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Isocaloric Carbohydrate Versus Carbohydrate-Protein Ingestion and Cycling Time-Trial Performance

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This study was designed to compare the effects of energy-matched carbohydrate (CHO) and carbohydrate-protein (CHO-PRO) supplements on cycling time-trial performance. Twelve competitive male cyclists and triathletes each completed 2 trials in a randomized and counterbalanced order that were separated by 5–10 d and applied in a double-blind manner. Participants performed a 45-min variable-intensity exercise protocol on a cycle ergometer while ingesting either a 9% CHO solution or a mixture of 6.8% CHO plus 2.2% protein in volumes providing 22 kJ/kg body mass. Participants were then asked to cycle 6 km in the shortest time possible. Blood glucose and lactate concentrations were measured every 15 min during exercise, along with measures of substrate oxidation via indirect calorimetry, heart rate, and ratings of perceived exertion. Mean time to complete the 6-km time trial was 433 ± 21 s in CHO trials and 438 ± 22 s in CHO-PRO trials, which represents a 0.94% (CI: 0.01, 1.86) decrement in performance with the inclusion of protein (p = .048). However, no other variable measured in this study was significantly different between trials. Reducing the quantity of CHO included in a supplement and replacing it with protein may not represent an effective nutritional strategy when the supplement is ingested during exercise. This may reflect the central ergogenic influence of exogenous CHO during such activity.

Keywords*:* sucrose, amino acids, exercise

Physical performance during exercise of moderate to high intensity is dictated to a large extent by the ability to maintain required rates of carbohydrate oxidation (Sherman, 1995). This assertion is widely supported by findings that physical performance can be enhanced through either maximizing the availability of endogenous carbohydrate before exercise (Bergstrom, Hermansen, Hultman, & Saltin, 1967; Karlsson & Saltin, 1971; Maughan & Poole, 1981; Williams, Brewer, & Walker, 1992) or providing exogenous sources of carbohydrate during exercise (Bjorkman, Sahlin, Hagenfeldt, & Wahren, 1984; Ivy, Res, Sprague, & Widzer, 2003; Mitchell et al., 1989; Tsintzas, Liu, Williams, Campbell, & Gaitanos, 1993; Tsintzas, Williams, Bobbis, & Greenhaff, 1996). Notably, the studies cited herein also support the ergogenic effects of carbohydrate ingestion across a variety of exercise conditions (i.e., running/cycling, prolonged/short-duration, continuous/intermittent; Tsintzas & Williams, 1998). The mechanism through which ingested carbohydrate imparts these benefits is almost certainly related to improved maintenance of euglycemia late in exercise, either directly or indirectly via increased oxidation of exogenous carbohydrate and potentially a reduced rate of endogenous glycogen degradation (Claassen et al., 2005; Coyle, Coggan, Hemmert, & Ivy, 1986; Tsintzas, Williams, Bobbis, & Greenhaff, 1995).

Further to these observations, subsequent research has extended current understanding such that a range of evidence-based nutritional recommendations is now available regarding the precise carbohydrate feeding strategy to maximize any metabolic or ergogenic advantages (Jeukendrup, 2004). A number of recent studies have examined whether the benefits of ingesting carbohydrate during exercise might be accentuated by the coingestion of a small quantity of protein (Osterberg, Zachwieja, & Smith, 2008; Romano-Ely, Todd, Saunders, & Laurent, 2006; Saunders, Kane, & Todd, 2004; van Essen & Gibala, 2006), with several more also reporting the metabolic impact of such nutritional intervention in terms of substrate oxidation during exercise (Ivy et al., 2003; Saunders, Luden, & Herrick, 2007; Valentine, Saunders, Todd, & St. Laurent, 2008). Three of these studies reported fatigue to occur 12–28% earlier during prolonged cycling when carbohydrate was ingested at a rate of 0.50–0.75 g/min as opposed to the same amount of carbohydrate but with added protein (Ivy et al., 2003; Saunders et al., 2004; Saunders et al., 2007). However, the practical implications of these findings are questionable given that exercising to the point of volitional fatigue cannot be assumed to provide an ecologically valid (i.e., real-life) reflection of competitive performance over a predefined distance. It is also difficult to determine

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whether the additional protein would have exerted any benefits in these studies had carbohydrate been ingested at a rate more consistent with current recommendations for optimizing physical performance, that is, ≥1 g/min (Jeukendrup).

Two successive investigations have addressed these issues by examining whether additional protein ingested during prolonged cycling can improve actual time-trial performance beyond that achieved when ingesting carbohydrate at a rate of 1 g/min (Osterberg et al., 2008; van Essen & Gibala, 2006). Notably, neither study observed any performance benefit of the additional protein, despite the fact that one of the studies even provided 25% more carbohydrate along with the added protein (Osterberg et al.). These findings notwithstanding, it remains difficult to identify the precise mechanism through which exercise capacity was enhanced in other studies (Ivy et al., 2003; Saunders et al., 2004; Saunders et al., 2007), given that the carbohydrate-protein mixtures ingested in those investigations made 20–25% more energy available for metabolism than the control solutions containing carbohydrate alone.

