Effect of an Isocaloric Carbohydrate–Protein–Antioxidant Drink on Cycling Performance

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ABSTRACT

ROMANO-ELY, B. C., M. K. TODD, M. J. SAUNDERS, and T. ST. LAURENT. Effect of an Isocaloric Carbohydrate–Protein–Antioxidant Drink on Cycling Performance. *Med. Sci. Sports Exerc.*, Vol. 38, No. 9, pp. 1608–1616, 2006. **Purpose:** Fourteen male cyclists were studied to compare the effect of carbohydrate–protein–antioxidant beverage (CHOPA) to an isocaloric carbohydrate-only (CHO) beverage on time to fatigue and muscle damage. **Methods:** Subjects performed two sets of rides to exhaustion on a cycle ergometer. In each set, the first ride was performed at 70% $\dot{V}O_{2peak}$, and the second was performed 24 h later at 80%. CHO or CHOPA was consumed every 15 min during exercise and immediately afterward. Plasma CK and LDH and muscle soreness were measured pre- and postexercise. **Results:** Time to fatigue was not different between CHO and CHOPA at 70% $\dot{V}O_{2peak}$ (95.8 ± 29.7 vs 98.1 ± 28.7 min), 80% $\dot{V}O_{2peak}$ (42.3 ± 18.6 vs 42.9 ± 21.8 min), or total performance time (138.1 ± 39.3 vs 140.9 ± 43.7 min). Postexercise CK was increased ($P < 0.05$) from baseline in CHO (203 ± 120 vs 582 ± 475 $U \cdot L^{-1}$) but not with CHOPA (188 ± 119 vs 273 ± 169 $U \cdot L^{-1}$). Similarly, LDH values increased over baseline in CHO (437 ± 46 vs 495 ± 64 $U \cdot L^{-1}$) but not with CHOPA (432 ± 40 vs 451 ± 43 $U \cdot L^{-1}$). Postexercise CPK and LDH were higher after the CHO trial than after the CHOPA trial. Median postexercise muscle soreness was higher in CHO (3.0 ± 5.0) than with CHOPA (1.0 ± 3.0). **Conclusion:** No differences in time to fatigue were observed between the beverages, despite lower total carbohydrate content in the CHOPA beverage. The CHOPA beverage attenuated postexercise muscle damage, as evidenced by CK and LDH values, compared with an isocaloric CHO beverage. **Key Words:** SPORT DRINKS, AEROBIC EXERCISE, ENDURANCE, FATIGUE, MUSCLE DAMAGE

Commercially available sport beverages containing carbohydrate and protein have gained popularity among athletes, in part because of evidence suggesting that the added protein improves endurance performance (4,9,12,19–21,23,24,29,31). Some of these beverages are designed as a supplemental fuel to be used during exercise. Others, which typically have higher nutrient concentrations, are taken immediately after exercise to facilitate recovery and improve subsequent performance.

Several groups have examined whether adding protein to sport beverages provides performance advantages over carbohydrate-only beverages (CHO) or a placebo (12,19, 21,23,29–31). Williams and associates reported marked increases in blood glucose, insulin response, and glycogen storage with the carbohydrate–protein supplementation, indicating the potential to improve time trial performance and recovery (29,30). In these studies, the beverages were mixed according to the manufacturer's directions, and the carbohydrate–protein beverage contained more carbohydrate and total calories than the CHO beverage. These factors suggest that the reported benefits may be independent of the protein that was added to the beverages.

Recently, Ivy et al. (12) and Saunders et al. (23) compared CHO and carbohydrate–protein beverages that were matched for carbohydrate calories. A greater time to fatigue was found in these studies as well. Although the carbohydrate content was matched, the additional protein provided 25% greater caloric intake during exercise and recovery in the carbohydrate–protein trials. Because protein contributes up to 15% of total energy expenditure in prolonged bouts of exercise (13), the protein calories in the carbohydrate–protein beverage may account for the improvements in performance. To better understand how adding protein affects endurance performance, CHO and the beverages with added protein should be matched for total calories.

Carbohydrate–protein beverages have also been associated with the attenuation of exercise-induced muscle damage. Review of the evidence, however, reveals important limitations. In two studies (21,23), postexercise creatine kinase (CK) was lower in the carbohydrate–protein trials than in the CHO trials. The validity of CK as a measure of muscle damage has been questioned (20) because exercise may cause the release of CK from nonmuscular sources (e.g., monocytes). One way to address this issue is to measure multiple markers of muscle damage. Moreover, it is sometimes unclear whether the test beverages were fortified with vitamins C and E even though there is evidence that these antioxidants may protect against muscle damage (3,16,25).

The purpose of the present study was to compare the effects of two commercially available beverages, matched for total calories, on cycling performance and multiple indicators

of muscle damage. A further aim was to administer the beverages in a design that balanced experimental control and accountability with an approximation of the day-to-day conditions experienced by competitive cyclists. The products that were compared included 1) a CHO beverage and 2) a carbohydrate–protein beverage that was fortified with vitamins C and E (CHOPA). The specific research questions were 1) does CHOPA alter time to fatigue during prolonged bouts of cycling compared with an isocaloric CHO beverage, and 2) does CHOPA alter biomarkers of postexercise muscle damage and muscle soreness compared with an isocaloric CHO beverage?

