

Citation for published version: Hearris, MA, Pugh, JN, Langan-Evans, C, Mann, SJ, Burke, L, Stellingwerff, T, Gonzalez, JT & Morton, JP 2022, 'C-glucose-fructose labeling reveals comparable exogenous CHO oxidation during exercise when consuming 120 g/h in fluid, gel, jelly chew, or coingestion: CHO oxidation from fluid, semi-solid and solid', *Journal of Applied Physiology*, vol. 132, no. 6, pp. 1394-1406. https://doi.org/10.1152/japplphysiol.00091.2022

DOI: 10.1152/japplphysiol.00091.2022

Publication date: 2022

Document Version Peer reviewed version

Link to publication

University of Bath

Alternative formats

If you require this document in an alternative format, please contact: openaccess@bath.ac.uk

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 2 3 4	13C-glucose-fructose labelling reveals comparable exogenous CHO oxidation during exercise when consuming 120 g/h in fluid, gel, jelly chew or co-ingestion
5	
6 7 8	Mark A Hearris ¹ , Jamie N Pugh ¹ , Carl Langan-Evans ¹ , Stephen J Mann ² , Louise Burke ³ , Trent Stellingwerff ^{4,5} , Javier T Gonzalez ^{6,7} , James P Morton ¹
9	
10	¹ Research Institute for Sport and Exercise Sciences
11	Liverpool John Moores University
12 13	Liverpool, UK
13	² Science in Sport PLC
14	London, UK
16	London, OK
17	³ Exercise and Nutrition Research Program
18	Mary MacKillop Institute for Health Research
19	Australian Catholic University
20	Melbourne, Australia
21	
22	⁴ Exercise Science, Physical and Health Education
23	University of Victoria
24	Victoria, Canada
25 26	
26 27	⁵ Pacific Institute for Sport Excellence Canadian Sport Institute-Pacific
27	Victoria, Canada
20 29	Victoria, Canada
30	⁶ Centre for Nutrition, Exercise and Metabolism
31	University of Bath
32	Bath, UK
33	
34	⁷ Department for Health,
35	University of Bath,
36	Bath, UK
37	
38	Running head: CHO oxidation from fluid, semi-solid and solid
39	
40 41	Address for correspondence: Prof James Morton
41 42	Prof James Morton 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
49	

- 50 Abstract
- 51

52 We examined the effects of carbohydrate (CHO) delivery form on exogenous CHO 53 oxidation, gastrointestinal discomfort, and exercise capacity. In a randomised repeated measures design (after 24 h of high CHO intake (8 $g \cdot kg^{-1}$) and pre-exercise meal (2 $g \cdot kg^{-1}$)), 54 nine trained males ingested 120 g CHO·h⁻¹ from fluid (DRINK), semi-solid gel (GEL), solid 55 56 jelly chew (CHEW), or a co-ingestion approach (MIX). Participants cycled for 180 min at 57 95% lactate threshold followed by an exercise capacity test (150% lactate threshold). Peak 58 rates of exogenous CHO oxidation (DRINK, 1.56 ± 0.16 ; GEL, 1.58 ± 0.13 ; CHEW, $1.59 \pm$ 0.08; MIX, 1.66 \pm 0.02 g·min⁻¹) and oxidation efficiency (DRINK, 72 \pm 8; GEL, 72 \pm 5; 59 CHEW, 75 \pm 5; MIX, 75 \pm 6%) were not different between trials (all P > 0.05). Despite 60 ingesting 120 g·h⁻¹, participants reported minimal symptoms of gastrointestinal distress 61 62 across all trials. Exercise capacity was also not significantly different (all P < 0.05) between 63 conditions (DRINK, 446 ± 350 ; GEL, 529 ± 396 ; CHEW, 596 ± 416 ; MIX, 469 ± 395 sec). 64 Data represent the first time that rates of exogenous CHO oxidation (via stable isotope 65 methodology) have been simultaneously assessed using feeding strategies (i.e., pre-exercise 66 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 67 68 maltodextrin or glucose: fructose) is a practically tolerable strategy to promote high CHO 69 availability and oxidation during exercise.

70

71 Keywords

- 72 Stable isotopes, fructose, maltodextrin, metabolism
- 73

74 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.

99 Introduction

100 The introduction of the muscle biopsy technique in the late 1960s (1) has allowed robust 101 documentation of the importance of muscle glycogen in determining exercise capacity and 102 performance in endurance events (2). In addition to high endogenous carbohydrate (CHO) 103 availability, consumption of CHO during exercise can also enhance exercise performance (3-104 5), an effect likely mediated by liver glycogen sparing (6), the maintenance of plasma glucose 105 concentrations and CHO oxidation rates (7) and/or via direct effects on the central nervous 106 system (8). Indeed, the provision of exogenous CHO during exercise can address the finite 107 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 $g \cdot h^{-1}$ (9) 108 while other contemporary reviews recommend CHO intakes rates of 100+ g·h⁻¹, if 109 110 gastrointestinal (GI) outcomes are individually tolerated (10). However, the latter 111 recommendations are based more on practitioner experience and field data, and have not yet 112 been tested using multiple forms of CHO sources, in ecologically valid conditions.

113

114 The oxidation of ingested CHO by skeletal muscle during exercise is thought to be limited by 115 CHO absorption through the intestinal membrane (11). In this regard, it is well established 116 that a mixture of multiple-source CHO blends (e.g. glucose polymers, glucose and fructose 117 etc.) are oxidised at 20-50% higher rates when compared to single source formulations (12-118 14). Indeed, whereas the exogenous oxidation of single-transportable CHO plateaus at ~ 60 g·h⁻¹, exogenous oxidation of multiple-transportable CHO continues to rise with CHO 119 ingestion up to 144 g·h⁻¹ (15). When ingesting CHO at a rate \geq 90 g·h⁻¹, the ratio at which 120 121 sources of CHO are co-ingested also influences their subsequent oxidation, whereby a 1:0.8 122 ratio of maltodextrin to fructose yields higher rates of oxidation when compared with an 123 isocaloric 2:1 ratio (16, 17). It is noteworthy, however, that for an individual to achieve high 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 oxidation efficiency of less than 100% (15). Taken together, these data suggest that to 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.

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 \cdot h⁻¹ using a 2:1 ratio of 131 132 glucose and fructose) during prolonged endurance exercise (20, 21), thus suggesting that 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 $g \cdot h^{-1}$ (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).

