³C enrichment of expired CO₂, δIng is the ¹³C enrichment of the ingested carbohydrate, δEXP_{bkg} is the ¹³C enrichment of expired CO₂ during the water trial. and k is the $\dot{V}CO_2$ with the oxidation of 1 g of glucose (0.7467 L CO₂·g⁻¹). For the MIX trial, the ¹³C enrichment of the ingested carbohydrate used the average ¹³C enrichment (55.98 % versus PDB) from the three separate forms being consumed given that they were all equivalently enriched (Table 1).

Statistical analysis

All statistical analyses were performed using SPSS Statistics Version 27 (IBM, US). Differences in mean and peak exogenous CHO oxidation, gastrointestinal symptoms, heart rate, RPE, energy expenditure, and exercise capacity were all analysed using one-way repeated-measures ANOVA. Mauchly's test for sphericity was used and, in cases where this assumption was violated, the Greenhouse-Geisser correction was applied. A two-way repeated measures ANOVA was used to analyse differences over time (e.g., 30-180 min) and between conditions for substrate utilisation and plasma metabolites. Where a significant main effect was observed, pairwise comparisons were analysed using post-hoc LSD tests to locate specific differences. All data in text, figures and tables are presented as means \pm SD with P values \leq 0.05 indicating statistical significance.

Results

Physiological responses

Heart rate and absolute oxygen uptake increased during exercise (time effect, P < 0.001 for both, Table 2) although no differences were apparent between trials (treatment effect, P =0.621, P = 0.155 and P = 0.596, respectively). RPE also increased during exercise (time effect, P < 0.001) and was significantly higher in the WATER trial compared with the GEL trial at 120 minutes (P = 0.018) and compared with all CHO feeding trials from 150 minutes onwards (P < 0.001 for DRINK, GEL and CHEW and P = 0.033 for MIX) (Table 2). Plasma glucose and lactate concentrations were not significantly different between forms of CHO ingestion (treatment effect, P = 0.749 and P = 0.426 respectively). Plasma glycerol and NEFA progressively increased during exercise (time effect, P < 0.001 for both) although concentrations were not significantly different between forms of CHO ingestion (treatment effect, P = 0.735 and P = 0.983, respectively) (Figure 2).

2	Λ	1	
.)	_	_	

345

Substrate utilisation

346 Rates of whole-body CHO oxidation (Figure 3A) progressively decreased during exercise 347 (time effect, P < 0.001), whereby rates of CHO oxidation were significantly lower during the 348 WATER trial when compared with all CHO feeding trials (trial effect, P < 0.001; interaction 349 effect, P < 0.001). Compared to the WATER trial, rates of whole-body CHO oxidation were 350 higher during DRINK (P = 0.024) and MIX (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and higher during GEL (P = 0.041) at 30 min, and 351 = 0.002) and CHEW trials (P = 0.005) from 60 min onwards. Rates of whole-body fat 352 oxidation (Figure 3C) progressively increased during exercise (time effect, P < 0.001), 353 whereby rates of fat oxidation were significantly higher during the WATER trial when 354 compared with all CHO feeding trials (trial effect, P < 0.001; interaction effect, P < 0.001). 355 Compared to the WATER trial, rates of whole-body fat oxidation were lower during both 356 DRINK (P = 0.035) and CHEW trials (P = 0.023) at 30 min, and lower during GEL (P < 0.025) 357 0.001) and MIX trials (P = 0.009) from 60 min onwards. The contribution of both CHO and 358 lipid towards energy expenditure throughout exercise is also presented in Figure 4.

359

360

Exogenous and endogenous CHO oxidation

- 361 There were no significant differences in mean exogenous CHO oxidation during hour 2
- 362 (DRINK, 1.40 ± 0.17 ; GEL, 1.36 ± 0.14 ; CHEW, 1.44 ± 0.11 ; MIX, 1.44 ± 0.13 g·min⁻¹;
- 363 treatment effect, P = 0.138) or hour 3 (DRINK, 1.50 ± 0.17 ; GEL, 1.52 ± 0.10 ; CHEW, 1.55
- 364 \pm 0.08; MIX, 1.6 \pm 0.13 g·min⁻¹; treatment effect, P = 0.092) between trials (Figure 5B).
- There was also no significant difference in oxidation efficiency between trials (DRINK, $72 \pm$
- 366 8; GEL, 72 ± 5 ; CHEW, 75 ± 4.6 ; MIX, $75 \pm 6\%$; treatment effect, P = 0.179) (Figure 5D).
- Furthermore, no significant differences in peak rates of exogenous CHO oxidation were
- 368 apparent between trials (DRINK, 1.56 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, 1.59 ± 0.08 ; MIX,

 $1.66 \pm 0.02 \text{ g} \cdot \text{min}^{-1}$; treatment effect, P = 0.189) (Figure 5C). We also highlight individual variability in the oxidation of each form across all participants in Figure 5C.

371

372 The contribution of exogenous CHO oxidation towards total energy expenditure was also 373 comparable between feeding forms during both hour 2 (DRINK, 41 ± 5; GEL, 41 ± 5; CHEW, 43 ± 7 ; MIX, 42 ± 7 %, P = 0.143) and hour 3 (DRINK, 44 ± 5 ; GEL, 46 ± 5 ; 374 375 CHEW, 47 ± 7 ; Mix, 48 ± 7 %, P = 0.329). The contribution of endogenous CHO oxidation 376 towards total energy expenditure was significantly higher in the WATER trial compared with 377 all CHO feeding forms during both hour 2 (WATER; $55 \pm 6\%$; DRINK, 28 ± 5 , P < 0.001; 378 GEL, 32 ± 8 , P = 0.002; CHEW, 33 ± 9 , P = 0.009; MIX, $31 \pm 8\%$, P = 0.001) and hour 3 379 (WATER; $40 \pm 6\%$; DRINK, 17 ± 6 ; GEL, 17 ± 5 ; CHEW, 17 ± 4 ; MIX, $15 \pm 5\%$, P < 0.001380 for all). The contribution of fat towards total energy expenditure was also significant higher 381 in the WATER trial compared with all feeding forms during both hour 2 (WATER; $45 \pm 6\%$; 382 DRINK, 31 ± 5 , P = 0.001; GEL, 27 ± 9 , P < 0.001; CHEW, 25 ± 7 , P < 0.001; MIX, 27 ± 10

385

386

387

388

389

390

391

392

393

383

384

Gastrointestinal discomfort

8; MIX, $38 \pm 6\%$, P < 0.001 for all) (Figure 5E and 3F).

Mean cumulative scores for each gastrointestinal symptom were negligible (< 2 out of a possible 60 for all) with no significant differences between conditions (treatment effect for nausea; P = 0.437, regurgitation; P = 0.580, fullness; P = 0.827. cramps; P = 0.422, gas; P = 0.757 and urge to defecate; P = 0.580). Similarly, peak scores for each symptom were low, with no participants reporting any symptoms > 3 in any trial and no significant differences between conditions (treatment effect for nausea; P = 0.827, regurgitation; P = 0.364, fullness; P = 0.187. cramps; P = 580, gas; P = 0.804 and urge to defecate; P = 0.422).

