

# Failure of Protein to Improve Time Trial Performance when Added to a Sports Drink

MARTIN VAN ESSEN and MARTIN J. GIBALA

*Exercise Metabolism Research Group, Department of Kinesiology, McMaster University, Hamilton, Ontario, CANADA*

## ABSTRACT

VAN ESSEN, M. and M. J. GIBALA. Failure of Protein to Improve Time Trial Performance when Added to a Sports Drink. *Med. Sci. Sports Exerc.*, Vol. 38, No. 8, pp. 1476–1483, 2006. **Introduction:** Recent studies have reported that adding approximately 2% protein to a carbohydrate sports drink increased cycle endurance capacity compared with carbohydrate alone. However, the practical implications of these studies work are hampered by the following limitations: (a) the rate of carbohydrate ingestion was less than what is considered optimal for endurance performance, and (b) the performance test (exercise time to fatigue) did not mimic the way in which athletes typically compete (i.e., a race in which a fixed distance or set amount of work is performed as quickly as possible). **Purpose:** We tested the hypothesis that adding 2% protein to a 6% carbohydrate drink (CHO-PRO) would improve 80-km cycling time trial performance, as compared with a 6% carbohydrate drink (CHO) and a nonenergetic sweetened placebo (PLAC). **Methods:** Ten trained male cyclists ( $24 \pm 2$  yr;  $\dot{V}O_{2\text{peak}} = 63 \pm 2$  mL·kg<sup>-1</sup>·min<sup>-1</sup>; mean  $\pm$  SE) performed an 80-km laboratory time trial (TT) on three occasions separated by 7 d. In a double-blind crossover manner, subjects ingested CHO-PRO, CHO, or PLAC at a rate of 250 mL every 15 min with no temporal, verbal, or physiological feedback. **Results:** Time to complete the TT was 4.4% lower ( $P < 0.002$ ) during CHO ( $135 \pm 9$  min) and CHO-PRO ( $135 \pm 9$ ) compared with PLAC ( $141 \pm 10$ ), with no difference between CHO and CHO-PRO ( $P = 0.92$ ). **Conclusion:** Ingesting 6% carbohydrate at a rate of 1 L·h<sup>-1</sup> (60 g·h<sup>-1</sup>) improved 80-km TT performance in trained male cyclists. However, adding 2% protein to a 6% carbohydrate drink provided no additional performance benefit during a task that closely simulated the manner in which athletes typically compete. **Key Words:** EXERCISE METABOLISM, CARBOHYDRATE, CYCLING PERFORMANCE

It is generally accepted that carbohydrate ingestion during exercise improves endurance performance in events lasting  $\geq 1$  h, likely via maintenance of euglycemia and a high rate of carbohydrate oxidation, although other mechanisms may be involved (7,18). The ergogenic effect of carbohydrate ingestion has mainly been demonstrated using time-to-fatigue tests, in which the goal is to sustain a given power output for as long as possible (6,8,9). A few studies have shown that carbohydrate feeding improves performance during tasks that more closely resemble athletic competition (e.g., a prolonged laboratory time trial) (2,21) or short time trials after prolonged periods of steady-state exercise (11,13), although this is not a universal finding (23). Nonetheless, most evidence suggests that carbohydrate feeding is ergogenic for endurance performance, and leading sports nutrition

organizations recommend consuming 30–60 g·h<sup>-1</sup> of carbohydrate during prolonged exercise (1,5,7). Given the importance of fluid balance, a convenient way for athletes to satisfy their hydration needs and meet this rate of carbohydrate ingestion is to drink 600–1400 mL·h<sup>-1</sup> of a 4–8% carbohydrate solution, preferably in small, frequent doses from the onset of activity (1,5,7).

It seems prudent for endurance athletes to ingest carbohydrate in amounts on the higher end of the recommended range because the oxidation of single carbohydrates, such as those typically found in sports drinks (e.g., glucose, sucrose, and glucose polymers such as maltodextrin), is optimal at ingestion rates near 1.0–1.2 g·min<sup>-1</sup> (18). Indeed, a recent comprehensive review of the literature by Jeukendrup (18) recommended a carbohydrate intake of approximately 60–70 g·h<sup>-1</sup> for optimal carbohydrate delivery. However, ingesting single carbohydrates in amounts  $> 60$ –70 g·h<sup>-1</sup> will not result in higher exogenous carbohydrate oxidation rates and is likely to be associated with gastrointestinal discomfort and, possibly, impaired performance (7,18). Therefore, investigators have examined whether the simultaneous ingestion of multiple types of carbohydrate (16,17) or coingestion of other macronutrients with carbohydrates (2,15,23,28,30) further enhances carbohydrate oxidation or endurance performance.

Recently, two studies reported that adding approximately 2% protein to a typical carbohydrate sports drink

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Address for correspondence: Martin J. Gibala, Ph.D., Department of Kinesiology IWC AB122, McMaster University, 1280 Main Street West, Hamilton, Ontario, L8S 4K1; E-mail: gibalam@mcmaster.ca.

