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Title: Carbohydrate supplementation and the influence of breakfast on fuel use in hypoxia

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Abstract

Purpose: This study investigated the effect of carbohydrate supplementation on substrate oxidation during exercise in hypoxia after pre-exercise breakfast consumption and omission.

Methods: Eleven men walked in normobaric hypoxia ($F_{iO_2} \sim 11.7\%$) for 90-min at 50% of hypoxic $\dot{V}O_{2max}$. Participants were supplemented with a carbohydrate beverage ($1.2g \cdot min^{-1}$ glucose) and a placebo beverage (both enriched with $U-^{13}C_6$ D-glucose) after breakfast consumption and after omission. Indirect calorimetry and isotope ratio mass spectrometry were used to calculate carbohydrate (exogenous and endogenous (muscle and liver)) and fat oxidation.

Results: In the first 60-min of exercise, there was no significant change in relative substrate oxidation in the carbohydrate compared with placebo trial after breakfast consumption or omission (both $p = 0.99$). In the last 30-min of exercise, increased relative carbohydrate oxidation occurred in the carbohydrate compared with placebo trial after breakfast omission (44.0 ± 8.8 vs. 28.0 ± 12.3 , $p < 0.01$) but not consumption (51.7 ± 12.3 vs. 44.2 ± 10.4 , $p = 0.38$). In the same period, a reduction in relative liver (but not muscle) glucose oxidation was observed in the carbohydrate compared with placebo trials after breakfast consumption (liver: $7.7 \pm 1.6\%$ vs. $14.8 \pm 2.3\%$, $p < 0.01$; muscle: $25.4 \pm 9.4\%$ vs. $29.4 \pm 11.1\%$, $p = 0.99$) and omission (liver: $3.8 \pm 0.8\%$ vs. $8.7 \pm 2.8\%$, $p < 0.01$; muscle: $19.4 \pm 7.5\%$ vs. $19.2 \pm 12.2\%$, $p = 0.99$). No significant difference in relative exogenous carbohydrate oxidation was observed between breakfast consumption and omission trials ($p = 0.14$).

Conclusion: In acute normobaric hypoxia, carbohydrate supplementation increased relative carbohydrate oxidation during exercise (> 60 min) after breakfast omission, but not consumption.

Key words: altitude, endogenous, exogenous, utilisation, endurance

Introduction

Hypoxia experienced at altitude induces a curvilinear decrement in endurance performance (1). As such, methods of overcoming this impairment in performance warrant investigation. The effect of carbohydrate supplementation on substrate oxidation in sea level conditions is well established (2-4) however differing effects have been observed in hypoxia (5-7). These responses may be explained by the contrasting use of pre-exercise nutritional status within experimental design (8, 9). The hormonal and substrate storage responses to breakfast consumption/omission which induce the aforementioned effect (10) likely also have implications for substrate oxidation during exercise with carbohydrate supplementation. As such, the effect of carbohydrate supplementation and the influence of pre-exercise nutritional status on substrate oxidation in hypoxia warrant further investigation.

After pre-exercise breakfast consumption, Péronnet et al (6) observed a greater relative contribution of carbohydrate oxidation to energy expenditure during exercise matched for relative intensities (77% altitude-specific $\dot{V}O_{2\max}$) in acute hypobaric hypoxia (445 mmHg or 4300 m) compared with normoxia following glucose ingestion ($1.75 \text{ g}\cdot\text{min}^{-1}$). This was attributed to a greater reliance on endogenous carbohydrate oxidation in hypoxia compared with normoxia. In contrast, O'Hara et al (5) utilised participants after pre-exercise breakfast omission and observed a significantly lower contribution of whole-body carbohydrate oxidation to energy expenditure during exercise matched for relative intensities (~74% altitude specific $\dot{V}O_{2\max}$) in hypoxia (terrestrial altitude ~3375 m) compared with normoxia after carbohydrate supplementation ($1.2 \text{ g}\cdot\text{min}^{-1}$ glucose, $0.6 \text{ g}\cdot\text{min}^{-1}$ fructose). A significant reduction in endogenous carbohydrate oxidation was observed in hypoxia compared with normoxia, derived from a reduced reliance on muscle glycogen. This varied response between studies appears to be derived from an altered reliance on endogenous carbohydrate stores in hypoxia, which may be determined by pre-exercise nutritional status. Exogenous carbohydrate

oxidation was not different between conditions in these studies, but reduced rates have been observed in hypoxia in females recently (11). Data that challenge the concept that breakfast may explain discrepancies in the literature have been published recently (12). Margolis et al (12) utilised participants after a 12 hour fast and observed an increased reliance on carbohydrate oxidation (derived from endogenous sources) during exercise in acute hypoxia compared with normoxia. These findings contrast those observed by O'Hara et al (5) who also utilised fasted participants. *Albeit*, Margolis et al (12) utilised exercise matched for absolute intensities in hypoxia compared with normoxia, during which it may be expected that an increased relative exercise intensity, rather than hypoxia *per se*, may induce an increased reliance on carbohydrate oxidation (9, 13).

To the authors' knowledge, only one study has investigated the effect of carbohydrate supplementation on substrate oxidation in hypoxia in a placebo-controlled fashion, utilising ¹³C tracer methods (7). Young et al (7) observed an increase in total carbohydrate oxidation during exercise (55% $\dot{V}O_{2max}$) with carbohydrate supplementation (1.00 g·min⁻¹ glucose, 0.82 g·min⁻¹ fructose) compared with placebo in acute hypoxia (terrestrial altitude ~4300 m). As expected, this increased reliance on carbohydrate oxidation in the carbohydrate group was derived from exogenous sources. However, as Young et al (7) utilised participants in the fasted state, the effects of carbohydrate supplementation are likely more pronounced than in fully fed participants. Interestingly, and in contrast to previous literature (5, 6) they also observed a reduced reliance on exogenous carbohydrate oxidation during exercise matched for absolute intensities in hypoxia compared with normoxia, questioning the efficacy of carbohydrate supplementation in such conditions. This finding has also been replicated in fasted participants recently (12). This is especially surprising given the use of exercise matched for absolute intensities in hypoxia vs. normoxia, and the increased propensity for carbohydrate oxidation during exercise at greater exercise intensities (14).

Carbohydrate supplementation has been demonstrated to improve time trial performance of sea level residents during energy deficit in hypoxia (15). However, later research by the same group observed no difference in time trial performance after carbohydrate supplementation in fed participants (*albeit* in moderate altitude residents) (16). In addition, recent literature has demonstrated no effect of carbohydrate supplementation on time trial performance in acute or chronic hypoxia when fasted and in energy deficit respectively (17). The effects of carbohydrate supplementation in both the fasted and fed state are yet to be determined using a within-study design, and an ecologically valid mode of exercise in relation to the severity of hypoxia. Therefore, the purpose of this study was to investigate the effect of carbohydrate supplementation on substrate utilisation (carbohydrate (exogenous and endogenous (muscle and liver glycogen)) and fat oxidation) and endurance performance in hypoxia after both breakfast consumption and omission. As a methodologically novel approach to this study, both placebo and carbohydrate beverages were enriched with ^{13}C glucose to allow the comparison of endogenous (muscle and liver) carbohydrate contributions between trials.

Methods

Participants

Eleven, physically active, healthy male volunteers (23 ± 3 years, 178.0 ± 7.0 cm, 76.6 ± 7.0 kg) provided written, informed consent to participate in this study. The study received institutional ethical approval (Leeds Beckett research ethics committee, application reference 46180) and was conducted in accordance with the Declaration of Helsinki. All participants were non-smokers, normotensive, free from food allergies and were not taking any medication. None of the participants had travelled to an altitude of >1500 m within the previous three months and were all currently residing at an altitude of <500 m.

A priori power analysis revealed that eight participants provided 80% power to detect differences in absolute whole body carbohydrate oxidation between carbohydrate and placebo groups during exercise in hypoxia, assuming an effect size of 0.97 (7) and an alpha of 0.05. In addition, a priori power analysis also revealed that eight participants provided 80% power to detect differences in absolute whole body carbohydrate oxidation between breakfast consumption and omission trials during exercise in hypoxia, assuming an effect size of 1.30 (8) and an alpha level of 0.05. Whilst the effect sizes extracted from previous literature were studies using two trials, effects sizes for relevant variables are likely similar, and a correction for four trials was made to match the experimental design of the present study. In excess of the required sample size, twelve participants were initially recruited to this study, however one participant dropped out due to injury, therefore eleven participants completed the study.