5%, P < 0.001) and hour 3 (WATER; $60 \pm 6\%$; DRINK, 39 ± 9 ; GEL, 37 ± 8 ; CHEW, $36 \pm 6\%$

Exercise capacity

CHO feeding at a rate of 120 g·h⁻¹ significantly improved exercise capacity (trial effect; P = 0.021) within all trials (DRINK, 446 ± 350 s, P = 0.004; GEL, 529 ± 396 s, P = 0.005; CHEW, 596 ± 416 s, P = 0.028; MIX, 470 ± 395 s, P = 0.035; Figure 6A) when compared with WATER only (231 ± 244 s). However, there were no significant differences in exercise capacity between the different feeding forms (P > 0.05 for all). When data were analysed for a trial order effect, there was a significant difference between conditions (trial effect, P = 0.044) which was explained by significant differences between Trial 1 (i.e. the WATER only familiarisation trial; 231 ± 244 sec) and all other trials (Trial 2, 475 ± 357, P < 0.001; Trial 3, 499 ± 400, P = 0.016; Trial 4, 458 ± 369, P = 0.034; Trial 5, 609 ± 429 sec, P = 0.024; Figure 6B) with no other pairwise differences apparent.

Discussion

Confirming our hypothesis, data demonstrate comparable rates of peak exogenous CHO oxidation when trained male cyclists consume 120 g·h⁻¹ in the form of a liquid (DRINK), semi-solid (GEL), solid (CHEW) or a co-ingestion approach (MIX). Importantly, we observed some of the highest peak rates of exogenous CHO oxidation (e.g. 1.66 g·min⁻¹ during the co-ingestion approach) reported in the literature, the result of which was not associated with any significant symptoms of gastrointestinal discomfort. When taken together, our data suggest that the consumption of 120 g·h⁻¹ CHO with a mixture of carbohydrate sources in a ratio close to unity, is a practically feasible and well-tolerated

protocol to achieve high CHO availability and oxidation during prolonged endurance exercise.

420

421

422

423

424

425

426

427

428

429

430

431

432

433

434

435

436

437

438

439

440

441

442

419

418

To address our aims, we studied a cohort of trained male cyclists who completed an exercise protocol previously studied in our laboratory (i.e. 3 h of steady-state cycling at 95% of lactate threshold followed by an exercise capacity test at 150% of lactate threshold) (24). Importantly, this exercise protocol was commenced after participants had consumed a high CHO diet (8 g·kg⁻¹ CHO for the previous 24 h) and CHO rich pre-exercise meal (2 g·kg⁻¹ CHO), nutritional strategies which are considered best practice in preparation for competitive endurance events or prolonged training (9). To quantify exogenous rates of CHO oxidation, we equivalently enriched all feeding forms with ¹³C-glucose and ¹³C-fructose during product manufacture to facilitate accurate estimates of exogenous oxidation rates. To our knowledge, this approach represents the first time that stable isotope tracers have been simultaneously incorporated into the fluid (DRINK), semi-solid (GEL) and solid (CHEW) CHO delivery forms that are typically ingested by endurance athletes during exercise. Furthermore, the incorporation of stable isotope tracers into the jelly chews, not only provides the first demonstration of the oxidation of this novel food form, but also circumvents previous technical difficulties associated with the assessment of other solid CHO sources (i.e., food bars). Indeed, such bars naturally consist of several different CHOs and other ingredients with different natural enrichments in ¹³C, thereby making the calculation of exogenous CHO oxidation challenging. Principles of stable isotope methods assume that the tracer represents the tracee of interest (i.e. ¹³C-glucose to trace ¹²C-glucose). While we employed ¹³C-glucose to trace maltodextrin, the hydrolysis of maltodextrin is rapid, and not rate limiting to absorption. Thus, maltodextrin displays equivalent digestion, absorption and exogenous oxidation kinetics to glucose (34), and enrichment with ¹³C-glucose thereby provides an appropriate method to study the oxidation of maltodextrin. This approach has been studied previously both in our laboratory (35) and elsewhere (36).

Another principle of the methodology of measurement of exogenous CHO oxidation relates to the appropriate background expired $^{13}\text{CO}_2$ enrichment. When ingesting CHO at natural abundance of ^{13}C , the optimal background is to perform a trial with identical CHO ingestion rates with an even low enrichment of ^{13}C (37). This increases the sensitivity to detect breath enrichment with naturally enriched carbohydrates, as the breath ^{13}C enrichment might still be as low as -23 $\delta^{13}\text{C}$ % versus PDB in the higher enriched trials (38). However, this issue becomes negligible when spiking the ingested CHO with sufficient amounts of >99% enriched ^{13}C -carbohydrates, as fluctuations in the background expired $^{13}\text{CO}_2$ enrichment are too small to influence the calculations of exogenous CHO oxidation in the presence of highly enriched ingested CHO. In the present study, breath enrichment reached in excess of $^{+10}$ $\delta^{13}\text{C}$ % versus PDB (Figure 5A), thereby illustrating the substantial enrichment achieved by spiking the ingested CHO with stable isotopes.

When considering prolonged endurance exercise >2.5 h in duration, contemporary nutrition guidelines recommend the intake of multiple-transportable CHO's at a rate of up to 90 g·h⁻¹ (9, 39, 40). However, where the aim is to achieve high rates of exogenous CHO oxidation (e.g. 1.5 g·min^{-1}), individuals should likely consume $100\text{-}120 \text{ g·h}^{-1}$ since oxidation efficiency is not uniform (13, 14). Indeed, the upper limits of reported CHO intakes during competition range from $107 \text{ to } 137 \text{ g·h}^{-1}$ (19). It has previously been reported that blends of maltodextrin and fructose (formulated in drink form at a ratio of 1:0.8) induce greater oxidation efficiency (74 \pm 7%), peak rates of exogenous CHO oxidation (~1.2 g·min⁻¹), and endurance performance when compared with 2:1 formulations (62 \pm 12% and ~1.0 g·min⁻¹,

respectively) ingested at a rate of 90 g·h⁻¹(17). In keeping with these data, our chosen formulation of maltodextrin and fructose in a 1:0.8 ratio also induced a similar oxidation efficiency, the values of which were comparable between forms (DRINK; 72, GEL; 72, CHEW; 75, MIX; 75%). However, in accordance with the higher CHO ingestion rate studied here (120 g·h⁻¹), we observed higher rates of peak exogenous CHO oxidation during our DRINK trial (i.e. 1.56 g·min⁻¹) than has been previously reported (~1.2 g·h⁻¹) when CHO was ingested at 90 g·h⁻¹ (17). Although previous researchers also reported peak rates of exogenous CHO oxidation of 1.53 g·min⁻¹ (range: 1.23-1.77 g·min⁻¹) when consuming 108 g per hour (of a beverage formulated in a 2:1 ratio) (41), examination of individual oxidation rates reported in the present study (see Figure 5C; range 1.25 -1.87 g·min⁻¹) suggest that higher rates of oxidation may be achieved with the strategy adopted here (i.e. 120 g per hour of a 1:0.8 ratio). When taken together, such data further support the suggestion that athletes should ingest multiple-transportable CHO's, co-ingested in ratios closer to unity (18), and at absolute intakes exceeding 90 g·h⁻¹ in order to optimise CHO availability and oxidation.