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improved cycle endurance capacity by approximately 30% compared with carbohydrate alone in trained cyclists (15,28). These findings are intriguing, but the practical implications of the work are hampered by the research designs used. First, the rate of carbohydrate ingestion was less than the optimal intake of 60–70 g·min<sup>-1</sup> recommended by Jeukendrup (18). Ivy et al. (15) had subjects with a mean body mass of 70 kg ingest carbohydrate at a rate of 47 g·h<sup>-1</sup>, whereas a 70-kg subject in the study by Saunders et al. (28) received 37 g·h<sup>-1</sup> of carbohydrate. Second, the nature of the exercise tests used by Ivy et al. (15) and Saunders et al. (28) (i.e., cycling at a fixed workload until fatigue) did not mimic the manner in which athletes typically compete (i.e., a race in which a fixed distance or a set amount of work is performed as quickly as possible).

The present study investigated whether the addition of 2% protein to a 6% carbohydrate drink would improve 80-km cycling time trial performance, as compared with a 6% carbohydrate drink and nonenergetic sweetened placebo, when ingested at a rate of 1 L·h<sup>-1</sup>. We recruited trained cyclists and had them ingest a carbohydrate drink during exercise at a rate (60 g·h<sup>-1</sup>) considered near optimal for improving endurance performance (18), then perform a cycling task (lasting approximately 2–2.5 h) that closely simulated “real-life” endurance exercise competition. Using a double-blind, placebo-controlled, repeated-measures crossover design, we tested the following hypotheses: (a) ingestion of a carbohydrate–protein drink and carbohydrate drink would improve time trial performance compared with a placebo; and (b) ingestion of a carbohydrate–protein drink would improve time trial performance compared with a carbohydrate drink. We also obtained venous blood samples during exercise to investigate potential differences in substrate metabolism between trials.

## METHODS

**Subjects.** Ten trained male cyclists with a background in either road cycling or triathlon (Table 1) volunteered to participate in the study. The subjects had been engaged in regular cycle training for 5.0 ± 0.8 yr (mean ± SE), and all had raced competitively at the provincial, national, or international levels. The purpose and potential risks of the experiment were explained to all subjects before their participation, and all provided written informed consent.

TABLE 1. Subject characteristics.

Subject	Sport	Age (yr)	Height (cm)	Weight (kg)	BMI (kg·m <sup>-2</sup> )	$\dot{V}O_{2peak}$ (mL·kg <sup>-1</sup> ·min <sup>-1</sup> )
1	Cycling	20	178	71	22.5	64
2	Cycling	23	191	81	22.3	63
3	Cycling	19	178	65	20.7	69
4	Cycling	28	186	78	22.7	52
5	Cycling	24	178	78	24.8	62
6	Triathlon	21	183	80	23.9	63
7	Cycling	38	173	65	21.7	65
8	Triathlon	25	183	80	23.9	66
9	Triathlon	25	183	84	25.1	66
10	Cycling	21	188	77	21.6	55
Mean ± SE		24 ± 2	182 ± 2	76 ± 2	22.9 ± 0.5	63 ± 2

BMI, body mass index.

The McMaster University and Hamilton Health Sciences Corporation research ethics board approved the experimental protocol.

**Study design.** The design was a double-blind, placebo-controlled, repeated-measures crossover study that compared the effects of a 6% carbohydrate drink, a 6% carbohydrate + 2% protein drink, and a nonenergetic sweetened placebo drink on 80-km cycling time trial performance and blood markers of energy metabolism. Fluid was ingested during exercise at a rate of 250 mL every 15 min, which ensured a carbohydrate delivery rate of 60 g·h<sup>-1</sup> during the two carbohydrate-supplemented trials. The experimental design did not include a fourth trial that was energetically matched to the 6% carbohydrate + 2% protein trial (i.e., 8% carbohydrate) because this rate of carbohydrate ingestion (80 g·h<sup>-1</sup>) would have exceeded the optimal recommended amount and could have caused gastrointestinal distress or been ergolytic (7,18). Following familiarization and baseline testing, all subjects performed three experimental trials in a randomized, counterbalanced manner, separated by 7 d, with one exception: subject 1 had only 5 d of recovery between his second and third trials because of a previously scheduled commitment. No evidence indicates that this exception influenced the study results, and the performance and blood data for subject 1 were consistent with the group means (see Results).

**Preexperimental procedures.** Before the actual experimental trials, all subjects performed an incremental cycle test to exhaustion on an electrically braked cycle ergometer (Lode Excalibur Sport V 2.0, Groningen, The Netherlands) to determine  $\dot{V}O_{2peak}$ . The test consisted of a 3-min warm-up at 100 W, followed by a ramp increase of 1 W every 2 s until volitional exhaustion, which was defined as the point at which pedal cadence fell below 50 rpm; according to the manufacturer’s specifications, the power output was not guaranteed below this cadence. Respiratory data were collected continuously during the test through a mouthpiece and analyzed online using a metabolic cart (Moxus modular oxygen uptake system, AEI Technologies, Pittsburgh, PA). Heart rate was collected continuously using a heart rate monitor (Polar A3, Lake Success, NY). Respiratory variables were averaged over 15-s collection intervals, and the average of the two highest 15-s values were used to determine  $\dot{V}O_{2peak}$ . At least two of the following three criteria also had to be satisfied for determination of  $\dot{V}O_{2peak}$ : (a) a plateau in  $\dot{V}O_2$  was demonstrated, (b) heart rate was within ± 5 bpm of the age-predicted maximal value, and (c) respiratory exchange ratio was > 1.1.

