



*Citation for published version:*

Narang, BJ, Wallis, GA & Gonzalez, JT 2021, 'The effect of calcium co-ingestion on exogenous glucose oxidation during endurance exercise in healthy men: A pilot study', *European Journal of Sport Science*, vol. 21, no. 8, pp. 1156-1164. <https://doi.org/10.1080/17461391.2020.1813336>

*DOI:*

[10.1080/17461391.2020.1813336](https://doi.org/10.1080/17461391.2020.1813336)

*Publication date:*

2021

*Document Version*

Peer reviewed version

[Link to publication](#)

This is an Accepted Manuscript of an article published by Taylor & Francis in *European Journal of Sport Science* on 12/09/2020, available online: <https://www.tandfonline.com/doi/full/10.1080/17461391.2020.1813336>

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1 **Title:**

2 The effect of calcium co-ingestion on exogenous glucose oxidation during endurance  
3 exercise in healthy men: A Pilot Study.

4

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19 **Abstract**

20 The benefits of high exogenous glucose availability for endurance exercise  
21 performance are well-established. Exogenous glucose oxidation rates are thought to be limited  
22 by intestinal glucose transport. Extracellular calcium in rodent intestine increases the  
23 translocation of the intestinal glucose transporter GLUT2 which, if translated to humans, could  
24 increase the capacity for exogenous glucose availability during exercise. Therefore, this pilot  
25 study aimed to explore the effect of calcium co-ingestion during endurance exercise on  
26 exogenous glucose oxidation in healthy men.

27 Eight healthy men cycled for 2 h at 50% peak power output, ingesting either 1.2 g·min<sup>-1</sup>  
28 dextrose alone (GLU) or with the addition of 2000 mg calcium (GLU+CAL), in a randomised  
29 crossover design. Expired breath samples were collected to determine whole-body and  
30 exogenous glucose oxidation.

31 Peak exogenous glucose oxidation during GLU was 0.83±0.15 g·min<sup>-1</sup>, and was not  
32 enhanced during GLU+CAL (0.88±0.11 g·min<sup>-1</sup>,  $p = 0.541$ ). The relative contributions of  
33 exogenous carbohydrate (19±3% vs. 20±2%,  $p = 0.434$ ), endogenous carbohydrate (65±3% vs.  
34 65±3%,  $p = 0.822$ ) and fat (16±3% vs. 15±3%,  $p = 0.677$ ) to total substrate utilisation did not  
35 differ between trials.

36 These results suggest the addition of calcium to glucose ingestion, at saturating glucose  
37 ingestion rates, does not appear to alter exogenous glucose oxidation during endurance exercise  
38 in healthy men.

39

40 **Key words**

41 Calcium; Carbohydrate; Sports nutrition; Endurance exercise; Metabolism; Intestinal  
42 absorption; Exogenous glucose oxidation;

43



## 45 **Introduction**

46           The importance of carbohydrate intake for optimal endurance performance, particularly  
47 in events lasting more than 90 minutes, is well-established (Vandenbogaerde and Hopkins  
48 2011). The intake of carbohydrate during exercise provides an exogenous fuel source, sparing  
49 hepatic (Gonzalez et al. 2015) and sometimes muscle (Tsintzas et al. 1995) glycogen stores in  
50 addition to maintaining euglycaemia (Karelis et al. 2010) and high carbohydrate oxidation rates  
51 (Coyle et al. 1986). It is thought that exogenous carbohydrate availability during exercise is  
52 limited by intestinal absorption (Gonzalez et al. 2017; Jeukendrup and Jentjens 2000).  
53 Identifying novel methods of enhancing intestinal carbohydrate absorption could thereby  
54 contribute to optimising carbohydrate availability during exercise.

55           The most recent guidelines regarding carbohydrate intake during exercise recommend  
56 an intake of 30 – 60 g·h<sup>-1</sup> and up to 90 g·h<sup>-1</sup> for endurance and ultra-endurance, respectively  
57 (Thomas et al. 2016). The former values are based on research identifying maximal intestinal  
58 absorption rates of glucose via active sodium-dependent cotransporters (SGLT1) and  
59 facilitative (passive) transporters (GLUT2) of ~1 g·min<sup>-1</sup> (Burke et al. 2011). The higher ultra-  
60 endurance recommendations are associated with glucose-fructose co-ingestion, as intestinal  
61 absorption of fructose into the enterocytes occurs via an alternative transporter to that of  
62 glucose (GLUT5) resulting in a greater capacity for overall carbohydrate uptake and  
63 subsequent oxidation (Gonzalez et al. 2017; Jeukendrup 2010; Rowlands et al. 2015).