METHODS

Subjects. Fourteen male volunteers between the ages of 18 and 33 yr completed this study. The subject number exceeded the minimum sample size needed to elucidate differences in the biochemical markers of muscle damage with a statistical power of 0.80, an estimated effect size of 1.0 SD units, a two-tailed alpha level of 0.05, and an intraclass correlation of 0.80 between repeated measures (15). To obtain subjects who were moderately fit, all subjects were required to 1) be presently training at least $4 \ d \cdot wk^{-1}$ and 2) demonstrate a maximal oxygen uptake ($\dot{V}O_{2peak}$) above 45 $mL \cdot kg^{-1} \cdot min^{-1}$. These inclusion criteria helped ensure that subjects cycled for at least 1 h at 70% $\dot{V}O_{2peak}$, or long enough to significantly reduce muscle glycogen stores.

All subjects completed a health status questionnaire to determine whether they had any health conditions that may limit physical activity or their ability to safely participate in the study. The James Madison University institutional review board approved the study design and procedures. All subjects provided written informed consent.

Testing procedures. The order and timeline of testing for this study is illustrated in Figure 1.

Phase 1: Physical fitness assessment testing. Cardiorespiratory fitness and body mass were assessed in the first phase of the study. During this assessment, subjects were first weighed on a digital scale to the nearest tenth of a kilogram and then performed a graded exercise test to determine $\dot{V}O_{2peak}$.

$\dot{V}O_{2peak}$ testing was conducted on an electronically braked cycle ergometer (Ergoline 800S, SensorMedics, Yorba Linda, CA). Subjects rode at a self-selected cadence above 50 rpm throughout the test. Each subject warmed up

for 3 min with light resistance before beginning the test. Workload was initially set at 150 W and then increased by 50 W every 2 min during the test until the subjects achieved volitional exhaustion. Oxygen uptake and respiratory exchange ratio were assessed at each stage during this test with a SensorMedics (Yorba Linda, CA) Vmax 229 metabolic cart. Heart rate was assessed with a Polar (Brooklyn, NY) heart rate monitor, and rating of perceived exertion (RPE) was measured using the 6–20 Borg scale. Tests were considered maximal when at least two of the following criteria were met: failure of heart rate to increase with an increase in exercise intensity, a plateau in oxygen uptake with increasing workload, a respiratory exchange ratio of greater than 1.15, and an RPE greater than 17 (1). Values obtained from these measures were also used to determine exercise intensities for subsequent testing.

Phase 2: Experimental rides with blinded treatment 1 (two sessions). In the second phase of the study, each subject performed two prolonged bouts of cycle ergometry to fatigue with 22–24 h of rest between bouts. To minimize within-subject diurnal variations, the time of day for each ride was held constant for individual subjects. During the rides, subjects ingested the treatment beverage at a rate of 2 $mL \cdot kg^{-1}$ body mass every 15 min. The first ride was at an intensity of 70% $\dot{V}O_{2peak}$. The second bout was performed under the same conditions, except at a higher intensity (80% $\dot{V}O_{2peak}$). The rationale for using two bouts of exercise is based on previously reported improvements in glycogen replenishment (9) and decreased muscle damage (23) observed with the administration of carbohydrate–protein beverages. These studies suggest that the potential differences in performance between CHO and CHOPA treatment beverages may be greater in a subsequent bout of exercise. Thus, a secondary purpose of the first performance ride was to induce a degree glycogen degradation that would reduce the performance potential for a subsequent ride. The purpose of the second ride was to provide a measure of performance in a state of partial glycogen depletion. Based on the proposed benefits of the CHOPA beverage, we hypothesized that differences in performance between beverages would be greater in the second ride.

$\dot{V}O_2$ and heart rate were assessed for 3 min at 30-min intervals during the exercise sessions. The mean of the 3-min $\dot{V}O_2$ values was used as the measure of exercise intensity. Workload was set and remained constant throughout the ride. Subjects were encouraged to continue
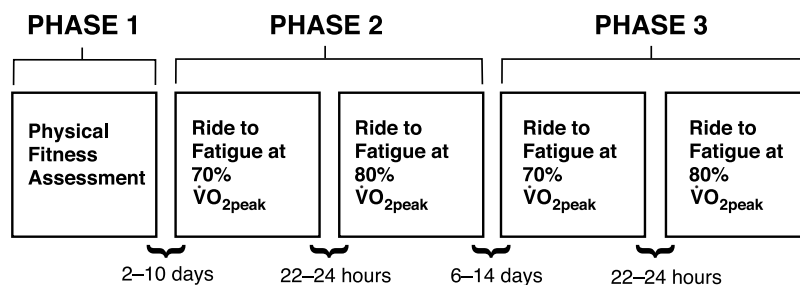

FIGURE 1—Time course of study protocol.

to cycle at the prescribed intensity until they dropped below a cadence of 50 rpm for a period of 20 s as a result of fatigue.