Experimental design

Participants were required to make a total of seven visits to the laboratory. The first visit involved pre-exercise screening, anthropometry, verbal familiarisation with testing procedures, sickle cell trait test and a baseline 12 lead ECG test (18). Further exclusion criteria included diabetes and thyroid disorders. The second and third visit required participants to be acutely exposed to normobaric hypoxia (fraction of inspired oxygen (F_{iO_2}): ~11.7% when considering water vapour partial pressure and daily fluctuations in barometric pressure (19)) equivalent to 4300 m (partial pressure of inspired oxygen (P_{iO_2}): 83 mmHg) in an environmental chamber (TISS, Alton, UK and Sporting Edge, Sheffield on London, UK). On visit 2, participants completed a sub-maximal and maximal exercise test to calculate walking speed required to elicit 50% $\dot{V}O_{2max}$ in normobaric hypoxia. On visit 3, participants completed two 30-minute sub-maximal walking tests at 50% $\dot{V}O_{2max}$ (pre and post breakfast) and a 3 km familiarisation time trial (see experimental trials). The 30-minute sub-maximal walking tests were used to measure background ^{13}C -enrichment of expired CO_2 observed in response to exercise (no

glucose or placebo beverage ingested) for use in the calculation of exogenous carbohydrate oxidation (see calculations). These two preliminary trials were separated by >48 hours.

On visits 4-7 (normobaric hypoxia equivalent to 4300 m), participants completed a 4-hour 30-minute experimental trial which included rest, followed by a 90 minute sub-maximal walking test (50% $\dot{V}O_{2max}$), 3 km time trial and a post exercise rest period (Figure 1). Two trials involved pre-exercise breakfast consumption followed by ingestion of a carbohydrate (B-CHO) or placebo beverage (B-PLA) and the other two trials involved pre-exercise breakfast omission followed by ingestion of a carbohydrate (F-CHO) or placebo beverage (F-PLA) during the 1-hour 30-minute sub-maximal walking test. The carbohydrate beverage trials involved ingestion of 1.2 g·min⁻¹ (108 g) of glucose (D-glucose, Thornton and Ross LTD, Huddersfield, UK). Stock glucose was enriched using 0.18 g of U-¹³C₆ D-glucose (Cambridge Isotope Laboratories Inc, Tewksbury, MA, USA), achieving an enrichment of $\delta^{13}C = 115.6\%$. As a methodological approach novel to hypoxia, the placebo beverage trials involved ingestion of a water solution, also enriched with 0.18 g U-¹³C₆ D-glucose (99 atom %). This allowed for the comparison of endogenous (muscle and liver) carbohydrate contributions between trials. All $\delta^{13}C$ measurements are quoted with reference to the internationally accepted standard for carbon isotope measurements, VPDB. The ¹³C abundance of stock glucose and ¹³C enrichment of spiked glucose was determined using liquid chromatography coupled to isotope ratio mass spectrometry (LC-IRMS); Isoprime, Cheadle, UK) (20). The enriched water solution has been shown to have negligible effects on substrate oxidation (21). Each beverage contained 25.7 mmol·L⁻¹ sodium chloride (2.25 g). These visits were separated by ≥ 7 days and pre-exercise nutritional status (breakfast consumption or omission) was randomised in a single blind fashion. The order of beverage ingestion was randomised in a double-blind fashion by a researcher independent to the study.

Diet and physical activity before testing

Participants recorded their food intake for the 24 hours before the second preliminary trial (visit 3) and were instructed to replicate this for each experimental trial. During this time participants were asked not to perform strenuous activity or consume caffeine or alcohol. Participants consumed a standardised meal prior to the second preliminary trial and all experimental trials which contained fusilli pasta, pasta sauce, cheese, semi-skimmed milk and an apple (1043 kcal, 55% carbohydrate, 30% fat, 15% protein). This meal was consumed to minimise the possibility of a second meal effect confounding glycaemic control or any other measured variables (22). A week prior to and throughout the duration of the study, participants were also asked to refrain from consuming carbohydrates derived from plants which utilise the C₄ photosynthetic cycle, in which there is a higher natural abundance of ¹³C (23). This ensured that background ¹³CO₂ abundance was less likely to be perturbed from oxidation of endogenous and dietary substance stores from naturally “enriched” C₄ origin.

Preliminary testing

On visit 2, participants completed a sub-maximal and maximal exercise test in normobaric hypoxia, as described previously (8). Briefly, the sub-maximal test involved walking at 10% gradient, with a 10kg backpack at a range of walking speeds. The maximal test involved running at a constant speed, dependant on fitness, aiming for an RPE of 12. The test began at 1% gradient and increased every minute until volitional exhaustion. These data were used to establish walking speeds that would elicit 50% $\dot{V}O_{2max}$ whilst carrying a 10kg rucksack at a 10% gradient.

On visit 3, participants arrived at the environmental chamber fasted (~12 hours overnight) and subsequently completed a 30-minute sub-maximal walking test. Participants were then permitted 15 minutes to consume a standardised breakfast (535 kcal, 58% carbohydrate, 24% fat, 18% protein) as detailed previously (8). After breakfast consumption, participants rested

for 1 hour, then repeated the 30-minute sub-maximal walking test. Expired gas was collected (see experimental trials) at the end of each sub-maximal walking test to measure background ^{13}C enrichment of expired gas without ingestion of the carbohydrate or placebo drink for calculation of exogenous carbohydrate oxidation (see calculations). After a 5-minute rest period, participants then completed a 3 km time trial to assess performance (see experimental trials).

Experimental trials

Participants entered the environmental chamber at 7:30am, following a 12 hour fast. Participants then rested for 30 minutes. At 30 minutes in the B-CHO and B-PLA trials, participants were allowed 15 minutes to consume a standardised breakfast (as per preliminary trial) but remained fasted in the F-CHO and F-PLA trials. At 45 minutes, participants in all trials rested for a further hour. At 1-hour 45 minutes participants completed a 1-hour 30 minute sub-maximal ($50\% \dot{V}\text{O}_{2\text{max}}$) walking test at a 10% gradient, carrying a 10kg rucksack, to mimic the demands of high altitude trekking. Within each nutritional sub-group (breakfast omission and breakfast consumption), one trial consumed a carbohydrate and one trial consumed a placebo beverage. Each beverage was consumed pre-exercise (600 ml) and every 15 minutes during exercise (150 ml). A total of 1.5 L of carbohydrate or placebo solution was consumed over the course of the trial. After a short rest period, participants then completed a self-paced 3 km time trial at 10% gradient, carrying a 10kg rucksack. A rolling start was employed, in which the speed required to elicit $50\% \dot{V}\text{O}_{2\text{max}}$ was utilised. Once the time trial had started, participants had full control over speed. Participants were informed of their distance every 500 m, but were blinded to their speed and time (24). Following exercise, participants rested for a further 30 minutes before returning to sea level conditions.

Measurements

217 Heart rate, SpO₂ and RPE

218 Heart rate and SpO₂ were measured every 15 minutes during rest. Heart rate, SpO₂ and RPE
219 were measured every 10 minutes throughout exercise.

220 Expired breath collection

221 Expired gas breath samples were collected using an online gas analysis system (Metalyser,
222 Cortex, Germany). These measurements were made intermittently throughout exercise (20 –
223 30 minutes, 50 minutes – 1-hour, 1-hour 5 minutes – 1-hour 15 minutes, 1 hour 20 minutes –
224 1-hour 30 minutes). Participants were fitted with a facemask 5 minutes prior to the collection
225 period whilst the participant was seated. In addition, samples of expired gas were collected in
226 duplicate via a mixing chamber in 12ml Labco Exetainers (SerCon Ltd, Crewe, UK) for the
227 analysis of ¹³C/¹²C in expired CO₂ during the final 60 seconds of each gas collection period
228 (pre-exercise, 60 min, 75 min and 90 min exercise).

229 Blood sampling

230 Venous blood samples were drawn for the analysis of plasma glucose, plasma lactate and serum
231 FFA at baseline (before entry to the chamber), 30 minutes (pre-prandial) and 1-hour 45 minutes
232 (post-prandial), as well as during the sub-maximal walking test at 30, 60, 75 and 90 minutes
233 and also after exercise at 4-hour 30 minutes (post-exercise). Samples for serum insulin were
234 drawn at all timepoints with the exclusion of 30 and 75 minutes of the sub-maximal walking
235 test. Samples for the analysis of ¹³C plasma glucose enrichment were drawn at 1-hour 45
236 minutes (post-prandial) and at 60, 75 and 90 minutes during sub-maximal exercise.

237 Analyses

238 Commercially available enzyme-linked immunosorbent assay kits were used to determine
239 serum concentrations of insulin (IBL, Hamburg, Germany). To eliminate interassay variation,
240 all samples from each participant were analysed on the same plate. Plasma glucose and lactate,

and serum FFA were measured photometrically with reagents from Instrumentation Laboratories (Lexington, MA) and Randox Laboratories (Crumlin, UK). The within batch CV were as follows: insulin 6.1%, glucose 1.6%, lactate 2.4% and FFA 3.8%.

The ^{13}C abundance of stock glucose and ^{13}C enrichment of spiked glucose was determined using liquid chromatography coupled to isotope ratio mass spectrometry (LC-IRMS; Isoprime, Cheadle, UK) using L-Fucose as an isotopic internal standard as previously described (20). The $^{13}\text{C}/^{12}\text{C}$ ratio in expired CO_2 was determined using isotope ratio mass spectrometry (IRMS; AP2003, GVI instruments Ltd, Manchester, UK). The isotopic ratio $^{13}\text{C}/^{12}\text{C}$ is derived against laboratory CO_2 (itself calibrated against VPDB) from the ion beam area ratio measurements with correction of the small contribution of $^{12}\text{C}^{16}\text{O}^{17}\text{O}$ at m/z 45 (25). The $^{13}\text{C}/^{12}\text{C}$ ratio in plasma glucose was determined using LC-IRMS as detailed previously (20).