With this in mind, the primary aim of the present study was to quantify rates of exogenous CHO oxidation from the individual ingestion of liquid, gel and jelly chew forms of CHO as well as the combination of the three forms. To this end, trained male cyclists consumed CHO at a rate of 120 g \cdot h⁻¹ (using a 1:0.8 ratio of maltodextrin or glucose to fructose) during three 148 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 149 150 the manufacturing process, thus representing the first study to incorporate dual stable isotope 151 tracers at high enrichment into both solid and semi-solid CHO sources ingested during 152 exercise. To assess the effects of CHO on exercise capacity, a time to exhaustion test (at 153 150% of lactate threshold) was also performed after the completion of the 3 h steady-state 154 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 $g \cdot h^{-1}$ 155 156 CHO would not cause negative gastrointestinal symptoms and 3) exercise capacity would not 157 differ between the various feeding forms and formats.

158

159 Methodology

160 **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).

165

166 **Participants**

167 Nine endurance trained, amateur male cyclists (mean \pm SD: age, 25 \pm 8 years; body mass, 168 75.6 \pm 7.0 kg; height, 179.1 \pm 4.7 cm) volunteered to participate in the study. Mean $\dot{V}O_{2max}$, 169 peak power output (PPO) and power output at lactate threshold were 64.9 \pm 6.8 ml·kg⁻¹·min⁻¹ 170 ¹, 438 \pm 79 W and 226 \pm 37 W, respectively. Subjects were defined as either highly-trained 171 (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 \pm 0.13 g.min⁻¹ with solid vs 1.14 \pm 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.

179

180 **Experimental overview**

181 In a repeated measures (> 6 days, but < 15 days apart), randomised, cross-over design, with 182 each experimental trial separated by a minimum of 7 days, participants completed a 183 prolonged endurance-based cycling exercise protocol, consisting of 180 min of submaximal 184 exercise (undertaken at 95% of lactate threshold) followed by an exercise capacity test to 185 exhaustion (undertaken at 150% of lactate threshold) on five separate occasions. The initial 186 trial (WATER; where water only was consumed during exercise) was performed to provide a 187 full familiarisation to the exercise protocol and to examine any background shifts in breath ¹³CO₂ appearance. In the following four randomised trials, subjects ingested CHO at a rate of 188 120 g·h⁻¹ from fluid (DRINK), gels (GEL), jelly chews (CHEW) or a combination of all three 189 190 delivery forms (MIX). Each experimental trial was commenced following 24-h of high CHO intake (8 $g \cdot kg^{-1}$) and 3 h after the consumption of a CHO rich pre-exercise meal (2 $g \cdot kg^{-1}$). 191 192 An overview of the experimental design and nutritional protocols is displayed in Figure 1.

193

194 **Preliminary testing**

195 At least 7 days prior to experimental trials, subjects performed a two-part incremental cycle

196 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 197 198 the test was commenced at 100 W and increased by 25 W at the end of each 4-minute stage. 199 A fingertip blood sample was collected during the final 30 seconds of each stage for the 200 determination of blood lactate concentrations (Biosen C-Line; EKF Diagnostics, Cardiff, UK) 201 and the lactate threshold (LT) was determined using the D_{max} method (25). Subjects 202 commenced the second part of the test at an intensity corresponding to that of the penultimate 203 stage completed in the previous part, whereby exercise intensity increased by 25 W every 204 minute until volitional exhaustion. The end time and power output at the point of exhaustion 205 were used to calculate PPO using the following equation (26):

206

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

207

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.

214

215 **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 13 C to minimise the background shift from glycogen stores during exercise. Foods with a high natural abundance of 13 C (e.g. corn and sugar cane) were also avoided in the standardised diet and pre-exercise breakfast. Twentyfour 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

228 180-min steady state cycling

229 On the morning of the main experimental trials, subjects reported to the laboratory at $\sim 10:00$ 230 h having consumed the pre-packaged, standardised breakfast provided (see above). Upon 231 arrival, an indwelling cannula (Safety Lock 22G; BD Biosciences, West Sussex, UK) was 232 inserted into the antecubital vein in the anterior crease of the forearm and a resting blood 233 sample drawn and subsequently flushed with ~5 ml of sterile saline (Kays Medical, 234 Liverpool, UK). Resting expired breath samples were collected in duplicate into evacuated 10 235 mL Exetainer tubes (Labco, High Wycombe, UK), sampled directly from the mixing chamber, to determine the ${}^{13}C/{}^{12}C$ ratio in CO₂ at rest. Following the collection of resting 236 237 measures, subjects completed a 10-min warm-up at 100 W and began the 180 min cycling 238 protocol at 95% LT (215 ± 35 W). This relative exercise intensity was chosen as it has been 239 suggested as an appropriate method of matching metabolic stress between subjects when 240 compared with exercising at a percentage of VO_{2max} (27). Heart rate (Polar H10; Polar, 241 Kempele, Finland), ratings of perceived exertion (RPE) (28) and cycling cadence were 242 obtained at 30 min intervals throughout. Expired gas was collected for a 5 min period at 30 243 min intervals to calculate whole body substrate utilisation. The final minute of this period 244 was used to collect expired gas into the evacuated Exetainer tubes to determine the ${}^{13}C/{}^{12}C$ ratio in CO2. Gastrointestinal (GI) symptoms (nausea, regurgitation, fullness, cramps, gas, 245

246 and urge to defecate) were recorded at 30-min intervals during exercise using a 0-10 visual 247 analogue scale (0 = no discomfort, and 10 = very severe discomfort) (29). Subjects were 248 instructed that a score > 4 should be regarded as a moderate symptom that was detrimental to 249 their ability to exercise. The sum of scores at each time point were collated for each 250 gastrointestinal symptom, resulting in maximum scores of 60 for each symptom. 251 Immediately following the 180 min submaximal cycle, subjects began the exercise capacity 252 test, where they cycled at 150% LT (339 ± 55 W) until task failure, defined as an inability to 253 maintain a cadence > 60 rpm. During the capacity test, the only information available to the 254 subjects was the fixed power output and pedal cadence and no performance results were 255 provided to subjects until they had completed all experimental trials. All exercise tests were 256 performed at the same time of day under normal laboratory conditions (20-22°C and 50-60% 257 humidity) using the same electrically braked cycle ergometer (Lode Excalibur Sport, 258 Groningen, Netherlands) and automated gas analyser (Moxus Modular Metabolic System; 259 AEI Technologies, IL, USA). During all exercise trials, subjects were cooled with a floor fan 260 to minimise thermal stress. Participants were not provided with any prior information from the 261 researchers that would influence their bias on which form of CHO would be superior for 262 exercise performance.

