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Carbohydrate and carbohydrate + protein for cycling time-trial performance

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Abstract

Carbohydrate intake during endurance exercise delays the onset of fatigue and improves performance. Two recent cycling studies have reported increased time to exhaustion when protein is ingested together with carbohydrate. The purpose of the present study was to test the hypothesis that ingestion of a carbohydrate + protein beverage will lead to significant improvements in cycling time-trial performance relative to placebo and carbohydrate alone. Thirteen cyclists completed 120 min of constant-load ergometer cycling. Thereafter, participants performed a time-trial in which they completed a set amount of work ($7 \text{ kJ} \cdot \text{kg}^{-1}$) as quickly as possible. Participants completed four experimental trials, the first for familiarization and then three randomized, double-blind treatments consisting of a placebo, carbohydrate, and carbohydrate + protein. Participants received 250 ml of beverage every 15 min during the constant-load ride. Time-trial performance for carbohydrate (37.1 min, $s=3.8$) was significantly ($P < 0.05$) faster than placebo (39.7 min, $s=4.6$). Time-trial performance for carbohydrate + protein (38.8 min, $s=5.5$) was not significantly different from either placebo or carbohydrate. Ingestion of a carbohydrate beverage during two hours of constant-load cycling significantly enhanced subsequent time-trial performance compared with placebo. The carbohydrate + protein beverage provided no additional performance benefit.

Keywords: *Cycling performance, protein, carbohydrate, hydration*

Introduction

The preponderance of research on carbohydrate intake during endurance exercise has shown that performance is improved and fatigue is delayed compared with a non-caloric placebo or water (Below, Mora-Rodriguez, Gonzales-Alonso, & Coyle, 1995; Coggan & Coyle, 1991; El-Sayed, Balmer, & Rattu, 1997; Febbraio, Chiu, Angus, Arkenstall, & Hawley, 2000; Fritzsche *et al.*, 2000; Jeukendrup, Brouns, Wagenmakers, & Saris, 1997). The precise mechanisms by which carbohydrate intake improves performance or delays fatigue have not been fully elucidated, but maintenance of blood glucose is likely involved (Coyle, 1992). Moderate- to high-intensity exercise lasting more than 1.5–2.0 h depletes muscle and liver glycogen stores and therefore forces greater reliance on exogenous carbohydrate to maintain blood glucose and exercise intensity. Although some research has suggested that carbohydrate intake can be ergogenic even when endogenous carbohydrate stores are sufficient such as during exercise lasting 45–60 min (Anantaraman,

Carmines, Gaesser, & Weltman, 1995; El-Sayed *et al.*, 1997; Jeukendrup *et al.*, 1997). Because ingesting fluid and carbohydrate during exercise has been shown to improve performance and maintain hydration under a variety of conditions, the American College of Sports Medicine (ACSM) recommends consuming 30–60 g of carbohydrate and enough fluid to stay within 2% of pre-exercise body weight (Sawka *et al.*, 2007).

Because there appears to be an upper limit to exogenous carbohydrate oxidation mediated by absorption mechanisms (Jentjens, Achten, & Jeukendrup, 2004; Jeukendrup & Jentjens, 2000), it has been hypothesized that the addition of other macronutrients to a carbohydrate drink can further improve performance. Medium-chain triglycerides (Angus, Hargreaves, Dancey, & Febbraio, 2000; Horowitz, Mora-Rodriguez, Byerley, & Coyle, 2000) and amino acids (Chinevere, Sawyer, Creer, Conlee, & Parcell, 2002; Madsen, MacLean, Keines, & Cristensen, 1996) have been explored as potential substrates during moderate- to high-intensity exercise with discouraging results.