Based on previous glucose infusion studies which bypass the limitations of the GI system and directly infuse glucose into the circulation, it is assumed that the maximal rate of exogenous CHO oxidation that working skeletal muscle can use is $\sim 1.8~\rm g\cdot min^{-1}$ (42), Notwithstanding potential effects of hyperinsulinemia and assuming an estimated oxidation efficiency of ~ 70 -75%, the ingestion of 140-150 g·h⁻¹ may be considered as the maximal worthwhile dose to achieve such high rates of oxidation. In support of this, the ingestion of 144 g·h⁻¹ (in a 1:1 ratio of glucose to fructose) has been previously shown to elicit peak exogenous oxidation rates of 1.75 g·min⁻¹ which remain the highest reported rates of oxidation within the literature (14). Nonetheless, given that the individual responses in the present study demonstrate peak oxidation rates of 1.8 g·min⁻¹ can be achieved with the ingestion of 120 g·h⁻¹ (Figure 5C) it

may be possible to achieve maximal rates of oxidation from lower dose of CHO amongst individuals with high oxidation efficiency. We also highlight the inter-individual variability in peak oxidation rates (Figure 5C) and suggest that individual responses should be considered when providing CHO intake recommendations to avoid the potential for large amounts of ingested CHO to remain within the intestine.

498

499

500

501

502

503

504

505

506

507

508

509

510

511

512

513

514

497

493

494

495

496

To our knowledge, there are only two previous reports that have compared exogenous rates of CHO oxidation from the ingestion of a drink, gel, or energy bar (20, 21). These studies reported comparable rates of peak exogenous CHO oxidation from a drink (1.42 g·min⁻¹) and gel form (1.44 g·min⁻¹) when CHO was ingested at a rate of 108 g·h⁻¹ in a 2:1 ratio (21). In contrast, lower rates (albeit not statistically significant) of peak exogenous CHO oxidation were reported when CHO was ingested in the form of a bar (1.25 g·min⁻¹) versus a drink (1.34 g·min⁻¹), when fed at a rate of 93 g·h⁻¹ and delivered in a 2:1 ratio (20). The present study provides novel data and an extension to our understanding by investigating CHO oxidation rates from an alternative solid form (i.e. jelly chews) that is typically used by athletes. To this end, we observed comparable rates of peak exogenous CHO oxidation between trials (DRINK; 1.56, GEL; 1.58, CHEW; 1.59, MIX; 1.66 g·min⁻¹), noting that these values are some of the highest reported in the current literature and provided almost half of the energy requirements of exercise just below lactate threshold. Furthermore, the inclusion of a co-ingestion trial (i.e. MIX) is a highly novel aspect of the present study and mimics the self-chosen protocols of fuelling observed in the real-life practices of endurance and ultraendurance athletes (19, 43).

515

516

517

Despite the consumption of 120 g·h⁻¹ CHO during exercise, it is noteworthy that the present participants reported trivial symptoms of gastrointestinal discomfort across all feeding forms.

These data are consistent with previous reports that demonstrate the ingestion of multiple source carbohydrates (as opposed to single source solutions) at a rate of 144 g·h⁻¹ is generally well tolerated during steady-state cycling (13, 14). However, in contrast to such studies who report "individual" cases of severe discomfort (e.g., stomach cramping and bloating), no gastrointestinal discomfort was reported by any of the present participants across individual trials. Although previous studies support the notion that gastrointestinal symptoms do not differ between liquid (e.g., drinks) and semi-solid (e.g., gels) form of CHO intake, greater feelings of nausea, stomach fullness and abdominal cramps have been previously reported with the ingestion of solid CHO sources such as energy bars (20, 22). Given that these symptoms are typically associated with the presence of other nutrients (such as fat, protein, and fibre), the lack of reported symptoms in solid form studied here (i.e. CHEW) could be attributed to relative absence of such nutrients and a ratio of glucose (polymers) to fructose that increases the oxidation efficiency of the ingested CHO. Importantly, we also observed minimal gastrointestinal symptoms during our MIX trial that combined all three CHO forms. These findings have important implications given that this pattern of ingestion reflects the real-world fuelling practices of elite endurance athletes, who typically ingest a mix of CHO forms across exercise durations of 3-6 hours (19). Although the exercise intensity in the present study was deliberately chosen to reflect the sub-maximal intensity of "riding in the bunch" during professional road races (i.e. below lactate threshold), it would also be beneficial to include frequent high-intensity efforts in future studies so as to assess the effects of high CHO ingestion on potential GI symptoms when exercising at intensities above lactate threshold.

540

541

542

518

519

520

521

522

523

524

525

526

527

528

529

530

531

532

533

534

535

536

537

538

539

To address the effects of CHO form on exercise capacity, participants completed an exercise capacity test undertaken at 150% of lactate threshold immediately after the completion of the

3 h steady-state sub-maximal exercise protocol. This protocol has been previously studied in our laboratory (also using trained male cyclists) where we observed a dose response effect of CHO feeding, in that the consumption of 90 g·h⁻¹ extended exercise capacity when compared with both 45 g·h⁻¹ and 0 g·h⁻¹ (24). This ergogenic effect was likely mediated by increased liver glycogen and/or plasma glucose availability associated wth the higher dose of CHO ingestion. Interestingly, we also observed that the consumption of 90 g·h⁻¹ delayed the point at which lipid oxidation comprises the largest proportion of energy production by ~10 and ~40 minutes when compared with 45 g·h⁻¹ and 0 g·h⁻¹, respectively (24). In consuming the higher absolute dose of 120 g·h⁻¹ in the present study, it is noteworthy that CHO remained the predominant source of substrate utilisation throughout exercise in all trials (Figure 4) and no statistical differences in exercise capacity were observed between feeding forms (Figure 6). These data are unsurprising given the similar rates of whole body and exogenous CHO oxidation and lack of gastrointestinal distress observed between feeding forms, two potential mechanisms by which feeding form may impact upon performance (22). Indeed, Guillochon and Rowlands (22) recently reported reductions in peak power that was associated with the increased gastrointestinal distress arising from repeated intake of solid CHO (e.g. bar). We readily acknowledge, however, that a potential lack of statistical power may have limited our ability to detect small differences in performance between trials given that the study was primarily powered to detect changes in exogenous CHO oxidation and the high interindividual variability in exercise capacity. Although the participants were given no prior information to influence their beliefs on what form of CHO may be superior for performance, we also acknowledge that our study design was not a true double blind design (i.e. participants and researchers were consciously aware of what form of CHO they were consuming). Furthermore, the inclusion of a known water only trial (that served as our familiarisation trial) does not allow us to assess the effects of CHO versus a true placebo trial.

543

544

545

546

547

548

549

550

551

552

553

554

555

556

557

558

559

560

561

562

563

564

565

566

Despite the high rates of peak exogenous CHO oxidation observed here, further studies are therefore required to assess the dose response effects on exercise performane and capacity when ingesting the feeding forms observed here. Indeed, it was recently demonstrated (albeit using 2:1 drink formulations) that consumption of 90 g·h⁻¹ induces higher peak power during a 30-minute time trial (completed after 3 hours of steady state exercise at 60% VO_{2peak}) when compared with both 80 g·h⁻¹ (3.7%) and 100 g·h⁻¹ (7.5%) (44).