Calculations

Substrate oxidation was calculated using relevant equations for exercise periods (26). The isotopic enrichment of the ingested glucose was expressed in standard $\delta^{13}\text{C}$ units (‰) relative to VPDB (25). Exogenous carbohydrate oxidation derived from the ingested glucose was calculated using the following equation (27):

$$\text{Exogenous carbohydrate oxidation (g} \cdot \text{min}^{-1}) = \dot{V}\text{CO}_2((R_{\text{exp}} - R_{\text{ref}}) \div (R_{\text{exo}} - R_{\text{ref}})) \div k$$

$\dot{V}\text{CO}_2$ is represented in $\text{L} \cdot \text{min}^{-1}$, R_{exp} is the measured isotopic composition in expired CO_2 during exercise at different time points, R_{ref} is the isotopic composition of expired CO_2 during exercise with the ingestion of placebo (background), R_{exo} is the measured isotopic enrichment of the ingested glucose, and k is the rate adjusted value for the complete oxidation of glucose (27). Changes in the background isotopic composition of expired CO_2 with a placebo beverage could not be determined from the experimental trials due to the enrichment of the placebo

beverage with U-¹³C₆D-glucose. Therefore, data from the preliminary trial (visit 3) in which expired gas was collected during exercise pre and post breakfast were utilised. The use of R_{ref} from expired CO₂ during exercise with placebo is typical of studies in this area (21). The ¹³C-enrichment of exogenous glucose is high, and the use of R_{ref} during exercise cancels the confounding effect of small fluctuations in background enrichment of expired CO₂ derived from the Western European diet. Endogenous carbohydrate oxidation was calculated by subtracting exogenous carbohydrate oxidation from total carbohydrate oxidation.

Computations were made on the assumption that, in response to exercise, ¹³C is not lost irreversibly in pools of tricarboxylic acid cycle intermediates and/or bicarbonate, and that ¹³CO₂ recovery in expired gases was complete or almost complete during exercise (28). Such computation has been shown to underestimate exogenous carbohydrate oxidation rates at the beginning of exercise because of the delay between ¹³CO₂ production in tissues and expired ¹³CO₂ at the mouth (29). As such, exogenous carbohydrate oxidation rates are presented from 60 minutes onwards during sub-maximal exercise, where it is expected that there would be isotopic equilibrium in the tissues and at the mouth.

Plasma glucose oxidation was computed at 60, 75 and 90 minutes during the sub-maximal walking test, based on the isotopic composition of plasma glucose (R_{glu}) using the following equation (30):

$$\text{Plasma glucose oxidation (g} \cdot \text{min}^{-1}) = VCO_2 ((R_{exp} - R_{ref}) \div (R_{glu} - R_{ref})) \div k$$

The oxidation rate of muscle glycogen (g·min⁻¹) either directly or through the lactate shuttle (31) was calculated by subtracting plasma glucose oxidation from total carbohydrate oxidation. Finally, the amount of glucose released from the liver was estimated as the difference between plasma glucose oxidation and exogenous carbohydrate oxidation. Liver glucose oxidation

values are representative of contributions from liver glycogen, gluconeogenesis, as well as residual glucose uptake from the gut derived from previous breakfast consumption.

Statistical analysis

Data are expressed as mean \pm standard deviation (SD) in text and mean \pm standard error (SE) in figures. All data were analysed using IBM SPSS statistics (v24 for Windows; SPSS; Chicago, IL). The trapezoid method was used to calculate AUC for substrate oxidation and hormone concentrations. The periods of AUC were defined as pre-prandial (0 - 30 minutes), post-prandial (45 - 1-hour 45 minutes), submaximal exercise (0 - 60 minutes and 60 - 90 minutes) and post exercise (4 hour- 4 hour 30 minutes). Two-way repeated measures ANOVA (time x trial) was used to determine differences between absolute and relative carbohydrate and fat oxidation, hormone concentrations, $\delta^{13}\text{CO}_2$ in expired gas and plasma glucose, and rates of oxidation of plasma glucose, liver glucose and muscle glycogen. One-way repeated measures ANOVA was used to determine differences between trials for absolute (AUC) and relative contributions of endogenous carbohydrate, liver glucose, muscle glycogen, plasma glucose (only absolute) oxidation, energy expenditure, heart rate, SpO_2 and RPE and time trial times. Where significant main effects were found, further post-hoc analysis was performed using Bonferroni correction for multiple comparisons. Paired sample t-tests were used to determine differences in relative and absolute exogenous carbohydrate oxidation and total absolute exogenous carbohydrate oxidation (AUC). Effect sizes are presented as Cohen's *d* and interpreted as ≤ 0.2 trivial, > 0.2 small, > 0.6 moderate, > 1.2 large, > 2 very large, > 4 extremely large (32).

Results

Maximal oxygen uptake and walking speeds

$\dot{V}O_{2\max}$ in hypoxia was $40.6 \pm 4.3 \text{ ml}\cdot\text{kg}\cdot\text{min}^{-1}$ and this elicited a walking speed of $2.9 \pm 0.5 \text{ km}\cdot\text{h}^{-1}$ in the experimental trials (B-CHO: $50.0 \pm 8.4\% \dot{V}O_{2\max}$; B-PLA: $49.0 \pm 8.1 \dot{V}O_{2\max}$; F-CHO: $49.3 \pm 8.3 \dot{V}O_{2\max}$; F-PLA: $49.0 \pm 8.1 \dot{V}O_{2\max}$, $p = 0.99$).

Energy expenditure

Energy expenditure was not significantly different between trials (B-CHO: $4003 \pm 671 \text{ kJ}$; B-PLA: $3648 \pm 726 \text{ kJ}$; F-CHO: $3768 \pm 598 \text{ kJ}$; F-PLA: $3563 \pm 621 \text{ kJ}$; all $p = 0.99$, $d \leq 0.32$).

Total Carbohydrate and Fat oxidation

Results for total carbohydrate and fat oxidation for the full exercise duration are described in supplemental digital content (see text document, supplemental digital content 1 for descriptive text and statistics). Due to the necessary partitioning of exercise (i.e. 0-60 min and 60-90 min) as a result of ^{13}C ingestion, exercise will be discussed in relation to the first 60 minutes, and last 30 minutes herein.

During the first hour of exercise, absolute (Table 1) and the relative contribution of carbohydrate oxidation to energy expenditure was significantly higher after breakfast consumption compared with omission in the carbohydrate (absolute: $p < 0.01$, $d = 1.10$; relative: $51.0 \pm 10.4\%$ vs. $38.1 \pm 7.8\%$, $p < 0.01$, $d = 1.42$) and placebo trials (absolute: $p < 0.01$, $d = 1.07$; relative: 47.8 ± 10.0 vs. 32.1 ± 12.3 , $p < 0.01$, $d = 1.41$). In addition, absolute and the relative contribution of carbohydrate oxidation to energy expenditure were not significantly different between carbohydrate and placebo trials after breakfast consumption (absolute: $p = 0.86$, $d = 0.35$; relative: $p = 0.99$, $d = 0.32$) or omission (absolute: $p = 0.99$, $d = 0.39$; relative: $p = 0.99$, $d = 0.60$). In the same period, absolute fat oxidation was significantly higher after breakfast omission compared with consumption in the placebo trials ($p < 0.01$, $d = 0.77$), but not in the carbohydrate trial ($p = 0.15$, $d = 0.65$). The relative contribution of fat oxidation to energy expenditure was significantly higher after breakfast omission compared

with consumption in the carbohydrate ($61.9 \pm 7.8\%$ vs. 49.0 ± 10.4 $p < 0.01$, $d = 1.42$) and placebo trials ($67.9 \pm 12.3\%$ vs. 52.2 ± 10.0 , $p < 0.01$, $d = 1.41$). In addition, absolute and the relative contribution of fat oxidation were not significantly different between carbohydrate and placebo trials after breakfast consumption (absolute: $p = 0.99$, $d = 0.13$; relative: $p = 0.99$, $d = 0.32$) or omission (absolute: $p = 0.99$, $d = 0.30$; relative: $p = 0.99$, $d = 0.60$).

During the last 30 minutes of exercise, absolute (Table 1) and the relative (Figure 2) contribution of carbohydrate oxidation was higher after breakfast consumption compared with omission in the placebo (absolute: $p = 0.02$, $d = 0.99$; relative: $p < 0.01$, $d = 1.43$) but not carbohydrate trials (absolute: $p = 0.20$, $d = 0.59$; relative: $p = 0.20$, $d = 0.73$). In addition, the absolute and relative contribution of carbohydrate oxidation was significantly higher in the carbohydrate compared with placebo trial after breakfast omission (absolute: $p = 0.02$, $d = 1.08$; relative: $p < 0.01$, $d = 1.52$) but not consumption (absolute: $p = 0.39$, $d = 0.58$, relative: $p = 0.38$, $d = 0.66$). In the same period, absolute and the relative contribution of fat oxidation to energy expenditure was higher after breakfast omission compared with consumption in the placebo (absolute: $p < 0.01$, $d = 0.91$; relative: $p < 0.01$, $d = 1.43$) but not carbohydrate trials (absolute: $p = 0.57$, $d = 0.51$; relative: $p = 0.20$, $d = 0.73$). In addition, absolute and the relative contribution of fat oxidation were significantly higher in the placebo compared with carbohydrate trials after breakfast omission (absolute: $p = 0.02$, $d = 0.90$; relative: $p < 0.01$, $d = 1.52$) but not consumption (absolute: $p = 0.77$, $d = 0.42$; relative: $p = 0.38$, $d = 0.66$).

