Cycling time trial performance is improved by carbohydrate ingestion during exercise regardless of a fed or fasted state

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Cycling TT performance is improved by carbohydrate ingestion during exercise regardless of a fed or fasted state.

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Running head: CHO intake, fasting and endurance performance

ABSTRACT

Purpose: We tested the hypothesis that carbohydrate ingestion during exercise improves time trial (TT) performance and that this carbohydrate-induced improvement is greater when carbohydrates are ingested during exercise in a fasted rather than a fed state.

Methods: Nine males performed 105 min of constant-load exercise (50% of the difference between the first and second lactate thresholds), followed by a 10-km cycling TT. Exercise started at 9 am, 3 h after either breakfast (FED, 824 kcal, 67% carbohydrate) or a 15-h overnight fast (FAST). Before exercise, after every 15 min of exercise and at 5 km of the TT, participants ingested 2 $mL.kg^{-1}$ body mass of a non-caloric sweetened solution containing either carbohydrate (8% of maltodextrin, CHO) or placebo (0% carbohydrate, PLA).

Results: Irrespective of the fasting state, when carbohydrate was ingested during exercise, the rating of perceived exertion (RPE) was lower throughout the constant-load exercise, while the plasma glucose concentration and carbohydrate oxidation were higher during the last stages of the constant-load exercise ($P<0.05$). Consequently, TT performance was faster when carbohydrate was ingested during exercise (18.5 ± 0.3 and 18.7 ± 0.4 min for the FEDCHO and FASTCHO conditions, respectively) than when the placebo was ingested during exercise (20.2 ± 0.8 and 21.7 ± 1.4 min for the FEDPLA and FASTPLA conditions, respectively), regardless of fasting.

Conclusion: These findings indicate that even when breakfast is provided before exercise, carbohydrate ingestion during exercise is still beneficial for exercise performance. However, ingesting carbohydrate during exercise can overcome a lack of breakfast.

Key-words: *fasting; liver glycogen; carbohydrate supplementation; endurance performance.*

INTRODUCTION

The importance of carbohydrate supplementation during exercise for endurance performance during prolonged exercise has been reported over many years.[1] However, most laboratory studies investigating the effect of carbohydrate ingestion during exercise on performance have been performed in an overnight fasted state in an effort to maintain a methodological control of the experiment (for review, see [2]). Therefore, the results of such studies may not be indicative of real life and provide incomplete information about the importance of carbohydrate ingestion during exercise on exercise performance.

Overnight fasting results in a substantial degree of liver glycogen depletion, while a carbohydrate-rich breakfast replenishes liver glycogen.[3] The reduction in pre-exercise liver glycogen by overnight fasting results in a greater decrease in blood glucose concentration during prolonged exercise, which likely contributes to the observed impairment of endurance performance, compared to when exercise is commenced following breakfast.[4,5] In addition, muscle glucose uptake during exercise is increased when carbohydrate is ingested during exercise in a fasted state compared to a fed state.[5] Therefore, we hypothesize that carbohydrate ingestion during exercise results in greater improvement in prolonged exercise performance when the exercise is commenced in a fasted rather than a fed state. However, we are not aware of any study that has directly tested this hypothesis.

Interestingly, Chryssanthopoulos et al[4] examined the effect of carbohydrate ingestion during exercise on time to task failure during an exercise performed at 70% of maximal oxygen uptake ($\dot{V}O_2max$) commencing in a fasted or fed state. Time to task failure was longer when combining carbohydrate ingestion during exercise with a pre-exercise meal than with carbohydrate ingestion during exercise but without a pre-exercise meal (fasting).[4] Unfortunately, conditions without carbohydrate ingestion during the exercise (placebo) were not included, which prevents determination of whether carbohydrate ingestion during exercise is more important when exercise begins after a fasting period. In a further study, however, the same research team compared carbohydrate and placebo ingestion during a task-to-failure exercise (70% $\dot{V}O_2max$) in a fed state.[6] Although time to exhaustion was longer with carbohydrate ingestion during the exercise than in the placebo group, whether the benefits of carbohydrate ingestion during exercise have different effects in a fasted compared to a fed state was not assessed in that study, as there were no fasting conditions. In addition, both studies used time to exhaustion at a constant workload as their performance measure. The time to exhaustion test is more variable than a time trial (TT) exercise, and a TT exercise is also more relevant to real world competitions.[7] Therefore, it is important to explore this question during more realistic scenarios of competition such as during a TT exercise.

The objective of the present study was to determine the impact of carbohydrate or placebo ingestion during a 105-min constant-load exercise followed by a 10-km cycling TT in both fed and fasted states. We hypothesized that carbohydrate ingestion during exercise would improve TT performance, but this carbohydrate-induced improvement would be greater when carbohydrate is ingested during exercise in a fasted rather than a fed state.