263

264 Carbohydrate feeding

During exercise, subjects consumed CHO at a rate of 120 $g \cdot h^{-1}$ 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 271 comfort during exercise (16, 17, 30). Furthermore, maltodextrin and glucose can be 272 considered as broadly interchangeable with respect to exogenous carbohydrate oxidation rates 273 during exercise, since the rate of hydrolysis and exogenous oxidation between carbohydrate 274 monomers and polymers are comparable (31, 32). Carbohydrate was ingested immediately 275 prior to exercise and subsequently at 20 min intervals during exercise in equal amounts of 40 276 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 277 278 CHO to ensure equivalent proportions in relation to their tracee and match the ratio of 279 maltodextrin (glucose) and fructose in each form (Table 1). The MIX condition provided an 280 equal mixture of all three forms and was consumed in the same order for all subjects (e.g., 281 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. 282 283 During all other trials, subjects consumed an equivalent amount of water (267 ml at each 284 feeding point) to ensure total fluid intake and the pattern of ingestion was matched across all 285 trials.

286

287 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.

295

296 Estimates of whole-body substrate oxidation and energy expenditure

297 Rates of whole-body CHO and fat oxidation $(g \cdot min^{-1})$ were calculated at 30 min intervals 298 during the 180 min submaximal cycle using the equations of Jeukendrup and Wallis (33). 299 Total energy expenditure was estimated for each trial assuming an energy yield of 17.57 kJ 300 and 39.33 kJ for 1 g of CHO and fat, respectively. Substrate utilisation data during the MIX 301 trial is missing for one participant due to a technical error and at the 180 min time point 302 during the WATER trial for two participants due to volitational fatigue.

303

$304 \quad {}^{13}\text{C}/{}^{12}\text{C}$ analysis of breath CO₂

305 Isotopic enrichment of breath samples was determined using gas chromatography isotope 306 ratio mass spectrometry (Iso-analytical, Crewe, UK). The ¹³C enrichment from breath 307 samples are expressed as δ^{13} C ‰ versus Pee Dee Belemnite (PDB). Exogenous carbohydrate 308 oxidation was calculated using the following formula

309

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

310

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).

317

318 Statistical analysis

319 All statistical analyses were performed using SPSS Statistics Version 27 (IBM, US). 320 Differences in mean and peak exogenous CHO oxidation, gastrointestinal symptoms, heart 321 rate, RPE, energy expenditure, and exercise capacity were all analysed using one-way 322 repeated-measures ANOVA. Mauchly's test for sphericity was used and, in cases where this 323 assumption was violated, the Greenhouse-Geisser correction was applied. A two-way 324 repeated measures ANOVA was used to analyse differences over time (e.g., 30-180 min) and 325 between conditions for substrate utilisation and plasma metabolites. Where a significant main 326 effect was observed, pairwise comparisons were analysed using post-hoc LSD tests to locate 327 specific differences. All data in text, figures and tables are presented as means \pm SD with P 328 values ≤ 0.05 indicating statistical significance.

329

330 Results

331

332 Physiological responses

333 Heart rate and absolute oxygen uptake increased during exercise (time effect, P < 0.001 for 334 both, Table 2) although no differences were apparent between trials (treatment effect, P =335 0.621, P = 0.155 and P = 0.596, respectively). RPE also increased during exercise (time 336 effect, P < 0.001) and was significantly higher in the WATER trial compared with the GEL 337 trial at 120 minutes (P = 0.018) and compared with all CHO feeding trials from 150 minutes 338 onwards (P < 0.001 for DRINK, GEL and CHEW and P = 0.033 for MIX) (Table 2). Plasma 339 glucose and lactate concentrations were not significantly different between forms of CHO 340 ingestion (treatment effect, P = 0.749 and P = 0.426 respectively). Plasma glycerol and 341 NEFA progressively increased during exercise (time effect, P < 0.001 for both) although 342 concentrations were not significantly different between forms of CHO ingestion (treatment 343 effect, P = 0.735 and P = 0.983, respectively) (Figure 2).

344

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 (P351 = 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 (DRINK, 1.40 ± 0.17 ; GEL, 1.36 ± 0.14 ; CHEW, 1.44 ± 0.11 ; MIX, 1.44 ± 0.13 g·min⁻¹; 362 363 treatment effect, P = 0.138) or hour 3 (DRINK, 1.50 ± 0.17 ; GEL, 1.52 ± 0.10 ; CHEW, 1.55364 \pm 0.08; MIX, 1.6 \pm 0.13 g·min⁻¹; treatment effect, P = 0.092) between trials (Figure 5B). 365 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). 367 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, 369 $1.66 \pm 0.02 \text{ g·min}^{-1}$; treatment effect, P = 0.189) (Figure 5C). We also highlight individual

370 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 ± 6%; DRINK, 17 ± 6; GEL, 17 ± 5; CHEW, 17 ± 4; MIX, 15 ± 5%, *P* < 0.001 380 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 ± 383 5%, P < 0.001) and hour 3 (WATER; 60 ± 6%; DRINK, 39 ± 9; GEL, 37 ± 8; CHEW, 36 ± 384 8; MIX, $38 \pm 6\%$, P < 0.001 for all) (Figure 5E and 3F).

385

386 Gastrointestinal discomfort

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). 394 395

Exercise capacity

CHO feeding at a rate of 120 g·h⁻¹ significantly improved exercise capacity (trial effect; P =397 0.021) within all trials (DRINK, 446 ± 350 s, P = 0.004; GEL, 529 ± 396 s, P = 0.005; 398 399 CHEW, 596 ± 416 s, P = 0.028; MIX, 470 ± 395 s, P = 0.035; Figure 6A) when compared 400 with WATER only $(231 \pm 244 \text{ s})$. However, there were no significant differences in exercise 401 capacity between the different feeding forms (P > 0.05 for all). When data were analysed for 402 a trial order effect, there was a significant difference between conditions (trial effect, P =403 0.044) which was explained by significant differences between Trial 1 (i.e. the WATER only 404 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 405 406 6B) with no other pairwise differences apparent.