Expired gas and plasma glucose

Results for $\delta^{13}\text{CO}_2$ enrichment in expired gas and plasma glucose are presented in supplemental digital content (see text, supplemental materials 1 for descriptive text; see figure, supplemental digital content 2 for visual representation of data)

Exogenous and endogenous carbohydrate oxidation

There was no significant difference in exogenous carbohydrate oxidation rates (Figure 3A) after breakfast consumption compared with omission in the carbohydrate trials at 60 min (0.27 ± 0.12 vs. 0.31 ± 0.07 g.min⁻¹), 75 min (0.34 ± 0.10 vs. 0.38 ± 0.10 g.min⁻¹) or 90 min (0.43 ± 0.13 vs. 0.43 ± 0.12 g.min⁻¹) ($p = 0.30$). The contribution of exogenous carbohydrate oxidation in the placebo trials was considered negligible (< 0.001 g.min⁻¹). The relative contribution of exogenous carbohydrate oxidation in the last 30 minutes of exercise was not significantly different between breakfast consumption and omission in the carbohydrate trials ($18.6 \pm 3.5\%$ vs. $20.8 \pm 3.4\%$, $p = 0.14$, $d = 0.59$, Figure 3A). The total absolute exogenous oxidation in the last 30 minutes of exercise was also not significantly different between breakfast consumption and omission in the carbohydrate trials ($p = 0.23$, $d = 0.28$, Table 2).

Total absolute endogenous carbohydrate oxidation in the last 30 minutes of exercise (Table 2) was significantly higher after breakfast consumption compared with omission in the carbohydrate ($p = 0.03$, $d = 0.95$) and placebo trials ($p = 0.02$, $d = 0.99$). There was no significant difference in endogenous carbohydrate oxidation in the last 30 minutes of exercise between the carbohydrate and placebo trials after breakfast consumption ($p = 0.18$, $d = 0.77$) or omission ($p = 0.99$, $d = 0.41$).

Oxidation of plasma glucose, liver glucose and muscle glycogen

Plasma glucose oxidation rates (Figure 3B) were significantly higher after breakfast consumption compared with omission in the placebo trials during exercise at 60, 75 and 90 min (all $p < 0.01$, $d > 1.51$) but not carbohydrate trials ($p > 0.31$, $d < 0.55$). Plasma glucose oxidation rate was higher in the carbohydrate compared with placebo trials after breakfast consumption at 75 min and 90 min ($p < 0.01$, $d > 2.25$) but not 60 min ($p = 0.09$, $d = 1.23$). Plasma glucose oxidation rate was higher in the carbohydrate compared with placebo trials after breakfast omission at 60, 75 and 90 min ($p < 0.01$, $d > 3.20$). Total absolute plasma glucose oxidation

during the last 30 minutes of exercise (Table 2) was significantly higher after breakfast consumption compared with omission in the placebo ($p < 0.01$, $d = 1.86$) but not carbohydrate trials ($p = 0.99$, $d = 0.34$). Total absolute plasma glucose oxidation was also significantly higher in the carbohydrate compared with placebo trials after breakfast consumption ($p < 0.01$, $d = 2.21$) and omission ($p < 0.01$, $d = 3.42$).

Liver glucose oxidation rates (Figure 3C) were significantly higher after breakfast consumption compared with omission at 60, 75 and 90 min in the carbohydrate ($p < 0.049$, $d > 1.55$) and placebo trials (all $p < 0.01$, $d > 1.51$). Liver glucose oxidation rates were also significantly higher in the placebo compared with carbohydrate trials at 60, 75 and 90 min after breakfast consumption ($p < 0.02$, $d > 1.83$) and omission ($p < 0.01$, $d > 1.83$). Relative and total absolute (Table 2) liver glucose oxidation was significantly higher after breakfast consumption compared with omission during the last 30 minutes of exercise in the carbohydrate (absolute: $p < 0.01$, $d = 2.03$; relative: $7.7 \pm 1.6\%$ vs. $3.8 \pm 0.8\%$, $p < 0.01$, $d = 3.20$) and placebo trials (absolute: $p < 0.01$, $d = 1.86$; relative: 14.8 ± 2.3 vs. $8.7 \pm 2.8\%$, $p < 0.01$, $d = 2.42$). Relative and total absolute liver glucose oxidation was significantly lower in the carbohydrate compared with placebo trials after breakfast consumption (relative: $p < 0.01$, $d = 3.67$; absolute: $p < 0.01$, $d = 2.31$) and omission (relative: $p < 0.01$, $d = 2.77$; absolute: $p < 0.01$, $d = 2.18$).

Muscle glycogen oxidation rates (Figure 3D) were significantly higher after breakfast consumption compared with omission at 60-min in the carbohydrate trials ($p = 0.04$, $d = 0.98$) but not placebo trials ($p = 0.13$, $d = 0.69$). Muscle glycogen oxidation rates were not significantly different at 60 min between carbohydrate and placebo trials after breakfast consumption ($p = 0.99$, $d = 0.03$) or omission ($p = 0.99$, $d = 0.06$). There was no significant difference between trials in muscle glycogen oxidation rates during exercise at 75 min ($p > 0.12$, $d < 0.64$), or 90 min ($p > 0.19$, $d < 0.57$). The relative, but not absolute (Table 2) contribution of muscle glycogen oxidation to energy expenditure during the last 30 minutes of

exercise was higher after breakfast consumption compared with omission in the placebo trials (relative: $29.4 \pm 11.1\%$ vs. 19.2 ± 12.2 , $p = 0.04$, $d = 0.87$; absolute: $p = 0.14$, $d = 0.65$) and approached significance in the carbohydrate trials (relative: $25.4.0 \pm 9.4\%$ vs. $19.4 \pm 7.5\%$, $p = 0.09$, $d = 0.71$; absolute: $p = 0.14$, $d = 0.64$). There was no significant difference in the relative or absolute contribution of muscle glycogen oxidation to energy expenditure during the last 30 minutes of exercise in the carbohydrate compared with placebo trials after breakfast consumption (relative: $p = 0.99$, $d = 0.38$; absolute: $p = 0.99$, $d = 0.30$) or omission (relative: $p = 0.99$, $d = 0.02$; absolute: $p = 0.99$, $d = 0.03$).

Blood biochemistry

A significant effect of time (all $p < 0.01$) was observed for all analytes (Figure 4). A significant effect of trial was observed for all analytes ($p < 0.01$), except lactate ($p = 0.17$). Further, a significant interaction effect of time x trial was also observed for all analytes (all $p < 0.01$). All significant pairwise statistical comparisons are presented in Figure 4.

3 km time trial performance

Time to completion (see figure, supplemental digital content 3 for visual representation of data) was not significantly different between trials (B-CHO: 2121 ± 230 seconds, B-PLA: 2154 ± 284 seconds, F-CHO: 2134 ± 289 seconds, F-PLA: 2209 ± 213 seconds; $p = 0.99$, $d \leq 0.30$).

Heart rate, SpO₂ and RPE

There were no significant differences between trials for SpO₂ ($p \geq 0.45$, $d \leq 0.51$), heart rate (all $p = 0.99$, $d \leq 0.36$) and RPE (all $p = 0.99$, $d \leq 0.36$) (see table, supplemental digital content 4 for data).

Discussion

This study investigated the effect of carbohydrate supplementation on substrate oxidation and time trial performance after both breakfast consumption and omission. In the first 60-min of exercise, carbohydrate supplementation had no effect on substrate oxidation after breakfast consumption or omission. However, in the final 30 min of exercise, carbohydrate supplementation increased the relative carbohydrate contribution to energy expenditure after breakfast omission, but *not* consumption. This was likely explained by an increased contribution of exogenous carbohydrate and a concomitant reduction in the relative contribution of muscle and liver glycogen oxidation to energy expenditure during exercise after breakfast omission compared with consumption in hypoxia. These findings suggest that sub-optimal concentrations of endogenous carbohydrate stores during exercise > 60 minutes may have been observed in the breakfast omission, but not consumption trials. Interestingly, a reduction in liver glucose oxidation suggests a liver glycogen sparing effect of carbohydrate supplementation during sub-maximal exercise, however there was no effect of carbohydrate supplementation on time trial performance in hypoxia.