In summary, the present data demonstrate comparable rates of exogenous CHO oxidation when CHO is ingested during exercise in a liquid, semi-solid or solid form, as well as a feeding strategy that combined all forms. When considering the high absolute rates of exogenous CHO oxidation, the maintainence of whole-body CHO oxidation, and the lack of gastrointestinal symptoms, data demonstrate that consumption of 120 g·h⁻¹ (as achieved via 1:0.8 formulations of maltodextrin or glucose: fructose) is a practically feasible and well-tolerated strategy to promote high CHO availability during exercise. Indeed, the present data represent some of the highest rates of exogenous CHO oxidation reported in the literature and were achieved via feeding forms and formats that are commonly adopted by elite endurance athletes.

	1	••	
Fu	nc	lın	g

This study was funded by a research grant from Science in Sport PLC.

587

588

589

590

591

592

593

594

595

596

597

598

599

600

601

602

603

604

585

Disclosures

JPM is a consultant for Science in Sport (SiS). SiS, Glaxosmithkline (GSK) and Lucozade Ribena Suntory (LRS) have previously funded JPM's research on glycogen metabolism and exercise. JTG is an investigator on research grants funded by BBSRC, MRC, British Heart Foundation, The Rank Prize Funds, The European Society for Clinical Nutrition and Metabolism (ESPEN), Lucozade Ribena Suntory, ARLA Foods Ingredients and Kenniscentrum Suiker and Voeding; and has completed paid consultancy for PepsiCo and SVGC. LMB is a consultant for Science in Sport, for which she does not receive remuneration. Her previous studies related to CHO and sports performance have been funded by the Australian Institute of Sport, Australian Catholic University and the Alliance for Potato Research and Education, and she has received honoraria for conference presentations by Gatorade Sport Science Institute (GSSI, Pepsico). TS is a consultant for Science in Sport (SiS), and has done previous consultancy work for Gatorade Sport Science Institute (GSSI, Pepsico) and was formerly employed by Nestec / PowerBar. TS's research is currently funded by Own The Podium i4G grants, 94Forward, B2ten, MITACS and the Wu Tsai Performance Alliance. JNP is a consultant for Aliment Nutrition. Aliment Nutrition have previously funded JNP's research on probiotic supplementation and substrate metabolism.

605

606

607

Author contributions

- 608 MAH, JNP, CLE, SJM, JTG and JPM designed the study. MAH performed experiments;
- MAH, JNP, JTG, and JPM analysed the data and interpreted the results. MAH, JNP, JTG and

- 610 JPM drafted the manuscript and CLE, SJM, TS and LB edited and revised the manuscript. All
- authors approved the final version and agree to be accountable for all aspects of the work in
- ensuring that questions related to the accuracy or integrity of any part of the work are
- appropriately investigated and resolved. All persons designated as authors qualify for
- authorship, and all those who qualify for authorship are listed.

615

616 References

- 617 1. Bergstrom J, Hermansen L, Hultman E, and Saltin B. Diet, muscle glycogen and
- 618 physical performance. *Acta Physiol Scand* 71: 140-150, 1967.
- 619 2. Hawley JA, Schabort EJ, Noakes TD, and Dennis SC. Carbohydrate-loading and
- 620 exercise performance. An update. *Sports Med* 24: 73-81, 1997.
- 621 3. **Currell K, and Jeukendrup AE**. Superior endurance performance with ingestion of
- multiple transportable carbohydrates. *Med Sci Sports Exerc* 40: 275-281, 2008.
- 623 4. **Rowlands DS, Swift M, Ros M, and Green JG**. Composite versus single transportable
- 624 carbohydrate solution enhances race and laboratory cycling performance. *Appl Physiol Nutr*
- 625 *Metab* 37: 425-436, 2012.
- 5. Triplett D, Doyle JA, Rupp JC, and Benardot D. An isocaloric glucose-fructose
- beverage's effect on simulated 100-km cycling performance compared with a glucose-only
- 628 beverage. *Int J Sport Nutr Exerc Metab* 20: 122-131, 2010.
- 629 6. Gonzalez JT, Fuchs CJ, Smith FE, Thelwall PE, Taylor R, Stevenson EJ, Trenell MI,
- 630 **Cermak NM, and van Loon LJ**. Ingestion of glucose or sucrose prevents liver but not muscle
- 631 glycogen depletion during prolonged endurance-type exercise in trained cyclists. Am J
- 632 Physiol Endocrinol Metab 309: E1032-1039, 2015.
- 633 7. Coyle EF, Coggan AR, Hemmert MK, and Ivy JL. Muscle glycogen utilization during
- 634 prolonged strenuous exercise when fed carbohydrate. J Appl Physiol (1985) 61: 165-172,
- 635 1986.
- 636 8. **Chambers ES, Bridge MW, and Jones DA**. Carbohydrate sensing in the human
- 637 mouth: effects on exercise performance and brain activity. *J Physiol* 587: 1779-1794, 2009.
- 638 9. Thomas DT, Erdman KA, and Burke LM. American College of Sports Medicine Joint
- Position Statement. Nutrition and Athletic Performance. *Med Sci Sports Exerc* 48: 543-568,
- 640 2016.
- 641 10. Stellingwerff T, and Cox GR. Systematic review: Carbohydrate supplementation on
- 642 exercise performance or capacity of varying durations. Appl Physiol Nutr Metab 39: 998-
- 643 1011, 2014.
- 644 11. Jeukendrup AE, and Jentjens R. Oxidation of carbohydrate feedings during
- prolonged exercise: current thoughts, guidelines and directions for future research. Sports
- 646 *Med* 29: 407-424, 2000.
- 647 12. Hulston CJ, Wallis GA, and Jeukendrup AE. Exogenous CHO oxidation with glucose
- 648 plus fructose intake during exercise. *Med Sci Sports Exerc* 41: 357-363, 2009.
- 649 13. Jentjens RL, Achten J, and Jeukendrup AE. High oxidation rates from combined
- 650 carbohydrates ingested during exercise. *Med Sci Sports Exerc* 36: 1551-1558, 2004.