To our knowledge, only Romano-Ely et al. (2006) and Valentine et al. (2008) have evaluated the ergogenic effects of ingesting a combined carbohydrate-protein supplement during exercise relative to an energy-matched carbohydrate control. In the former study, ingesting carbohydrate at a rate of 1 g/min during prolonged cycling resulted in a similar exercise time to fatigue as did ingestion of an energy-matched 4:1 mixture of carbohydrate and protein, although it should be noted that the latter supplement also included antioxidants (Romano-Ely et al.). Similarly, Valentine et al. applied a comprehensive research design to evaluate cycling time to fatigue with ingestion of 1.3 g/min carbohydrate with added protein during exercise relative to both carbohydrate- and energy-matched controls (all containing antioxidants). That study also supported the pattern of the others cited previously in that the additional protein was no more effective than an energy-matched quantity of carbohydrate.

Overall, it is apparent that coingesting carbohydrate and protein during prolonged exercise can potentially postpone fatigue relative to the carbohydrate fraction alone, but only if the additional protein increases the energy content of the supplement and/or the carbohydrate fraction is ingested in suboptimal quantities. What remains to be established, however, are the ergogenic (i.e., time-trial) and metabolic (i.e., substrate-oxidation) effects of ingesting a carbohydrate-protein mixture relative to an energy-matched supplement providing carbohydrate alone at the recommended rate (i.e., ³1 g/min). This is of further interest given that all existing studies in this field have examined prolonged exercise (i.e., ~90–210 min) in which carbohydrate availability is likely to directly limit physical performance, whereas the current investigation was the first to examine exercise of shorter duration (i.e., ~60 min). Examination of these supplements under such circumstances is therefore intended to provide mechanistic insight regarding whether the potential ergogenic benefits of including protein in a supplement are entirely attributable to improved carbohydrate availability. In addition, although carbohydrate ingestion alone has been shown to improve even shorter duration or intermittent exercise performance (Tsintzas & Williams, 1998), the current study will extend understanding regarding the practical application of supplements including protein during variable-intensity exercise of less than 90 min in duration. Given the balance of the evidence cited, we hypothesized that reducing the quantity of carbohydrate included in a supplement and replacing it with protein would not significantly improve physical performance when the supplement was ingested during a competitive race simulation before a cycling time trial.

Methods

Participants

Twelve highly trained male cyclists and triathletes took part in this study (age 23.4 ± 3.2 years, body mass [BM] 72.5 ± 5.2 kg, VO_{2max} 64.3 ± 6.4 ml × kg BM^{-1} × min^{-1}). These individuals had 2.9 ± 2.1 years of competitive time-trial experience and habitually performed 11 ± 5 hr/week of training. Participants were briefed regarding the nature of the study, and they provided written informed consent in keeping with the requirements of the University of Bath Research Ethics Approval Panel, which approved this study.

Preliminary Measurements

Preliminary tests were conducted to determine each participant's maximal oxygen uptake (VO_{2max}). Participants completed an incremental test to exhaustion on a Monark cycle ergometer (model 824 E). The protocol included a 5-min warm-up at self-selected intensities followed by consecutive 3-min stages, at the end of which the load on the ergometer was increased by 0.5 kg. Data were included on the basis that the participant attained a respiratory-exchange ratio of ³1.15, a rating of perceived exertion (RPE) >17, and an increase in oxygen uptake (VO_2) £5 ml × kg BM^{-1} × min^{-1} in response to an increased load. Data were then used to calculate work intensities for the subsequent experimental trials. Another test was then performed 1 week before participants' first trials to confirm that calculated cycling power outputs were equivalent to the appropriate percentage of VO_{2max} and to ensure that participants were fully familiar with the time-trial distance to be performed. Specifically, although all participants were already accustomed to road races of similar distance to that of the trial, each individual in this study also completed one 15-min block of the variable-intensity exercise protocol followed by an attempt at the time trial exactly as would be performed during the actual experiment. Familiarization trials have been shown to be important for accurate assessment of

time-trial performance, even with experienced, highly trained cyclists (Laursen, Shing, & Jenkins, 2003). In this regard, investigations in our laboratory have revealed that a single familiarization is sufficient to reduce the coefficient of variation for test–retest reliability for time trials in this population to 1–2%. All participants continued their habitual training throughout the study period but refrained from strenuous exercise and avoided both alcohol and caffeine consumption during the 24 hr before any main trial.

Experimental Design

Participants performed two main trials in a randomized, counterbalanced order that were separated by 5–10 days and applied in a double-blind manner (i.e., supplements were prepared by an individual unattached to the study and provided in unmarked containers to those who interacted with participants during experiments). Over the 24 hr preceding the first of these trials, each participant was asked to weigh and record his usual diet before subsequently adhering to the same dietary intake over the same period before the second trial. Each trial consisted of a 10-min warm-up followed by a 45-min variable-intensity protocol and a 6-km time trial. Consequently, total exercise time was approximately 62 min. Fifteen minutes before the start of the warm-up and every 15 min throughout the variable-intensity exercise protocol (but not during the 6-km time trial), participants ingested either a solution containing carbohydrate alone (CHO) or a solution matched for available energy content but containing a 3:1 mixture of carbohydrate and protein (CHO-PRO).