METHODS

Participants

Nine healthy males (28 ± 6 years old, 178.9 ± 4.6 cm, 79.8 ± 9.6 kg, 10.6 ± 4.7% body fat, $\dot{V}O_2$max 3.26 ± 0.33 L.min^{-1} and 41.2 ± 3.7 mL.min.kg^{-1}) volunteered to participate in this study. Participants exercised ~8 h.week^{-1} including cycling recreationally ~80 km.week^{-1} and were classified as physically active according to the International Physical Activity Questionnaire.[8] The required sample size was estimated using G*Power software (Heinrich-Heine-University Düsseldorf, version 3.1.9.2, Düsseldorf, Germany). The input parameters used were an alpha of 0.05 and a desired power of 0.80. Because no previous study has been conducted using a similar experimental design with multiple comparisons, an expected partial eta squared of 0.15 (moderate) for the interaction between state and supplement was used.[9] Therefore, the required sample size was estimated to be eight participants. Participants were informed of all risks and benefits before providing written informed consent and then answered a Physical Activity Readiness Questionnaire to exclude potential cardiovascular risks. The Ethics Research Committee of the Federal University of Pernambuco approved this study (approval number: 790.169).

Experimental procedures

Participants visited the laboratory on six different occasions (Fig. 1). During the first visit, participants completed a 24-h diet recall form and performed anthropometric measurements, a cycling graded exercise test to exhaustion (GXT) and, after a recovery period, familiarization with TT procedures. During the second visit, at least three days later, participants completed an additional 24-h diet recall form and a full familiarization session with the experimental protocol. Participants had the opportunity to practice a 10-km TT twice (one after the GXT and another after the constant-load exercise in the second visit) to reduce

the influence of learning on TT performance.[10] From the third to sixth visits, participants performed the experimental sessions, with a 1-week washout period (Fig. 1). Two experimental trials were performed in the morning in a fed state (FED, i.e., three hours after breakfast), and two trials were performed in the morning in a fasted state (FAST, i.e., after a 15-h overnight fast with no breakfast). Participants ingested a carbohydrate or a placebo solution immediately before and periodically during the exercise. Therefore, four experimental conditions were performed: FEDPLA, FEDCHO, FASTPLA and FASTCHO. Experimental sessions were performed using an incomplete, counterbalanced measures "balanced Latin Square" design. The carbohydrate and placebo drinks consumed during the exercise were offered in a double-blind matter.

All tests started at the same time of day (9 am) in a stable temperature and relative humidity environment (21.1 ± 1.2 °C and 35.0 ± 2.8%, respectively). A fan was placed in front of the participant to minimize thermal stress. All trials were performed on a bicycle coupled to a cyclosimulator (Computrainer Pro^{TM}, RacerMate®, Seattle, USA). The seat height was adjusted for each participant during the first visit and reproduced for all subsequent sessions. Exercise and food ingestion were controlled two days before each experimental test to maintain muscle and hepatic glycogen at similar levels across the conditions. Participants were also asked to refrain from alcohol and caffeine 24 h before each experimental session.

Graded exercise test (GXT) with lactate threshold

A previously validated GXT, starting with 50 W and increasing 30 W every 4 min, with a 1-min rest interval was performed until voluntary exhaustion.[11] Blood capillary puncture was performed during rest intervals. Pedal cadence was maintained between 70-80

rpm throughout the GTX. Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured breath-by-breath using an on-line gas analyzer (Cortex Metalyzer 3B, Cortex Biophysik Leipzig, Germany). The O_2 and CO_2 sensors were calibrated according to the manufacturer's specifications before each test with gas containing known concentrations of O_2 (12%) and CO_2 (5%). The volume of expired air was measured by a bidirectional flow sensor that was calibrated before each test with a 3-L syringe. Heart rate (HR) was continually monitored using a HR transmitter coupled to the gas analyzer.

We considered the $\dot{V}O_2$max to be the highest 60-s average value registered during the test. The first lactate threshold (LT1) was identified by two experienced researchers who visually identified the first work rate at which the lactate concentration suddenly increased above the resting level.[12,13] The two researchers agreed in 100% of the cases. The second lactate threshold (LT2) was identified using a modified Dmax-method,[14] which has been demonstrated to provide a valid estimate of the maximal steady state of lactate.[15] Briefly, the lactate-work rate curve was adjusted with a third order polynomial regression from LT1 until peak power output, and the maximal perpendicular distance to a straight line connecting LT1 to peak power output was assumed as LT2.