The two treatments contained equal fluid volumes and equal calories. The CHOPA beverage was mixed according to package instructions to contain 140 calories per 12 fluid ounces. The CHO beverage was mixed to provide an equal number of calories per unit volume. As a result, the CHOPA beverage contained 20% fewer carbohydrate calories than the CHO beverage (see Table 1). Subjects were also given one 10-mL·kg^{-1} serving of recovery treatment beverage to consume during the 15 min immediately following the first bout (i.e., 70% $\dot{V}O_{2peak}$) of exercise.

Subjects were instructed to keep dietary records for 24 h prior to the first ride and during the 24 h between the two rides. Instructions for completing the diet records were given orally and in written form as an attachment to the record log sheets. Specifically, subjects were told to record all foods and beverages and to keep intake as similar as possible before, during, and between trials. They were also asked to limit food intake for at least 4 h prior to each ride and to avoid using other supplements throughout the study. Diet records were analyzed using the Food Processor® program for calorie, carbohydrate, and protein content. The subjects' dietary records for each blinded treatment were compared to evaluate similarity between trials.

Venous blood samples and subjective ratings of soreness were collected before the first exercise session (baseline), before the second exercise session (24 h), and 2 d (72 h) after the second exercise session. Clinical diagnostic procedures (10) and data from our lab (23) show that CK is significantly elevated at between 12 and 24 h after muscle damage. These (10) and other sources (5) indicate that LDH values can be expected to be elevated between 72 and 96 h following skeletal muscle damage. Therefore, CK was compared in blood samples that were collected at baseline and at 24 h, and LDH was compared in the samples taken at baseline and at 72 h. Muscle soreness ratings were analyzed across the three time periods (i.e., baseline, 24 h, and 72 h).

Phase 3: Experimental rides with blinded treatment 2 (two sessions). Subjects returned after a 1- to 2-wk washout period and repeated the performance rides described in phase 2. The only difference in protocol from phase 2 was the administration of an alternate beverage. For example, if the subject consumed the CHOPA drink during treatment 1, they consumed the CHO drink during treatment 2. During the period between the two rides in phase 3, subjects were instructed to follow a diet as similar as possible to the diet followed during the same period in phase 2.

Given the possibility of order effects (e.g., attenuation of muscle damage during the second trial period due to a repeated-bout effect), the order of treatments was randomized and counterbalanced, such that half of the subjects consumed the CHOPA drink during the first trial and half consumed it during the second trial. In addition to analyzing the data for beverage-related treatment effects, the data were analyzed according to the order of the trials to further rule out a repeated-bout effect. A double-blind design was used to insure that subjects and the researcher conducting the trials did not know which drink was consumed during the study.

Beverage formulation. The two treatment beverages were matched for caloric content so that both treatments provided the same amount of available energy (i.e., total calories). The CHOPA supplement was Accelerade® (PacificHealth Laboratories, Inc) during exercise and Endurox® (PacificHealth Laboratories, Inc) postexercise. These supplements provide carbohydrates in the form of sucrose, trehalose, fructose, and maltodextrin, as well as whey protein. The CHOPA products were formulated according to package instructions because this formulation has been associated with performance benefits (23,30). The CHO beverage was Gatorade® (Gatorade, Inc), which provides carbohydrates in the form of sucrose and dextrose. The CHO beverage was mixed at a higher concentration than company recommendations, but still below 10% carbohydrate, which is the level determined to potentially cause gastric distress and absorption problems (24,28). None of the subjects reported any gastrointestinal discomfort during the study. Additional nutrient information about these products is provided in Table 1.

The CHOPA beverage contained 7.5% carbohydrate and 1.8% protein, and the CHO was a 9.3% carbohydrate solution. The recovery beverage concentration was doubled (15% carbohydrate and 3.6% protein in CHO+P and 18.6% carbohydrate in CHO). Gastrointestinal distress is much less likely during recovery than during exercise because digestive tract blood flow is less restricted and the body can better digest and absorb highly concentrated foods and drinks. All drinks were orange flavored and colored so that the difference in products would not be easily discernible. Beverages were administered in opaque bottles.

TABLE 1. Beverage formulation.

Drink	Calories per Serving	Carbohydrate per Serving (g)	Protein per Serving (g)	Other Percentage of RDA
CHOPA during exercise	139 per 12 oz 3.2 g·8 mL^{-1}	26 g·12 oz^{-1} 0.6 g·8 mL^{-1}	6.5 g·12 oz^{-1} 0.15 g·8 mL^{-1}	200% vitamin E 200% vitamin C per 12 oz
CHO during exercise	140 per 12 oz 3.2 g·8 mL^{-1}	35 g·12 oz^{-1} 0.8 g·8 mL^{-1}	0 g 0 g	None
CHOPA after exercise	277 per 12 oz 7.75 g·10 mL^{-1}	53 g·12 oz^{-1} 1.49 g·10 mL^{-1}	14 g·12 oz^{-1} 0.39 g·10 mL^{-1}	1330% vitamin E 780% vitamin C per 12 oz
CHO after exercise	276 per 12 oz 7.75 g·10 mL^{-1}	69 g·12 oz^{-1} 1.96 g·10 mL^{-1}	0 g 0 g	None

For a reference 75-kg man, the beverages delivered 600 mL·h^{-1} (approximately 20 fluid ounces) of fluid during exercise, totaling 240 calories. The CHOPA drink supplied 45 g of carbohydrate and 11.25 g of protein per hour, and the CHO beverage supplied 60 g of carbohydrate and no protein. The additional calories in the CHOPA beverage came from a small amount of fat present in the formulation (1 g per 12-oz serving, or 1.7 g·600 mL^{-1}.)