Several days after the  $\dot{V}O_{2peak}$  test, subjects performed an 80-km laboratory cycling time trial to become familiar with the testing procedures to be used during the main experimental trials (see below). We included this control because previous work has demonstrated that a familiarization ride is important for the accurate assessment of time trial performance, even with highly trained cyclists who use their own bicycles (22). The familiarization time trial was identical in all respects to the main experimental time trials (see below), except that no blood samples were

obtained and subjects received only water during the ride. The experimental trials commenced 7–10 d following the familiarization time trial.

**Physical activity and nutritional controls.** All subjects were engaged in regular cycle training at the time of the experiment (February–March 2005), mainly using indoor stationary trainers, but none had commenced their competitive racing season. The subjects agreed to keep their weekly training schedule as constant as possible for the duration of the experiment, but given the within-subject design (each subject served as his own control), training was not standardized between subjects. Subjects were instructed to standardize any workout performed 24–48 h before an experimental trial and to perform no physical activity, aside from activities of daily living, for 24 h before each trial. Subjects were also advised to keep their habitual diet as constant as possible over the course of the experiment. Strict nutritional controls were used for 24 h before each experimental trial, and all subjects were provided with a standardized food parcel that provided 12.7 MJ of energy, derived from 66% carbohydrate, 21% fat, and 17% protein. The food parcel was formulated to be palatable to all subjects based on their expressed likes and dislikes and contained cereal, bagels, jam, sandwiches, fruit juices, milk, and energy bars. Subjects were instructed to consume only the food and drink contained in the food parcel, with the exception of additional water *ad libitum*. All experimental trials for a given subject were performed at the same time of day (either 9:00 a.m., 1:00 p.m., or 5:00 p.m.), and the final preexercise meal was consumed 2–3 h before an experimental trial. The timing of the final preexercise meal was standardized for a given subject and depended on his usual routine before competition. Subjects maintained diaries throughout the experiment, and self-reported compliance with the physical activity and nutritional controls was excellent.

**Experimental trials.** On arrival at the laboratory, a 22-gauge catheter (Infusion Therapy Systems, Sandy, UT) was inserted into an antecubital vein, secured with a sterile dressing, and maintained patent with a sterile saline drip. Subjects were also fitted with a heart rate monitor (Polar A3). After a resting blood sample was obtained, subjects performed a standardized warm-up that consisted of 10 min on a stationary cycle ergometer (Monark 814E, Vansbro, Sweden) at a work intensity of 150 W. The time trial performance tests were completed on a CompuTrainer bicycle trainer (Pro Lab 3D Edition, Racermate, Seattle, WA) using each subject's own bicycle, which enabled the trainer to operate as a stand-alone electronic bicycle ergometer. The subject's bicycle was mounted on the CompuTrainer before each trial, and the unit was calibrated according to the manufacturer's instructions. The CompuTrainer was connected to a laptop computer loaded with RacerMate Interactive 3D software and interfaced with a projector that displayed an image of a cyclist riding on a panoramic three-dimensional race course. The image was projected on a wall approximately 2 m in front of the CompuTrainer so that the subject was able to view "himself" cycling on the course. A 20-km course was specifically designed for the experiment using

the custom course creation option of the Pro 3D Software. The time trial consisted of four laps of the 20-km course, which consisted of mainly flat to rolling terrain, interspersed with several hills with a maximal grade of 4%. The computer software permitted the subject to experience the "feel of the road", and changes in course terrain were transmitted into changes in resistance on the CompuTrainer. Subjects had to adjust their gear selection in response to changes in course terrain, just as they would when cycling on a road outdoors. A 10-mL syringe of venous blood was drawn after 20, 40, and 60 km, and on completion of each experimental trial. The blood sampling protocol was based on distance covered rather than elapsed time to prevent the subject from inferring knowledge about his performance time. This meant, however, that the precise timing of blood sampling (i.e., elapsed time in minutes) differed between treatments for a given subject. Heart rate was collected continuously during exercise (Polar A3). All trials were performed at a temperature of 20–23°C, and an electric fan circulated air to minimize heat stress.

The subjects were instructed to complete the time trial as quickly as possible but received no verbal, temporal, or physiological feedback during the ride. The only feedback that subjects received during the time trial was distance covered, which was displayed in one corner of the projected image, and verbal encouragement to consume the experimental beverages, as described below. The subjects were also permitted to listen to music while cycling, provided this decision was constant for all three trials (i.e., music or no music). Subjects who decided to listen to music were permitted to bring their own digital music files into the laboratory. Music files were played on a laboratory computer, and the same playlist was used during each trial for a given subject. Importantly, during each trial the songs were played using a random setting to prevent the subjects from gaining a sense of pacing (e.g., by comparing distance covered with a particular point in the song playlist).