Recently, two studies (Ivy, Res, Sprague, & Widzer, 2003; Saunders, Kane, & Todd, 2004) have shown that the addition of protein to a carbohydrate beverage extended time to fatigue compared with carbohydrate alone. Both studies utilized cycling time-to-exhaustion, which, although a frequent measure of performance, has been shown to have poor reproducibility (Jeukendrup, Saris, Brouns, & Kenster, 1996). In contrast, cycling studies that require a set amount of work to be completed as quickly as possible (i.e. a time-trial) or involve the accomplishment of the greatest amount of work in a set period of time are closer to the competitive task and are more reproducible. In addition, recent studies (Ivy *et al.*, 2003; Saunders *et al.*, 2004) examining the benefit of carbohydrate and protein during exercise have provided carbohydrate at a rate that was at the lower end ($\sim 30 \text{ g} \cdot \text{h}^{-1}$) of what is generally recommended, and this may have been the reason for the observed benefit for carbohydrate plus protein. In these studies, carbohydrate intake was matched but energy was not.

We hypothesized that when carbohydrate is provided during prolonged exercise at a reasonably high rate ($\sim 60 \text{ g} \cdot \text{h}^{-1}$), the addition of protein will not provide an extra performance benefit. Therefore, we sought to determine: (1) if a carbohydrate beverage would improve time-trial cycling performance after 2 h of constant-load ergometer exercise compared with a non-caloric, flavoured placebo when consumed at a rate to deliver one litre of fluid and 60 g carbohydrate per hour; and (2) if the addition of protein to a carbohydrate beverage would further improve performance over carbohydrate alone. We chose to use commercially available sports drinks for comparison in this study for the practical implications this topic has for cyclists.

Methods

Participants

Thirteen trained, male cyclists aged 27–36 years (mean 31.2 years, $s = 2.4$) volunteered to participate in the study. They had a mean peak oxygen uptake

($\dot{V}O_{2\text{peak}}$) of $56.0 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ($s = 6.9$), a mean body mass of 73.4 kg ($s = 9.0$), and a mean body mass index of $22.6 \text{ kg} \cdot \text{m}^{-2}$ ($s = 1.9$). The mean cycling experience of the participants was 5 years ($s = 3.1$), and they were either road cyclists or triathletes. Data was collected over the course of 10 months. Consequently, not all participants were in the same phase of their training cycles. However, because all trials were completed within 4 weeks, it was assumed that the participants began and completed all trials at the same relative fitness. The experimental protocol was approved by a Human Subjects Review Committee and all athletes gave written informed consent before participation.

Study design

This was a randomized, placebo-controlled, and double-blind study in which each participant completed all treatments serving as his own control. Participants were weighed before and after each trial to calculate percent change in body mass. Each trial consisted of 120 min of constant-load ergometer cycling at a workload 5% below that which elicited the onset of blood lactate accumulation. Thereafter, participants performed a time-trial in which they had to complete a set amount of work ($7 \text{ kJ} \cdot \text{kg}^{-1}$) as quickly as possible. Each participant completed a familiarization trial and three randomized, double-blind treatments. The three experimental trials were a 6% carbohydrate beverage (Gatorade Thirst Quencher, The Gatorade Company, Chicago, IL), a carbohydrate + protein beverage (7.5% carbohydrate and 1.6% protein) (Accelerade, Pacific Health Laboratories, Matawan, NJ), and a non-caloric, artificially sweetened and flavoured placebo containing the same electrolyte profile as the 6% carbohydrate beverage. Composition of the beverages is shown in Table I. They were served in opaque bottles, and each beverage was flavoured differently in an attempt to prevent taste and mouth feel characteristic comparisons between the beverages. Participants received a fixed volume of 250 ml every 15 min during the steady-state ride starting at

Table I. Composition of the experimental beverages.

Test beverage	Type of carbohydrate*	Protein*	Na (mmol·l ⁻¹)*	K (mmol·l ⁻¹)*	Flavour	Osmolality (mOsm)
Carbohydrate	Glucose (2%), fructose (2%), sucrose (2%)	None	18	3	x	365
Carbohydrate + protein	Glucose (1.7%), sucrose (1.3%), trehalose (2.8%), fructose (1.7%)	Whey (1.6%)	23	4	x	460
Placebo	None (aspartame)	None	18	3	x	54

*All values based on analysis and % weight by volume.