64           An alternative method of enhancing intestinal absorptive capacity may be to upregulate  
65 the intrinsic activity of the various intestinal transporter proteins. As SGLT1 becomes saturated  
66 at relatively low intestinal glucose concentrations (Chaudhry et al. 2012), GLUT2 translocation  
67 is particularly important under conditions of high glucose availability. Despite early belief that  
68 intestinal absorption of both glucose (Pappenheimer and Reiss 1987) and calcium (Bronner  
69 2003) under these conditions occurred primarily through paracellular flow, more recent

70 research appears to demonstrate a facilitative role of calcium in transcellular glucose uptake  
71 (Mace et al. 2007). Indeed, the translocation of GLUT2 requires cytoskeletal rearrangement  
72 and the expression of protein kinase-C (PKC)  $\beta$ II, both of which are calcium-dependent. Mace  
73 and colleagues (2007) found the co-presence of calcium to greatly enhance cytoskeletal  
74 rearrangement and PKC  $\beta$ II expression when perfusing the lumen of rodent intestine with 75  
75 mmol·L<sup>-1</sup> of glucose for 30 minutes, suggesting a facilitative role for calcium in intestinal  
76 glucose uptake may exist. In addition, these authors found increased extracellular calcium at  
77 physiological concentrations to facilitate the secretion of gut peptides from intestinal  
78 enteroendocrine cells (Mace et al. 2012). Subsequent human research has consistently found  
79 that co-ingestion of calcium with other nutrients increases postprandial gut peptide  
80 concentrations (Chen et al. 2019; Gonzalez & Stevenson, 2014). This provides support for a  
81 role of dietary calcium in the regulation of human intestinal cell signalling. However, to date,  
82 the effects of calcium ingestion exogenous glucose oxidation rates during prolonged exercise  
83 are unknown. If calcium co-ingestion can enhance the absorption and oxidation of exogenous  
84 glucose, there may be a role for calcium in contemporary nutritional guidelines to enhance  
85 carbohydrate availability during endurance exercise performance.

86 The aim of the present pilot study was to explore the effect of calcium co-ingestion  
87 during endurance exercise on exogenous glucose oxidation in healthy men. It was hypothesised  
88 that exogenous glucose oxidation rates would be higher with calcium-glucose co-ingestion  
89 compared to glucose ingestion alone.

90

## 91 **Materials and Methods**

### 92 **Participants**

93 Following written informed consent, nine healthy male volunteers participated in the  
94 study between July and September 2019. Inclusion criteria were age (18 – 35 y), body mass

95 index ( $18.5 - 30 \text{ kg}\cdot\text{m}^2$ ) and physical activity levels (self-reported  $\geq 30$  min moderate intensity  
96  $\geq$  three times per week). Participants were excluded if they had been habitual smokers in the  
97 previous five years, or if they had a history of metabolic disorders or medications that would  
98 pose undue risk or introduce bias into the outcome measures. Due to the failure to complete a  
99 main trial (inability to sustain the required exercise intensity), one participant was excluded  
100 from the analysis leaving a total sample of  $n = 8$ . Participant characteristics are presented in  
101 **Table 1**.

102

### 103 **Experimental Design**

104 Participants visited the laboratory on three occasions to complete a preliminary test  
105 followed by two main trials in a randomised, single-blind, counterbalanced crossover design.  
106 All trials were separated by a minimum 5-day washout period. The research was conducted at  
107 the University of Bath after ethical approval was granted by the Research Ethics Approval  
108 Committee for Health (REF: EP 18/19 047), and in accordance with the Declaration of Helsinki.