During recovery, the CHOPA beverage provided 750 mL of fluid containing 584 calories, 112 g of carbohydrate, and 29 g of protein for a 75-kg man. The 750 mL of CHO beverage supplied approximately 584 calories from 146 g of carbohydrate. These amounts were similar to package instructions for the CHOPA beverages but more concentrated than the recommendations for the CHO beverage.

Creatine kinase and lactate dehydrogenase measurements. Venipuncture blood samples were obtained from an antecubital vein at baseline and at 24 and 72 h after the first exercise bout. Samples were collected in heparinized Vacutainer® tubes and centrifuged within 10 min of collection. Following the extraction from the whole blood, plasma used for the CK measures was refrigerated, and plasma used for LDH measures was stored at room temperature. Samples were stored in capped tubes. Analyzes were conducted within 3 d of collection. Baseline and 24-h samples were analyzed for CK. Baseline and 72-h samples were analyzed for LDH. Analysis was performed on a Vitro DT 60II blood enzyme analyzer using standardized enzyme reaction procedures (Johnson and Johnson). Reconstituted lyophilized calibration standards, purchased from the manufacturer, were used to calibrate the Vitro DT 60II before CK and LDH analysis. Standard concentrations of CPK were 45, 525, and 1700 U·L^{-1}, and LDH was 30, 250, and 800 U·L^{-1}. All enzyme analysis methods were in accordance with recommendations and instructions provided with the Vitro DT 60II. Enzyme values were adjusted for changes in plasma volume according to the procedures described by Dill and Costill (8).

Subjective muscle soreness scale. A 7-point Likert scale of muscle soreness was given to each subject at baseline, 24 h, and 72 h after the first exercise bout (27). The scale ranged from 0 (no soreness present) to 6 (a severe pain that limits ability to move). Postexercise values were compared with baseline values and between treatment rides.

Dietary analysis. Subjects completed dietary record forms for a 24-h period prior to each experimental ride and during the days of the first and second exercise bouts. Subjects were given specific instructions to maintain a similar diet during the two trials. Amount of calories, carbohydrates, and protein were calculated using the Food Processor® program (ESHA Research, Salem, OR).

Statistical analysis. This study employed a within-subject repeated-measures design that contrasted the impact of two nutritional treatments on cycling performance and markers of muscle damage. Mean $\dot{V}O_2$ values during each trial were compared using a paired-sample t-test to assure that the rides were at similar intensities

between treatments. Calorie, carbohydrate, and protein intake, as well as time to fatigue for the two performance rides and combined time to fatigue, were also compared between trials using paired-samples t-tests. Time to fatigue data were also analyzed according to the order of the trials (i.e., independent of treatment) to rule out a repeated-bout effect. A paired-sample t-test was used for this analysis.

Plasma concentrations of CK and LDH were compared using 2×2 repeated-measures ANOVA. The CK and LDH data were also analyzed according to the order of the trials (i.e., independent of treatment) to rule out a repeated-bout effect. Within-subject and between-treatment comparisons of CK and LDH were made using the Newman–Keuls multiple comparison technique. Data from these analyses are reported as mean ± standard deviation.

Muscle soreness, which was measured using a Likert scale, was analyzed with nonparametric statistics. Between-treatment comparisons were made with a Wilcoxon matched-pair test. These data are reported as median ± range. Alpha was set at $P < 0.05$ for all analyses.

RESULTS

Physical and descriptive characteristics of the subjects. Fourteen male subjects between the ages of 18 and 33 (mean = 24.0 ± 4.1 yr) completed the study. All subjects engaged in endurance exercise at least four times per week and had a tested $\dot{V}O_{2max}$ over 45 mL·kg^{-1}·min^{-1} (mean = 59.8 ± 11.9).

Performance trial workloads and dietary analyses. All subjects completed trials at 70 and 80% $\dot{V}O_{2peak}$ at a constant workload measured in watts. Mean power output (W) and $\dot{V}O_2$ values, as absolute values and as percentage of max, are summarized in Table 2 for each trial. There were no significant differences in $\dot{V}O_2$ values between treatment trials at either 70 or 80% intensity. Additionally, diet records reflected no differences in total calories, carbohydrate content, or protein content for the 24-h period prior to the trial and the 22- to 24-h period between rides 1 and 2. Nutrient contents of treatment beverages were not included in the analysis. These data are presented in Table 3.