15 min for a total of one litre of fluid per hour. No fluid was given during the time-trial.

Participants cycled in thermoneutral conditions (23°C, 40% relative humidity) and body mass was measured before and after exercise to assess the change in body mass between trials. Heart rate and rating of perceived exertion (RPE) were measured every 15 min during the 120-min constant-load ride. Each participant kept a diet record the day before their first randomized trial and repeated that diet for each subsequent trial. All participants reported to the laboratory at 05.45 h for each trial after an overnight fast.

Preliminary testing

Peak oxygen consumption. Before the trials, $\dot{V}O_{2\text{peak}}$ was determined for each participant. They performed an incremental cycle test to exhaustion on an electrically braked cycle ergometer (LODE Excalibur Sport V 2.0 Groningen, Netherlands). The test consisted of a 5-min warm-up at 100 W, followed by an increase of 50 W every 2.5 min until they reached 250 W. Thereafter, workload was increased by 25 W every minute until volitional exhaustion (participants were unable to continue pedalling while seated). Heart rate (Polar Electro, Inc) and respiratory data were collected continuously using a portable metabolic cart (Cosmed K4b²). The $\dot{V}O_{2\text{peak}}$ was used to calculate submaximal workloads for the blood lactate test.

Blood lactate test. To set a workload for the experimental trials, participants also completed an incremental exercise test with assessment of blood lactate response. For this test workloads were calculated to elicit 50–90% $\dot{V}O_{2\text{peak}}$ in 5% increments. Upon arrival, participants had a venous catheter inserted into a forearm vein and a resting blood sample was taken and analyzed for lactate (Instrumentation Laboratories, Lexington, MA). Participants then began cycling at 50% $\dot{V}O_{2\text{peak}}$ for 3 min. Blood samples were taken at the end of a 3-min stage and workload was increased to the next 5% increment. Respiratory data were collected and heart rate was measured continuously throughout the test. The test was terminated after cycling for 3 min at a workload representing 90% $\dot{V}O_{2\text{peak}}$.

Calculation of workload. The blood lactate results were plotted versus workload and a power output (in watts) equivalent to 5% below that which elicited a blood lactate concentration of 4 mmol·l⁻¹ was calculated for the 120-min constant-load ride on the stationary cycle ergometer. For the time-trial portion of the trial, the cycle ergometer was changed from a hyperbolic mode (workload independent of cadence)

to the linear mode (workload dependent upon cadence). A linear factor, calculated as work rate at 85% $\dot{V}O_{2\text{peak}}$ divided by (100 rev·min⁻¹)² was entered into the ergometer controller (Cox *et al.*, 2000).

Familiarization trial. The first trial for all participants was the familiarization trial, which served two purposes: (1) to allow the participants to experience what the experimental trials would be like and (2) for adjustments in workload to be made, if necessary, so participants could complete the 120-min constant-load ride. At minimum, participants were required to complete the constant-load cycling at a workload that was above their lactate threshold (lactate concentration of 1 mmol·l⁻¹ above baseline). If unable to do so, they were eliminated from the study. Respiratory measurements were taken during the constant-load ride to ensure that participants were exercising at an intensity at which carbohydrate was the primary fuel oxidized ($R \geq 0.90$). Once workload was set, participants rode at this intensity for the three randomized experimental trials. Trials were separated by 5–7 days to allow sufficient recovery between trials but also complete all trials while at the same relative fitness level. Participants were permitted to see their cadence during the constant load ride.

Time-trials

Following the constant-load ride, participants were given a 2-min rest while the bike was converted from the hyperbolic to the linear mode. The amount of work for the time-trial was kept constant throughout the trials based on the participants' body mass on the day of the familiarization trial (7 kJ/kg). Participants were blind to their cadence and no feedback or encouragement was given during the time-trial; however, they were told when they had completed 25%, 50%, 75%, and 100% of the trial. Heart rate, revolutions per minute, and accumulated work was recorded every 3 min.