109

### 110 **Preliminary Trial**

111 Upon arrival for preliminary testing, participant height (Holtain Ltd., Pembrokeshire,  
112 UK) and body mass (BC-543 Monitor, Tokyo, Japan) were recorded to the nearest 0.1 cm and  
113 0.1 kg, respectively. Participants were fitted with a heart rate monitor (Polar Electro Oy,  
114 Kempele, Finland), before providing 5-min resting expired gas samples in 200-litre Douglas  
115 Bags (Hans Rudolph, Kansas City, USA). Resting heart rate was recorded and blood lactate  
116 concentrations (Nova Biomedical, Waltham, USA) measured with capillary fingertip blood  
117 samples, before participants adjusted the saddle and handlebar positions of an electronically-  
118 braked cycle ergometer (Lode, Groningen, Netherlands) to preference. These settings were  
119 recorded and replicated in the main trials.

120 Participants then completed a graded exercise test to volitional exhaustion at a self-  
121 selected cadence, with an initial power output of 50 W which was increased by 50 W every  
122 four minutes for four stages. A 60-s expired gas sample was collected at the end of each stage,  
123 during which heart rate, blood lactate concentration and ratings of perceived exertion (RPE;  
124 Borg 1970) were recorded. From the fifth stage until volitional exhaustion, exercise intensity  
125 was increased by 20 W every 60 s while heart rate, blood lactate concentration and RPE  
126 continued to be collected at regular intervals. Participants indicated when they felt they were  
127 approximately 60 s from exhaustion, at which point a final expired gas sample was collected  
128 and they were verbally encouraged by the researchers. Peak power output ( $W_{\max}$ ) was  
129 calculated as the power output at the highest completed stage, plus a fraction of the subsequent  
130 increment that reflected the duration of the final stage the participant completed. Peak oxygen  
131 uptake ( $\dot{V}O_{2\text{peak}}$ ) was determined by analysing the final expired gas sample.

132

### 133 **Main Trials**

134 Participants were asked to abstain from caffeine, alcohol and strenuous exercise in the  
135 24 h before the main trials. They also attempted to replicate their diets as closely as possible in  
136 this time period, arrived in the laboratory after a minimum 8 h fast, and at a similar time of day  
137 for both trials ( $\pm 30$  min within participants). A 5-min resting expired gas sample was collected,  
138 and resting heart rate recorded, before blood lactate and glucose concentrations (Abbott  
139 Diabetes Care, Maidenhead, UK) were measured with capillary fingertip blood samples.  
140 Participants also indicated their baseline levels of gut discomfort on a Likert scale ranging from  
141 1 “No Gut Discomfort” to 10 “Maximal Gut Discomfort”. Finally, participants provided a 20-  
142 s single-breath sample in a 10 mL Exetainer tube (Labco Ltd, Lampeter, UK) by exhaling into  
143 a discard bag (Quintron Inc, Milwaukee, USA).



144 The exercise bouts consisted of 2 h continuous cycling at 50%  $W_{max}$ , during which  
145 participants ingested either glucose only (GLU) or glucose-calcium (GLU+CAL) beverages.  
146 In both trials, participants were provided with 144 g naturally high  $^{13}C$  abundance dextrose  
147 (MyProtein, Northwich, UK), with 7500 mg of a calcium-enriched milk mineral supplement  
148 (24% calcium, 12.5% phosphorous, 8% lactose, 3% milk protein; Arla Foods Ingredients, Viby  
149 J, Denmark) added to GLU+CAL to provide 2000 mg of calcium phosphate. A milk mineral  
150 supplement was used in the present study as it reflects a typical source of dietary calcium and  
151 has previously been shown to elicit effects on gut hormones in humans (Chen et al. 2019).  
152 These ingredients were evenly distributed and dissolved in eight 100 ml boluses of tap water,  
153 to be consumed at the onset of exercise and every 15 minutes throughout. Drinks were provided  
154 in opaque bottles to facilitate blinding to the independent variable.

155 Every 15 minutes during exercise, immediately before the next drink was consumed,  
156 60-s expired gas samples were collected in Douglas Bags while heart rate, RPE and gut  
157 discomfort ratings were recorded. 10-s single-breath samples were then collected in Exetainers  
158 and the next test drink was ingested. Blood glucose and blood lactate concentrations were  
159 subsequently measured.

160 Blinding success was assessed at the end of participants' final trials, by asking whether  
161 they could tell a difference between the two drinks and whether they could identify in which  
162 trial they were given the calcium. Of the eight participants, four correctly differentiated  
163 between trials, one was only able to identify a difference and three perceived the drinks to be  
164 identical.