- 651 14. Jentjens RL, and Jeukendrup AE. High rates of exogenous carbohydrate oxidation
- from a mixture of glucose and fructose ingested during prolonged cycling exercise. Br J Nutr
- 653 93: 485-492, 2005.
- 654 15. Gonzalez JT, Fuchs CJ, Betts JA, and van Loon LJ. Glucose Plus Fructose Ingestion for
- 655 Post-Exercise Recovery-Greater than the Sum of Its Parts? Nutrients 9: 2017.
- 656 16. **O'Brien WJ, and Rowlands DS**. Fructose-maltodextrin ratio in a carbohydrate-
- 657 electrolyte solution differentially affects exogenous carbohydrate oxidation rate, gut
- comfort, and performance. *Am J Physiol Gastrointest Liver Physiol* 300: G181-189, 2011.
- 659 17. **O'Brien WJ, Stannard SR, Clarke JA, and Rowlands DS**. Fructose-maltodextrin ratio
- governs exogenous and other CHO oxidation and performance. *Med Sci Sports Exerc* 45:
- 661 1814-1824, 2013.
- 662 18. Rowlands DS, Houltham S, Musa-Veloso K, Brown F, Paulionis L, and Bailey D.
- Fructose-Glucose Composite Carbohydrates and Endurance Performance: Critical Review
- and Future Perspectives. *Sports Med* 45: 1561-1576, 2015.
- 665 19. Pfeiffer B, Stellingwerff T, Hodgson AB, Randell R, Pottgen K, Res P, and
- 666 **Jeukendrup AE**. Nutritional intake and gastrointestinal problems during competitive
- endurance events. *Med Sci Sports Exerc* 44: 344-351, 2012.
- 668 20. **Pfeiffer B, Stellingwerff T, Zaltas E, and Jeukendrup AE**. Oxidation of solid versus
- liquid CHO sources during exercise. *Med Sci Sports Exerc* 42: 2030-2037, 2010.
- 670 21. Pfeiffer B, Stellingwerff T, Zaltas E, and Jeukendrup AE. CHO oxidation from a CHO
- 671 gel compared with a drink during exercise. Med Sci Sports Exerc 42: 2038-2045, 2010.
- 672 22. **Guillochon M, and Rowlands DS**. Solid, Gel, and Liquid Carbohydrate Format Effects
- on Gut Comfort and Performance. *Int J Sport Nutr Exerc Metab* 27: 247-254, 2017.
- 674 23. McKay AKA, Stellingwerff T, Smith ES, Martin DT, Mujika I, Goosey-Tolfrey VL,
- 675 Sheppard J, and Burke LM. Defining Training and Performance Caliber: A Participant
- 676 Classification Framework. Int J Sports Physiol Perform 1-15, 2022.
- 677 24. Fell JM, Hearris MA, Ellis DG, Moran JEP, Jevons EFP, Owens DJ, Strauss JA, Cocks
- 678 **M, Louis JB, Shepherd SO, and Morton JP**. Carbohydrate improves exercise capacity but
- does not affect subcellular lipid droplet morphology, AMPK and p53 signalling in human
- 680 skeletal muscle. *J Physiol* 599: 2823-2849, 2021.
- 681 25. Cheng B, Kuipers H, Snyder AC, Keizer HA, Jeukendrup A, and Hesselink M. A new
- approach for the determination of ventilatory and lactate thresholds. *Int J Sports Med* 13:
- 683 518-522, 1992.
- 684 26. Kuipers H, Verstappen FT, Keizer HA, Geurten P, and van Kranenburg G. Variability
- of aerobic performance in the laboratory and its physiologic correlates. *Int J Sports Med* 6:
- 686 197-201, 1985.
- 687 27. **Baldwin J, Snow RJ, and Febbraio MA**. Effect of training status and relative exercise
- 688 intensity on physiological responses in men. Med Sci Sports Exerc 32: 1648-1654, 2000.
- 689 28. Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 14: 377-
- 690 381, 1982.
- 691 29. Wilson PB. Frequency of Chronic Gastrointestinal Distress in Runners: Validity and
- Reliability of a Retrospective Questionnaire. *Int J Sport Nutr Exerc Metab* 27: 370-376, 2017.
- 693 30. Rowlands DS, Thorburn MS, Thorp RM, Broadbent S, and Shi X. Effect of graded
- 694 fructose coingestion with maltodextrin on exogenous 14C-fructose and 13C-glucose
- oxidation efficiency and high-intensity cycling performance. J Appl Physiol (1985) 104: 1709-
- 696 1719, 2008.

- 697 31. **Gray GM, and Ingelfinger FJ**. Intestinal absorption of sucrose in man: interrelation of
- 698 hydrolysis and monosaccharide product absorption. *J Clin Invest* 45: 388-398, 1966.
- 699 32. Moodley D, Noakes TD, Bosch AN, Hawley JA, Schall R, and Dennis SC. Oxidation of
- 700 exogenous carbohydrate during prolonged exercise: the effects of the carbohydrate type
- and its concentration. Eur J Appl Physiol Occup Physiol 64: 328-334, 1992.
- 702 33. **Jeukendrup AE, and Wallis GA**. Measurement of substrate oxidation during exercise
- by means of gas exchange measurements. Int J Sports Med 26 Suppl 1: S28-37, 2005.
- 704 34. **Rehrer NJ, van Kemenade M, Meester W, Brouns F, and Saris WH**. Gastrointestinal
- 705 complaints in relation to dietary intake in triathletes. *Int J Sport Nutr* 2: 48-59, 1992.
- 706 35. Pugh JN, Wagenmakers AJM, Doran DA, Fleming SC, Fielding BA, Morton JP, and
- 707 Close GL. Probiotic supplementation increases carbohydrate metabolism in trained male
- 708 cyclists: a randomized, double-blind, placebo-controlled crossover trial. Am J Physiol
- 709 Endocrinol Metab 318: E504-E513, 2020.
- 710 36. Stellingwerff T, Godin JP, Beaumont M, Tavenard A, Grathwohl D, van Bladeren PJ,
- 711 Kapp AF, le Coutre J, and Damak S. Effects of pre-exercise sucralose ingestion on
- 712 carbohydrate oxidation during exercise. *Int J Sport Nutr Exerc Metab* 23: 584-592, 2013.
- 713 37. **Odell OJ, Podlogar T, and Wallis GA**. Comparable Exogenous Carbohydrate
- Oxidation from Lactose or Sucrose during Exercise. *Med Sci Sports Exerc* 52: 2663-2672,
- 715 2020.
- 716 38. Barber JFP, Thomas J, Narang B, Hengist A, Betts JA, Wallis GA, and Gonzalez JT.
- 717 Pectin-Alginate Does Not Further Enhance Exogenous Carbohydrate Oxidation in Running.
- 718 *Med Sci Sports Exerc* 52: 1376-1384, 2020.
- 719 39. **Burke LM, Jeukendrup AE, Jones AM, and Mooses M**. Contemporary Nutrition
- 720 Strategies to Optimize Performance in Distance Runners and Race Walkers. Int J Sport Nutr
- 721 *Exerc Metab* 29: 117-129, 2019.
- 722 40. **Jeukendrup AE**. Nutrition for endurance sports: marathon, triathlon, and road
- 723 cycling. *J Sports Sci* 29 Suppl 1: S91-99, 2011.
- 724 41. Wallis GA, Rowlands DS, Shaw C, Jentjens RL, and Jeukendrup AE. Oxidation of
- 725 combined ingestion of maltodextrins and fructose during exercise. *Med Sci Sports Exerc* 37:
- 726 426-432, 2005.
- 42. Hawley JA, Bosch AN, Weltan SM, Dennis SC, and Noakes TD. Glucose kinetics
- 728 during prolonged exercise in euglycaemic and hyperglycaemic subjects. *Pflugers Arch* 426:
- 729 378-386, 1994.
- 730 43. **Stellingwerff T**. Competition Nutrition Practices of Elite Ultramarathon Runners. *Int J*
- 731 *Sport Nutr Exerc Metab* 26: 93-99, 2016.
- 732 44. King AJ, O'Hara JP, Arjomandkhah NC, Rowe J, Morrison DJ, Preston T, and King R.
- Liver and muscle glycogen oxidation and performance with dose variation of glucose-
- 734 fructose ingestion during prolonged (3 h) exercise. Eur J Appl Physiol 119: 1157-1169, 2019.