In addition to a standard remuneration paid to all subjects on completion of the study, additional incentives were used to motivate subjects to complete all three time trials as quickly as possible. A monetary bonus was given to the rider who posted the fastest average time for the three experimental trials. A bonus was also given to the rider who sustained the highest relative intensity as measured by the highest percent of heart rate reserve that was maintained over the course of the three trials. Finally, the subjects were divided into two teams, matched as closely as possible based on the familiarization time trial results, and the fastest overall team also received a monetary bonus. Subjects did not receive any information regarding individual or team standings until study completion. In addition, all monetary bonuses were based on the mean of the three time trials to encourage a maximal effort during each time trial. Providing performance incentives simulated a competitive environment among the subjects and helped maintain motivation over the course of the experiment.

**Experimental beverages and drink administration.** The three experimental beverages were formulated by Gatorade

TABLE 2. Composition of experimental beverages.

Ingredient	Grams of Ingredient per Liter of Water		
	Placebo	CHO	CHO-PRO
Sucrose	0	60.000	60.000
Whey protein	0	0	20.000
Sodium chloride	0.6730	0.6730	0.6730
Sodium citrate	0.5963	0.5963	0.5963
Monopotassium phosphate	0.4447	0.4447	0.4447
Citric acid	2.7641	2.7641	2.7641
Flavor	0.5115	0.5115	0.5115
Aspartame	0.2250	0	0

CHO, carbohydrate trial; CHO-PRO, carbohydrate + protein trial.

(Barrington, IL), contained the same amount of electrolytes, and were similarly flavored (Table 2). The only difference between the beverages was that one was artificially sweetened (PL), one contained 6% carbohydrate in the form of sucrose (CHO), and one contained 6% carbohydrate plus 2% whey protein (CHO-PRO). The source of the whey protein isolate was Lactodan (Arla Foods, Basking Ridge, NJ). The three beverages were delivered as dry powders in sealed packages, identified by code numbers to ensure blinding, and were subsequently stored in sealed and locked containers at room temperature in the laboratory. Aliquots of test beverages were carefully weighed and dissolved in water according to the manufacturer's instructions on the day of each experimental trial. The drinks were stored in translucent containers, each containing 250 mL of fluid, and served slightly chilled.

The test beverages were administered at a rate of 250 mL every 15 min during exercise, and subjects commenced drinking from the outset of the time trial. An investigator handed the subject one container at a time and verbally encouraged the subject to drink at an appropriate rate (e.g., "You should finish drinking the fluid in that container within the next 5 km"). In this manner, the investigator was able to monitor the subject's rate of fluid ingestion to ensure that 250 mL was consumed every 15 min, but the protocol did not enable the subject to keep track of his specific performance time. After the subject had finished drinking the fluid in a container, it was removed from view and replaced with another filled drink container. The investigator then continued verbally to prompt the subject to drink at the appropriate rate. Thus, although the subject's drinking rate may have varied within a given 15-min interval, the protocol was designed to ensure that subjects ingested exactly 250 mL of fluid every 15 min.

**Blood analyses.** Venous blood samples were immediately transferred into ice-chilled vacutainer tubes that contained either heparin (4 mL), EDTA (4 mL), or no additive (2 mL). Heparinized blood (20  $\mu$ L) was immediately used to determine blood lactate using an automated lactate analyzer (Accutrend Lactate, Roche Diagnostics, Mannheim, Germany). An additional 200  $\mu$ L of heparinized blood was added to 1000  $\mu$ L of ice-chilled perchloric acid, the mixture was centrifuged, and the supernatant was removed and stored at  $-20^{\circ}\text{C}$  for subsequent determination of blood glucose using an enzymatic method (25) adapted for fluorometry (Hitachi F-2500, Hitachi Instruments, Japan). The

remainder of blood in the heparinized tube was centrifuged, and one portion of the separated plasma was stored on ice and analyzed for ammonia on a Roche analyzer (F. Hoffman-La Roche Ltd, Basel, Switzerland). The remaining plasma was stored at  $-20^{\circ}\text{C}$  for subsequent analysis of plasma free fatty acids (Wako NEFA C test kit, Wako Chemicals). Blood in the tubes treated with EDTA was stored on crushed ice and analyzed for hemoglobin and hematocrit using an automated Coulter Gen-S analyzer (Beckman-Coulter Inc., Fullerton, CA). Finally, blood in the tubes with no additive was allowed to clot, was centrifuged, and the supernatant was collected and stored at  $-20^{\circ}\text{C}$  for subsequent analysis of serum insulin using a radioimmunoassay kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA).

**Calculations.** Average power output over the course of the time trial was calculated using the Interactive 3D software that was interfaced with the CompuTrainer. Average heart rate was calculated based on the continual recording of heart rate throughout the entire time trial. Relative work intensity was calculated based on the concept of heart rate reserve (19). Changes in plasma volume were calculated using the method described by Dill and Costill (10) based on changes in hematocrit and hemoglobin.

**Statistical analyses.** Variables that consisted of a single measurement per trial were examined using a one-factor (condition) repeated-measures analysis of variance (ANOVA). Variables that included multiple measures per trial were analyzed using a two-factor (condition  $\times$  time) ANOVA. The level of significance for all analyses was set at  $P \leq 0.05$ . When a significant main effect or interaction was identified, data were subsequently analyzed using a Tukey *post hoc* test. All data are presented as means  $\pm$  SE unless otherwise noted.