Diet and exercise control during the two days before each experimental trial

During the two days preceding each experimental trial, participants followed a standardized diet and exercise protocol (Fig. 1). Water was consumed during the two days preceding the first experimental trial ad libitum and replicated during the two days preceding the following experimental trials. The diet on the first of these two days comprised six meals: breakfast, morning snack, lunch, afternoon snack, dinner and supper. This diet had the same energy and macronutrient composition as the participants' usual macronutrient consumption. The usual macronutrient consumption was measured from the two 24-h diet recall forms

completed at visit 1 and 2. The nutritional values of foods reported on the 24-h diet recalls (kcal, carbohydrate, protein and fat) were analyzed using a computer program designed to perform nutritional calculations (Nutwin, Nutrition Support Program, version 1.5, Department of Health Informatics, Federal University of São Paulo, Brazil). The usual energy and macronutrient intake amounts of participants were 2,866 ± 410 kcal (36.7 ± 8.2 kcal.kg^{-1}), 57 ± 6% carbohydrates (409.9 ± 91.1 g, 5.3 ± 1.5 g.kg^{-1}), 22 ± 3% lipids (70.6 ± 12.1 g, 0.9 ± 0.2 g.kg^{-1}) and 21 ± 3% protein (147.3 ± 15.9 g, 1.9 ± 0.4 g.kg^{-1}). Participants also performed standardized control exercises on this day (60 min of constant-load exercise at 50% of the difference between LT1 and LT2).

On the second day, no exercise was performed, and participants followed the same diet as that of the previous day, except that their dinner at 6 pm was replaced by a standardized shake. The shake consisted of 32 g of skim milk, 32 g of whole milk, 42 g of maltodextrin, 12 g of oats, 30 g of chia, 8 g Brazilian chestnuts (two units) and 80 g of banana (two units) diluted in 300 mL of water [650 kcal, 60% (98.4 g) carbohydrate, 25% (16.9 g) lipids and 15% (24.8 g) protein]. No supper was consumed. Altogether, this second day involved consumption of 2,874 ± 409 kcal (36.8 ± 8.2 kcal.kg^{-1}), 58 ± 4% carbohydrates (420.8 ± 73.6 g, 5.4 ± 1.4 g.kg^{-1}), 23 ± 3% lipids (73.3 ± 13.4 g, 0.9 ± 0.2 g.kg^{-1}) and 19 ± 4% protein (132.7 ± 19.9 g, 1.7 ± 0.4 g.kg^{-1}). The standardized shake that we used has been shown previously to fully load liver glycogen content for individuals of similar body mass.[3] Participants then fasted overnight. This diet and exercise control was rigorously repeated before each experimental trial.

Experimental trials

Participants arrived at the laboratory at 5:45 am, and then at 6 am and 9 am, capillary blood samples (50 µL) were collected from the earlobe to measure plasma glucose concentrations. During the FED conditions, immediately after capillary blood collection, participants ingested a shake for breakfast. The shake consisted of 48 g of skim milk, 40 g of whole milk, 85 g of dextrose, 8 g Brazilian chestnuts (two units) and 90 g of guava (one unit) diluted in 300 mL of water [824 kcal, 67% (137.0 g) carbohydrate, 19% (16.9 g) fat and 14% (29.8 g) protein]. This macronutrient content ingested at 6 am has been shown to be sufficient to restore liver glycogen 3 hours after ingestion to levels close to the pre-fasting level in individuals of similar body mass.[3] In the FAST conditions, participants did not have any food and remained fasted until 9 am, resulting in a total of 15 h of fasting. We chose this fasting protocol because a 15-h overnight fast has been shown to reduce hepatic but not muscle glycogen stores.[3]

The trials started at 9 am and consisted of 105 min of cycling at 50% of the difference between LT1 and LT2, followed immediately by a 10-km TT. We chose this kind of protocol because previous work by our group was able to detect differences in TT performance with carbohydrate ingestion during exercise using a similar protocol.[16] In addition, we chose to perform constant-load exercise using LTs rather than percentage of $\dot{V}O_2max$ to better match metabolic stress across participants.[17] Participants were instructed to complete the 10-km TT as quickly as possible and were free to choose their pedaling rate and establish their own pacing strategies. However, participants received visual feedback only for distance completed and not for exercise time, power output, pedal frequency, or physiological parameters. Participants ingested a beverage (2 $mL.kg^{-1}$ of body mass) containing carbohydrate (maltodextrin, CHO) or placebo (0% carbohydrate, PLA) immediately before exercise, every 15 min throughout the constant-load exercise and at the 5^{th} km point during the 10-km TT. At

the 5-km point, the mouthpiece was removed, the beverage was ingested, and the mouthpiece was rapidly replaced (~10 s). The placebo beverage was prepared from a commercially available non-caloric artificial sweetener (aspartame, 0.2 mL, Finn, Cosmed, Cosmetics and Medicines Industry, Barueri, São Paulo, Brazil), yellow dye (0.3 mL, Mix, food industry, São Bernardo do Campo, São Paulo, Brazil) and orange essence (0.2 mL, Arcolor, Arco-Ires Brasil, Industry trade in food products, São Paulo, São Paulo, Brazil) diluted to 1000 mL with water. The CHO beverage contained 80 g of a commercially available unflavored maltodextrin (Neonutri, Poços de Caldas, Brazil) per 1000 mL of the same solution. A staff member of the laboratory, who was not directly involved in the study, prepared the carbohydrate and placebo solutions. We demonstrated during a pilot study involving six participants that the carbohydrate and placebo solutions were indistinguishable in smell, flavor, sweetness, color and viscosity (all $P>0.05$ with a *t*-test applied using a 10-cm visual analogue scale). In addition, participants were asked after each experimental trial to guess whether they ingested the carbohydrate or the placebo. Participants were able to guess correctly 50% of the time, indicating the success of the blinding protocol.