Statistical analysis

SPSS version 11.5 was used to analyze the data. One-way analysis of variance (ANOVA) using a general linear model was used to determine differences between mean values. Two-way ANOVA with repeated measures was used to determine differences between trials at each time point (*post-hoc* = Duncan). Data are reported as the mean and standard deviation (*s*). Statistical significance was set at $P < 0.05$. Power was determined for mean differences for time-trial performance using Cohen's effect size, *d* (mean difference as a proportion of the average standard deviation for repeated measures).

Coefficients of variation (CVs) were calculated for familiarization and treatment time-trial performance to further characterize measurement reliability.

Results

Heart rate, rating of perceived exertion, and percent dehydration

There was no difference at any 15-min time point during the constant-load ride for RPE or heart rate between the trials. Percent dehydration was 2.2% ($s=0.8$), 2.4% ($s=0.9$), and 2.3% ($s=0.8$) for carbohydrate, carbohydrate + protein, and placebo respectively. There was no difference between trials. The mean workload for the 120-min constant-load ride was 216 W ($s=35.2$).

Time-trial performance

Time-trial performance for carbohydrate (37.1 min, $s=3.8$) was significantly ($P < 0.05$) faster than placebo (39.7 min, $s=4.6$). Time-trial performance for carbohydrate + protein (38.8 min, $s=5.5$) was not significantly different from either placebo or carbohydrate (see Figure 1 for grouped time trial data). Mean power output during the time-trial for carbohydrate (234.8 W, $s=33.4$) was significantly higher ($P < 0.05$) than in the placebo trial (221.3 W,

$s=32.8$). Power output for carbohydrate + protein (228.9 W, $s=38.2$) was not different from either carbohydrate or placebo. Twelve of 13 participants recorded their fastest time-trial time when they were drinking carbohydrate (7/13) or carbohydrate + protein (5/13). There was no order effect of the treatments. The total amount of carbohydrate given during the carbohydrate and carbohydrate + protein trial was 120 g and 150 g respectively. The carbohydrate + protein beverage also provided 32 g of protein.

Discussion

It is widely accepted that carbohydrate ingestion improves performance by delaying the onset of fatigue. This was also the case in our experiment, with the carbohydrate–electrolyte beverage improving time-trial performance by 6% over the non-caloric placebo. As summarized by Coyle (1992), this effect was most likely due to maintenance of blood glucose during exercise. Even though carbohydrate + protein had more calories, given that it contained protein and a higher concentration of carbohydrate, performance during this treatment was directionally but not statistically different from placebo. We would expect blood glucose concentrations also to be maintained with carbohydrate + protein, so it is surprising a more robust performance benefit was not evident. However, it should be noted that two of the participants had their worst time-trial effort when consuming carbohydrate + protein, so a greater variability in inter-individual response provides a partial explanation for the lack of a statistically significant benefit.

While several investigators have touted reasons why protein, when added to a carbohydrate drink consumed during exercise should improve endurance and performance, few have discussed potential unfavourable effects of the addition of protein. One or a combination of the following may be reasons why some do not attain an added performance benefit when consuming a protein-containing carbohydrate drink during exercise. First, additional calories can slow down gastric emptying (Murray, Bartoli, Stofan, Horn, & Eddy, 1999). The total caloric load of carbohydrate + protein was 51% greater than that of carbohydrate. This could have put the participants at a greater risk of gastrointestinal distress wherein effort may have been diminished. Second, some but not all studies have shown carbohydrate–protein ingestion to result in a greater insulin response. While theoretically this could drive exogenous carbohydrate oxidation, higher insulin concentrations will suppress lipolysis leading to a greater reliance on muscle glycogen. Inefficient use of muscle glycogen might produce suboptimal