## RESULTS

**Time trial performance data.** Average time to complete the 80-km time trial was 4.4% faster during both CHO

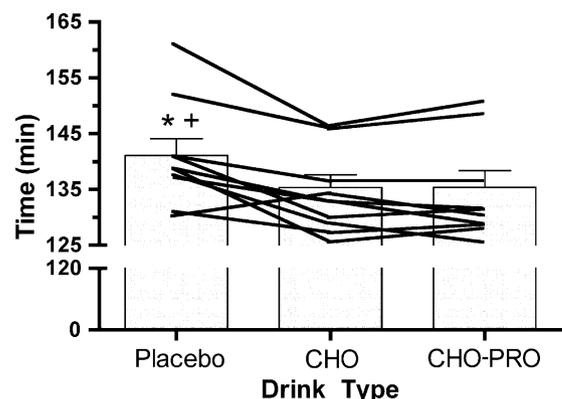
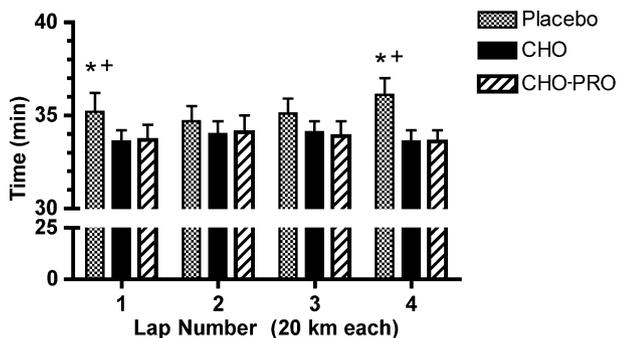
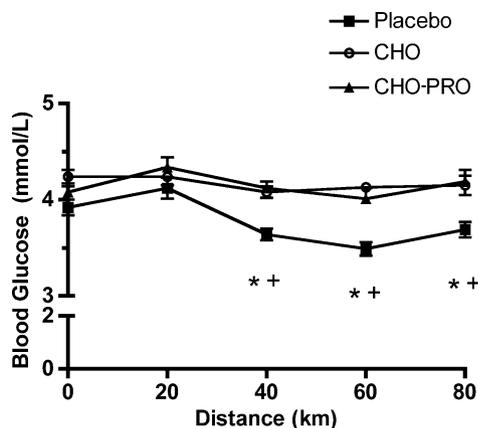


FIGURE 1—Time required to complete a simulated 80-km cycling time trial when subjects ingested a placebo, 6% carbohydrate (CHO), or 6% carbohydrate + 2% protein solution (CHO-PRO) during exercise. Solid lines represent individual data, and bars are means  $\pm$  SE;  $N = 10$ . \*  $P < 0.05$ , placebo vs CHO; +  $P < 0.05$ , placebo vs CHO-PRO.



**FIGURE 2**—Time required to complete each 20-km lap of a simulated 80-km cycling time trial when subjects ingested a placebo, 6% carbohydrate (CHO), or 6% carbohydrate + 2% protein solution (CHO-PRO) during exercise. Values are means  $\pm$  SE;  $N = 10$ . \*  $P < 0.05$ , placebo vs CHO; +  $P < 0.05$ , placebo vs CHO-PRO.

( $135 \pm 2$  min,  $P = 0.001$ ) and CHO-PRO ( $135 \pm 2$  min;  $P = 0.002$ ) compared with PLAC ( $141 \pm 3$  min), but no difference was seen between the two carbohydrate-supplemented trials ( $P = 0.92$ ) (Fig. 1). The individual responses were consistent, and 9 of the 10 subjects posted faster times during CHO and CHO-PRO compared with PLAC (Fig. 1). Average time to complete laps 1 and 4 were faster ( $P \leq 0.03$ ) during CHO and CHO-PRO compared with PLAC, but no differences were found between trials for laps 2 or 3 (Fig. 2). Average power output for 80 km was higher for CHO ( $253 \pm 12$  W;  $P = 0.02$ ) and CHO-PRO ( $250 \pm 9$  W;  $P = 0.03$ ) compared with PLAC ( $231 \pm 11$  W), with no difference between CHO and CHO-PRO ( $P = 0.87$ ). Average heart rate during CHO ( $159 \pm 3$  bpm) was higher ( $P = 0.04$ ) versus PLAC ( $155 \pm 2$ ). Average heart rate during CHO-PRO ( $159 \pm 4$ ) was not different compared with the other two treatments ( $P = 0.96$  vs CHO,  $P = 0.07$  vs PLAC). Ratings of perceived exertion were higher during exercise compared with at rest ( $P < 0.05$ ), but no differences were found between trials at any point (data not shown).



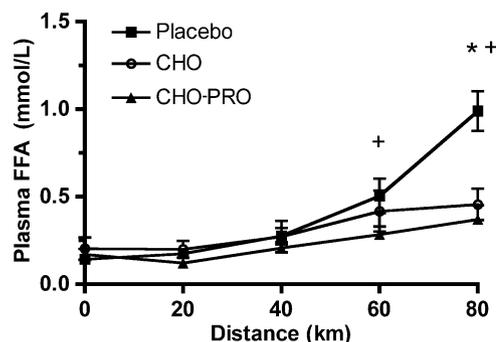
**FIGURE 3**—Blood glucose concentration at rest and during an 80-km cycling time trial when subjects ingested a placebo, 6% carbohydrate (CHO), or 6% carbohydrate + 2% protein solution (CHO-PRO) during exercise. Values are means  $\pm$  SE;  $N = 10$ . \*  $P < 0.05$ , placebo vs CHO; +  $P < 0.05$ , placebo vs CHO-PRO.