The finding that the relative contribution of carbohydrate oxidation to energy expenditure was increased after breakfast consumption compared with omission in hypoxia is consistent with the well-established response to feeding in normoxia (33). The reduction in plasma glucose concentrations in the post-prandial state typically observed at the onset of exercise in normoxia was also replicated in hypoxic conditions in the present study, as observed previously (8). A concomitant reduction in FFA availability and oxidation was also observed in the present study after breakfast consumption compared with omission, likely due to the inhibitory effect of insulin on lipolysis (34). Interestingly, there was no effect of carbohydrate supplementation on whole body substrate oxidation after breakfast consumption or omission in the first hour of exercise. This finding is likely explained by the non-glycogen limiting duration of exercise (35).

Carbohydrate supplementation induced a higher relative contribution of carbohydrate oxidation to energy expenditure during the last 30-min of exercise in hypoxia after breakfast omission but *not* consumption. This increased reliance on carbohydrate oxidation was associated with greater plasma glucose concentration and oxidation, derived from the exogenous carbohydrate source. The findings observed in fasted participants are in agreement with the normoxic (4) and hypoxic literature (7). This increased contribution of carbohydrate oxidation likely only occurred in the final 30 minutes of exercise due to the depletion of endogenous carbohydrate stores in such conditions, as discussed previously (35). Data from the final 30-min of exercise in present study support this hypothesis by demonstrating significant reductions in total endogenous carbohydrate oxidation after breakfast omission compared with consumption in both the carbohydrate and placebo trials. These reduced contributions were derived from significant, *moderate* reductions in muscle glycogen utilisation in the placebo trial, and non-significant, ($p = 0.09$), *moderate* reductions in the carbohydrate trials. In addition, significant, *large/very large* reductions in liver glucose oxidation were also observed after breakfast omission compared with consumption in the carbohydrate and placebo trials. Further, the absence of feeding both pre and during exercise in the placebo trial after breakfast omission resulted in a low insulin concentration and likely facilitated an increased reliance on fat oxidation during exercise in this trial (34).

Whilst the effect of hypoxia on substrate oxidation during exercise with carbohydrate supplementation has been investigated in fed participants previously (6), the effect of carbohydrate supplementation on substrate oxidation during exercise in hypoxia, in a placebo-controlled fashion has not. Our data demonstrate that carbohydrate supplementation has no effect on substrate oxidation during exercise (≤ 90 minutes) after breakfast consumption. Similar relative contributions of exogenous carbohydrate oxidation to energy expenditure were observed in the breakfast consumption compared with omission trial. As such, the absence of

change in the relative contribution of carbohydrate oxidation between the carbohydrate and placebo trial after breakfast consumption is likely due to maintained contributions of endogenous carbohydrate oxidation in the fed state (36). In support of this statement, the relative contribution of liver glucose and muscle glycogen oxidation was significantly higher after breakfast consumption compared with omission in the placebo trials, subsequently negating the effects of carbohydrate supplementation.

Findings from Young et al (7) suggest that the oxidation of exogenous carbohydrate may be suppressed during exercise in hypoxia compared with normoxia. Whilst unable to confirm the suppression of exogenous carbohydrate oxidation, the rates observed in the present study (breakfast consumption and omission means (60 – 90 min): 0.35 and 0.37 g·min⁻¹) are lower than that observed by O'Hara et al (5) (~0.92 g·min⁻¹), O'Hara et al (11) (0.82 g·min⁻¹) and Péronnet et al (6) (0.43 g·min⁻¹) but higher than that observed by Young et al (7) (~0.19 g·min⁻¹). The lower exogenous carbohydrate oxidation rates in the present study compared with O'Hara et al (5), O'Hara et al (11) and Péronnet et al (6) are likely due to reduced exercise intensity (50% vs. 74% and 77% $\dot{V}O_{2\max}$ respectively). Maximal exogenous carbohydrate oxidation rates typically occur during exercise at up to 64% $\dot{V}O_{2\max}$ (37), therefore, it is possible the exercise intensity utilised in the present study (50% $\dot{V}O_{2\max}$) did not elicit maximal exogenous carbohydrate oxidation rates. In contrast, the higher exercise intensities employed by others were likely sufficient to elicit maximal rates. In addition, the carbohydrate supplement utilised by some (5, 11) included both glucose and fructose, which has been demonstrated to increase exogenous oxidation compared with glucose alone due to the use of distinct intestinal transporters (38). Young et al (7) supplemented participants with a glucose and fructose solution, however only the glucose was labelled with a ¹³C isotopic tracer and thus fructose oxidation could not be quantified. As such, the low exogenous glucose oxidation rates may be expected, as ingestion rates of glucose alone was just ~1.0 g·min⁻¹ compared with 1.2

g·min⁻¹ in the present study, despite a slightly higher exercise intensity employed by Young et al (7) (~60% $\dot{V}O_{2max}$ vs. 50% $\dot{V}O_{2max}$).

Young et al (7) suggested that the exogenous carbohydrate oxidation suppression observed in acute hypoxia compared to normoxia infers that exogenous carbohydrate supplementation does not spare endogenous glucose stores to the same degree as in normoxia and therefore provides less of an advantage in hypoxic than normoxic conditions. Whilst the present study cannot determine this response in comparison with normoxic conditions, these data suggest that a sparing of endogenous carbohydrate may occur in hypoxia. In this regard, significant reductions in liver glucose oxidation were observed in the carbohydrate compared with placebo trials after both breakfast consumption and omission. This indication of an endogenous glucose sparing effect seems exclusive to liver glycogen stores, as no significant differences in muscle glycogen oxidation were observed between the carbohydrate and placebo trials after breakfast consumption or omission. A liver glycogen sparing effect is consistent with some (4, 39) but not all (3) normoxic literature. These findings are likely associated with partial attenuation of liver glycogenolysis and gluconeogenesis (4), however these measurements were beyond the scope of the present study.

Interestingly, carbohydrate supplementation had no effect on 3 km time trial performance. This is in agreement with Bradbury et al (17) who observed no difference in time trial performance (2 mile walk) with a carbohydrate compared with placebo beverage in acute and chronic hypoxia (4300 m). However, these studies are in contrast to Fulco et al (15), who observed an increase in endurance performance with carbohydrate supplementation after 3 days hypoxic exposure (4300 m) (CHO: 80.1 ± 7 min vs. PLA: 104.9 ± 9.0 min). Differences between findings are likely due to variance in experimental design. In this regard, the intensity of the exercise utilised in the present study may not have been sufficient to deplete endogenous substrate stores to critical levels, therefore suppressing the effect of the supplement. Assuming

a rate of glycogenolysis of $\sim 0.7 \text{ mmol} \cdot \text{kg} \cdot \text{min}^{-1}$ during exercise at 50% $\dot{V}\text{O}_{2\text{max}}$ (40) a muscle glycogen depletion of $\sim 63 \text{ mmol} \cdot \text{kg} \cdot \text{min}^{-1}$ likely occurred in the present study (expected concentration at the end of sub-maximal exercise $\sim 87 \text{ mmol} \cdot \text{kg}^{-1}$). As such, it seems likely that muscle glycogen concentration did not reach a critical threshold ($< 70 \text{ mmol} \cdot \text{kg}^{-1}$ wet weight) in which it may be expected calcium release from the sarcoplasmic reticulum is impaired, and subsequently peak power output reduced (41). This hypothesis may also explain the null findings observed by Bradbury et al (17), as they utilised similar experimental design. Bradbury et al (17) also demonstrated no effect of carbohydrate supplementation on endurance performance in sea level conditions, contradicting their hypothesis that hypoxia *per se*, confounds the ergogenic effect of carbohydrate supplementation. In contrast, although Fulco et al, (2005) utilised a shorter sub-maximal exercise period (45 minutes), the time trial utilised was longer in duration and therefore participants likely spent more time exercising at higher intensities, thus depleting endogenous substrate stores. Further research is required to elucidate the effects of carbohydrate supplementation in conditions directly applicable to real world scenarios (i.e. day long mountaineering treks).

Despite the novel findings reported in this study, several limitations must be acknowledged. Firstly, participants in this study were young males, and these findings remain to be elucidated in other populations. For example, women have shown differing metabolic responses in hypoxia (42) and normoxia (43), as well as a varied response to carbohydrate supplementation (11). In addition, this study was conducted in simulated normobaric hypoxia and caution should be applied when considering the practical application of these findings to terrestrial altitude. Finally, calculations estimating liver glucose oxidation may also comprise residual gut uptake of glucose derived from pre-exercise breakfast consumption, *albeit* in small quantities. Future research should investigate the effects of carbohydrate supplementation on endurance performance of a longer duration ($\geq 60 \text{ min}$) after breakfast consumption and omission. In

addition, both the optimal dose and composition should be elucidated in hypoxia, in both males and females.

In conclusion, breakfast consumption increased carbohydrate oxidation during the first 60 minutes of exercise regardless of carbohydrate supplementation. However, carbohydrate supplementation matched the effects of pre-exercise breakfast consumption during the final 30 minutes of exercise by increasing carbohydrate oxidation after breakfast omission, but *not* consumption. The reduction in liver glucose oxidation following carbohydrate supplementation suggested a liver glycogen sparing effect was present, however no difference was observed in muscle glycogen oxidation. No effect of carbohydrate supplementation on 3 km time trial performance was observed after breakfast consumption or omission. These data provide novel information regarding the use of carbohydrate supplementation in hypoxia for populations in differing states of energy balance (i.e. fasted or fed). These findings should be considered in the design of nutritional interventions for mountaineers, military personnel and athletes exposed to high altitude. Specifically, the findings observed after breakfast omission in the present study may be applicable to individuals experiencing attenuated energy intake as a result of hypoxia-induced appetite suppression (44, 45). Carbohydrate supplementation may be a useful nutritional strategy to induce alterations in substrate oxidation for these individuals. Whilst no changes in endurance performance were observed in the present study, this requires further investigation in chronic hypoxia, in which exercise duration, and subsequent glycogen depletion is potentiated.

