Title:

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

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Abstract

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

Eight healthy men cycled for 2 h at 50% peak power output, ingesting either 1.2 $g \cdot min^{-1}$ dextrose alone (GLU) or with the addition of 2000 mg calcium (GLU+CAL), in a randomised crossover design. Expired breath samples were collected to determine whole-body and exogenous glucose oxidation.

Peak exogenous glucose oxidation during GLU was 0.83±0.15 $g \cdot min^{-1}$, and was not enhanced during GLU+CAL (0.88±0.11 $g \cdot min^{-1}$, p = 0.541). The relative contributions of exogenous carbohydrate (19±3% vs. 20±2%, p = 0.434), endogenous carbohydrate (65±3% vs. 65±3%, p = 0.822) and fat (16±3% vs. 15±3%, p = 0.677) to total substrate utilisation did not differ between trials.

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

Key words

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

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Introduction

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

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

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

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

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

Materials and Methods

Participants

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

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

Experimental Design

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

Preliminary Trial

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

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

Main Trials

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

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

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

$$\text{Exogenous Carbohydrate Oxidation} = \dot{V}CO_2 \cdot \left(\frac{\delta Exp - \delta Exp_{bkg}}{\delta Ing - \delta Exp_{bkg}}\right)\left(\frac{1}{k}\right)$$

in which δExp is the ^{13}C enrichment of expired gas, δIng is the ^{13}C enrichment of the ingested solution, δExp_{bkg} is the background ^{13}C enrichment of expired gas determined using a water trial, and *k* is the $\dot{V}CO_2$ produced by the oxidation of 1 g of glucose (0.7467 L $CO_2 \cdot g^{-1}$). As a water trial was not conducted in this study, δExp_{bkg} was estimated using the mean values observed in a similar experiment in which endurance trained men completed 2 h of treadmill exercise at 60% $\dot{V}O_{2peak}$ (Barber et al. 2020).

Statistical Analysis

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

193 $g \cdot min^{-1}$, 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 $^{13}CO_2$ 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_{max} (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**

Rates of oxygen consumption and carbon dioxide production increased during exercise in both conditions (both $p < 0.001$), with no treatment effect (both $p > 0.529$) or trial*time interaction effect (both $p > 0.185$; **Figure 1A** and **1B**). Expired $^{13}CO_2$ enrichments increased during exercise in both conditions ($p < 0.001$), with no treatment effect ($p = 0.471$) or trial*time interaction effect ($p = 0.555$; **Figure 1C**). Concurrently, the rate of exogenous carbohydrate oxidation increased over time ($p < 0.001$), with no main effect of trial ($p = 0.346$) or trial*time interaction ($p = 0.500$; **Figure 1D**). Peak exogenous carbohydrate oxidation rates did not differ between trials (0.83±0.15 g·min^{-1} vs. 0.88±0.11 g·min^{-1} with GLU vs. GLU+CAL, respectively, $p = 0.541$), the iAUC values also did not differ between conditions (59.6±15.2 g·120 min vs. 64.3±14.5 g·120 min with GLU vs. GLU+CAL, respectively, $p = 0.390$), and the total amounts of exogenous carbohydrate oxidised throughout the exercise bouts were also unaffected by the co-ingestion of calcium with glucose, compared to glucose alone (58.7±10.2 g vs. 64.9±9.2 g with GLU vs. GLU+CAL, respectively, $p = 0.309$). When expressed relative to total substrate oxidation, the relative contributions of exogenous carbohydrate (19±3% vs. 20±2% with GLU vs. GLU+CAL, respectively, $p = 0.434$), endogenous carbohydrate (65±3% vs. 65±3% with GLU vs. GLU+CAL, respectively, $p = 0.822$) and fat (16±3% vs. 15±3% with GLU vs. GLU+CAL, respectively, $p = 0.677$) did not significantly differ between trials.

Blood Metabolite Concentrations

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

Subjective Ratings

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

Discussion

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

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

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

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

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

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

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

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

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

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

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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 ($kg \cdot m^2$)	23.8±1.9
$\dot{V}O_2max$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	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 ($mmol \cdot L^{-1}$)	5.1±0.3
Resting Blood Lactate Concentration ($mmol \cdot L^{-1}$)	0.9±0.3

Note: Data are mean±SD. BMI, Body mass index; $\dot{V}O_2max$, Maximum oxygen uptake.

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FIGURE LEGENDS

Figure 1. Rates of oxygen consumption (**A**) and carbon dioxide production (**B**). Breath $^{13}CO_2$ enrichment (**C**), and rates of exogenous carbohydrate oxidation (**D**), during moderate-intensity cycling with the ingestion of glucose only (GLU) or glucose plus calcium ingestion (GLU+CAL) in healthy men. Data are mean±SD. n = 8.

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