**TABLE 3.** Blood data at rest and during exercise.

Compound	Condition	Rest	20 km	40 km	60 km	80 km
Insulin (mIU·L <sup>-1</sup> )	Placebo	19.9 $\pm$ 4.7	5.9 $\pm$ 0.9	3.2 $\pm$ 0.3	3.0 $\pm$ 0.2	1.8 $\pm$ 0.3
	CHO	21.4 $\pm$ 3.7	7.3 $\pm$ 1.4	5.6 $\pm$ 0.7	5.6 $\pm$ 1.4	3.1 $\pm$ 0.3
	CHO-PRO	15.8 $\pm$ 3.2	7.7 $\pm$ 1.9	5.9 $\pm$ 1.1	4.8 $\pm$ 0.7	5.8 $\pm$ 1.6
Lactate (mmol·L <sup>-1</sup> )	Placebo	1.7 $\pm$ 0.1	4.1 $\pm$ 0.6	3.1 $\pm$ 0.3	2.5 $\pm$ 0.3	3.5 $\pm$ 0.5
	CHO	1.5 $\pm$ 0.1	4.0 $\pm$ 0.4	3.5 $\pm$ 0.5	2.7 $\pm$ 0.2	5.0 $\pm$ 0.3*
	CHO-PRO	1.6 $\pm$ 0.1	3.6 $\pm$ 0.6	3.2 $\pm$ 0.5	2.8 $\pm$ 0.4	4.6 $\pm$ 0.6
Ammonia ( $\mu$ mol·L <sup>-1</sup> )	Placebo	54 $\pm$ 5	93 $\pm$ 6	99 $\pm$ 8	104 $\pm$ 12	138 $\pm$ 25
	CHO	47 $\pm$ 4	88 $\pm$ 6	98 $\pm$ 5	109 $\pm$ 6	168 $\pm$ 21
	CHO-PRO	63 $\pm$ 10	97 $\pm$ 15	105 $\pm$ 7	122 $\pm$ 10	162 $\pm$ 16

Values are means  $\pm$  SE;  $N = 10$ . CHO, carbohydrate trial; CHO-PRO, carbohydrate + protein trial. For all variables, main effects for time were such that values during exercise differed compared with at rest ( $P < 0.05$ ). No differences were noted between treatments at any time point, except for lactate as indicated (\*  $P < 0.05$  vs placebo).

**Blood data.** Blood glucose concentration remained relatively constant during CHO and CHO-PRO, with no differences between trials (Fig. 3). In contrast, blood glucose concentration gradually declined from rest during PLAC and was lower ( $P < 0.05$ ) compared with CHO and CHO-PRO at 40, 60, and 80 km. Serum insulin concentration decreased during exercise in all three trials and was lower at 20, 40, 60, and 80 km compared with at rest (main effect,  $P < 0.05$ ), but no interactions occurred (Table 3). Blood lactate concentration was higher ( $P < 0.05$ ) at 20 and 80 km compared with at rest in all three trials, but the only difference between trials occurred at the end of exercise, when blood lactate concentration was higher ( $P < 0.05$ ) in CHO versus PLAC (Table 3). Plasma free fatty acid (FFA) concentration remained relatively constant during exercise in CHO and CHO-PRO but was higher at the end of exercise compared with at rest ( $P < 0.01$ ; Fig. 4). In contrast, a sharp increase in plasma FFA concentration was seen during PLAC toward the latter part exercise. Plasma FFA concentration during PLAC was higher versus CHO-PRO at 60 km ( $P = 0.01$ ) and higher versus CHO and CHO-PRO at 80 km (both  $P = 0.0001$ ). Plasma ammonia concentration increased progressively during exercise and was higher at 20, 40, 60, and 80 km compared with at rest, but no differences were seen between trials (Table 3). The change in plasma



**FIGURE 4**—Plasma free fatty acid (FFA) concentration at rest and during an 80-km cycling time trial when subjects ingested a placebo, 6% carbohydrate (CHO), or 6% carbohydrate + 2% protein solution (CHO-PRO) during exercise. Values are means  $\pm$  SE;  $N = 10$ . \*  $P < 0.05$ , placebo vs CHO; +  $P < 0.05$ , placebo vs CHO-PRO.

volume during exercise was not different between trials (PLAC:  $-3.0 \pm 1.0\%$ ; CHO:  $-3.9 \pm 1.3\%$ ; CHO-PRO:  $-3.7 \pm 1.0\%$ ).