Experimental Protocol

Each participant arrived in the laboratory at the same time of day for both trials after a 12-hr overnight fast. After informed consent to take part in the study was confirmed, BM was recorded and baseline 5-min gas and capillary-blood samples were obtained. Blood was sampled from the fingertip and analyzed immediately for glucose and lactate concentrations. Participants also provided a subjective rating (scale 1–10) of their perceived degree of baseline muscle soreness at this time as has been described previously (Thompson, Nicholas, & Williams, 1999). Fifteen minutes before commencing exercise, participants were provided with 7 ml/kg BM of one of the two experimental solutions and given 5 min to consume this volume. The exercise protocol began with a 10-min warm-up on an electronically braked SRM cycle ergometer (Schoberer Rad Mebtechnik) at an intensity corresponding to 60% VO_{2max}, followed by a 45-min variable-intensity exercise protocol. This variable-intensity exercise protocol was performed as three 15-min exercise blocks at intensities between 60% and 90% VO_{2max}, which has been shown to effectively deplete the glycogen content of both Type I and Type II muscle fibers (Kuipers, Keizer, Brouns, & Saris, 1987). In addition to achieving some degree of glycogen depletion, this protocol was intended to partially simulate the conditions of a competitive road race, during which intensity is decidedly variable in nature (Vogt et al., 2006). A schematic illustration of the precise design and timings adopted during this protocol is presented in Figure 1. Blood and 1-min expired-gas samples, heart rate, and subjective RPEs were taken at the end of the warm-up and at the end of each 15-min block, thus always after 3 min of steady-state exercise at 60% VO_{2max}. After these measurements, participants ingested an additional 2.5 ml/kg BM of the prescribed supplement and were again required to consume this within 5 min. Voluntary ingestion of other fluid (water) was permitted ad libitum during participants' first trials and then matched in each participant's second trial. There was a short (i.e., <1-min) rest interval on completion of the 45-min variable-intensity exercise protocol to allow the ergometer flywheel to be reset before participants began the 6-km time trial. The SRM ergometer was used for main trials to permit sufficient control and standardization over the exercise protocol, while also allowing participants to ride as they would during their usual training. Similar to previous investigations, time was blinded to participants during the time trial to prevent bias between trials, but distance was visible and also relayed verbally. Participants received verbal encouragement throughout the time trial, and a final capillary-blood sample was collected within 30 s of completion. Follow-up assessments of each participant's reported degree of perceived muscle soreness (scale of 1–10) were made 24 and 48 hr after the time trial.

Solution Composition

The CHO and CHO-PRO solutions were provided in equal volumes for each treatment (i.e., 1,053 ± 75 ml in total), and both made an estimated 22 kJ/kg BM available for metabolism (total energy intake 1,586 ± 113 kJ). The total amount of carbohydrate (sucrose) ingested was therefore 95 ± 7 g in CHO trials (i.e., 9%) and 72 ± 5 g in CHO-PRO trials (i.e., 6.8%), which equates to ingestion rates of approximately 1.4 and 1.1 g/min, respectively (with minor variations resulting from differences in exercise time). These ingestion rates were selected so that neither supplement would fail to meet current recommendations for optimizing physical performance, that is, ≥1 g/min (Jeukendrup, 2004). Equivalent energy content was achieved between trials by including 22 ± 2 g, or 2.2%, of whey-protein isolate in the CHO-PRO solution (i.e., 23 ± 2 g in total), which equates to an ingestion rate of approximately 0.4 g/min. The amino acid profile of this whey-protein isolate is presented in Figure 2. Pretesting was conducted to ensure that these solutions were successfully matched for taste (orange and passion fruit), consistency, and odor. Successful double-blinding was verified via an exit interview in which none of the participants was able to identify which solution they had ingested in each trial.