Capillary blood samples (50 µL) from the earlobe for determination of plasma glucose and lactate concentrations were obtained immediately before, at 15, 60 and 105 min of constant-load exercise, and then immediately after the TT. The rating of perceived exertion (RPE, 15-point Borg's scale) was obtained every 15 min during the constant-load exercise and at the end of the TT. Gas exchange ($\dot{V}O_2$ and $\dot{V}CO_2$) and HR were measured over the 5 min before the 15-, 30-, 45-, 60-, 75-, 90- and 105-min intervals of constant-load exercise and continuously throughout the TT. Body mass was measured before each trial.

Analytical techniques

Capillary blood samples were immediately transferred to micro tubes containing EDTA and centrifuged at 4000 rpm for 10 min at 4 °C. Following centrifugation, plasma lactate and glucose concentrations were immediately analyzed in duplicate using a UV spectrophotometer (Quimis®, 239 model Q798U2V5, São Paulo, Brazil) with commercial kits (Labtest, Labtest Diagnostic S.A., Vista Alegre/Lagoa Santa, Brazil). The intra-assay coefficients of variation were 2.8 ± 0.6% and 1.8 ± 1.4% for the plasma lactate and glucose concentrations, respectively.

Fat and carbohydrate oxidation rates

The respiratory exchange ratio (RER) and fat and carbohydrate oxidation rates at rest and during the constant-load exercise were calculated using the mean $\dot{V}O_2$ and $\dot{V}CO_2$ values over the 5 min collection periods. Fat and carbohydrate oxidation rates were calculated from the non-protein respiratory quotient[18] using the following stoichiometric equations:

$$\text{Fat oxidation rate} = (1.67 \cdot \dot{V}O_2) - (1.67 \cdot \dot{V}CO_2) \quad \text{(equation 1)}$$

$$\text{Carbohydrate oxidation rate} = (4.55 \cdot \dot{V}CO_2) - (3.21 \cdot \dot{V}O_2) \quad \text{(equation 2)}$$

where $\dot{V}O_2$ and $\dot{V}CO_2$ are in $L.min^{-1}$, and oxidation rates are in $g.min^{-1}$.

The area under the curve was calculated to measure the total oxidized amounts (g) of carbohydrate and fat during the constant-load exercise. The average of $\dot{V}O_2$, $\dot{V}CO_2$ and RER for the entire 10-km TT were also calculated; however, fat and carbohydrate oxidation rates were not calculated because the RER exceeded 1.0.

Statistical Analysis

The data are presented as the mean and standard error of the mean (mean ± SEM). Three-way ANOVA with repeated measures was used to compare plasma glucose concentrations pre and post breakfast [factors: feeding (FAST *vs.* FED), supplementation during exercise (placebo *vs*. carbohydrate), and time (pre *vs.* and post breakfast)]. Three-way ANOVA with repeated measures was also used to verify the effect of pre-exercise feeding (FAST *vs.* FED), supplementation during exercise (placebo *vs*. carbohydrate) and time (rest and exercise time points) on $\dot{V}O_2$, $\dot{V}CO_2$, RER, HR, plasma glucose and lactate concentrations, carbohydrate and fat oxidation rates, and RPE response during the constant-load exercise. The effect of pre-exercise feeding (FAST *vs.* FED), supplementation during exercise (placebo *vs*. carbohydrate) and distance (from 1 to 10 km) on power output during the 10-km TT was also verified with three-way ANOVA with repeated measures. Two-way ANOVA with repeated measures was used to verify the effect of pre-exercise feeding (FAST *vs.* FED) and supplementation during exercise (placebo *vs*. carbohydrate) on exercise performance, total carbohydrate and fat oxidized, and mean or end physiological variables during the 10-km TT. One-way ANOVA with repeated measures was used to determine whether pre-exercise body mass was different across the conditions. When ANOVA revealed a significant main effect or interaction, a *Bonferroni* post hoc test was performed. Significance was accepted at $P \leq 0.05$. All analyses were performed using Statistica software (version 10.0; Tulsa, USA).