## DISCUSSION

The main finding from the present study was that the addition of 2% protein to a 6% carbohydrate solution did not improve 80-km time trial performance compared with 6% carbohydrate alone when ingested during exercise at a rate of  $1 \text{ L}\cdot\text{h}^{-1}$ . These data are in contrast to recent reports by others (15,28) that used time-to-fatigue tests and concluded that the addition of protein to a typical carbohydrate sports drink improved endurance performance. Rather, our findings demonstrate that when trained athletes ingest carbohydrate during exercise in an amount considered near optimal for exogenous carbohydrate oxidation (18), protein does not improve performance during a task that closely simulates athletic competition.

The discrepancy between the present study and recent findings by Ivy et al. (15) and Saunders et al. (28) is likely related to methodological differences. We feel, however, that our results are much more applicable to the manner in which athletes typically compete. When attempting to assess the performance ability of endurance athletes, laboratory-based tests should be valid, reliable, sensitive to small changes in performance, and, ideally, they should closely simulate the athlete's competitive task in the field (14,22). Previous work by Saunders et al. (28) and Ivy et al. (15) assessed performance using cycling time-to-fatigue tests at work intensities of 75 and 85%  $\dot{V}O_{2\text{peak}}$ , respectively, and reported performance improvements ranging from 29% (28) to 36% (15) when protein was added to a carbohydrate drink compared with carbohydrate alone. The practical applications of their work are constrained, however, by the fact that endurance athletes do not typically compete in events that require sustaining a fixed power output for as long as possible. The performance test used in the present study was a laboratory time trial, in which subjects rode their own bicycles and attempted to complete a simulated 80-km race course as fast as possible.

The assessment of time trial performance in well-trained endurance cyclists who use their own bicycles on a stationary trainer is highly reproducible (coefficient of variation (CV)  $\leq 1\%$ ), provided that the athletes perform a familiarization time trial (22,24). Therefore, our research design included this important control to improve the likelihood of detecting significant differences between trials. Given the demanding nature of our study and the fact that all subjects were highly trained endurance cyclists who had extensive experience riding on stationary trainers, we did not specifically assess the reproducibility of our performance test by including a second familiarization session. If we compare the data from our familiarization time trial (during which subjects ingested plain water) versus the placebo trial (during which subjects ingested a

flavored, nonenergetic drink), the CV was 2.8%, determined using the method error technique described by Sale (27). This value compares favorably with data from Laursen et al. (22), who reported a CV of 3.0% when they compared a familiarization 40-km time trial with two subsequent 40-km time trials in trained cyclists. The fact that we included a familiarization trial likely reduced the experimental trial CV to  $\leq 1\%$ , as suggested by the data of Laursen et al. (22) and Palmer et al. (24), and we are confident that our research design was sensitive to detect small differences between treatments. Indeed, our statistical analyses revealed a 4.4% improvement in time trial performance when subjects ingested carbohydrate compared with placebo ( $P < 0.002$ ), which compares favorably with the carbohydrate-mediated improvement in performance over a 100-km time trial reported by Angus et al. (2). We found no additional effect of protein, however, and the mean time to complete the time trial during the CHO and CHO-PRO trials was virtually identical (135 min;  $P = 0.92$ ), with six subjects posting times with  $\leq 3$  min of difference between trials, two performing  $> 3$  min better during CHO, and two performing  $> 3$  min better during CHO-PRO (Fig. 1).

The other major difference between the present study and those that reported an ergogenic effect of adding protein to a carbohydrate sports drink (15,28) is the rate of carbohydrate ingested during exercise. A recent comprehensive review of the literature by Jeukendrup (18) concluded that exogenous carbohydrate oxidation is maximal when a single carbohydrate is ingested at a rate  $60\text{--}70 \text{ g}\cdot\text{h}^{-1}$ , which meets or exceeds the upper limit of the range typically recommended by leading sports nutrition organizations (1,5,7). Consequently, we had our subjects ingest carbohydrate at a rate of  $60 \text{ g}\cdot\text{h}^{-1}$  ( $0.79 \text{ g}\cdot\text{kg}^{-1} \text{ body mass}\cdot\text{h}^{-1}$ , based on mean subject mass of 76 kg), which is 18 and 49% higher than the rate of carbohydrate feeding in the studies by Ivy et al. (15) and Saunders et al. (28), respectively. Ivy et al. (15) had subjects with a mean body mass of 70 kg ingest carbohydrate at a rate of  $47 \text{ g}\cdot\text{h}^{-1}$  ( $0.67 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ), whereas a 70-kg subject in the study by Saunders et al. (28) would have received carbohydrate at a rate of  $37 \text{ g}\cdot\text{h}^{-1}$  ( $0.53 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ). Thus, whereas Ivy et al. (15) and Saunders et al. (28) fed their subjects carbohydrate in amounts within the range recommended by sports nutrition organizations (1,5,7), the amounts were probably less than optimal for attaining peak rates of exogenous carbohydrate oxidation (18). In addition, the rate of fluid ingestion in the studies by Ivy et al. (15) and Saunders et al. (28) was lower than in the present study. We chose a rate of fluid intake ( $1 \text{ L}\cdot\text{h}^{-1}$ ) that was in the middle of the broad range recommended by sports nutrition organizations (i.e.,  $600\text{--}1400 \text{ mL}\cdot\text{h}^{-1}$ ) (1,5,7), whereas the study by Ivy et al. (15) provided subjects with the minimal recommended amount ( $600 \text{ mL}\cdot\text{h}^{-1}$ ), and Saunders et al. (28) provided less than what is recommended ( $504 \text{ mL}\cdot\text{h}^{-1}$  for a 70-kg subject).