- 407
- 408

409 **Discussion**

410 Confirming our hypothesis, data demonstrate comparable rates of peak exogenous CHO oxidation when trained male cyclists consume 120 $g \cdot h^{-1}$ in the form of a liquid (DRINK), 411 412 semi-solid (GEL), solid (CHEW) or a co-ingestion approach (MIX). Importantly, we 413 observed some of the highest peak rates of exogenous CHO oxidation (e.g. 1.66 g·min⁻¹ 414 during the co-ingestion approach) reported in the literature, the result of which was not 415 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 416 417 carbohydrate sources in a ratio close to unity, is a practically feasible and well-tolerated

418 protocol to achieve high CHO availability and oxidation during prolonged endurance419 exercise.

420

421 To address our aims, we studied a cohort of trained male cyclists who completed an exercise 422 protocol previously studied in our laboratory (i.e. 3 h of steady-state cycling at 95% of lactate 423 threshold followed by an exercise capacity test at 150% of lactate threshold) (24). 424 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⁻¹ 425 426 CHO), nutritional strategies which are considered best practice in preparation for competitive 427 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 428 429 manufacture to facilitate accurate estimates of exogenous oxidation rates. To our knowledge, 430 this approach represents the first time that stable isotope tracers have been *simultaneously* 431 incorporated into the fluid (DRINK), semi-solid (GEL) and solid (CHEW) CHO delivery 432 forms that are typically ingested by endurance athletes during exercise. Furthermore, the 433 incorporation of stable isotope tracers into the jelly chews, not only provides the first 434 demonstration of the oxidation of this novel food form, but also circumvents previous 435 technical difficulties associated with the assessment of other solid CHO sources (i.e., food 436 bars). Indeed, such bars naturally consist of several different CHOs and other ingredients 437 with different natural enrichments in ¹³C, thereby making the calculation of exogenous CHO 438 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 439 440 to trace maltodextrin, the hydrolysis of maltodextrin is rapid, and not rate limiting to 441 absorption. Thus, maltodextrin displays equivalent digestion, absorption and exogenous oxidation kinetics to glucose (34), and enrichment with ¹³C-glucose thereby provides an 442

443 appropriate method to study the oxidation of maltodextrin. This approach has been studied444 previously both in our laboratory (35) and elsewhere (36).

445

446 Another principle of the methodology of measurement of exogenous CHO oxidation relates to the appropriate background expired ¹³CO₂ enrichment. When ingesting CHO at natural 447 448 abundance of ¹³C, the optimal background is to perform a trial with identical CHO ingestion 449 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 ¹³C enrichment might still be 450 as low as -23 δ^{13} C ‰ versus PDB in the higher enriched trials (38). However, this issue 451 452 becomes negligible when spiking the ingested CHO with sufficient amounts of >99% enriched ¹³C-carbohydrates, as fluctuations in the background expired ¹³CO₂ enrichment are 453 454 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 +10 455 δ^{13} C ‰ versus PDB (Figure 5A), thereby illustrating the substantial enrichment achieved by 456 457 spiking the ingested CHO with stable isotopes.

458

459 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 $\text{g}\cdot\text{h}^{-1}$ 460 461 (9, 39, 40). However, where the aim is to achieve high rates of exogenous CHO oxidation (e.g. 1.5 $g \cdot min^{-1}$), individuals should likely consume 100-120 $g \cdot h^{-1}$ since oxidation efficiency 462 463 is not uniform (13, 14). Indeed, the upper limits of reported CHO intakes during competition range from 107 to 137 $g \cdot h^{-1}$ (19). It has previously been reported that blends of maltodextrin 464 465 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 466 performance when compared with 2:1 formulations (62 \pm 12% and ~1.0 g·min⁻¹, 467

respectively) ingested at a rate of 90 g·h⁻¹(17). In keeping with these data, our chosen 468 469 formulation of maltodextrin and fructose in a 1:0.8 ratio also induced a similar oxidation 470 efficiency, the values of which were comparable between forms (DRINK; 72, GEL; 72, 471 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 472 DRINK trial (i.e. 1.56 g·min⁻¹) than has been previously reported (~1.2 g·h⁻¹) when CHO was 473 474 ingested at 90 g·h⁻¹(17). Although previous researchers also reported peak rates of exogenous 475 CHO oxidation of 1.53 g·min⁻¹ (range: 1.23-1.77 g·min⁻¹) when consuming 108 g per hour (of 476 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 477 478 oxidation may be achieved with the strategy adopted here (i.e. 120 g per hour of a 1:0.8 479 ratio). When taken together, such data further support the suggestion that athletes should 480 ingest multiple-transportable CHO's, co-ingested in ratios closer to unity (18), and at absolute intakes exceeding 90 $g \cdot h^{-1}$ in order to optimise CHO availability and oxidation. 481

482

483 Based on previous glucose infusion studies which bypass the limitations of the GI system and 484 directly infuse glucose into the circulation, it is assumed that the maximal rate of exogenous CHO oxidation that working skeletal muscle can use is $\sim 1.8 \text{ g} \cdot \text{min}^{-1}$ (42), Notwithstanding 485 486 potential effects of hyperinsulinemia and assuming an estimated oxidation efficiency of ~70-75%, the ingestion of 140-150 $g \cdot h^{-1}$ may be considered as the maximal worthwhile dose to 487 achieve such high rates of oxidation. In support of this, the ingestion of 144 $g \cdot h^{-1}$ (in a 1:1 488 489 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 490 491 (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 492

493 may be possible to achieve maximal rates of oxidation from lower dose of CHO amongst 494 individuals with high oxidation efficiency. We also highlight the inter-individual variability 495 in peak oxidation rates (Figure 5C) and suggest that individual responses should be 496 considered when providing CHO intake recommendations to avoid the potential for large 497 amounts of ingested CHO to remain within the intestine.

498

499 To our knowledge, there are only two previous reports that have compared exogenous rates 500 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 501 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 502 503 contrast, lower rates (albeit not statistically significant) of peak exogenous CHO oxidation were reported when CHO was ingested in the form of a bar $(1.25 \text{ g} \cdot \text{min}^{-1})$ versus a drink 504 (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 505 506 study provides novel data and an extension to our understanding by investigating CHO 507 oxidation rates from an alternative solid form (i.e. jelly chews) that is typically used by 508 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 509 510 values are some of the highest reported in the current literature and provided almost half of 511 the energy requirements of exercise just below lactate threshold. Furthermore, the inclusion 512 of a co-ingestion trial (i.e. MIX) is a highly novel aspect of the present study and mimics the 513 self-chosen protocols of fuelling observed in the real-life practices of endurance and ultra-514 endurance athletes (19, 43).