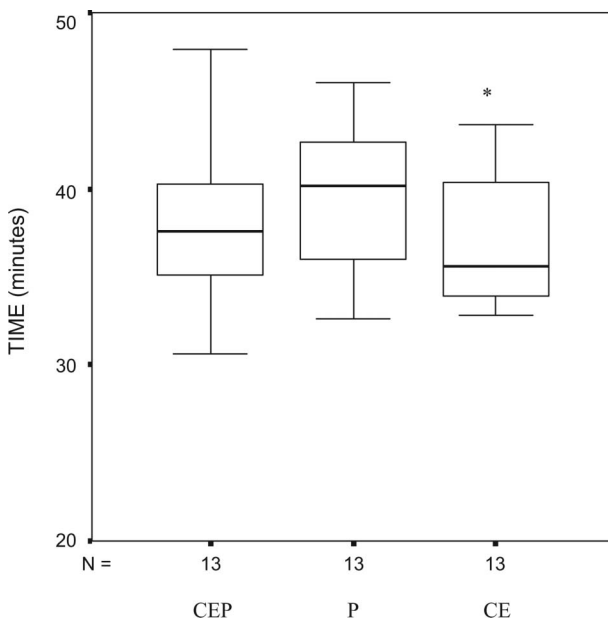


Figure 1. Grouped time-trial median and standard deviations of each treatment. *Carbohydrate–electrolyte (CE) significantly different than placebo (P) but not carbohydrate–electrolyte + protein (CEP). The thick line represents the median, whereas the top and bottom of the box represent the 75th and the 25th percentile respectively. The whiskers represent the 5th and 95th percentiles.

efforts. Lastly, ingested protein requires metabolism and the necessary transamination and deamination reactions generate urea and ammonia. In particular, ammonia accumulation in cerebral spinal fluid may provoke central fatigue (Nybo, Dalsgaard, Steenberg, Moller, & Secher, 2005).

The prior discussion is somewhat presumptive since we did not measure blood, muscle or respiratory responses. While this is clearly a limitation, our intentions are to first assess performance responses and then move on to mechanistic studies and observations. Too often studies are conducted under distracting conditions such that participant effort, mentally and physically, is influenced by numerous experimental procedures that may or may not explain the performance result. Thus, we limited experimental interaction with the participants so that 100% of their attention could be devoted to the performance task at hand, even during the 120 min preceding the time-trial. Consequently, we feel the performance measurements made for this study are robust and of high quality.

Our results support those of Van Essen and Gibala (2006), who reported no improvement in 80-km cycling performance when protein was added to a carbohydrate–electrolyte beverage. Their study, which also provided cyclists with 60 g of carbohydrate and one litre of fluid per hour, showed parity between the two carbohydrate beverages with both trials resulting in significantly faster times than placebo. The beverages used in their study were matched for carbohydrate concentration but were not matched for calories. Despite this, the cyclists did not perform better with the addition of protein. Using a variety of protocols, others (Davis, Welsh, De Volve, & Alderson, 1999; Madsen *et al.*, 1996; Watson, Shirreffs, & Maughan, 2004) have shown that the addition of branched-chain amino acids to a carbohydrate beverage did not enhance performance over the beverage that contained only carbohydrate. These results are not surprising because, although branched-chain amino acids are oxidized, they are typically not utilized to a large extent until muscle glycogen is lowered and blood glucose is no longer able to meet the needs for carbohydrate oxidation in muscle. Failure of the ingestion of branched-chain amino acids to consistently and significantly improve endurance performance reinforces the unlikelihood that protein would be beneficial, as many of its constituent amino acids are not directly oxidized by the muscle.