RESULTS

10-km time trial (TT)

The time to cover 10-km was shorter (main effect of supplement, $P=0.019$, Table 1) when carbohydrate was ingested during exercise (FEDCHO and FASTCHO) than when the placebo was ingested during exercise (FEDPLA and FASTPLA), regardless of a fed or fasted

state (no interaction of feeding *vs.* supplement, P=0.260). Accordingly, the $\dot{V}O_2$, $\dot{V}CO_2$, and RER and end plasma glucose and lactate concentrations were all higher with carbohydrate ingestion during exercise (FEDCHO and FASTCHO) than with placebo ingestion during exercise (FEDPLA and FASTPLA) (Table 1). Power output was higher during the initial (first 2 km) and final (last 2 km) stages than during the middle stages (main effect of distance, P=0.001, Fig. 2) and was higher throughout the trial in the FEDCHO and FASTCHO conditions in relation to the FEDPLA and FASTPLA conditions (main effect of supplement, P=0.002), regardless of whether breakfast was eaten (no interaction of feeding *vs.* supplement, P=0.268 or of feeding *vs.* supplement *vs.* distance, P=0.799). The end RPE was similar between conditions (P>0.05).

Physiological and perceptual responses to the 105-min constant-load exercise

The 105-min constant-load exercise bouts were performed at 162 ± 19 W (60.8 ± 0.2%, $\dot{V}O_2$max). The $\dot{V}O_2$ and $\dot{V}CO_2$ increased from rest to 15 min (P<0.01) and then remained stable (P>0.05) until the end of submaximal exercise in all conditions (Fig. 3A and 3B, respectively). The carbohydrate oxidation rate and RER increased from rest to 15 min (P<0.05) in all conditions, but values were higher in the FEDPLA and FEDCHO conditions than in the FASTPLA and FASTCHO conditions throughout the exercise (main effect of pre-exercise feeding, P=0.011 and 0.018, Fig. 3C and 3D, respectively). The carbohydrate oxidation rate was also higher with carbohydrate ingestion during exercise (FEDCHO and FASCHO conditions) at 30, 90 and 105 min (supplementation *vs.* time interaction, P=0.017), and the RER was higher with carbohydrate ingestion during exercise at 45 and 105 min (supplementation *vs.* time interaction, P=0.008). However, total carbohydrate oxidization was higher in the FEDPLA (263.9 ± 15.7 g) and FEDCHO (265.8 ± 11.2 g) conditions than in the FASTPLA (232.9 ± 17.5 g) and FASTCHO (243.4 ± 9.9 g) conditions (main effect of pre-

exercise feeding, P=0.008). The fat oxidation rate was higher in the FASTPLA and FASTCHO conditions than in the FEDPLA and FASTCHO conditions throughout the submaximal exercise (main effect of pre-exercise feeding, P=0.010, Fig. 3E). Consequently, total fat oxidized was higher in the FASTPLA (19.8 ± 2.2 g) and FASTCHO (17.4 ± 2.1 g) conditions than in the FEDPLA (9.1 ± 2.3 g) and FEDCHO (9.4 ± 4.4 g) conditions (main effect of pre-exercise feeding, P=0.010). The HR increased from rest to 15 min (P<0.01) and then remained stable and similar among trials until 105 min in all conditions (P>0.05, Fig. 3F).

Pre-exercise feeding *vs.* time (P=0.012) and supplementation *vs.* time (P=0.005) interactions were found for plasma glucose concentration (Fig. 4A). The plasma glucose concentration was higher in the FEDPLA and FEDCHO conditions than in the FASTPLA and FASTCHO conditions at rest (P<0.05) and decreased from rest to 15 min in the FASTPLA, FEDPLA and FEDCHO conditions (P<0.05), but not in the FASTCHO condition (P>0.05). The plasma glucose concentration in the FASTCHO condition was also higher than in the FASTPLA and FEDPLA conditions at 15 and 60 min (P<0.01). At 60 min, the plasma glucose concentration was higher in the FEDCHO than in the FASTPLA condition (P<0.05). At the end of the submaximal exercise (105 min), the plasma glucose concentration was higher (P<0.01) when carbohydrate was ingested during exercise (FEDCHO and FASTCHO conditions) than when the placebo was ingested during exercise (FEDPLA and FASTCHO conditions).

A pre-exercise feeding *vs.* supplementation *vs.* time interaction (P=0.020) was observed for plasma lactate concentration (Fig. 4B). The plasma lactate concentration increased from rest to 15 min exercise in all conditions (P<0.01), but the lactate concentration was higher at 15 min in the FEDCHO than in the FEDPLA condition (P<0.05). The plasma lactate concentration was reduced significantly from 15 to 105 min in the FEDCHO,

FASTCHO and FASTPLA conditions (P<0.05) but remained unaltered in the FEDPLA condition (P>0.05).

A supplementation *vs.* time interaction (P=0.022) was observed for RPE, with values increasing more slowly when carbohydrate was ingested during exercise (FEDCHO and FASTCHO conditions) (Fig. 4C).

Pre- and post-breakfast effects on plasma glucose concentration

The plasma glucose concentration significantly increased from 6 to 9 am in the FEDPLA (+ 0.30 ± 0.21 mmol.L^{-1}) and FEDCHO (+ 0.74 ± 0.24 mmol.L^{-1}) conditions but not in the FASTPLA (- 0.26 ± 0.19 mmol.L^{-1}) and FASTCHO (- 0.22 ± 0.13 mmol.L^{-1}) conditions (pre-exercise feeding *vs*. time interaction, P=0.006).