515

516 Despite the consumption of 120 $g \cdot h^{-1}$ CHO during exercise, it is noteworthy that the present 517 participants reported trivial symptoms of gastrointestinal discomfort across all feeding forms. 518 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 519 520 well tolerated during steady-state cycling (13, 14). However, in contrast to such studies who 521 report "individual" cases of severe discomfort (e.g., stomach cramping and bloating), no 522 gastrointestinal discomfort was reported by any of the present participants across individual 523 trials. Although previous studies support the notion that gastrointestinal symptoms do not 524 differ between liquid (e.g., drinks) and semi-solid (e.g., gels) form of CHO intake, greater 525 feelings of nausea, stomach fullness and abdominal cramps have been previously reported 526 with the ingestion of solid CHO sources such as energy bars (20, 22). Given that these 527 symptoms are typically associated with the presence of other nutrients (such as fat, protein, 528 and fibre), the lack of reported symptoms in solid form studied here (i.e. CHEW) could be 529 attributed to relative absence of such nutrients and a ratio of glucose (polymers) to fructose 530 that increases the oxidation efficiency of the ingested CHO. Importantly, we also observed 531 minimal gastrointestinal symptoms during our MIX trial that combined all three CHO forms. 532 These findings have important implications given that this pattern of ingestion reflects the 533 real-world fuelling practices of elite endurance athletes, who typically ingest a mix of CHO 534 forms across exercise durations of 3-6 hours (19). Although the exercise intensity in the 535 present study was deliberately chosen to reflect the sub-maximal intensity of "riding in the 536 bunch" during professional road races (i.e. below lactate threshold), it would also be 537 beneficial to include frequent high-intensity efforts in future studies so as to assess the effects 538 of high CHO ingestion on potential GI symptoms when exercising at intensities above lactate 539 threshold.

540

541 To address the effects of CHO form on exercise capacity, participants completed an exercise 542 capacity test undertaken at 150% of lactate threshold immediately after the completion of the 543 3 h steady-state sub-maximal exercise protocol. This protocol has been previously studied in 544 our laboratory (also using trained male cyclists) where we observed a dose response effect of CHO feeding, in that the consumption of 90 $g \cdot h^{-1}$ extended exercise capacity when compared 545 with both 45 $g \cdot h^{-1}$ and 0 $g \cdot h^{-1}$ (24). This ergogenic effect was likely mediated by increased 546 547 liver glycogen and/or plasma glucose availability associated wth the higher dose of CHO ingestion. Interestingly, we also observed that the consumption of 90 $g \cdot h^{-1}$ delayed the point 548 at which lipid oxidation comprises the largest proportion of energy production by ~ 10 and 549 ~40 minutes when compared with 45 $g \cdot h^{-1}$ and 0 $g \cdot h^{-1}$, respectively (24). In consuming the 550 higher absolute dose of 120 $g \cdot h^{-1}$ in the present study, it is noteworthy that CHO remained the 551 552 predominant source of substrate utilisation throughout exercise in all trials (Figure 4) and no 553 statistical differences in exercise capacity were observed between feeding forms (Figure 6). 554 These data are unsurprising given the similar rates of whole body and exogenous CHO 555 oxidation and lack of gastrointestinal distress observed between feeding forms, two potential 556 mechanisms by which feeding form may impact upon performance (22). Indeed, Guillochon 557 and Rowlands (22) recently reported reductions in peak power that was associated with the 558 increased gastrointestinal distress arising from repeated intake of solid CHO (e.g. bar). We 559 readily acknowledge, however, that a potential lack of statistical power may have limited our 560 ability to detect small differences in performance between trials given that the study was 561 primarily powered to detect changes in exogenous CHO oxidation and the high inter-562 individual variability in exercise capacity. Although the participants were given no prior 563 information to influence their beliefs on what form of CHO may be superior for performance, 564 we also acknowledge that our study design was not a true double blind design (i.e. 565 participants and researchers were consciously aware of what form of CHO they were 566 consuming). Furthermore, the inclusion of a known water only trial (that served as our 567 familiarisation trial) does not allow us to assess the effects of CHO versus a true placebo trial. 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).

574

575 In summary, the present data demonstrate comparable rates of exogenous CHO oxidation 576 when CHO is ingested during exercise in a liquid, semi-solid or solid form, as well as a 577 feeding strategy that combined all forms. When considering the high absolute rates of 578 exogenous CHO oxidation, the maintainence of whole-body CHO oxidation, and the lack of gastrointestinal symptoms, data demonstrate that consumption of 120 $g \cdot h^{-1}$ (as achieved via 579 580 1:0.8 formulations of maltodextrin or glucose: fructose) is a practically feasible and well-581 tolerated strategy to promote high CHO availability during exercise. Indeed, the present data 582 represent some of the highest rates of exogenous CHO oxidation reported in the literature and 583 were achieved via feeding forms and formats that are commonly adopted by elite endurance 584 athletes.

585 Funding

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

587

588 Disclosures

589 JPM is a consultant for Science in Sport (SiS). SiS, Glaxosmithkline (GSK) and Lucozade 590 Ribena Suntory (LRS) have previously funded JPM's research on glycogen metabolism and 591 exercise. JTG is an investigator on research grants funded by BBSRC, MRC, British Heart 592 Foundation, The Rank Prize Funds, The European Society for Clinical Nutrition and 593 Metabolism (ESPEN), Lucozade Ribena Suntory, ARLA Foods Ingredients and 594 Kenniscentrum Suiker and Voeding; and has completed paid consultancy for PepsiCo and 595 SVGC. LMB is a consultant for Science in Sport, for which she does not receive 596 remuneration. Her previous studies related to CHO and sports performance have been funded 597 by the Australian Institute of Sport, Australian Catholic University and the Alliance for 598 Potato Research and Education, and she has received honoraria for conference presentations 599 by Gatorade Sport Science Institute (GSSI, Pepsico). TS is a consultant for Science in Sport 600 (SiS), and has done previous consultancy work for Gatorade Sport Science Institute (GSSI, 601 Pepsico) and was formerly employed by Nestec / PowerBar. TS's research is currently 602 funded by Own The Podium i4G grants, 94Forward, B2ten, MITACS and the Wu Tsai 603 Performance Alliance. JNP is a consultant for Aliment Nutrition. Aliment Nutrition have 604 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;

609 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

611 authors approved the final version and agree to be accountable for all aspects of the work in

612 ensuring that questions related to the accuracy or integrity of any part of the work are

613 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.