165

## 166 **Substrate Oxidation**

167 All expired gas samples collected in Douglas Bags were analysed for  $O_2$  and  $CO_2$   
168 concentration using paramagnetic and infrared transducers, respectively (Servomex Group Ltd,

169 Crowborough, UK). A two-point calibration was conducted on these sensors using gas  
170 cylinders of known concentration prior to each trial. Douglas Bags were subsequently  
171 evacuated using a dry gas meter (Harvard Apparatus, Holliston, USA) to determine the total  
172 volume of expired gas collected during the sampling period. Total carbohydrate and fat  
173 oxidation rates were calculated using the stoichiometric equations proposed by Jeukendrup and  
174 Wallis (2005), under the assumption that protein metabolism was negligible.

175 The single-breath samples collected at rest and during exercise were analysed using  
176 continuous flow ratio mass spectrometry. The isotopic enrichments of the samples were  
177 expressed as  $\delta$  per millilitre difference between the  $^{13}\text{C}/^{12}\text{C}$  ratio of the sample and a known  
178 laboratory reference standard (Craig 1957), before exogenous carbohydrate oxidation was  
179 calculated using the following formula (Pirnay et al. 1995):

$$180 \quad \text{Exogenous Carbohydrate Oxidation} = \dot{V}\text{CO}_2 \cdot \left( \frac{\delta\text{Exp} - \delta\text{Exp}_{\text{bkg}}}{\delta\text{Ing} - \delta\text{Exp}_{\text{bkg}}} \right) \left( \frac{1}{k} \right)$$

181 in which  $\delta\text{Exp}$  is the  $^{13}\text{C}$  enrichment of expired gas,  $\delta\text{Ing}$  is the  $^{13}\text{C}$  enrichment of the  
182 ingested solution,  $\delta\text{Exp}_{\text{bkg}}$  is the background  $^{13}\text{C}$  enrichment of expired gas determined using a  
183 water trial, and  $k$  is the  $\dot{V}\text{CO}_2$  produced by the oxidation of 1 g of glucose ( $0.7467 \text{ L CO}_2 \cdot \text{g}^{-1}$ ).  
184 As a water trial was not conducted in this study,  $\delta\text{Exp}_{\text{bkg}}$  was estimated using the mean values  
185 observed in a similar experiment in which endurance trained men completed 2 h of treadmill  
186 exercise at 60%  $\dot{V}\text{O}_{2\text{peak}}$  (Barber et al. 2020).

187

## 188 **Statistical Analysis**

189 An *a priori* sample size estimation was obtained using previous research comparing the  
190 effect of glucose-fructose co-ingestion on exogenous carbohydrate oxidation rates relative to  
191 the ingestion of glucose alone (Trommelen et al. 2017). Peak exogenous carbohydrate  
192 oxidation rates for the glucose-fructose and glucose-only conditions were  $1.40 \text{ g} \cdot \text{min}^{-1}$  and  $0.96$

193 g·min<sup>-1</sup>, respectively, resulting in an effect size of Cohen's *d*: 2.32. Based on these data, five  
194 participants were required to detect an effect at the 5% significance level with >95% statistical  
195 power.

196 Data were processed and analysed using Microsoft Excel 2016 and SPSS v26 (IBM,  
197 Armonk, USA). The incremental area under the curve (iAUC) relative to baseline was  
198 calculated for exogenous carbohydrate oxidation (Narang et al. 2020), using the data from the  
199 second hour of exercise to account for the delayed <sup>13</sup>CO<sub>2</sub> production in isotope enrichment  
200 methodologies. The paired differences of all variables were determined to be sufficiently  
201 normally distributed for parametric inferential tests to be conducted. Thus, all data expressed  
202 over time were analysed with two-way repeated measures ANOVA (trial\*time), and summary  
203 statistics were compared using paired-samples *t*-tests. In the case of statistically significant *F*-  
204 ratios, Ryan-Holm Bonferroni *post hoc* tests were applied to locate differences. All values are  
205 presented as mean±95%CI. For all statistical analyses, significance was accepted at *P* < 0.05.