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Conflict of interest

The authors report no competing interests. The results of the present study do not constitute endorsement by ACSM. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Contributions

AG, KD, RK and JOH conceived and designed the study. AG and JR collected the data. AG, DM and TP analysed the data. AG analysed the data and wrote the manuscript. All authors provided critical feedback on the manuscript before submission.

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Figure 1. Schematic of full experimental trial

Figure 2. The relative (% energy yield) contribution to energy expenditure during the last 30 minutes of exercise in all trials. B-CHO = breakfast consumption and carbohydrate supplementation, B-PLA = breakfast consumption and placebo supplementation, F-CHO = breakfast omission and carbohydrate supplementation, F-PLA = breakfast omission and placebo supplementation. * represents significant difference in relative carbohydrate oxidation, † represents significant difference in relative liver glucose oxidation, ‡ represents significant difference in relative muscle glycogen oxidation ($p < 0.05$)

Figure 3. Oxidation rates of exogenous carbohydrate, liver glucose, plasma glucose and muscle glycogen during the final 30 minutes of exercise. Values are mean \pm SE. B-CHO = breakfast consumption and carbohydrate supplementation, B-PLA = breakfast consumption and placebo supplementation, F-CHO = breakfast omission and carbohydrate supplementation, F-PLA = breakfast omission and placebo supplementation. (a) indicates a significant difference between breakfast consumption and omission in the carbohydrate trials, (b) represents a significant difference between breakfast consumption and omission in the placebo trials, (c) indicates a significant difference between the carbohydrate and placebo trial after breakfast consumption, (d) indicates a significant difference between the carbohydrate and placebo trial after breakfast omission

Figure 4. Plasma glucose, serum FFA, plasma lactate and serum insulin concentrations over the full experimental trial. Values are mean \pm SE. The thin arrow represents the timing of breakfast in the breakfast consumption trials. The black rectangle represents the exercise period. B-CHO = breakfast consumption and carbohydrate supplementation, B-PLA = breakfast consumption and placebo supplementation, F-CHO = breakfast omission and carbohydrate supplementation, F-PLA = breakfast omission and placebo supplementation. (a) indicates a significant difference between breakfast consumption and omission in the carbohydrate trials, (b) represents a significant difference between breakfast consumption and

omission in the placebo trials, (c) indicates a significant difference between the carbohydrate and placebo trial after breakfast consumption, (d) indicates a significant difference between the carbohydrate and placebo trial after breakfast omission. Significance $p < 0.05$.

List of tables

Table 1. Area under the curve (AUC) values for absolute carbohydrate and fat oxidation during exercise in all trials

Table 2. Carbohydrate oxidation from various sources during the last 30 minutes of exercise in all trials

Supplemental digital content

Supplemental digital content 1.docx – Text document with supporting results for total carbohydrate and fat oxidation and $\delta^{13}\text{C}$ in expired gas and plasma glucose at rest and exercise

Supplemental digital content 2.docx – Figure presenting $\delta^{13}\text{C}$ in expired gas and plasma glucose at rest and exercise

Supplemental digital content 3.docx – Figure presenting time trial performance

Supplemental digital content 4.docx – Table presenting heart rate, SpO_2 and RPE data

Figure 1.

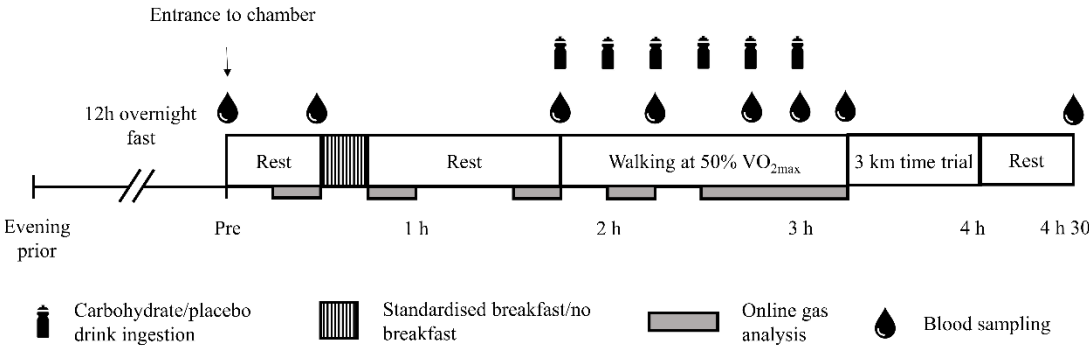
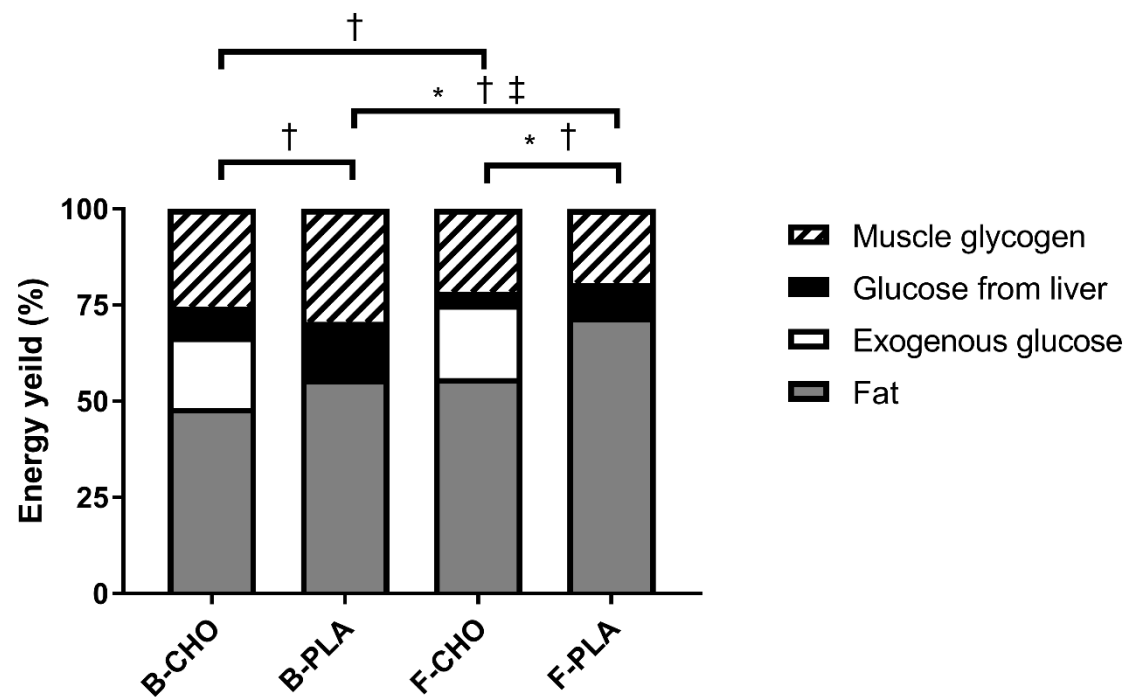
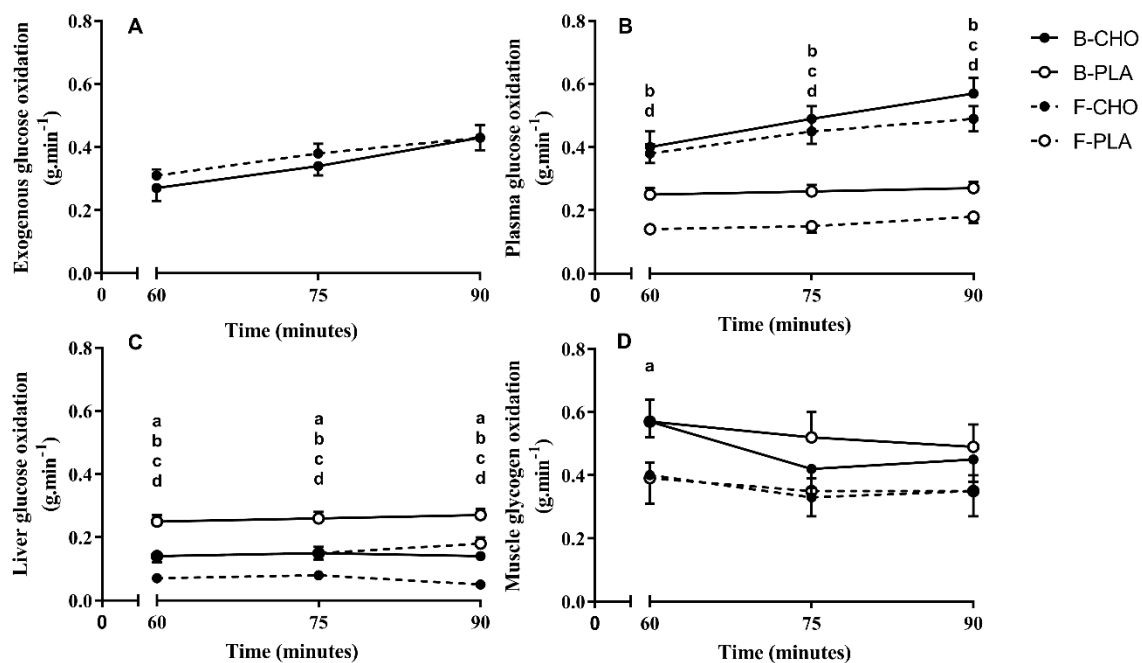