6213.Currell K, and Jeukendrup AE. Superior endurance performance with ingestion of622multiple transportable carbohydrates. *Med Sci Sports Exerc* 40: 275-281, 2008.

Rowlands DS, Swift M, Ros M, and Green JG. Composite versus single transportable
 carbohydrate solution enhances race and laboratory cycling performance. *Appl Physiol Nutr 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
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
639 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: 998643 1011, 2014.

644 11. Jeukendrup AE, and Jentjens R. Oxidation of carbohydrate feedings during
645 prolonged exercise: current thoughts, guidelines and directions for future research. *Sports*646 *Med* 29: 407-424, 2000.

Hulston CJ, Wallis GA, and Jeukendrup AE. Exogenous CHO oxidation with glucose
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 652 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 O'Brien WJ, and Rowlands DS. Fructose-maltodextrin ratio in a carbohydrate-16. 657 electrolyte solution differentially affects exogenous carbohydrate oxidation rate, gut 658 comfort, and performance. Am J Physiol Gastrointest Liver Physiol 300: G181-189, 2011. 659 O'Brien WJ, Stannard SR, Clarke JA, and Rowlands DS. Fructose-maltodextrin ratio 17. 660 governs exogenous and other CHO oxidation and performance. Med Sci Sports Exerc 45: 661 1814-1824, 2013. 662 Rowlands DS, Houltham S, Musa-Veloso K, Brown F, Paulionis L, and Bailey D. 18. 663 Fructose-Glucose Composite Carbohydrates and Endurance Performance: Critical Review 664 and Future Perspectives. Sports Med 45: 1561-1576, 2015. 665 Pfeiffer B, Stellingwerff T, Hodgson AB, Randell R, Pottgen K, Res P, and 19. 666 Jeukendrup AE. Nutritional intake and gastrointestinal problems during competitive 667 endurance events. Med Sci Sports Exerc 44: 344-351, 2012. 668 Pfeiffer B, Stellingwerff T, Zaltas E, and Jeukendrup AE. Oxidation of solid versus 20. 669 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 673 on Gut Comfort and Performance. Int J Sport Nutr Exerc Metab 27: 247-254, 2017. 674 McKay AKA, Stellingwerff T, Smith ES, Martin DT, Mujika I, Goosey-Tolfrey VL, 23. 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 679 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 682 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 685 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 692 Reliability of a Retrospective Questionnaire. Int J Sport Nutr Exerc Metab 27: 370-376, 2017. 693 Rowlands DS, Thorburn MS, Thorp RM, Broadbent S, and Shi X. Effect of graded 30. 694 fructose coingestion with maltodextrin on exogenous 14C-fructose and 13C-glucose 695 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 Moodley D, Noakes TD, Bosch AN, Hawley JA, Schall R, and Dennis SC. Oxidation of 32. 700 exogenous carbohydrate during prolonged exercise: the effects of the carbohydrate type 701 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 703 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 Pugh JN, Wagenmakers AJM, Doran DA, Fleming SC, Fielding BA, Morton JP, and 35. 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 714 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 Wallis GA, Rowlands DS, Shaw C, Jentjens RL, and Jeukendrup AE. Oxidation of 41. 725 combined ingestion of maltodextrins and fructose during exercise. Med Sci Sports Exerc 37: 726 426-432, 2005. 727 Hawley JA, Bosch AN, Weltan SM, Dennis SC, and Noakes TD. Glucose kinetics 42. 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 King AJ, O'Hara JP, Arjomandkhah NC, Rowe J, Morrison DJ, Preston T, and King R. 44. 733 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. 735 736 737 738 739

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 $g \cdot h^{-1}$ 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

765exchange ratio (RER) during exercise in the WATER, DRINK, GEL, CHEW and MIX trials.766^aSignificant difference from 30 min, ^bsignificant difference from 60 min, ^csignificant767difference from 90 min, ^dsignificant difference from 120 min, ^esignificant difference from768150 min, P < 0.05. *Significant difference from water, P < 0.05.

769

770 Figure 4

Rates of energy provision from carbohydrate and fat oxidation during the (A) WATER, (B)
DRINK, (C) GEL, (D) CHEW and (E) MIX trials.

773

774 **Figure 5**

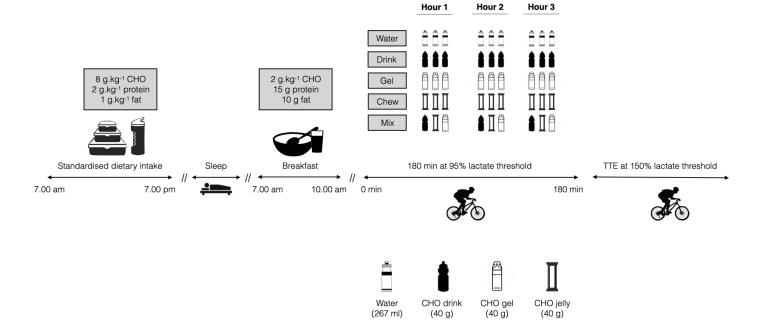
(A) Breath ¹³CO₂ enrichment and (B) exogenous CHO oxidation during 180 minutes of 775 776 exercise during the WATER, DRINK, GEL, CHEW and MIX trials. ^aSignificant difference 777 from 30 min, ^bsignificant difference from 60 min, ^csignificant difference from 90 min, 778 ^dsignificant difference from 120 min, P < 0.05. (C) Individual participant's peak exogenous 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