206

## 207 **Results**

### 208 **Exercise Intensity**

209 The prescribed workload of 50% *W*<sub>max</sub> (178±23 W) resulted in similar exercise  
210 intensities between trials when expressed relative to  $\dot{V}O_{2peak}$  (63.7±2.7% vs. 63.9±2.8% with  
211 GLU vs. GLU+CAL, respectively, *p* = 0.745). Mean exercising heart rate (140±6 bpm vs.  
212 142±7 bpm with GLU vs. GLU+CAL, respectively, *p* = 0.266), mean RPE (13.2±0.7 vs.  
213 13.1±0.7 with GLU vs. GLU+CAL, respectively, *p* = 0.704) and total energy expenditure  
214 (1530±147 kcal vs. 1539±148 kcal with GLU vs. GLU+CAL, respectively, *p* = 0.630) did not  
215 significantly differ between trials.

216

### 217 **Expired Breath and Substrate Oxidation**

218 Rates of oxygen consumption and carbon dioxide production increased during exercise  
219 in both conditions (both  $p < 0.001$ ), with no treatment effect (both  $p > 0.529$ ) or trial\*time  
220 interaction effect (both  $p > 0.185$ ; **Figure 1A** and **1B**). Expired  $^{13}\text{CO}_2$  enrichments increased  
221 during exercise in both conditions ( $p < 0.001$ ), with no treatment effect ( $p = 0.471$ ) or trial\*time  
222 interaction effect ( $p = 0.555$ ; **Figure 1C**). Concurrently, the rate of exogenous carbohydrate  
223 oxidation increased over time ( $p < 0.001$ ), with no main effect of trial ( $p = 0.346$ ) or trial\*time  
224 interaction ( $p = 0.500$ ; **Figure 1D**). Peak exogenous carbohydrate oxidation rates did not differ  
225 between trials ( $0.83 \pm 0.15 \text{ g} \cdot \text{min}^{-1}$  vs.  $0.88 \pm 0.11 \text{ g} \cdot \text{min}^{-1}$  with GLU vs. GLU+CAL, respectively,  
226  $p = 0.541$ ), the iAUC values also did not differ between conditions ( $59.6 \pm 15.2 \text{ g} \cdot 120 \text{ min}$  vs.  
227  $64.3 \pm 14.5 \text{ g} \cdot 120 \text{ min}$  with GLU vs. GLU+CAL, respectively,  $p = 0.390$ ), and the total amounts  
228 of exogenous carbohydrate oxidised throughout the exercise bouts were also unaffected by the  
229 co-ingestion of calcium with glucose, compared to glucose alone ( $58.7 \pm 10.2 \text{ g}$  vs.  $64.9 \pm 9.2 \text{ g}$   
230 with GLU vs. GLU+CAL, respectively,  $p = 0.309$ ). When expressed relative to total substrate  
231 oxidation, the relative contributions of exogenous carbohydrate ( $19 \pm 3\%$  vs.  $20 \pm 2\%$  with GLU  
232 vs. GLU+CAL, respectively,  $p = 0.434$ ), endogenous carbohydrate ( $65 \pm 3\%$  vs.  $65 \pm 3\%$  with  
233 GLU vs. GLU+CAL, respectively,  $p = 0.822$ ) and fat ( $16 \pm 3\%$  vs.  $15 \pm 3\%$  with GLU vs.  
234 GLU+CAL, respectively,  $p = 0.677$ ) did not significantly differ between trials.

235

### 236 **Blood Metabolite Concentrations**

237 Blood glucose concentrations did not differ between trials ( $p = 0.106$ ) or across time ( $p$   
238  $= 0.147$ ), and there was no trial\*time interaction effect ( $p = 0.761$ ; **Figure 2A**). Blood lactate  
239 concentrations increased during both trials ( $p = 0.018$ ) but did not differ between GLU and  
240 GLU+CAL ( $p = 0.955$ ), and no time\* trial interaction effect was observed ( $p = 0.590$ ; **Figure**  
241 **2B**).

242

## 243 **Subjective Ratings**

244 Gut discomfort ratings did not differ between trials ( $p = 0.854$ ), did not change across  
245 time ( $p = 0.119$ ) and displayed no trial\*time interaction ( $p = 0.750$ ; **Figure 2C**). RPE increased  
246 throughout exercise ( $p < 0.001$ ; **Figure 2D**) but did not differ between trials (main effect of  
247 trial,  $p = 0.704$ ; trial\*time interaction,  $p = 0.278$ ).