736

735

737

738

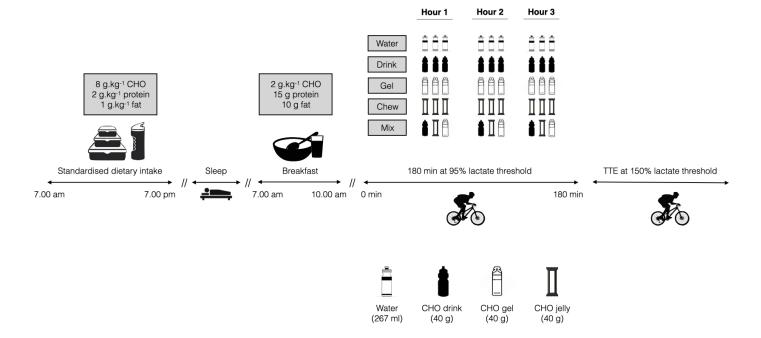
740	Table 1
741	Nutritional composition and mean enrichment of each carbohydrate form. pH and osmolality
742	for GEL trial is measured when 3 gels (equivalent to 120 g CHO) are mixed with 800 ml
743	water as per trial conditions.
744	
745	Table 2
746	Heart rate, RPE, absolute oxygen consumption and energy expenditure during 180 min
747	steady-state cycling.
748	
749	Figure 1
750	Schematic overview of the experimental protocol employed in each trial. Following 24 h of a
751	high CHO diet, subjects consumed a high CHO pre-exercise meal before undertaking 180
752	min steady-state submaximal exercise during which they consumed 120 g·h ⁻¹ CHO from fluid
753	(DRINK), gels (GEL), jelly chews (CHEW) or a co-ingestion approach (MIX), followed by a
754	time to exhaustion (TTE) exercise capacity test. TTE; time to exhaustion.
755	
756	Figure 2
757	(A) Plasma glucose, (B) lactate, (C) glycerol and (D) NEFA at rest and during exercise in the
758	DRINK, GEL, CHEW and MIX trials. ^a Significant difference from 0 min, ^b significant
759	difference from 30 min, ^c significant difference from 60 min, ^d significant difference from 90
760	min, ^e significant difference from 120 min, ^f significant difference from 150 min, $P < 0.05$.
761	
762	Figure 3
763	(A) Rates of whole-body CHO oxidation during exercise, (B) total CHO oxidation, (C) rates
764	of whole-body fat oxidation during exercise, (D) total fat oxidation, and (E) respiratory

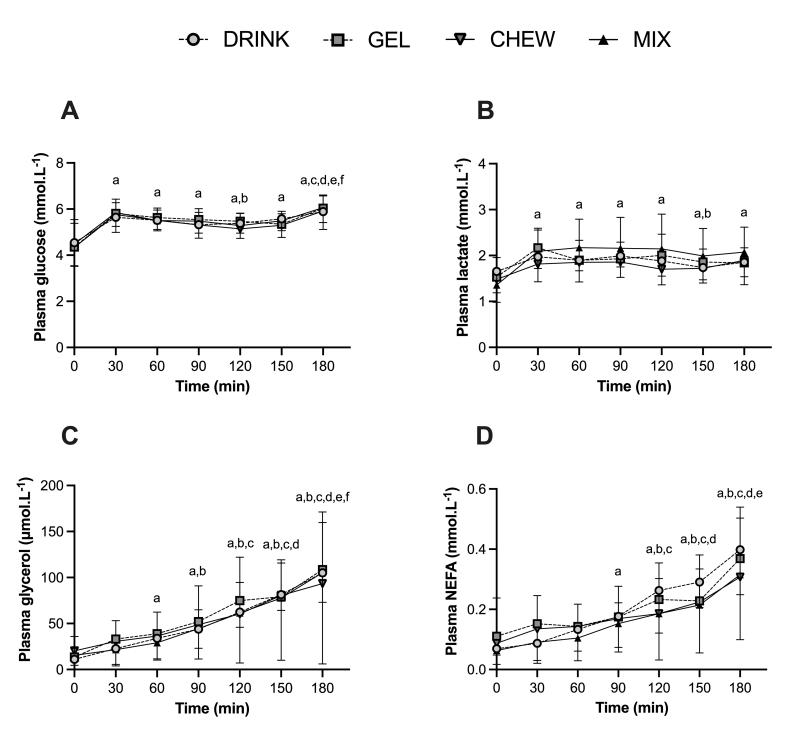
765 exchange ratio (RER) during exercise in the WATER, DRINK, GEL, CHEW and MIX trials. ^aSignificant difference from 30 min, ^bsignificant difference from 60 min, ^csignificant 766 767 difference from 90 min, ^d significant difference from 120 min, ^e significant difference from 768 150 min, P < 0.05. *Significant difference from water, P < 0.05. 769 770 Figure 4 771 Rates of energy provision from carbohydrate and fat oxidation during the (A) WATER, (B) 772 DRINK, (C) GEL, (D) CHEW and (E) MIX trials. 773 774 Figure 5 (A) Breath ¹³CO₂ enrichment and (B) exogenous CHO oxidation during 180 minutes of 775 776 exercise during the WATER, DRINK, GEL, CHEW and MIX trials. aSignificant difference 777 from 30 min, bsignificant difference from 60 min, csignificant difference from 90 min, difference from 120 min, P < 0.05. (C) Individual participant's peak exogenous 778 779 CHO oxidation during exercise and (D) mean oxidation efficiency. N = 8 for MIX trial 780 (missing individual data point for participant 2). Substrate contributions to total energy 781 expenditure during the (E) second and (F) third hour of exercise. †Significant difference 782 between water and all other feeding trials, P < 0.05. 783 784 Figure 6 785 (A) Exercise capacity time (time to exhaustion) during the WATER only (familiarisation), 786 DRINK, GEL, CHEW and MIX trials and (B) trial order exercise capacity time. 787 *Significantly different from WATER, P < 0.05. Bars represent group means and circles