Body mass and total fluid intake

No difference was found across the experimental conditions for pre-exercise body mass (FEDPLA: 80.3 ± 4.8 kg, FEDCHO: 80.6 ± 4.9 kg, FASTPLA: 80.6 ± 5.0, FASTCHO: 79.9 ± 5.0, P=0.165). The total fluid intake during the trials was 1,436 ± 57 mL.

DISCUSSION

It was hypothesized that carbohydrate ingestion during exercise improves TT performance but that this carbohydrate-induced improvement is greater when carbohydrate was ingested during exercise in a fasted state rather than a fed state. However, contrary to our hypothesis, exercise performance was improved with carbohydrate ingestion during exercise regardless of a fed or fasted state. Similarly, the plasma glucose concentration and carbohydrate oxidation were higher and the RPE was lower during exercise when

carbohydrate was ingested during exercise, an effect that was independent of a fed or fasted state. These results suggest that the improvement in TT performance may have been due to changes in metabolic and perceptive responses during the previous constant-load exercise when carbohydrate was ingested during exercise.

Contrary to our hypothesis, carbohydrate ingestion improved exercise performance similarly in both the fed and fasted state (Table 1). The power output was significantly higher at the beginning of the trial in the FEDCHO and FASTCHO conditions than in the FEDPLA and FASTPLA conditions (Fig. 2). In fact, power output increased from ~160 W (~65% $\dot{V}O_2$max) during the constant-load exercise to ~200 W (~82% $\dot{V}O_2$max) during the TT for the FEDCHO and FASTCHO conditions (Fig. 2). However, the increase was much more discrete in the FEDPLA and FASTPLA conditions (from ~160 W, ~65% $\dot{V}O_2$max to ~165 W, ~68% $\dot{V}O_2$max). It has been determined that both muscle and liver glycogen are substantially depleted after > 90 min of exercise at ~65% of $\dot{V}O_2$max.[19,20,21,22] Therefore, this suggests that the TT in the present study would have started with substantially reduced muscle and liver glycogen stores. Carbohydrate ingestion during exercise at ~65% of $\dot{V}O_2$max has minimal effects on muscle glycogen sparing but considerably spares liver glycogen.[5] Thus, the increase in exercise intensity at the onset of the TT in the FEDCHO and FASTCHO conditions (from ~65% to ~82% of $\dot{V}O_2$max) may have been due to a higher glucose oxidation rate,[23] perhaps due to higher liver glucose output from the spared liver glycogen.[5] This result reinforces the importance of prior carbohydrate ingestion during prolonged exercise to increase power output during a subsequent TT.

Although total carbohydrate oxidization during the constant-load exercise was not affected by carbohydrate ingestion during exercise, consistent with elevated plasma glucose concentrations, the carbohydrate oxidation rate was higher during the last stages of the

constant-load exercise in the FEDCHO and FASTCHO conditions (Fig. 3C). Previous studies have shown that the carbohydrate oxidation rate is significantly higher during the latter stages of prolonged exercise with carbohydrate ingestion during exercise compared with placebo ingestion.[24,20] We showed previously that ingesting carbohydrate throughout exercise is more beneficial for exercise performance than ingesting the same amount late in exercise.[16] We have suggested that periodic ingestion of carbohydrate during the exercise may attenuate liver glycogen depletion.[16] In the present study, the increased carbohydrate oxidation rates at 90 and 105 min of exercise with carbohydrate ingestion during exercise highlights the importance of exogenous carbohydrate to spare liver glycogen and/or maintain muscle glucose oxidation when liver glycogen is likely reduced (i.e., in the last stage of prolonged exercise).

The progressive increase in RPE during constant-load exercise was attenuated by carbohydrate ingestion during exercise (FEDCHO and FASTCHO conditions), regardless of a fed or fasted state (Fig. 4C). The lower values of RPE with carbohydrate ingestion during exercise might be related to a direct effect of carbohydrate on the central nervous system[25,26] and/or a favorable metabolic *milieu* (i.e., higher plasma glucose concentration and increased carbohydrate oxidation rate). The presence of carbohydrate in the mouth stimulates a group of sensory receptors that activates some areas of the brain that are associated with reward and motor control,[25] ultimately reducing RPE.[27] Interestingly, this central effect of carbohydrate supplementation during exercise seems to be more pronounced when endogenous carbohydrate availability is low.[28] Therefore, it seems reasonable to assume that any central effect of carbohydrate ingestion during exercise would become more important as the exercise progresses and endogenous carbohydrate availability decreases. However, as plasma glucose concentration and carbohydrate oxidation were also better maintained during the latter stages of the exercise with carbohydrate ingestion during exercise, this treatment could

reduce the perturbation in muscle energy homeostasis, ultimately reducing afferent signals to the brain.[29,30] It is possible that a combined central and peripheral effect of carbohydrate ingestion explains why RPE differences between carbohydrate and non-carbohydrate conditions increased as the exercise progressed in the present study.