Although the data from Ivy et al. (15) and Saunders et al. (28) suggest that the addition of protein to a carbohydrate sports drink can extend endurance time to fatigue, the basic physiological mechanism for such an effect is unclear. We

speculate that if an effect exists for adding protein to carbohydrate on endurance performance, it may only be manifested if suboptimal amounts of carbohydrate are ingested during exercise. By analogy, perhaps the effect is similar to that seen for muscle glycogen resynthesis when carbohydrate is ingested with or without protein during recovery from prolonged exercise. As recently summarized by Burke et al. (3), a reasonable interpretation of the recovery literature is that “most evidence suggests that feeding a high amount of carbohydrate at frequent intervals negates the benefits of added protein... (but) co-ingestion of protein with carbohydrate will increase the efficiency of muscle glycogen storage when the amount of carbohydrate ingested is below the threshold for maximal glycogen synthesis”. We show here that adding 2% to a 6% carbohydrate drink is not ergogenic for time trial performance, as compared with 6% carbohydrate alone, despite the differences in energy content. Perhaps this is because the 1 g·min<sup>-1</sup> of carbohydrate provided in the CHO trial met or exceeded the threshold for peak exogenous carbohydrate oxidation. Both Ivy et al. (15) and Saunders et al. (28) dismissed the possibility that the improved performance with carbohydrate plus protein versus carbohydrate alone in their studies was related to differences in energy intake. A subsequent study by Saunders et al. (26) that has appeared in abstract form reported no difference in endurance time to fatigue at 70%  $\dot{V}O_{2peak}$  when a carbohydrate–protein drink was compared with an isoenergetic carbohydrate drink. Other purported mechanisms to explain the data of Ivy et al. (15) and Saunders et al. (28), as hypothesized by these investigators, include alterations in insulin stimulation, more efficient use of muscle glycogen, retention of Krebs cycle intermediates, maintenance of plasma amino acid concentrations as they relate to central fatigue, and faster fuel-medium transport across the lining of the intestine. In the study by Ivy et al. (15), however, the improved performance with protein plus carbohydrate compared with carbohydrate alone was not associated with differences in insulin concentration, and some of the other potential mechanisms are questionable from a basic science perspective, including retention of Krebs cycle intermediates (12) and the central fatigue hypothesis (29). Additional studies are warranted to evaluate the mechanisms responsible for the purported increase in cycle endurance capacity (time to fatigue) when protein is added to a carbohydrate drink (15,28).

A final interesting observation from the present study was that subjects performed better on the first 20-km lap of the time trial during CHO and CHO-PRO compared with placebo. It was not surprising to find that the final 20-km lap of the time trial was faster during the two carbohydrate-supplemented trials, because other studies have shown that improved endurance performance with carbohydrate feeding is associated with maintenance of euglycemia and a high rate of carbohydrate oxidation late in exercise (2,6,8,9). In the present study, blood glucose concentration was lower and plasma FFA concentration was higher toward the end of exercise during the placebo trial compared with CHO and CHO-PRO, and thus it appears that the improved time

trial performance with carbohydrate feeding was related, in part, to classically proposed mechanisms (7,18). No difference was seen in blood glucose, plasma FFA, or the concentration of any other metabolite over the first 20 km, but subjects performed better during CHO and CHO-PRO compared with placebo. All of our subjects were well fed, and exogenous carbohydrate availability does not typically limit performance under these conditions during exercise lasting < 45 min (1,7), which suggests that the performance differences during lap 1 were unrelated to blood glucose availability. Carter et al. (4) recently showed that a 40-km time trial performance (lasting approximately 1 h) improved when subjects rinsed their mouth (but did not swallow) a 6% carbohydrate solution compared with a placebo rinse. The authors concluded that, over this distance, the mechanism responsible for the improved performance appeared to involve an increase in central drive or motivation, possibly mediated via pharyngeal receptors that sensed the presence of carbohydrate in the mouth.

In conclusion, the present study showed that when trained cyclists ingested carbohydrate during exercise at a rate considered optimal for exogenous carbohydrate oxidation (18), the addition of protein did not further enhance performance during a time trial task that closely simulated athletic competition. These data are in contrast to recent reports (15,28) that have concluded that adding protein to a carbohydrate sports drink dramatically improved endurance performance, based on time-to-fatigue tests. Nonetheless, the protein–carbohydrate drink did not hinder performance in the present study, and additional work is warranted to determine whether coingestion of protein with carbohydrate confers any metabolic advantage compared with carbohydrate alone. Koopman et al. (20) recently reported that the combined ingestion of protein and carbohydrate during ultraendurance exercise improved whole-body net protein balance compared with carbohydrate alone. Research in this area is extremely limited, however, and it remains to be determined whether the addition of protein to a carbohydrate sports drink favorably alters skeletal muscle protein metabolism or the adaptive response to chronic training. From a purely applied perspective, we show here that trained cyclists derive no performance benefit from adding protein to a carbohydrate sports drink that they ingest during prolonged exercise at a rate considered near optimal for exogenous carbohydrate oxidation.

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