788

789

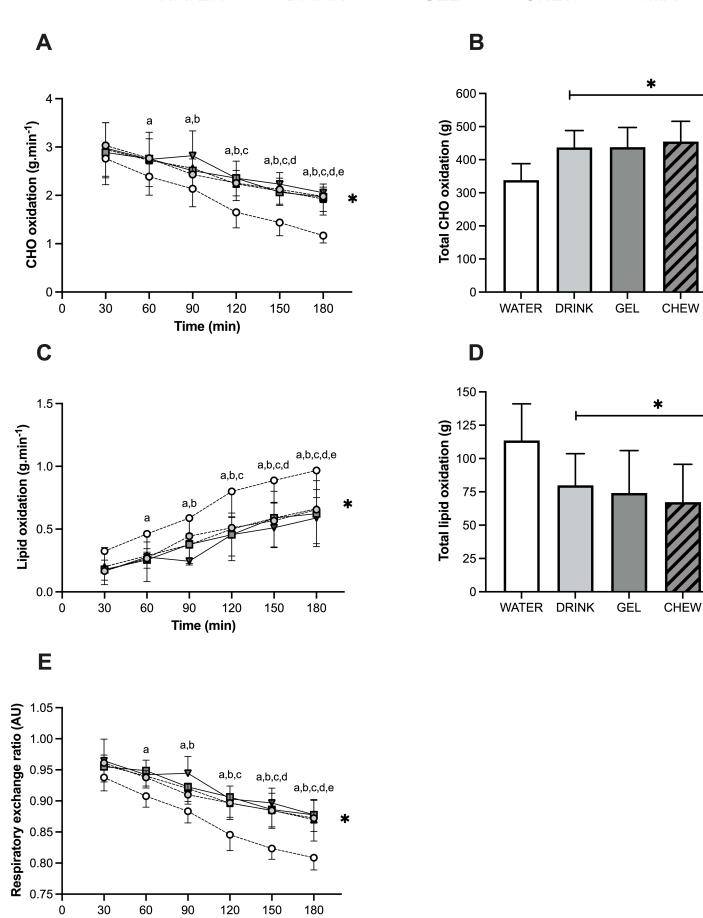
represent individual data points.



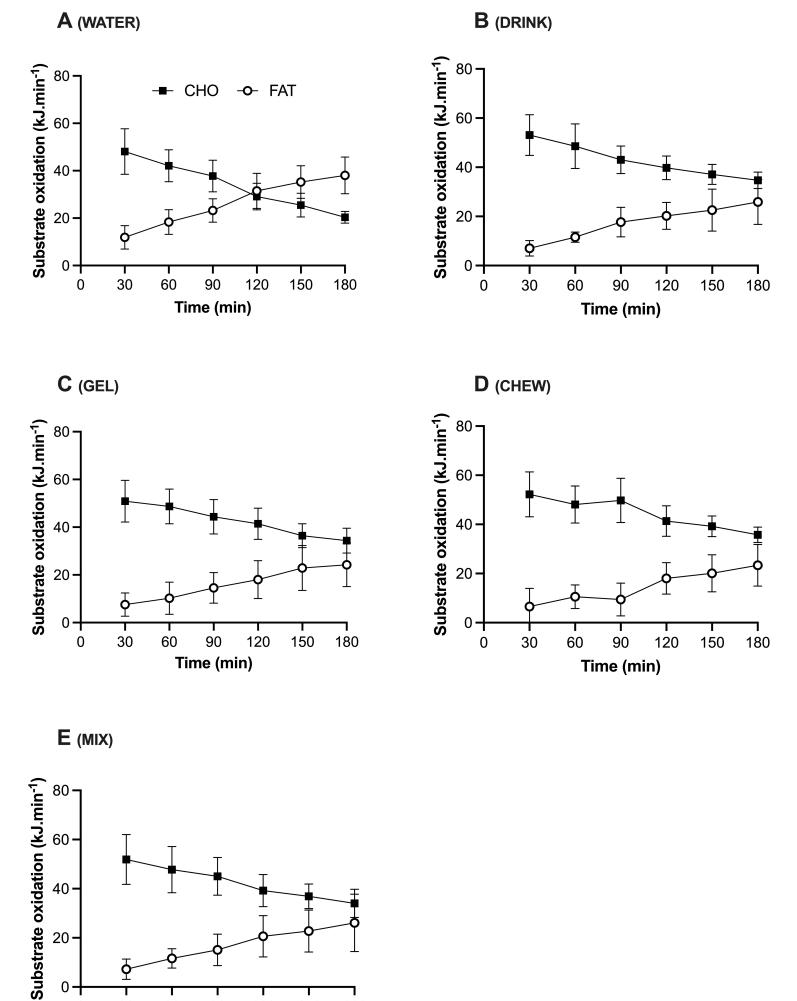


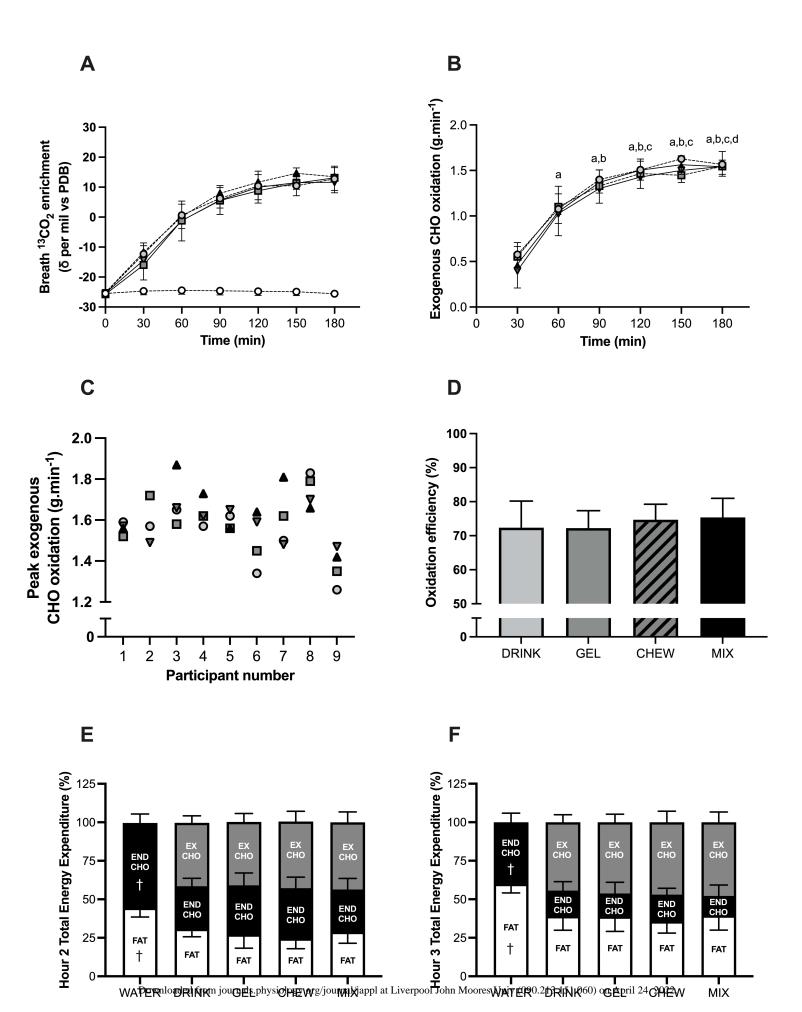
 MIX

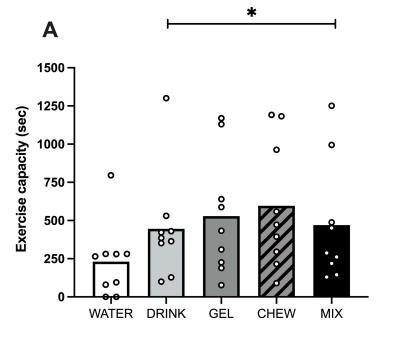
 MIX



Time (min)