The plasma glucose concentration in the present study increased from 6 to 9 am in the FEDPLA and FEDCHO conditions but not in the FASTPLA and FASTCHO conditions. The higher plasma glucose concentration at the beginning of exercise in the FEDPLA and FEDCHO conditions seemed to have promoted reactive hypoglycemia early in exercise (i.e., a larger reduction at 15 min in both FED trials, Fig. 4A). Reactive hypoglycemia may have occurred due to the high content of carbohydrate ingested at breakfast (137 g of carbohydrate). We adopted this meal regimen because a previous study reported that participants of similar body mass fed breakfast of the same composition showed a large increase in liver glycogen three hours later.[3] Reactive hypoglycemia occurs when exercise starts a few hours after a carbohydrate-rich meal, with elevated blood insulin levels triggering a larger muscle glucose uptake due the additive effect of insulin-dependent (meal) and insulin-independent (exercise) mechanisms.[31,32] However, the influence of reactive hypoglycemia on exercise performance is controversial, with some studies showing an impairment[33,34] and others showing no effect.[35,36] It seems that reactive hypoglycemia is an individual phenomenon, as some individuals with very low glycemic levels (< 3.5 $mmol.L^{-1}$) demonstrate no symptoms of hypoglycemia, while some individuals with normal glycemic levels (3.7 to 4.6 $mmol.L^{-1}$) report symptoms of hypoglycemia.[36] As the present study was not directly designed to investigate reactive hypoglycemia, it is not possible to fully disregard a possible negative influence of reactive hypoglycemia on exercise performance. It is noteworthy, however, that no participant reported symptoms of reactive hypoglycemia in the present study. The apparent reactive hypoglycemia was also reversed by carbohydrate

ingestion during exercise as plasma glucose concentrations increased throughout the exercise. This finding suggests that reactive hypoglycemia can be prevented by carbohydrate ingestion during exercise.

Some limitations of the present study must be recognized. The first breakpoint of the lactate-power curve was visually identified. Any error in this identification could impact the determination of exercise intensity of the constant-load exercise. However, the percentage of $\dot{V}O_2$max during the constant-load exercise was not largely variable across participants (60.8 ± 0.2%). We believe this lack of variation may have had no impact on our results. We have also used a non-protein respiratory quotient to calculate carbohydrate and fat oxidation. When fasting before exercise, amino acid oxidation during exercise increases.[37,38] However, amino acid oxidation is still low after several hours of fasting; [37,38] therefore, it is improbable that considerable error has occurred in our calculations of carbohydrate and fat oxidation. Additionally, we did not provide a calorie deficient meal for the no breakfast condition. The perception of ingesting breakfast *per se* can also influence exercise performance.[39] However, as the present study showed that performance was improved with carbohydrate ingestion during exercise regardless of a fed or fasted state, this result reinforces the importance of carbohydrate ingestion during exercise to maintain the plasma glucose concentration and carbohydrate oxidation rate. It is also important to mention that a 105-min steady-state exercise followed by a 10-km TT in cyclists who are not well-trained may not entirely reproduce a real-world scenario. Therefore, further studies should be performed to test this experimental approach during an exercise protocol simulating escape and attacks in experienced well-trained cyclists. In addition to controlling initial muscle glycogen levels, participants followed a rigorous diet and exercise regimen during the two days before each experimental trial. While this controlled diet-exercise regime should have guaranteed similar initial muscle glycogen levels across experimental trials, athletes usually adopt a

carbohydrate-rich diet (~80% carbohydrates) two or three days before a competition to increase their muscle glycogen stores. Therefore, further studies should replicate our approach using a pre-exercise carbohydrate-rich diet. Finally, water ingestion before trials was not strictly controlled and therefore there may have been an effect of the pre-exercise hydration status. Therefore, further studies should replicate our approach controlling pre-exercise hydration status.

In conclusion, cycling TT performance is improved by carbohydrate ingestion during exercise regardless of whether the exercise is begun in a fed or fasted state. The improved exercise performance with carbohydrate ingestion during exercise may be related to metabolic (higher plasma glucose concentration and higher carbohydrate oxidation rate) and perceptive (reduced RPE) alterations at the end of previous constant-load exercise, which enabled a larger power output during the following TT. Finally, our results suggest that breakfast may not be critical for exercise performance if carbohydrates are offered at adequate amounts during exercise.