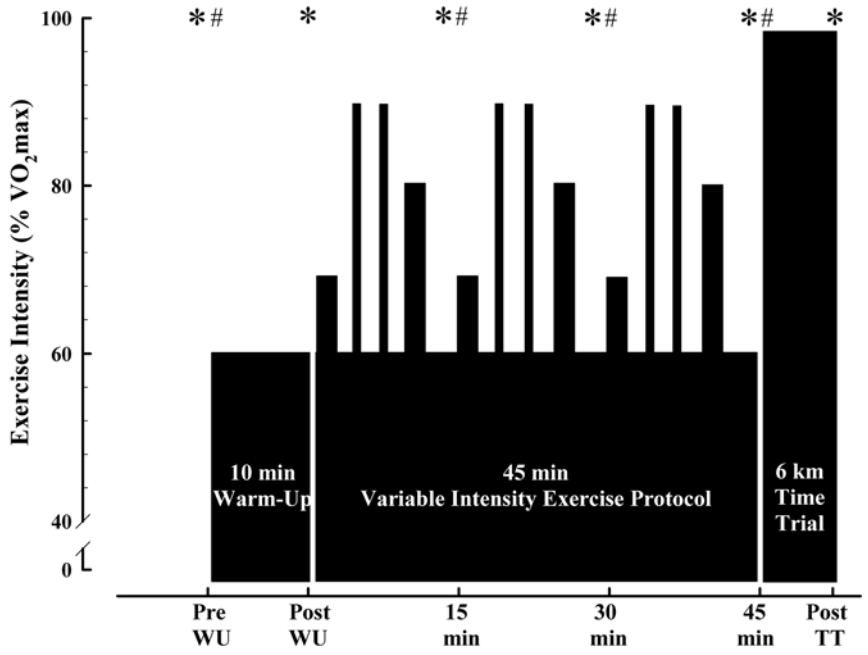

Figure 1 — Schematic of the exercise protocol. *Collection of blood and expired-gas samples and ratings of perceived exertion. #Ingestion of carbohydrate or carbohydrate-protein. WU = warm-up; TT = time trial.

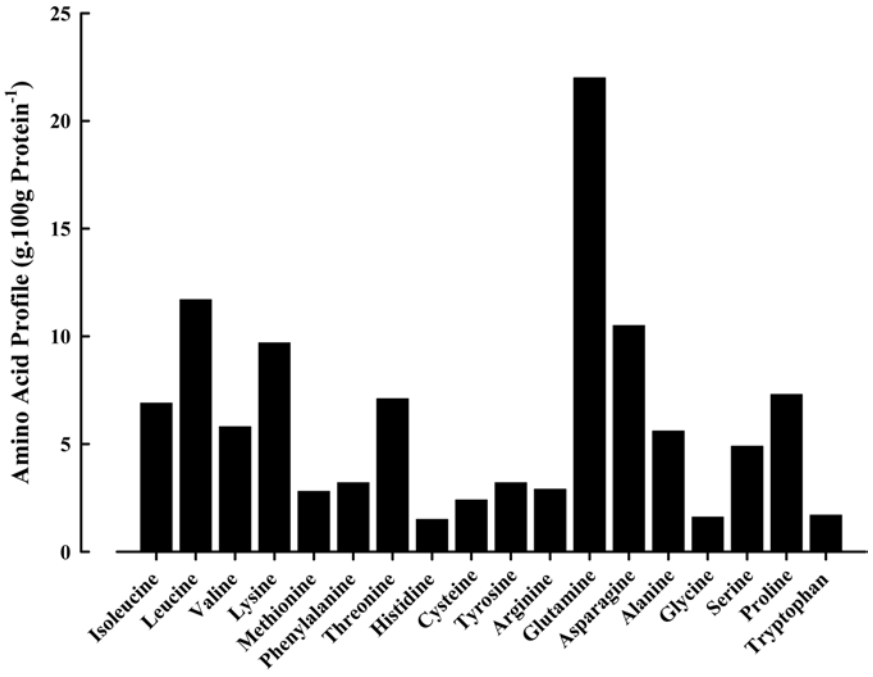

Figure 2 — Amino acid profile of the whey-protein isolate that was added to 0.8 g · kg body mass^{-1} · hr^{-1} of carbohydrate in the carbohydrate-protein solution.

Sampling and Analysis

Capillary blood samples (~1 ml) were taken from the fingertip and collected in heparinized containers. All blood samples were analyzed immediately for concentrations of glucose and lactate using an automated glucose and lactate analyzer (YSI 2300 STAT Plus). Expired-gas samples were collected using a Douglas bag, and the fractions of expired O_2 and CO_2 were assessed using paramagnetic and infrared analyzers, respectively (Servomex 1440, UK). Total volumes expired were determined using a dry gas meter (Harvard Apparatus, UK), and the temperatures of expired gases were measured with a digital thermometer (model C, Edale Instruments, UK). These analyzers were calibrated before each test with gases of known composition and volume within the physiological range, as certified by prior gravimetric analysis (British Oxygen Company, UK). Rates of oxygen uptake and carbon dioxide production (VO_2 and VCO_2) were then used to calculate rates of carbohydrate and lipid oxidation (g/min) using the following formulas:

$$\text{Carbohydrate oxidation} = (4.212 \times VCO_2) - (3.005 \times VO_2)$$

$$\text{Lipid oxidation} = (1.754 \times VO_2) - (1.754 \times VCO_2)$$

However, the combination of sustained high rates of carbohydrate ingestion with moderate- to high-intensity exercise resulted in high or exclusive reliance on carbohydrate oxidation for most participants (i.e., respiratory-exchange ratios 0.99–1.05), in which case lipid oxidation was assumed to be negligible and carbohydrate oxidation could be calculated without reference to VCO_2 (i.e., based solely on VO_2).