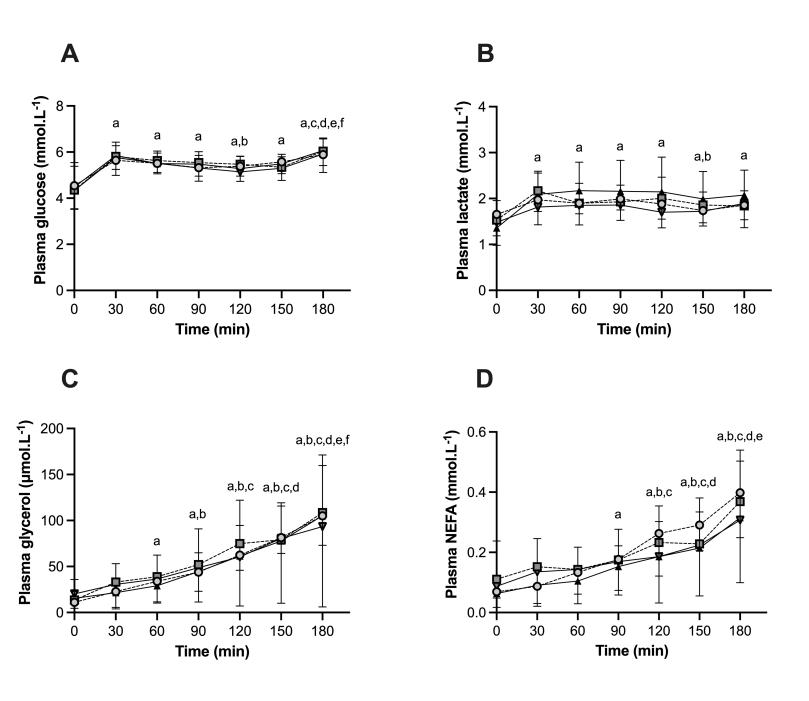
784 Figure 6

(A) Exercise capacity time (time to exhaustion) during the WATER only (familiarisation), DRINK, GEL, CHEW and MIX trials and (B) trial order exercise capacity time. *Significantly different from WATER, P < 0.05. Bars represent group means and circles represent individual data points.

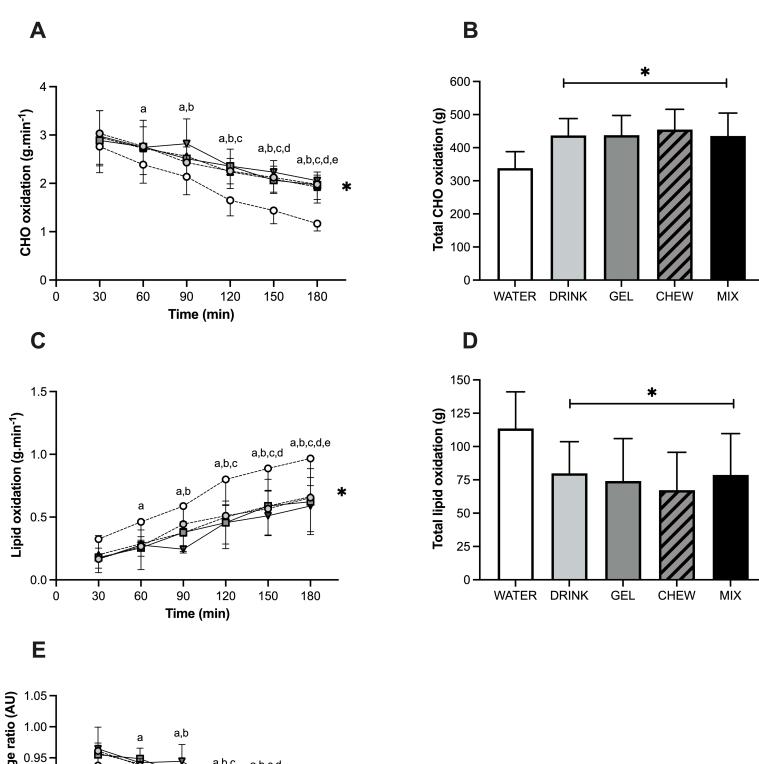
789

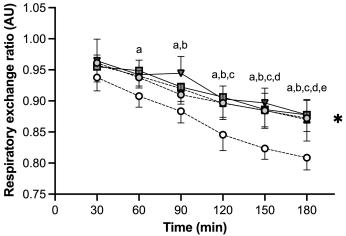


-• DRINK -■- GEL -▼- CHEW -▲ MIX



-O-- WATER -O-- DRINK -□- GEL -▼- CHEW -▲-- MIX





A (WATER)

Substrate oxidation (kJ.min⁻¹)

80

60

40

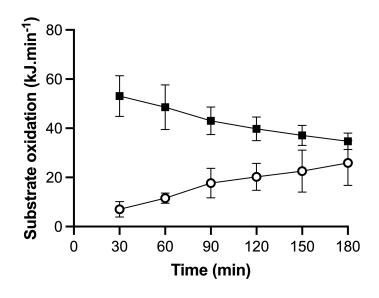
20

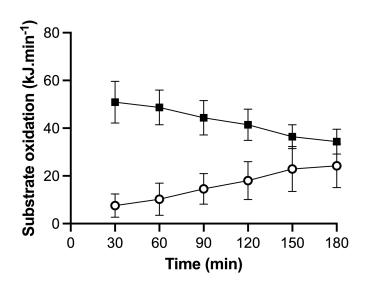
0

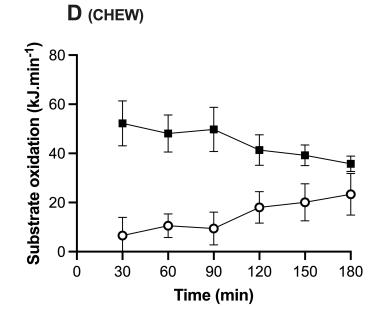
0 30 60 90 120 150 180 Time (min)