248

## 249 **Discussion**

250 The present study aimed to identify whether the co-ingestion of calcium with glucose  
251 would facilitate exogenous carbohydrate oxidation during submaximal exercise in healthy men.  
252 These data suggest that when ingesting glucose at rates that are in accordance with guidelines  
253 for optimising glucose availability during exercise (i.e.  $1.2 \text{ g}\cdot\text{min}^{-1}$ ) the co-ingestion of calcium  
254 with glucose does not further enhance exogenous carbohydrate oxidation rates.

255 The limit to exogenous carbohydrate availability during exercise could, in theory, be  
256 attributed to the rates of gastric emptying, intestinal absorption, passage via the liver or the rate  
257 of glucose uptake by exercising muscle. Since gastric emptying rates have been found to exceed  
258 the rates of exogenous glucose oxidation (Rehrer et al. 1992b), and the intravenous infusion of  
259 glucose results in greater rates of exogenous oxidation than those typically observed with oral  
260 ingestion (Hawley et al. 1994), gastric emptying and muscle uptake of glucose are unlikely to  
261 be limiting factors. In addition, similar maximal intestinal glucose absorption rates have been  
262 observed at rest (Duchman et al. 1997) and during intense exercise (Fordtran and Saltin 1967),  
263 suggesting an increased requirement for an exogenous fuel source does not result in facilitated  
264 absorption at the intestinal level. Therefore, the availability of orally ingested glucose during  
265 endurance exercise appears to be limited by the rate at which glucose is absorbed by the  
266 intestine (Fuchs et al. 2019; Gonzalez et al. 2017; Jeukendrup and Jentjens 2000).

267           The typical intestinal glucose absorption pathway consists of an active component  
268 mediated by SGLT1 at the apical membrane of the enterocyte, followed by the passive transport  
269 of glucose across the basolateral membrane via GLUT2 (Röder et al. 2014). When luminal  
270 glucose concentrations are high, transport across the brush border membrane is thought to be  
271 facilitated by apical GLUT2 insertion (Chaudhry et al. 2012), resulting in a greater capacity for  
272 glucose uptake into the enterocyte. Thus, any factor that can influence apical GLUT2  
273 expression has the potential to alter the absorption and subsequent metabolism of exogenous  
274 glucose. The putative role for calcium in apical GLUT2 insertion relates to both cytoskeletal  
275 rearrangement of the enterocyte (Turner 2000) and SGLT1-dependent expression of PKC  $\beta$ II  
276 (Hug and Sarre 1993). Mace and colleagues (2007) demonstrated the necessity of calcium for  
277 myosin light chain kinase (MLCK) activity in isolated rat intestine, and in turn showed a  
278 facilitative role for MLCK activity in intestinal glucose absorption. Furthermore, these authors  
279 demonstrated a decrease in PKC  $\beta$ II expression in a calcium-deplete rat intestine (Mace et al.  
280 2007; Morgan et al. 2007). However, despite the putative effect of calcium on intestinal glucose  
281 absorption, the present study shows that the addition of high-dose calcium to  $1.2 \text{ g} \cdot \text{min}^{-1}$   
282 glucose does not enhance exogenous carbohydrate oxidation during endurance exercise.

283           The total calcium dose administered in the carbohydrate beverages approached the  
284 upper tolerable limit for adults of 2500 mg, according to recent reference intake guidelines  
285 (EFSA 2012). The 2000 mg dose provided in this study is a considerably greater quantity than  
286 the median 929 mg male daily intake observed in a cross-sectional study of UK national dietary  
287 habits (Whitton et al. 2011) and equates to the calcium content of approximately 1.6 L of cow's  
288 milk. While this dose is only likely to be achieved with conscious nutritional planning and  
289 supplementation, it is a notable strength of this study as it allows the potential effect of all  
290 tolerable doses to be excluded. As intestinal concentrations of calcium and glucose were not  
291 directly measured in the present study, the exact microenvironment subjected to the