Time to fatigue. Time to fatigue, to the nearest hundredth of a minute, was recorded for each trial (Fig. 2). With both treatments, time to fatigue was shorter in trials at 80% $\dot{V}O_{2peak}$ compared with trials at 70% $\dot{V}O_{2peak}$. However, time to fatigue was not significantly different between trials supplemented with a CHO beverage compared with the CHOPA beverage at 70% intensity (CHO: 95.8 ± 29.7 min, CHOPA: 98.1 ± 28.7 min; $P = 0.77$), 80% intensity (CHO: 42.3 ± 18.6 min, CHOPA: 42.9 ± 21.8 min; $P = 0.91$), or as a total over the 2 d (CHO: 138.1 ± 39.3 min, CHOPA: 140.9 ± 43.7 min; $P = 0.77$). When time to fatigue values were analyzed according to the trial order (i.e., independent of treatment), no between-trial differences were found. The values for the 70% trial were 95.1 ± 27.5 min compared with 99.7 ± 31.0

min ($P = 0.56$). For the 80% trial, the values were 41.2 ± 20.1 and 44.4 ± 20.3 min ($P = 0.68$). Combined times for the CHO treatment were 136.3 ± 38.1 min and 144.1 ± 44.3 min for CHOPA ($P = 0.54$).

Muscle damage: CK and LDH. The 24-h CK values were significantly elevated ($P < 0.05$) over baseline values during the CHO trial (202.6 ± 120.0 vs 582.0 ± 474.9 $U \cdot L^{-1}$) but not the CHOPA trial (187.5 ± 119.0 vs 272.9 ± 169.4 $U \cdot L^{-1}$). Postexercise CK values were significantly higher ($P < 0.05$) in the CHO trial than in the CHOPA trial, despite no differences in baseline values between trials. These data are illustrated in Figure 3.

Similarly, 72-h LDH values were significantly elevated ($P < 0.05$) over baseline values during the CHO trial (437.4 ± 46.2 vs 495.1 ± 63.7 $U \cdot L^{-1}$) but not in the CHOPA trial (432.4 ± 39.6 vs 450.7 ± 43.1 $U \cdot L^{-1}$). Postexercise concentrations of LDH were significantly higher ($P < 0.05$) after the CHO trial than after the CHOPA trial, with no differences in baseline values. These data are illustrated in Figure 4.

When CK values were analyzed according to the trial order (i.e., independent of treatment), no between-trial differences were found ($P = 0.62$). The 24-h CK values were 399.2 ± 399.8 $U \cdot L^{-1}$ after the first trial and 455.6 ± 379.7 $U \cdot L^{-1}$ after the second trial. The 72-h LDH results similarly showed no order effect ($P = 0.67$).

Subjective soreness ratings. Peak muscle soreness occurred 24 h postexercise. Whereas baseline values were not different between treatments, peak muscle soreness ratings were significantly higher ($P < 0.05$) in the CHO trial (median = 3.0; range = 1.0–5.0) than the CHOPA trial (median = 1.0; range = 0.0–2.0) at 24 h. Muscle soreness data are illustrated in Figure 5.

DISCUSSION

The primary objectives of the present study were to compare the effects of commercially available, isocalorically matched CHO and CHOPA beverages on time to fatigue during prolonged cycling, biochemical markers of muscle damage, and subjective ratings of muscle soreness. The participants' $\dot{V}O_{2peak}$ values placed them well above the 90th percentile of males in their age bracket (1), an expected result because all of the subjects competed in endurance cycling events. These subjects were representative of the type of athletes that might benefit from sports nutrition products such as CHOPA beverages (12,23).

Time to fatigue was not different between treatments, a finding that is in agreement with one study (17) but in

TABLE 2. $\dot{V}O_2$ data from performance rides.

Performance Ride	CHO	CHOPA
Ride 1 (70% $\dot{V}O_{2peak}$)		
Actual $\dot{V}O_2$ (mL·kg^{-1}·min^{-1})	41.5 ± 7.5	41.3 ± 7.4
Percent of $\dot{V}O_{2peak}$	69.7 ± 4.7	69.5 ± 4.9
Ride 2 (80% $\dot{V}O_{2peak}$)		
Actual $\dot{V}O_2$ (mL·kg^{-1}·min^{-1})	47.5 ± 9.5	48.0 ± 8.9
Percent of $\dot{V}O_{2peak}$	79.6 ± 4.4	80.6 ± 3.3

TABLE 3. Dietary analysis before and between trails.

	Trial	24 h prior to Trial	22- to 24-h Period between Ride 1 and 2
Total Calories	CHO	2856 ± 411	2914 ± 545
	CHOPA	2902 ± 510	2795 ± 467
Carbohydrate (g)	CHO	415 ± 56	411 ± 51
	CHOPA	422 ± 43	409 ± 60
Protein (g)	CHO	110 ± 29	113 ± 35
	CHOPA	106 ± 19	108 ± 27

contrast to others that compared carbohydrate–protein beverages with CHO (12,19,23,30). With one exception (19), studies associated with differences in time to fatigue used carbohydrate–protein beverages that had more total calories than the CHO beverage. For example, Ivy et al. (12) and Saunders et al. (23) used a carbohydrate–protein beverage that contained 25% more calories than was used in the CHO beverage. Williams et al. (30) used a recovery carbohydrate–protein beverage that contained almost four times as many calories as the CHO beverage. In each case, it is plausible that the additional calories served as an energy substrate that directly enhanced performance. Because protein typically contributes a small proportion to total energy demands during exercise, utilization of protein added to CHO beverages could spare carbohydrate reserves, allowing athletes to perform for longer periods before exhaustion occurs. The present study and one reported by Millard-Stafford et al. (17) support this conclusion. In these two studies there were no significant differences between times to fatigue when the comparison beverages were matched for total calories.