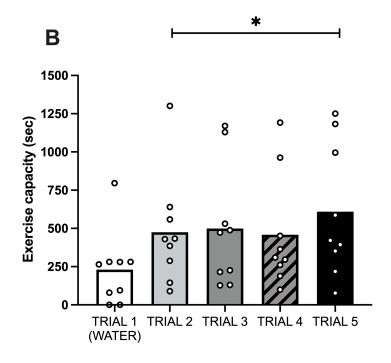


Table 1

Nutrition per 120 g CHO	DRINK	GEL	CHEW
Energy (kcal)	480	480	492
Fat (g)	0	0	0.1
Carbohydrate (g)	120	120	120
maltodextrin (g)	66.7	66.7	0
glucose (g)	-	-	66.7
fructose (g)	53.3	53.3	53.3
Protein (g)	0.0	0.0	0.3
Sodium (g)	0.00	0.05	0.12
Fibre (g)	0	0	5.7
Osmolality (mOsmol.kg ⁻¹)	380	386	-
pН	6.5	4.3	-
Mean enrichment δ ¹³ C vs PDB (‰)	57.33	56.38	54.23

	Time (min)					
	30	60	90	120	150	180
Heart rate (beats.min ⁻¹)						
WATER	136 ± 13	140 ± 13^a	$143\pm12^{a.b}$	$146\pm10^{a,b,c}$	$150\pm10^{a,b,c,d}$	$150\pm10^{a,b,c,d,e}$
DRINK	140 ± 10	143 ± 16^a	$147\pm16^{a.b}$	$149\pm16^{a,b,c}$	$151\pm16^{a,b,c,d}$	$153\pm14^{a,b,c,d,e}$
GEL	139 ± 15	142 ± 16^a	$142\pm16^{a,b}$	$147\pm16^{a,b,c}$	$148\pm16^{a,b,c,d}$	$150\pm15^{a,b,c,d,e}$
CHEW	137 ± 13	141 ± 13^a	$144\pm13^{\mathrm{a,b}}$	$147\pm12^{a,b,c}$	$149\pm13^{a,b,c,d}$	$151\pm13^{a,b,c,d,e}$
MIX	141 ± 16	$144\pm17^{\rm a}$	$146\pm16^{a,b}$	$150\pm18^{a,b,c}$	$152\pm17^{a,b,c,d}$	$154\pm19^{a,b,c,d,e}$
RPE (AU)						
WATER	10 ± 2	11 ± 1	12 ± 1^{a}	$13\pm2^{a,b}$	$14\pm2^{a,b,c,d}^*$	$16 \pm 2^{a,b,c,d}$ *
DRINK	11 ± 1	11 ± 1	12 ± 1^{a}	$12\pm1^{a,b}$	$13\pm2^{a,b,c,d}{}^*$	$13\pm2^{a,b,c,d}{}^*$
GEL	10 ± 2	11 ± 1	11 ± 2^{a}	$11\pm2^{a,b}^{*}$	$12\pm2^{a,b,c,d}{}^*$	$14\pm3^{a,b,c,d}{}^*$
CHEW	11 ± 1	11 ± 1	12 ± 1^a	$12\pm1^{a,b}$	$13 \pm 1^{a,b,c,d}$ *	$13\pm1^{a,b,c,d}{}^*$
MIX	11 ± 2	11 ± 2	11 ± 2^{a}	$12\pm2^{a,b}$	$13 \pm 1^{a,b,c,d}$ *	$14\pm2^{a,b,c,d}{}^*$
$\dot{V}O_2$ (L.min ⁻¹)						
WATER	2.81 ± 0.48	2.84 ± 0.48	$2.89\pm0.47^{\mathrm{a.b}}$	$2.93\pm0.45^{a.b}$	$2.94\pm0.45^{a.b}$	$2.85\pm0.45^{\mathrm{a.b}}$
DRINK	2.82 ± 0.48	2.85 ± 0.49	$2.90\pm0.46^{a.b}$	$2.89\pm0.45^{a.b}$	$2.88 \pm 047^{a.b}$	$2.94\pm0.51^{a.b}$
GEL	2.76 ± 0.48	2.78 ± 0.48	$2.81\pm0.45^{a.b}$	$2.85\pm0.48^{a.b}$	$2.86\pm0.49^{a.b}$	$2.84\pm0.51^{a.b}$
CHEW	2.74 ± 0.41	2.78 ± 0.40	$2.81\pm0.42^{a.b}$	$2.85\pm0.42^{a.b}$	$2.86\pm0.46^{a.b}$	$2.86\pm0.46^{a.b}$

MIX	2.83 ± 0.49	2.86 ± 0.52	$2.90\pm0.53^{a.b}$	$2.92 \pm 0.56^{a.b}$	$2.93\pm0.52^{\mathrm{a.b}}$	$2.97 \pm 0.56^{a.b}$	
Energy expenditure (kJ.min ⁻¹)							
WATER	59.2 ± 10.8	59.2 ± 10.0	59.5 ± 10.0	58.7 ± 9.3	59.0 ± 9.4	54.9 ± 6.8	
DRINK	60.0 ± 10	60.1 ± 10.4	60.1 ± 9.4	60.0 ± 9.2	59.5 ± 9.5	60.6 ± 10.0	
GEL	58.5 ± 10.0	58.8 ± 9.8	58.9 ± 9.3	59.3 ± 9.6	59.2 ± 9.8	58.5 ± 10.0	
CHEW	58.3 ± 8.5	58.7 ± 8.3	59.4 ± 8.8	59.3 ± 8.6	59.4 ± 9.2	59.1 ± 9.0	
MIX	59.3 ± 10.7	59.4 ± 11.3	60.0 ± 11.3	59.8 ± 11.7	59.7 ± 10.8	60.1 ± 11.4	

^asignificant difference from 30 min time point,

^bsignificant difference from 60 min time point,

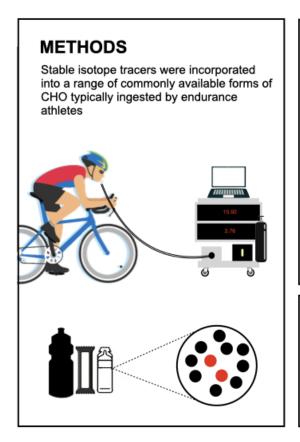
^csignificant difference from 90 min time point,

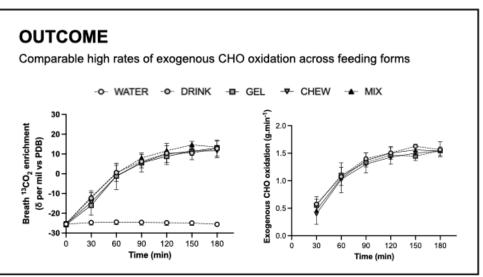
^dsignificant difference from 120 min time point and,

[°]significant difference from 150 min time point, P < 0.05

^{*}significant difference from water, P < 0.05

13-C-glucose-fructose labelling reveals comparable exogenous CHO oxidation during exercise when consuming 120 g/h in fluid, gel, jelly chew or co-ingestion





CONCLUSION

We demonstrate comparable high rates of exogenous CHO oxidation from fluid, semi-solid, solid or a combination of forms with negligible gastrointestinal symptoms. Considering the sustained high rates of total and exogenous carbohydrate oxidation, and relative lack of gastrointestinal symptoms, consuming 120 g CHO·h-¹ appears a well-tolerated strategy to promote high CHO availability during exercise.