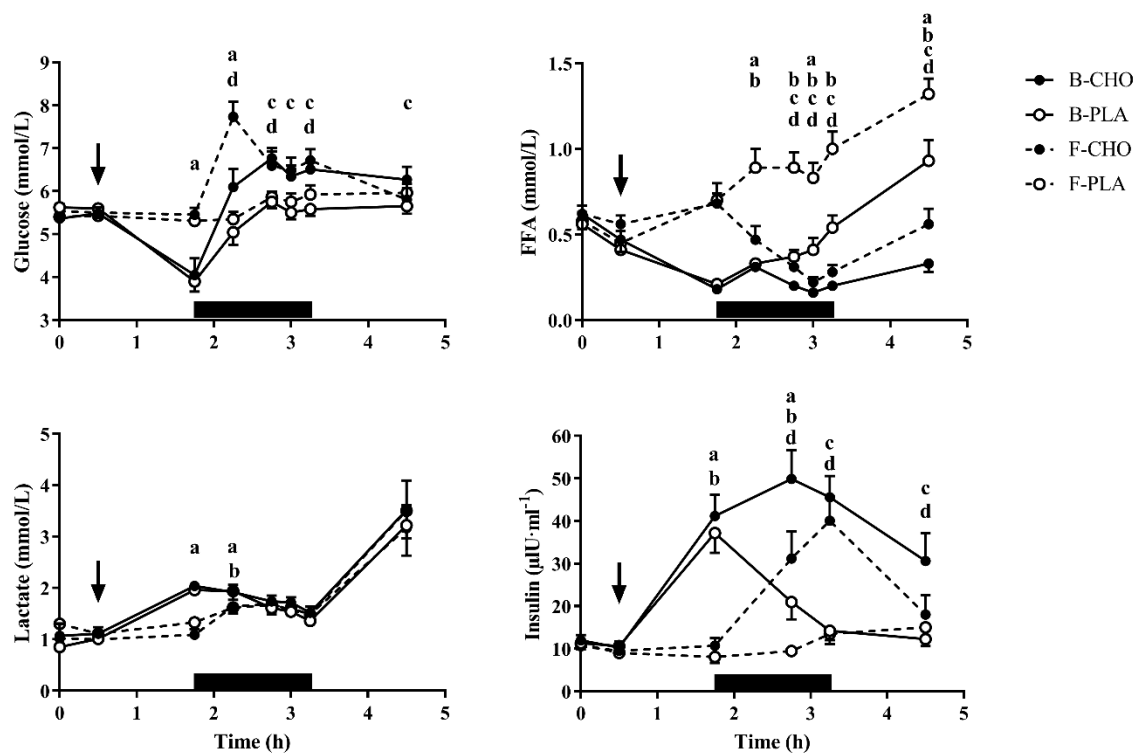
Figure 2.



785 **Figure 3.**



797 **Figure 4.**



808 **Table 1.**

| | Carbohydrate oxidation (g) | | | Fat oxidation (g) | | |
|--------------|----------------------------|--------------|-------------------------|-------------------|-------------|-------------------------|
| | 0-90 min | 0-60 min | 60-90 min | 0-90 min | 0-60 min | 60-90 min |
| B-CHO | 76.72±22.11* | 48.27±13.54* | 28.45±8.84 | 31.52±9.28 | 20.09±5.73 | 11.43±3.68 |
| B-PLA | 67.17±21.20* | 43.57±13.39* | 23.60±7.96* | 33.91±10.22* | 20.84±6.20* | 13.07±4.04* |
| F-CHO | 57.18±16.21 | 33.38±9.40 | 23.80±6.92 [†] | 37.12±9.24 | 23.81±5.66 | 13.31±3.64 [†] |
| F-PLA | 44.14±22.81 | 28.71±14.50 | 15.43±8.48 | 42.29±10.00 | 25.60±6.23 | 16.69±3.91 |

809 **B-CHO = breakfast consumption and carbohydrate supplementation, B-PLA = breakfast consumption and placebo, F-CHO = breakfast**
810 **omission and carbohydrate supplementation, F-PLA = breakfast omission and placebo. * denotes significant difference to alternative**
811 **nutritional status with the same supplement. † denotes significant difference to alternative supplement within the same nutritional**
812 **status**

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817 **Table 2.**

| | Exogenous oxidation (g) | Endogenous oxidation (g) | Muscle glycogen (g) | Glucose from the liver (g) | Plasma glucose (g) |
|--------------|----------------------------|-----------------------------|------------------------|-------------------------------|--------------------|
| B-CHO | 10.35±3.22 | 18.10±6.21* | 13.83±5.43 | 4.27±1.42*† | 14.62±4.48† |
| B-PLA | 0.02±0.01 | 23.58±7.96* | 15.77±7.61 | 7.81±1.65* | 7.83±1.65* |
| F-CHO | 11.22±2.95 | 12.58±5.34 | 10.51±4.91 | 2.07±0.73† | 13.29±3.33† |
| F-PLA | 0.02±0.01 | 15.41±8.48 | 10.70±8.04 | 4.71±1.69 | 4.73±1.69 |

818 **B-CHO = breakfast consumption and carbohydrate supplementation, B-PLA = breakfast consumption and placebo supplementation,**
819 **F-CHO = breakfast omission and carbohydrate supplementation, F-PLA = breakfast omission and placebo supplementation. * denotes**
820 **significant difference to alternative nutritional status with the same supplement. † denotes significant difference to alternative**
821 **supplement within the same nutritional status**

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Supplementary digital content 1.

Total carbohydrate and fat oxidation (full duration exercise)

Over the full duration of exercise, absolute (Table 1) and the relative contribution of carbohydrate oxidation to energy expenditure was greater after breakfast consumption compared with omission in the carbohydrate (absolute: $p < 0.01$, $d = 1.02$; relative: $51.2 \pm 11.0\%$ vs. $40.3 \pm 8.1\%$, $p < 0.01$, $d = 1.15$) and placebo trials (absolute: $p < 0.01$, $d = 1.05$; relative: $46.5 \pm 10.1\%$ vs. $30.5 \pm 12.2\%$, $p < 0.01$, $d = 1.43$). No significant differences in absolute or the relative contribution of carbohydrate oxidation to energy expenditure were observed between the carbohydrate and placebo trials after breakfast consumption (absolute: $p = 0.56$, $d = 0.44$; relative: $p = 0.92$, $d = 0.45$) or omission (absolute: $p = 0.36$, $d = 0.67$; relative: $p = 0.17$, $d = 0.97$). In the same period, absolute and the relative contribution of fat oxidation to energy expenditure was significantly higher after breakfast omission compared with consumption in the placebo trials (absolute: $p < 0.01$, $d = 0.83$, relative: $69.5 \pm 12.2\%$ vs. $53.5 \pm 10.1\%$, $p < 0.01$, $d = 1.44$). The relative, but not absolute contribution of fat oxidation was higher after breakfast omission compared with consumption in the carbohydrate trials (relative: 59.7 ± 8.1 vs. 48.8 ± 11.0 , $p < 0.01$, $d = 1.15$; absolute: $p = 0.25$, $d = 0.60$). No significant differences in the absolute or relative contribution of fat oxidation to energy expenditure were observed between carbohydrate and placebo trials after breakfast consumption (absolute: $p = 0.99$, $d = 0.25$; relative: $p = 0.92$, $d = 0.45$) or omission (absolute: $p = 0.41$, $d = 0.54$; relative: $p = 0.17$, $d = 0.97$).