An often discussed explanation for the performance improvements sometimes seen with carbohydrate–protein beverages is that the added protein may facilitate greater carbohydrate uptake by increasing insulin levels. Although postexercise insulin has been reported to be elevated following the use of carbohydrate–protein beverages (12,19,29,30), we are aware of no evidence showing that this occurs during exercise. Ivy et al. (12) observed elevated insulin values in carbohydrate–protein trials compared with placebo; however, the insulin levels were
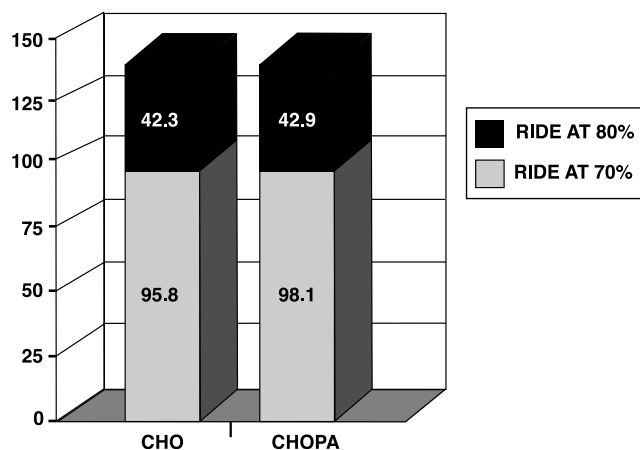

FIGURE 2—Time to fatigue at 70% $\dot{V}O_{2peak}$ and 80% $\dot{V}O_{2peak}$, and 2-d total for CHO and CHOPA trials.
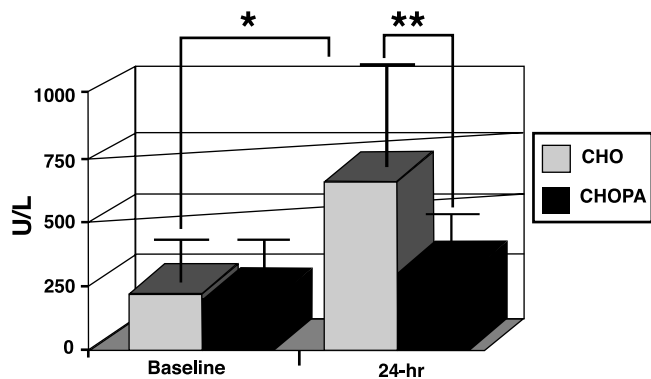
FIGURE 3—CK concentrations in CHO and CHOPA trials at baseline and at 24 h. * $P < 0.05$ over time; ** $P < 0.05$ between treatments.

FIGURE 5—Median muscle soreness ratings in CHO and CHOPA trials at baseline, 24 h, and 72 h. * $P < 0.05$ between treatments.

no different than those found during the CHO trial and, therefore, could not explain the differences in performance. Williams et al. (30) found a difference in insulin between treatments, but the carbohydrate–protein treatment contained more than twice as many carbohydrate calories as the CHO. Saunders et al. (23) did not measure insulin, and although other researchers have demonstrated that adding protein to carbohydrate beverages enhances the insulin response and replenishment of muscle glycogen, the effect on performance was not evaluated (31).

Niles et al. (19) also reported that a carbohydrate–protein beverage was associated with greater postexercise insulin increases than an isocaloric CHO beverage; however, in contrast to the present study, time to fatigue following a glycogen-depleting regime was greater with the carbohydrate–protein beverage. Fundamental differences in design may explain the discrepancy. The present study was designed to mimic day-to-day training and dietary practices common among competitive cyclist, whereas Niles et al. (19) appear to have designed a study intended to maximize the treatment effect. Niles et al. (19)
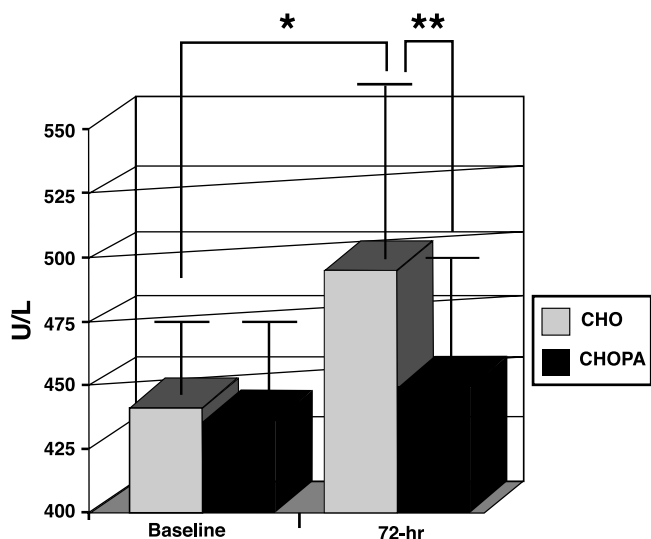
facilitated glycogen depletion with a low-carbohydrate diet (i.e., 35–40% of total calories) that began 48 h prior to an exhaustive exercise bout, and the run to exhaustion occurred within 2 h of ingesting the recovery beverage, presumably at a time when insulin levels were estimated to peak (31). Subjects in the present study cycled to exhaustion on two separate days. The first ride was at 70% $\dot{V}O_{2peak}$, considerably lower than the intensity used by Niles et al. (19), and the conditions prior to the second ride were not comparable with the conditions used by these (19) or other researchers (12,30). Finally, whereas Niles et al. (19) showed an increased time to fatigue, the high-intensity run times (i.e., CHO = 7.4 min, carbohydrate–protein = 9 min) were considerably less than the cycling times seen the in present study.