Statistical Analyses

A paired two-tailed *t* test was used to identify differences in time-trial performance between treatments, and a linear mixed model for repeated measures (Treatment × Time) was used to identify differences over time, with participants and trial order entered as random and fixed effects, respectively. In addition, simple summary statistics were calculated for the glycemic responses in each participant to inform interesting questions related to both the overall availability of blood glucose (area under the curve) and peak glucose concentrations after ingestion of each supplement (Hopkins, Marshall, Batterham, & Hanin, 2009; Matthews, Altman, Campbell, & Royston, 1990). These statistical analyses were performed using SPSS for Windows version 14.0 (SPSS, Inc., Chicago, USA). All data are expressed as means and standard deviations, with the magnitude of effect and associated statistical uncertainty for the outcome measures expressed in text as a percentage difference and confidence intervals where required. Despite the establishment of significant treatment effect with regard to time-trial performance in the current study with just 12 participants, a post hoc power analysis was applied and revealed that this sample size would provide a 67% probability of detecting the observed difference of 4.1 s between treatments with a standard deviation of differences of 6.4 s using a paired two-tailed *t* test with an alpha level of .05 (i.e., similar future investigations would require ~23 participants to achieve a 90% power of detecting such a difference statistically).

Results

Mean time-trial performance was 0.94% (0.01, 1.86) longer when the CHO-PRO supplement had been ingested relative to the energy-matched CHO supplement (p = .048; Figure 3), with no consistent difference between participants' first and second trials (i.e., 0.75% [–0.24, 1.75] such that no trial-order effects were apparent, p = .13). Blood glucose concentrations increased from baseline to the end of warm-up before decreasing over the ensuing 15 min and then increasing gradually over the remainder of the protocol (time: F = 14; p < .001). Although there was no significant Treatment × Time interaction for this variable (Figure 4), summary statistics indicate that the overall availability of blood glucose (area under the curve) was 18 mmol/L · 60 min (4, 32) lower (p = .02) in CHO-PRO trials (273 ± 36 mmol/L · 60 min) than in CHO trials (291 ± 30 mmol/L · 60 min). Similarly, peak glucose concentrations in each participant were 0.5 mmol/L (0.3, 0.9) lower (p = .04) in CHO-PRO trials (5.2 ± 0.8 mmol/L) than in CHO trials (5.7 ± 0.7 mmol/L). Blood lactate concentrations were not different between the CHO and CHO-PRO treatments at any time point (Figure 5). Concentrations were slightly elevated from basal levels with the onset of exercise and remained so throughout the variable-intensity exercise protocol. A more substantial increase in blood lactate concentrations was subsequently observed during the time trial, with eventual postexercise concentrations of 9.30 mmol/L across both treatments (time: F = 117; p < .001).

Data pertaining to substrate selection and oxidation rates are presented in Table 1. Mean rates of whole-body lipid oxidation averaged over the entire exercise protocol were 0.05 ± 0.03 g/min with the CHO treatment and 0.04 ± 0.02 g/min with the CHO-PRO treatment. Whole-body carbohydrate oxidation increased substantially with the onset of exercise (time: F = 578; p < .001), with absolute rates averaged across the entire exercise protocol of 3.38 ± 0.13 g/min with the CHO treatment and 3.43 ± 0.17 g/min with the CHO-PRO treatment.

There was a progressive rise in RPE from the start of the warm-up and throughout the variable-intensity exercise protocol in both the CHO and the CHO-PRO trials (time: F = 1250; p < .001). As can be seen in Table 1, RPE was significantly higher during CHO-PRO trials than CHO trials (treatment: F = 6.9; p = .01) but with no interaction of treatment and time apparent. Every participant reported maximal RPE immediately after the time trial. Participants' subjective ratings of perceived soreness were not substantially elevated relative to baseline either 24 hr or 48 hr after the time trial, and neither were they different between treatments (Table 1).

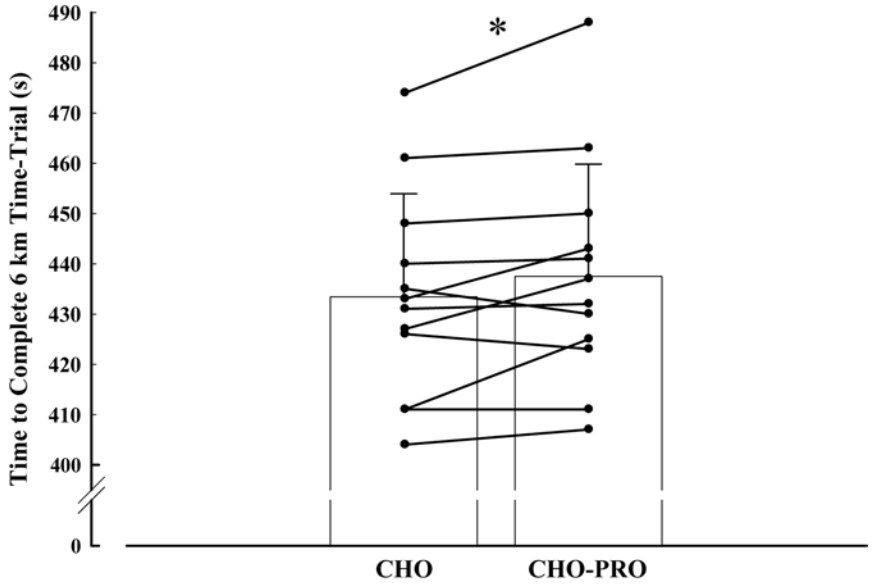

Figure 3 — Time-trial performance with ingestion of either carbohydrate (CHO) or carbohydrate-protein (CHO-PRO). *Values different between treatments (p = .048).