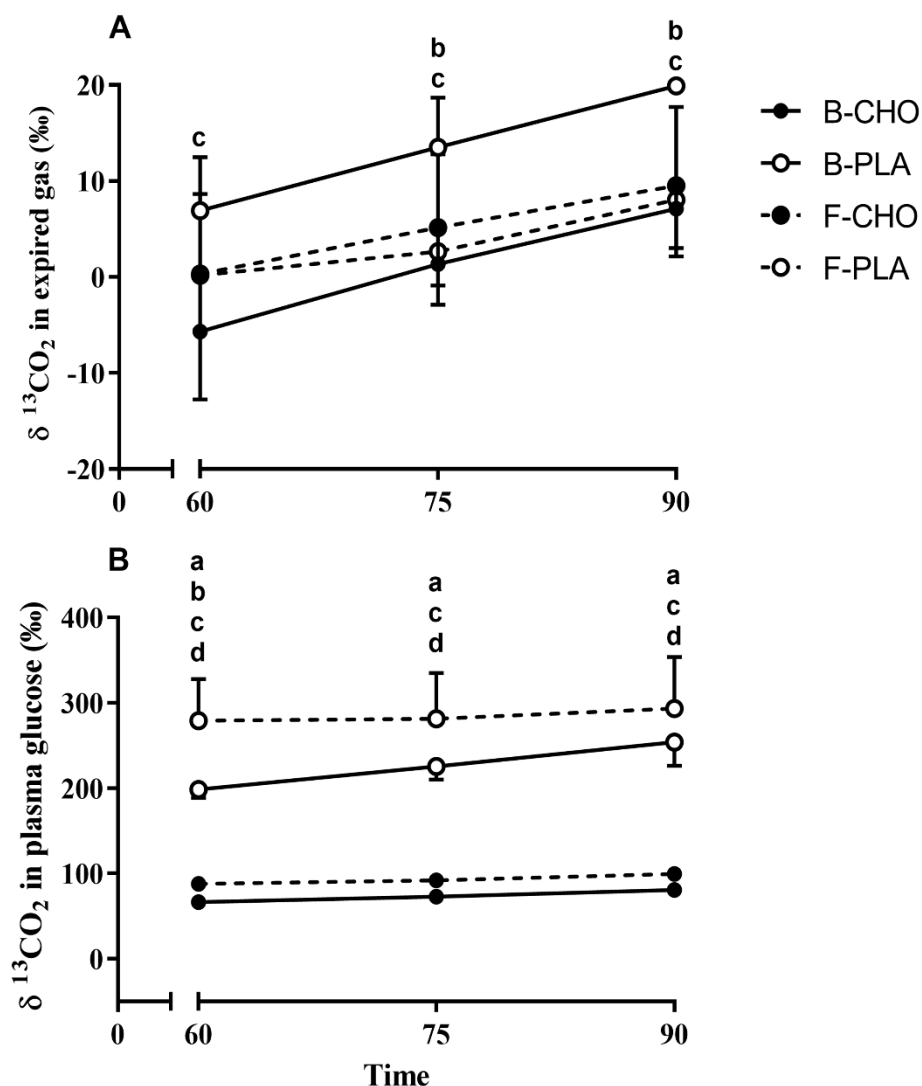
850

851 Expired gas and plasma glucose

852 Background $\delta^{13}\text{CO}_2$ enrichment in the preliminary trials was $-26.49 \pm 0.58\text{‰}$ and $-26.69 \pm$
853 0.51‰ during exercise at 50% $\dot{V}\text{O}_{2\text{max}}$ after breakfast omission and consumption respectively.
854 The $\delta^{13}\text{CO}_2$ in expired gas (Figure 3A) at rest before exercise and ingestion of the supplement
855 beverage was not significantly different between conditions ($p = 0.99$). The $\delta^{13}\text{CO}_2$ in expired
856 gas significantly increased over time from baseline through exercise following consumption of
857 each respective supplement (all $p < 0.01$, Figure 3A). The $\delta^{13}\text{CO}_2$ in expired gas was
858 significantly greater after breakfast consumption compared with omission in the placebo trials
859 at 75 min ($p = 0.02$, $d = 1.42$) and 90 min ($p < 0.01$, $d = 1.84$), but not at 60 min ($p = 0.14$, $d =$
860 0.96). There was no significant difference in the $\delta^{13}\text{CO}_2$ in expired gas between breakfast
861 consumption and omission in the carbohydrate trials at any time point ($p > 0.35$, $d < 0.93$). The
862 $\delta^{13}\text{CO}_2$ in expired gas was higher in the placebo compared with carbohydrate trials at 60, 75
863 and 90 min after breakfast consumption (all $p < 0.01$, $d < 2.60$) but not omission (all $p = 0.99$,
864 $d < 0.31$).

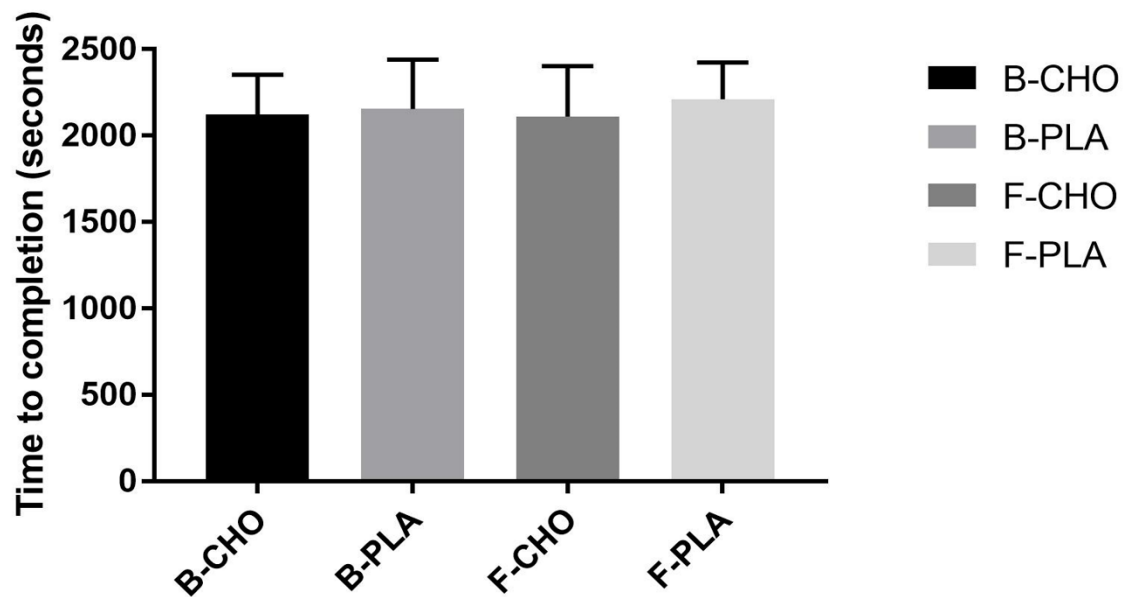
865 The $\delta^{13}\text{C}$ in plasma glucose (Figure 3B) at rest before exercise and ingestion of a supplement
866 was also not significantly different between condition ($p = 0.99$). Plasma $\delta^{13}\text{C}$ -glucose
867 significantly increased over time from baseline through exercise following consumption of
868 each respective supplement (all $p < 0.01$, Figure 3B). Plasma $\delta^{13}\text{C}$ -glucose was significantly
869 higher in the placebo compared with carbohydrate trials after both breakfast consumption (all
870 $p < 0.01$, $d < 17.22$) and omission (all $p < 0.01$, $d < 6.76$) at 60, 75 and 90 minutes. Plasma
871 $\delta^{13}\text{C}$ -glucose was significantly higher after breakfast consumption compared with omission in
872 the carbohydrate trials at 60, 75 and 90 minutes (all $p < 0.01$, $d < 3.24$). Plasma $\delta^{13}\text{C}$ -glucose
873 was also significantly higher after breakfast consumption compared with omission in the

placebo trials at 60 minutes ($p < 0.01$, $d = 2.75$) and approached significance at 75 ($p = 0.06$, $d = 1.64$), but not at 90 minutes ($p = 0.82$, $d = 0.89$).



SDC 2. Changes in $\delta^{13}\text{C}$ in expired gas (A) and plasma glucose (B) at rest (0 min) and during the 90-minute walking protocol. Values are mean \pm SD. Significance $p < 0.05$ (a) indicates a significant difference between breakfast consumption and omission in the carbohydrate trials, (b) represents a significant difference between breakfast consumption and omission in the placebo trials, (c) indicates a significant difference between the carbohydrate and placebo trial after breakfast consumption, (d) indicates a significant difference between the carbohydrate and placebo trial after breakfast omission. B-CHO = breakfast consumption and carbohydrate supplementation, B-PLA = breakfast consumption and placebo supplementation, F-CHO = breakfast omission and carbohydrate supplementation, F-PLA = breakfast omission and placebo supplementation

Supplementary digital content 3.



SDC 3. 3-km time trial performance in all trials. Values are presented as mean \pm SD. B-CHO = breakfast consumption and carbohydrate supplementation, B-PLA = breakfast consumption and placebo supplementation, F-CHO = breakfast omission and carbohydrate supplementation, F-PLA = breakfast omission and placebo supplementation

Supplementary digital content 4.

SDC 4. Mean SpO₂, heart rate and RPE across the full duration of all trials.

| | SpO ₂ (%) | Heart rate (bpm) | RPE |
|--------------|----------------------|------------------|------|
| B-CHO | 83±3 | 87±7 | 11±2 |
| B-PLA | 83±3 | 86±6 | 11±2 |
| F-CHO | 81±4 | 87±7 | 11±1 |
| F-PLA | 81±3 | 84±8 | 11±2 |

B-CHO = breakfast consumption and carbohydrate supplementation, B-PLA = breakfast consumption and placebo supplementation, F-CHO = breakfast omission and carbohydrate supplementation, F-PLA = breakfast omission and placebo supplementation.