Millard-Stafford et al. (17) compared the effects of a carbohydrate–protein beverage with an isocaloric CHO beverage and reported time to fatigue results similar to those found in the present study, thus supporting the position that a much of the performance difference observed in other research (12,23,30) was due to utilization of added protein. An alternate view assumes that when protein calories are substituted for carbohydrates, a resulting attenuation of the insulin response favors greater hepatic glucose output, but the position that the added protein calories are used as an energy substrate is further supported by data from Colombani et al. (6). These researchers found that amino acid levels, urea, and urinary total nitrogen were elevated with a carbohydrate–protein supplementation during marathon running when compared with a CHO treatment.

In the present study, the CHOPA beverage contained the same number of total calories and 25% fewer carbohydrate calories than the CHO beverage. Under these conditions, performance time during the CHOPA trial was nearly identical to that observed in the CHO trial, thus indicating that when matched for total calories, carbohydrate–protein beverages are equally effective as CHO beverages in providing metabolic benefits during exercise.

In future studies, more valid comparisons could be made between carbohydrate–protein and CHO beverages if two

FIGURE 4—LDH concentrations in CHO and CHOPA trials at baseline and at 72 h. * $P < 0.05$ over time; ** $P < 0.05$ between treatments.
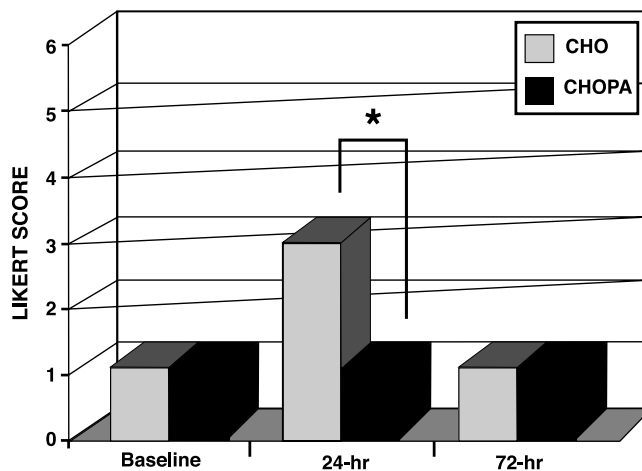
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CHO treatments are used, one that is isocarbohydrate and one that is isocaloric to the carbohydrate–protein beverage. Also, because the total amount of carbohydrate ingested during exercise in the studies reviewed here (12,17,19,23) ranged from 35 to 50 $g \cdot h^{-1}$, considerably less than the maximal exogenous carbohydrate oxidation rates of approximately 60 $g \cdot h^{-1}$ or more (22), it remains to be determined whether performance benefits will exist when carbohydrate is consumed at levels that approach maximal carbohydrate uptake. Adding carbohydrate calories beyond this rate should have no additional benefit, whereas adding protein may elicit improved performance if it functions as an energy substrate during endurance activity (12,19,23,30).

In the present study, CK was significantly elevated 24 h following the CHO trial (187%, $P < 0.05$) but not following the CHOPA trial. Postexercise CK was significantly lower (53%, $P < 0.05$) in the CHOPA trial compared with CHO, indicating that the added protein, antioxidants, or a combination of these nutrients may provide protection against muscle damage.

Although further research is needed to elucidate the apparent protective mechanisms, previous studies have shown attenuated postexercise CK following the administration of carbohydrate–protein beverages. Ready et al. (21) demonstrated that a sport drink containing carbohydrate and protein reduced CK by 36% following exercise compared with a CHO beverage. Interpretation of these findings is difficult because the carbohydrate–protein beverage contained more carbohydrate and total calories than the CHO. Moreover, it is not clear whether the protein beverage also contained antioxidants, which have, in some instances, been demonstrated to provide a protective effect against muscle damage (3,16,25).

Saunders et al. (23) compared CHO and CHOPA beverages that were matched for carbohydrate calories. Using an exercise protocol similar to the one in the present study, these researchers observed 83% lower CK values following the CHOPA trials. Although the present study demonstrates that CHOPA effectively reduces markers of muscle damage independent of the effects of increased caloric availability, the effect size is smaller than that reported by Saunders et al. (23). The higher magnitude of CK may be explained by the fact that in their study, blood samples were taken 12–15 h after the first exercise bout. Because CK generally peaks between 12 and 24 h postexercise (10), it is possible that these researchers captured the "peak" of the treatment effect. In addition, Saunders et al. (23) acknowledged that the CK concentrations they observed were higher than most published reports for cycling activity. This could have been due to a lower training status among some of their participants, as evidenced by lower mean $\dot{V}O_{2peak}$ values than observed in the present study, combined with the fact that their subjects worked at a slightly higher intensity (i.e., 75% $\dot{V}O_{2peak}$) during the first ride than did participants in this study.