-**o**- FAT

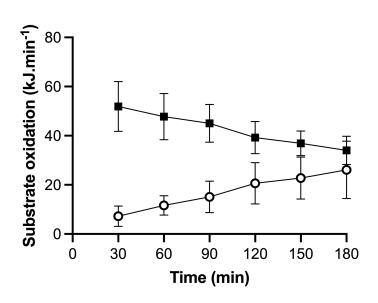
CHO



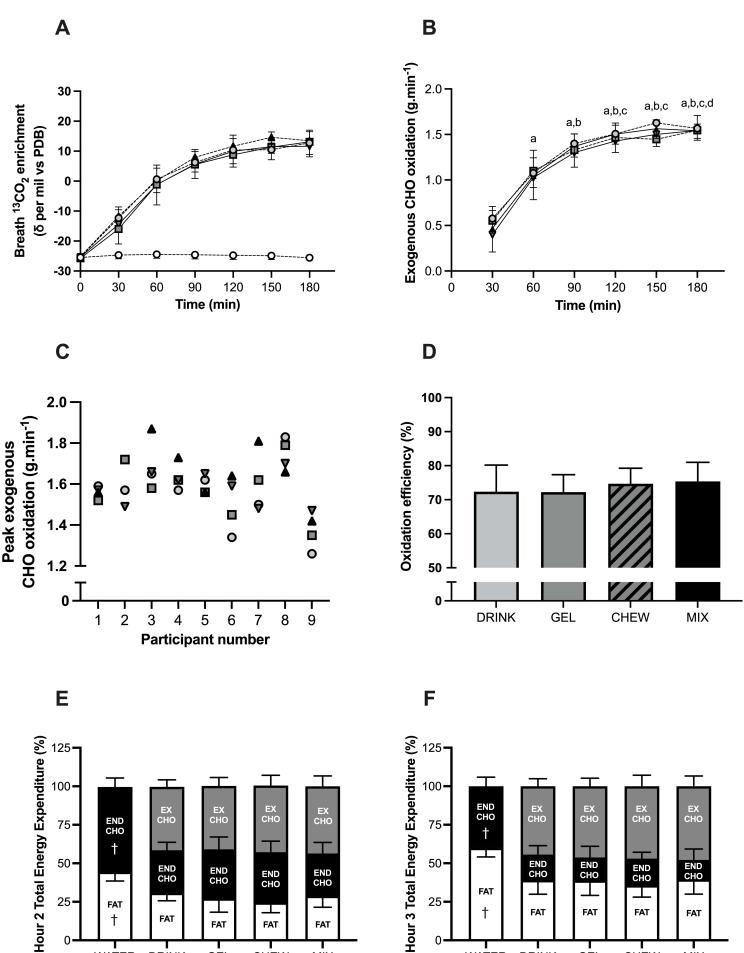








B (DRINK)



FAT

GEL

FAT

CHEW

FAT

міх

FAT

DRINK

FAT

†

WATER

25

0

Т

FAT

MIX

25.

0

FAT

t

WATER

FAT

DRINK

FAT

GEL

FAT

CHEW

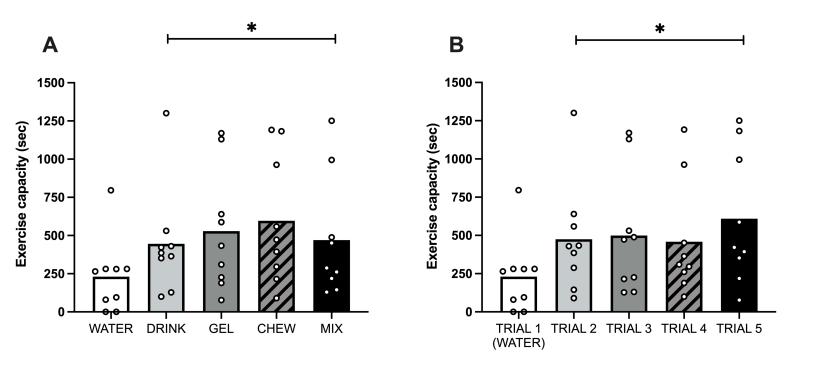


Table 1

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	-
pH	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}	$143\pm12^{a.b}$	$146 \pm 10^{a,b,c}$	$150\pm10^{\text{a,b,c,d}}$	$150\pm10^{a,b,c,d,e}$
DRINK	140 ± 10	$143\pm16^{\rm a}$	$147\pm16^{a.b}$	$149\pm16^{a,b,c}$	$151\pm16^{a,b,c,d}$	$153\pm14^{a,b,c,d,e}$
GEL	139 ± 15	142 ± 16^a	$142\pm16^{a,b}$	$147\pm16^{a,b,c}$	$148 \pm 16^{\text{a,b,c,d}}$	$150 \pm 15^{a,b,c,d,e}$
CHEW	137 ± 13	141 ± 13^{a}	$144 \pm 13^{a,b}$	$147\pm12^{a,b,c}$	$149\pm13^{a,b,c,d}$	$151\pm13^{a,b,c,d,e}$
MIX	141 ± 16	$144 \pm 17^{\rm a}$	$146\pm16^{a,b}$	$150\pm18^{a,b,c}$	$152\pm17^{a,b,c,d}$	$154\pm19^{a,b,c,d,e}$
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}	$11 \pm 2^{a,b}$ *	$12\pm2^{a,b,c,d\ *}$	$14\pm3^{a,b,c,d\ *}$
CHEW	11 ± 1	11 ± 1	12 ± 1^{a}	$12\pm1^{a,b}$	$13\pm1^{a,b,c,d\ *}$	$13\pm1^{a,b,c,d\ *}$
MIX	11 ± 2	11 ± 2	11 ± 2^{a}	$12\pm2^{a,b}$	$13\pm1^{a,b,c,d\ *}$	$14\pm2^{a,b,c,d\ *}$
[.]VO₂ (L.min ⁻¹)						
WATER	2.81 ± 0.48	2.84 ± 0.48	$2.89\pm0.47^{a.b}$	$2.93\pm0.45^{a.b}$	$2.94\pm0.45^{a.b}$	$2.85\pm0.45^{a.b}$
DRINK	2.82 ± 0.48	2.85 ± 0.49	$2.90\pm0.46^{a.b}$	$2.89\pm0.45^{a.b}$	$2.88\pm047^{a.b}$	$2.94\pm0.51^{a.b}$
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	$2.90\pm0.53^{a.b}$	$2.92\pm0.56^{a.b}$	$2.93\pm0.52^{a.b}$	$2.97\pm0.56^{a.b}$
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,

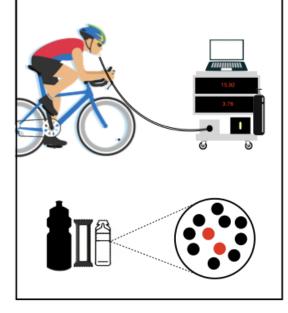
^esignificant difference from 150 min time point, P < 0.05

*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

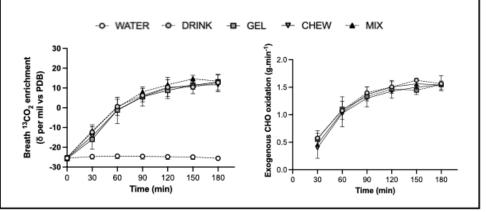
METHODS

Stable isotope tracers were incorporated into a range of commonly available forms of CHO typically ingested by endurance athletes



OUTCOME

Comparable high rates of exogenous CHO oxidation across feeding forms



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.