292 enteroendocrine cells is unclear. However, intestinal calcium concentrations in the range 0.2 to  
293 3.0 mmol·L<sup>-1</sup> appeared to increase the release of glucagon-like peptide-1 in rodent intestine  
294 (Mace et al. 2012), a range of concentrations similar to the 0.25 to 2.0 mmol·L<sup>-1</sup> typically  
295 observed in the small intestine of humans after a high calcium-containing meal (Fordtran and  
296 Locklear 1966). Therefore, though not directly measured, the dosage of calcium provided in  
297 the present study is likely to have increased intestinal calcium concentrations to those  
298 previously observed to facilitate gut peptide secretion by the enteroendocrine cells. Evidence  
299 has found calcium to delay gastric emptying (Shafer et al. 1985), suggesting a potential  
300 facilitative role at the level of the intestine may have been washed out by a decreased gastric  
301 emptying rate in the calcium trial. The effect of calcium on gastric emptying during exercise  
302 with the present feeding pattern has not been established, and the absence of direct luminal  
303 calcium and glucose concentration measurement means this suggestion remains speculative.  
304 Moreover, as gastric emptying is not limiting in this context (Rehrer et al. 1992b) it is unlikely  
305 that any calcium-induced delay would outstrip a potential benefit to intestinal absorption.

306         The rate of glucose ingestion employed in the present study approximately reflects the  
307 theoretical maximum intestinal glucose absorption rate of 1.2 g·min<sup>-1</sup>. This was chosen to  
308 ensure SGLT1 was saturated, isolating any effect to the putative role of GLUT2. While this  
309 approximately reflects typical endurance athlete practice in line with nutritional guidelines  
310 (Burke et al. 2011), the potential for a role for calcium at lower rates of glucose ingestion is  
311 worthy of consideration. Many athletes are prone to gastrointestinal discomfort when  
312 consuming large amounts of carbohydrate during exercise (Rehrer et al. 1992a), particularly  
313 when ingestion rates exceed the rate of intestinal absorption leading to an accumulation of  
314 carbohydrate in the intestine (de Oliveira and Burini 2014). Therefore, to prevent  
315 gastrointestinal discomfort limiting performance, these individuals are likely to consume  
316 carbohydrate at reduced rates. While effectively reducing gastrointestinal symptoms, this

317 practice also leads to a suboptimal ingestion of carbohydrate from the perspective of  
318 maximising carbohydrate availability. Theoretically, as SGLT1 saturation is proposed to have  
319 a calcium-independent role on GLUT2 translocation to the apical membrane (Kellett and  
320 Helliwell 2000), a scenario in which SGLT1 remains unsaturated may allow calcium to have  
321 an independent effect. Therefore, further studies investigating the potential for calcium to  
322 increase the rate of intestinal glucose absorption during exercise at suboptimal exogenous  
323 carbohydrate ingestion rates may demonstrate a facilitative role.

324         A key limitation in this study was the lack of low  $^{13}\text{C}$  enrichment conditions, to allow  
325 accurate quantification of absolute exercising exogenous carbohydrate oxidation rates. The  
326 calculations used to quantify this variable with isotope ratio mass spectrometry are normalised  
327 to background enrichment (Pirnay et al. 1995), which is typically determined through an  
328 identical exercise bout conducted with the ingestion of carbohydrate with a low natural  
329 abundance of  $^{13}\text{C}$ , or during a trial with the ingestion of water alone (Barber et al. 2020; Rehrer  
330 et al. 1992b; Trommelen et al. 2017). As additional conditions could not be performed within  
331 the constraints of this project, these calculations were instead normalised to the mean values  
332 observed in the background trial of a recent study (Barber et al. 2020). This study was also  
333 conducted in the laboratories at the University of Bath and recruited participants from a similar  
334 target population as the present study. The duration (2 h) and intensity (60%  $\dot{V}\text{O}_{2\text{peak}}$ ) of the  
335 exercise bouts were also comparable. While this approach may reduce the accuracy of  
336 estimating absolute exogenous carbohydrate oxidation rates, the background enrichment is  
337 typically applied equally to both conditions so interpretations of between-trial differences  
338 would be unaffected. Furthermore, the peak exogenous glucose oxidation rates reported ( $\sim 0.8$   
339  $\text{g}\cdot\text{min}^{-1}$ ) are in good agreement with what would be expected when ingesting glucose at a rate  
340 of  $1.2 \text{ g}\cdot\text{min}^{-1}$  during cycling exercise (Gonzalez et al. 2017).