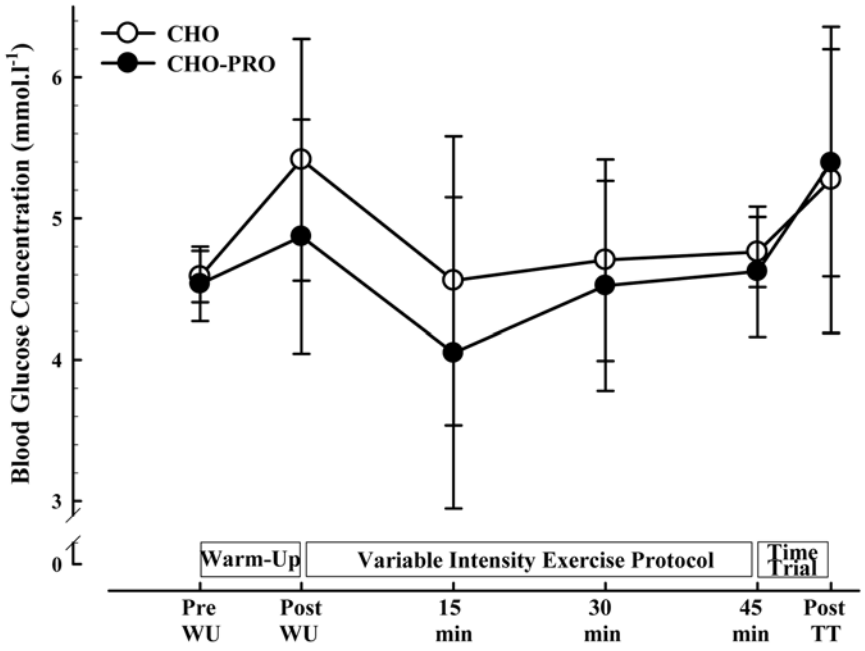

Figure 4 — Blood glucose concentrations during warm-up, variable-intensity exercise protocol, and 6-km time trial with ingestion of either carbohydrate (CHO) or carbohydrate-protein (CHO-PRO). WU = warm-up; TT = time trial.

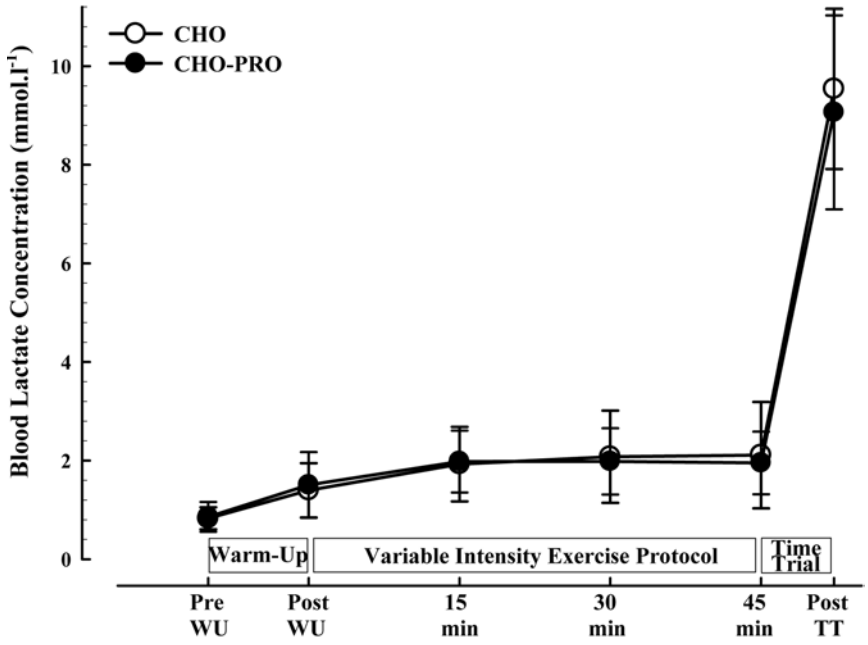

Figure 5 — Blood lactate concentrations during warm-up, variable-intensity exercise protocol, and 6-km time trial with ingestion of either carbohydrate (CHO) or carbohydrate-protein (CHO-PRO). WU = warm-up; TT = time trial.