We chose not to match carbohydrate or calorie content but to test the same commercially available products that were used by Saunders *et al.* (2004), who found a longer time to exhaustion with a carbohydrate + protein beverage compared with carbohydrate alone. Although they matched carbo-

hydrate content between the beverages by concentrating the carbohydrate beverage, the carbohydrate dose that the participants received per hour was still lower than what we gave our cyclists. Therefore, one reason for the discrepant findings could be the relatively low fluid volume and carbohydrate dose given to the participants tested by Saunders *et al.* (30 g of carbohydrate per hour and 400 ml fluid). Madsen *et al.* (1996) studied the effects of a carbohydrate beverage, carbohydrate plus branched-chain amino acids, and a placebo on a simulated 100-km time-trial. They found no difference between treatments when they gave 550–700 ml of fluid per hour and 30 g of carbohydrate prior to exercise, 27.5 g during the first hour of the time-trial, and 35 g each subsequent hour. The low dose of carbohydrate given to these cyclists (27.5–35.0 g·h⁻¹) may have been inadequate to realize a performance benefit during prolonged exercise. Conversely, Angus *et al.* (2000) studied the effects of medium-chain triglycerides + carbohydrate versus carbohydrate and placebo and found that 100-km time-trial performance was significantly faster with both carbohydrate beverages than with placebo but that medium-chain triglycerides provided no additional benefit over carbohydrate alone. They provided their cyclists with 60 g of carbohydrate per hour delivered in one litre of fluid. Taken together, it appears that ingesting ~60 g of carbohydrate per hour during exercise is necessary to derive a consistent performance benefit. Furthermore, at this level of intake, there appears to be little justification for adding fatty acids, protein or amino acids for further enhancement of performance.

The time-trial protocol we implemented for this study was similar to that used previously (Cox *et al.*, 2000). Simulated time-trials in which cyclists are required to complete a certain amount of work in the shortest time possible or the most work in a given amount of time have been shown to be more reliable and reproducible for research than studies that employ a ride-to-exhaustion protocol. Jeukendrup *et al.* (1996) reported a coefficient of variation of 26.6% (range 17.4–39.5%) in a time-to-exhaustion protocol. In the same study, they observed a much smaller coefficient of variation of 3.5% for a protocol that implemented a preload followed by a time-trial. Krebs and Powers (1989) also found time-to-exhaustion to be an unreliable measure of performance with high within-participant variability (range 5.2–55.9%), while Hickey and colleagues (Hickey, Costill, McConell, Widrick, & Tanaka, 1992) reported a coefficient of variation of 1% in participants repeating a simulated 40-km time-trial. In our hands, the coefficient of variation for repeated time-trial efforts is between 3 and 5%. The reason for

better reproducibility has not been clearly elucidated but it likely involves pacing against subjective feelings of fatigue with a clear endpoint in mind. Regardless, a highly reproducible performance test protocol is necessary to assess with accuracy the ergogenic effects of hydration and nutrition interventions.

Our participants performed one familiarization trial in which they received only water to drink. We found that the randomized placebo treatment resulted in a faster performance time than the water familiarization, indicating that the effect of carbohydrate was strong enough to overcome a “placebo effect”. Within the context of our randomization scheme, it seems that one familiarization trial was adequate because of the significant difference between placebo and carbohydrate. Although the treatments were not counterbalanced – five of the 13 participants received carbohydrate first, six placebo first, and two received carbohydrate + protein first – we did not find a statistically significant order effect. However, any influence of order would have favoured carbohydrate + protein.

The fact remains, two studies (Ivy et al., 2003; Saunders et al., 2004) have shown that ingestion of a carbohydrate–protein mixture during prolonged exercise extends time to fatigue, yet there are no mechanistic data available to explain this observation. It is possible that when carbohydrate is consumed in suboptimal amounts during exercise that protein provides an ergogenic effect for the muscle or brain during time-to-exhaustion trials. Additional studies are warranted to evaluate mechanisms and whether an increase in time to fatigue is observed when protein is ingested during exercise together with an optimal amount of carbohydrate. Regardless, the results of the present investigation are much more applicable to the manner in which most endurance athletes train and compete.

In conclusion, our data show that ingesting carbohydrate during prolonged moderate- to high-intensity exercise delays fatigue and results in significantly faster time-trial performance than a non-caloric beverage. The addition of protein provided no further benefit.

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