PERSPECTIVE

Our results provide evidence that breakfast may not be critical for exercise performance if carbohydrates are ingested during exercise.[5] These findings are of practical relevance, as they suggest that athletes may not need to have a breakfast in the morning prior to an event. It is important to consider that our findings relate to the conditions of our experiment and may not be as relevant to longer or more intense exercise.[40] Our sample did not consist of well-trained cyclists; therefore, it will be important to confirm that similar results occur in these individuals. Further studies should also confirm that muscle glucose uptake is similar during exercise when carbohydrate is ingested during exercise irrespective of whether breakfast is consumed. Infusion of glucose tracers before and during exercise and

assessment of muscle and liver glycogen will be required for this purpose. It would also be interesting to determine whether differences are observed in central drive during submaximal exercise using the current protocol.

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FIGURE CAPTIONS

FIGURE 1. Experimental design. First visit: graded exercise test (GXT) followed by familiarization (FAM) with the 10-km time trial (TT). Second visit: a complete FAM session with the experimental protocol [105 min constant load (CL) + 10 km TT]. EXP 1 to 4: the four experimental conditions performed in a counterbalanced order: FAST + PLA during the exercise, FAST + CHO during the exercise, FED + PLA during the exercise, and FED + CHO during the exercise. Black bars represent two days before an experimental trial in which participants followed their usual diet (~2,800 kcal) and performed standardized, 60-min cycling training at 50% of the difference between the first and second lactate thresholds. Gray bars represent the day before an experimental trial in which participants followed their usual diet and replaced their last meal by a shake at 6 pm (650 kcal). No exercise was performed on this day. A second shake (breakfast) was ingested at 6 am on the experimental day in the FED condition only (824 kcal). Times at which beverage was ingested and blood samples were taken during the experimental trial are shown at the bottom of the figure. CHO: carbohydrate; PLA: placebo.

FIGURE 2. Power output during the 10-km cycling time trial. Values are the means ± SEM. Details of the main effect of distance are omitted for clarity. The dashed horizontal line represents mean power output during the trial for each condition.

FIGURE 3. Oxygen uptake (A), carbon dioxide production (B), carbohydrate oxidation rate (C), respiratory exchange ratio (D), fat oxidation rate (E), and heart rate (F) during a 105-min constant-load exercise in the FAST and FED conditions, with and without carbohydrate supplementation. Values are the means ± SEM. ‡ Values are significantly higher in the

carbohydrate than in the placebo conditions (P<0.05). Details of time and pre-exercise feeding effects are omitted for clarity.

FIGURE 4. Plasma glucose concentration (A), plasma lactate concentration (B), and rating of perceived exertion (C) during a 105-min constant-load exercise in the FAST and FED conditions, with and without carbohydrate supplementation. Values are the means ± SEM. # Values in both FED conditions are significantly higher than those in both FAST conditions (P<0.05). * Values in the FASTPLA, FEDPLA and FEDCHO conditions are significantly lower than those at rest (P<0.05). ϕ Values in the FASTCHO condition are significantly higher than those in the FASTPLA and FEDPLA conditions (P<0.05). Ɛ Values in the FEDCHO condition are significantly higher than those in the FASTPLA (P<0.05) condition. ‡ Values in both carbohydrate conditions are significantly higher (in A) and lower (in C) than those in both placebo conditions (P<0.05) § Values are significantly higher in the FEDCHO than in the FEDPLA condition (P<0.05). Details of time and pre-exercise feeding effects are omitted for clarity.

TABLE 1. Performance, physiological parameters, and rating of perceived exertion during the 10-km TT.

	FASTPLA	FASTCHO	FEDPLA	FEDCHO
Time (min)	21.7 ± 1.4	18.7 ± 0.4‡	20.2 ± 0.8	18.5 ± 0.3‡
Power output (W)	154 ± 7	197 ± 7‡	173 ± 9	198 ± 9‡
$\dot{V}O_2$ (L.min^{-1})	2.23 ± 0.04	2.67 ± 0.05‡	2.28 ± 0.08	2.71 ± 0.06‡
$\dot{V}CO_2$ (L.min^{-1})	2.18 ± 0.06	2.78 ± 0.10‡	2.48 ± 0.12	2.79 ± 0.12‡
RER	0.96 ± 0.01	1.04 ± 0.02‡	1.02 ± 0.02	1.08 ± 0.02‡
Glucose (mmol.L^{-1})	3.1 ± 0.2	4.5 ± 0.2‡	3.7 ± 0.2	4.7 ± 0.2‡
Lactate (mmol.L^{-1})	3.4 ± 0.6	4.2 ± 0.7‡	3.5 ± 0.7	4.2 ± 0.4‡
RPE	19 ± 0.7	18 ± 0.6	19 ± 0.3	18 ± 0.6

Values are mean ± SEM. FED: fed state; FAST: fast state; PLA: placebo; CHO: carbohydrate. Oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and respiratory exchange ratio (RER) are mean of the entire trial, while glucose, lactate and rating of perceived exertion (RPE) are values at the end of the trial. There was a main effect of supplement for all variables (P<0.05), except to RPE (P=0.46). ‡ Significantly different from PLA conditions.