341 The present study investigated the study aims in only eight participants, and therefore  
342 it is possible that the study could be underpowered. However, the values obtained ( $0.83\pm 0.15$   
343  $\text{g}\cdot\text{min}^{-1}$  and  $0.88\pm 0.11 \text{ g}\cdot\text{min}^{-1}$  for GLU and GLU+CAL, respectively) result in difference  
344 between treatments of less than  $3 \text{ g}\cdot\text{h}^{-1}$ , which is unlikely to provide substantial changes to  
345 endurance performance. For example, data suggest that the increase in exogenous carbohydrate  
346 oxidation rates by increasing the fructose:maltodextrin ratio of a drink from 0.5:1.0 to 0.8:1.0  
347 is  $>10 \text{ g}\cdot\text{h}^{-1}$ , and increases endurance performance by  $>3\%$  (O'Brien et al. 2013). Assuming a  
348 linear relationship between exogenous carbohydrate oxidation and performance, the difference  
349 in exogenous carbohydrate oxidation rates in the present study would relate to a change in  
350 performance of  $<1\%$ . Nevertheless, the effect size generated by this pilot study could be used  
351 to adequately power future studies to definitely establish whether calcium influences  
352 exogenous carbohydrate oxidation rates.

353 In conclusion, according to the present data, the addition of calcium to a glucose-  
354 containing beverage does not appear to increase exogenous carbohydrate oxidation during  
355 prolonged submaximal exercise in healthy men. Therefore, this pilot study suggests that there  
356 is unlikely to be a meaningful role for co-ingestion of calcium with carbohydrate for optimising  
357 exogenous carbohydrate availability, at least when ingesting glucose at  $1.2 \text{ g}\cdot\text{min}^{-1}$ . Further  
358 research may however be required to test this hypothesis with a greater statistical power.

359

### 360 **Acknowledgements**

361 This work was supported by a Nutrition Society Summer Studentship award. J.T.G. has  
362 received research funding and/or has acted as a consultant for Arla Foods Ingredients,  
363 Lucozade Ribena Suntory, Kenniscentrum Suiker and Voeding, and PepsiCo. G.A.W has  
364 received research funding and/or has acted as a consultant for GlaxoSmithKline Ltd, Sugar  
365 Nutrition UK, Lucozade Ribena Suntory Ltd, Dairy Management Inc. and Volac International

366 Ltd. **Author Contributions:** B.J.N., G. A. W. and J. T. G.: conception and design, data analysis  
367 and interpretation. B.J.N. and J.T.G.: manuscript writing. B.J.N.: data collection. All authors  
368 read and approved the final manuscript.

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507 **Tables****Table 1.** Participant Characteristics ( $n = 8$ ).

Variable	Mean±SD
Age (y)	25±2
Height (cm)	178±7
Body Mass (kg)	75.1±7.4
BMI (kg·m <sup>2</sup> )	23.8±1.9
$\dot{V}O_2\text{max}$ (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	55.0±7.7
Maximal Power Output (W)	356±65
Maximal Power Output Relative to Body Mass (W/kg)	4.7±0.7
Resting Heart Rate (bpm)	52±6
Resting Blood Glucose Concentration (mmol·L <sup>-1</sup> )	5.1±0.3
Resting Blood Lactate Concentration (mmol·L <sup>-1</sup> )	0.9±0.3

**Note:** Data are mean±SD. BMI, Body mass index;  $\dot{V}O_2\text{max}$ , Maximum oxygen uptake.

508

509



510 **FIGURE LEGENDS**

511 **Figure 1.** Rates of oxygen consumption (**A**) and carbon dioxide production (**B**). Breath  $^{13}\text{CO}_2$   
512 enrichment (**C**), and rates of exogenous carbohydrate oxidation (**D**), during moderate-intensity  
513 cycling with the ingestion of glucose only (GLU) or glucose plus calcium ingestion  
514 (GLU+CAL) in healthy men. Data are mean $\pm$ SD.  $n = 8$ .

515

516 **Figure 2.** Blood glucose (**A**), and lactate concentrations (**B**), and ratings of gastrointestinal  
517 discomfort (**C**), and perceived exertion (**D**) during moderate-intensity cycling with the  
518 ingestion of glucose only (GLU) or glucose plus calcium ingestion (GLU+CAL) in healthy  
519 men. Data are mean $\pm$ SD.  $n = 8$ .