Table 1 Substrate Metabolism and Ratings of Perceived Exertion and Muscle Soreness During and After Recovery From Warm-Up, Variable-Intensity Exercise, and Subsequent Cycling Time-Trial With Ingestion of Carbohydrate or Carbohydrate-Protein, *M* ± *SD*

			Variable-Intensity Exercise Protocol				
	Pre-warm-up	Post-warm-up	15 min	30 min	45 min	24 hr after	48 hr after
Carbohydrate oxidation (g/min)							
carbohydrate	0.55 ± 0.04	3.57 ± 0.13	3.38 ± 0.11	3.27 ± 0.13	3.51 ± 0.17		
carbohydrate-protein	0.55 ± 0.05	3.53 ± 0.15	3.46 ± 0.17	3.50 ± 0.17	3.57 ± 0.27		
CI for effect of protein	(–0.14, 0.15)	(–0.42, 0.49)	(–0.45, 0.30)	(–0.70, 0.22)	(–0.54, 0.43)		
Lipid oxidation (g/min)							
carbohydrate	0.01 ± 0.01	0.02 ± 0.01	0.05 ± 0.02	0.09 ± 0.04	0.06 ± 0.03		
carbohydrate-protein	0.02 ± 0.01	0.03 ± 0.03	0.05 ± 0.04	0.05 ± 0.04	0.07 ± 0.04		
CI for effect of protein	(–0.03, 0.01)	(–0.09, 0.06)	(–0.10, 0.10)	(–0.10, 0.18)	(–0.13, 0.11)		
Respiratory-exchange ratio							
carbohydrate	1.05 ± 0.03	1.04 ± 0.01	1.01 ± 0.01	0.99 ± 0.01	1.01 ± 0.01		
carbohydrate-protein	1.03 ± 0.03	1.02 ± 0.01	1.01 ± 0.01	1.00 ± 0.01	1.00 ± 0.01		
CI for effect of protein	(–0.06, 0.10)	(–0.03, 0.06)	(–0.03, 0.04)	(–0.04, 0.03)	(–0.03, 0.04)		
Rating of perceived exertion (6–20)[a]							
carbohydrate	—	9 ± 1	11 ± 1	12 ± 1	13 ± 1		
carbohydrate-protein	—	10 ± 1	12 ± 1	13 ± 1	14 ± 2		
CI for effect of protein	—	(–1.6, 0.4)	(–1.2, 0.2)	(–1.8, 0.2)	(–1.9, 0.6)		
Rating of perceived muscle soreness (1–10)							
carbohydrate	2.3 ± 0.4	—	—			2.8 ± 0.3	2.6 ± 0.6
carbohydrate-protein	1.6 ± 0.2	—	—			2.7 ± 0.3	2.5 ± 0.5
CI for effect of protein	(–0.3, 1.7)	—	—			(–0.4, 0.6)	(–1.0, 1.2)

Note. CI = confidence interval.

[a]Effect of treatment: $F = 7.8$, $p = .01$.

Mean heart rates during the variable-intensity exercise protocol were not different between CHO and CHO-PRO, averaging 151 ± 9 beats/min across both treatments. The mean heart rates at the end of the time trial were also not different between treatments, reaching values of 190 ± 12 beats/min with CHO and 190 ± 9 beats/min with CHO-PRO. Although not formally assessed, 2 participants reported severe gastrointestinal discomfort after the time trial after ingesting the CHO-PRO solution. There was no significant fixed effect of trial order on the response of any variable measured in this study.

Discussion

The main finding of this investigation is that ingesting carbohydrate alone in sufficient quantities during a competitive race simulation resulted in a significantly enhanced time-trial performance relative to an energy-matched mixture of carbohydrate and protein. Our hypothesis that performance would not be improved with reduced carbohydrate intake, despite replacing the energy deficit with protein, is therefore clearly supported. Although the absolute difference in time-trial performance between treatments only represents ~1%, this equates to approximately 60 m in terms of distance (i.e., 0.24 laps of a typical indoor velodrome) and may therefore be worthwhile in a competitive environment (although this effect must obviously be balanced against the test–retest reliability of this measure for a given individual when applied to any given race). Nonetheless, aside from the tendency for blood glucose concentrations to be higher early in exercise with CHO, no metabolic effects were apparent between treatments that could account for the observed performance outcome.

The divergent blood glucose responses with the onset of exercise after ingestion of each solution could be the result of a reduced rate of glucose appearance in the circulation or an increased rate of glucose disposal. Regarding glucose disposal, a large number of studies have reported increased insulin secretion during recovery from exercise when participants ingested carbohydrate with added protein (Berardi, Price, Noreen, & Lemon, 2006; Betts et al., 2005; Betts, Williams, Boobis, & Tsintzas, 2008; Betts, Williams, Duffy, & Gunner, 2007; Jentjens, van Loon, Mann, Wagenmakers, & Jeukendrup, 2001; Kaastra et al., 2006; Rotman, Slotboom, Kreis, Boesch, & Jequier, 2000; Van Hall, Saris, van de Schoor, & Wagenmakers, 2000; Van Hall, Shirreffs, & Calbet, 2000; van Loon, Saris, Kruijshoop, & Wagenmakers, 2000; van Loon, Kruijshoop, Verhagen, Saris, & Wagenmakers, 2000; Zawadzki, Yaspelkis, & Ivy, 1992), which can promote increased glucose uptake and glycogen storage in some situations (Berardi et al.; Ivy et al., 2002; Van Hall, Saris, et al., 2000; van Loon, Saris, et al., 2000; Zawadzki et al.). During exercise, however, the catecholamine-mediated suppression of insulin secretion can be expected to limit insulinemic responses similarly with both treatments (Hunt & Ivy, 2002), as supported by the insulin concentrations reported in other studies on this topic (Ivy et al., 2003; van Essen & Gibala, 2006).

In contrast, a reduced rate of glucose appearance in the circulation represents a far more plausible explanation for the attenuated elevation in blood glucose concentration when ingesting the CHO-PRO mixture. This is mainly because a smaller quantity of carbohydrate was included in this supplement but also because the presence of protein may have delayed gastric emptying such that even the carbohydrate that was ingested appeared more slowly from the gastrointestinal tract (Dangin et al., 2001). In support of the latter, a recent study showed that additional protein can reduce the glycemic response to a supplement ingested during recovery (even if it does not displace carbohydrate from the solution) but that this is not the result of an increased rate of glucose clearance from the circulation (Kaastra et al., 2006).

The precise mechanisms through which the subtle differences in blood glucose availability may have affected physical performance are not easily discernable. It is unlikely that this effect is related to improved maintenance of euglycemia, given that any differences in blood glucose concentrations between treatments during the variable-intensity exercise protocol were no longer apparent by the onset of the time trial. However, this does not discount the possibility that increased oxidation of exogenous carbohydrate early in exercise might have spared endogenous glycogen for use during the subsequent time trial, although substantial glycogen depletion is unlikely to have occurred in the relatively short exercise duration in the current study, particularly when carbohydrate ingestion rates were maintained above 1 g/min with both treatments. Our previous study in this area showed that the addition of protein to a carbohydrate solution ingested during a 4-hr recovery from prolonged exercise can result in increased extramuscular carbohydrate oxidation during subsequent exercise but without altering the rate of muscle glycogen degradation (Betts et al., 2008). Although whole-body carbohydrate oxidation was not different between trials during the variable-intensity exercise protocol in the current study, substrate selection was not assessed during the time trial to preserve the ecological validity of this test. It therefore remains a possibility that the carbohydrate supplement resulted in higher rates of exogenous carbohydrate oxidation during the time trial and consequently improved performance.

Given that RPE was marginally higher in CHO-PRO trials in the current study, it should also be acknowledged that the availability of blood glucose may have dictated subsequent performance during the time trial via alterations in central nervous system activation (Davis & Bailey, 1997). Indeed, brain carbohydrate metabolism is known to increase in the transition from rest to exercise (Ide, Schmalbruch, Quistorff, Horn, & Secher, 2000), which, in combination with the gradual depletion of endogenous carbohydrate reserves during prolonged exercise, may act as a feed-forward signal to mediate cerebral sensitivity to fluctuations in blood glucose availability (Claassen et al., 2005). In this way, the differences in blood glucose concentration during the initial variable-intensity exercise protocol may well have sensitized the central nervous system to compromised carbohydrate availability and therefore limited performance during the

time trial even in the absence of frank glycogen depletion or hypoglycemia. It has also been speculated that a similar interaction between ingested protein and the central nervous system might explain the potential for CHO-PRO ingestion to improve physical performance (Ivy et al., 2003), although the current findings are clearly not consistent with this suggestion. It should also be acknowledged that perceived exertion and therefore exercise performance might have been influenced to some extent by the gastrointestinal discomfort reported by some participants in the CHO-PRO trials.

Similar to all previous investigations in this area (Ivy et al., 2003; Osterberg et al., 2008; Romano-Ely et al., 2006; Saunders et al., 2004; Saunders et al., 2007; Valentine et al., 2008; van Essen & Gibala, 2006), a limitation of the current study is that a fairly restricted selection of metabolic data was gathered with which to inform discussion regarding potential mechanisms of action. This approach was adopted primarily to preserve the ecological validity of the cycling performance test but also because the evidence currently available has yet to implicate a specific mechanism to be explored in relation to shorter duration exercise. In the current study we therefore simply attempted to inform potential mechanisms by examining whether the documented benefits of ingested carbohydrate during shorter duration exercise (Tsintzas & Williams, 1998) might also be apparent when coingesting protein. Future investigations with a more reductionist, basic-science approach are therefore required to complement existing applied data to explore specific mechanisms.

In conclusion, the results of this study indicate that reducing the quantity of carbohydrate included in a supplement and replacing it with protein may not be an effective nutritional strategy when the supplement is ingested during 60 min of variable-intensity cycling, possibly reflecting the central ergogenic influence of exogenous carbohydrate during such activity. The precise mechanism underpinning this effect remains unclear but may be related to a delayed rate of carbohydrate appearance from the gastrointestinal tract resulting from the combination of reduced carbohydrate intake and the presence of protein.

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