In contrast to the present study, Millard-Stafford et al. (17) reported no difference in postexercise CK values between isocalorically matched carbohydrate–protein and CHO treatments. While acknowledging that others have shown significant increases in CK following exercise to exhaustion, the researchers suggested that the blunted response seen in their study may have been associated with a repeated-bout effect. A lack of sufficient statistical power may have also contributed to the null finding because CK generally has a high variability and data were analyzed for only eight subjects. It is difficult to further assess this possible explanation because the actual CK values, standard deviations, and P values were not provided. However, CK values associated with the present study and other research from our lab (23) suggest that 12–15 subjects are needed to insure that statistical power is adequate to detect differences.

The validity of CK as a marker of muscle damage has been questioned by some researchers. As seen in other studies (5,17,23), CK can be highly variable, with coefficients of variation (COV) of 200% or more. Data with this much variability may also be subject to weak intraclass correlations from one treatment to the next. To help address these concerns, a within-subject design was used in the present study, and although our CK values had COV ranging from 59 to 82%, we found an intraclass correlation of 0.84 ($P < 0.05$) between baseline measures. This value was comparable with unreported data (0.89; $P < 0.05$) from similar research done in our laboratory (23). Another criticism of CK as a marker of muscle damage is evidence indicating that vigorous exercise is associated with the release of CK from sources (e.g., monocytes) other than muscle (20). One approach that may help overcome this weakness, as well as the limitations associated with highly variable data, would be to measure multiple indicators of muscle damage. Therefore, LDH, a second biochemical marker of muscle damage that has COV ranging from 10 to 75% (5) and muscle soreness were measured in this study to corroborate CK data.

Postexercise LDH levels were increased significantly (13%, $P < 0.05$) in CHO trials but not in the CHOPA trials. In addition, postexercise LDH levels were significantly lower following the CHOPA trial in comparison with CHO (9%, $P < 0.05$). This observation was consistent with the CK data, further supporting the interpretation that the CHOPA beverage may protect against muscle damage. Notably, the COV for the LDH data ranged from 9 to 13%. The time course and magnitude of change in the soreness ratings also corresponded well with the CK data; the median soreness at 24 h following the CHO treatment (3.0 ± 5.0) was three times higher than the median values reported 24 h following CHOPA.

Although the mechanisms of muscle damage were not directly addressed in the present study, it is plausible that the lower CK and LDH values found with the CHOPA treatment resulted from decreased muscle tissue breakdown for energy or an attenuation of oxidative damage. Unlike previous studies (21,23), where exercise volume differed between treatments, the differences in the markers of muscle damage found here were not likely due to

volume differences because the intensity and duration of exercise were almost identical between trials. It is also unlikely that the lower CK and LDH values seen with the CHOPA treatment are attributable to a repeated-bout effect because the two trials were counterbalanced and analysis of the data revealed no differences due to trial order. Thus, this study suggests that the attenuation of CK and LDH was caused by CHOPA, although it remains to be determined whether the response is attributable to the protein, antioxidants, or a combination of these nutrients.

Evidence from other studies indicates that both protein and antioxidants may provide protective effects. Miller et al. (18), for example, found that postexercise amino acid uptake was significantly greater after carbohydrate–protein supplementation than with carbohydrate or protein alone. Levenhagen et al. (14) reported that protein synthesis and net protein balance were greater when a carbohydrate–protein supplement was consumed immediately after exercise as opposed to 3 h later. Others suggest that antioxidants may play a role in the attenuation of muscle damage (3,11,16,25), although Thompson et al. (26), who utilized a protocol comparable with the present study, reported that vitamin C ingestion following a 90-min run had no effect on muscle recovery. The role of protein and antioxidants could be better clarified if administered separately and in combination in a single study that included measures of oxidative stress as well as direct observation of muscle damage.

Potential confounding factors influencing time to fatigue and markers of muscle damage include supplement differences unrelated to protein and antioxidants, as well as within-subject variations in training or diet. To match the two commercial beverages used in this study for total calories without simultaneously changing the nutrient content, small differences in the formulations that are independent of carbohydrate and protein calories may have influenced the results. Specifically, the carbohydrate found in the CHO beverage contained sucrose and dextrose, and the CHOPA beverage contained sucrose, trehalose, fructose, and maltodextrin. The CHOPA drink also contains one gram of fat per serving, whereas there was no fat in the CHO beverage.

A systematic effort was made to keep training and diet constant and to account for any differences that might be present. Subjects were instructed to perform similar training sessions in the 2 d leading up to the trials and to perform no additional exercise during the 22- to 24-h layover between the rides at 70 and 80% $\dot{V}O_{2peak}$. Exercise logs showed that subjects were compliant with these instructions. Subjects were also asked to maintain similar diets in the days preceding and during the trials and to limit food intake in the 4 h prior to the experimental sessions. Although diet records are reported to underestimate actual dietary intake, they are reported to be reliable (7) and sensitive enough to detect changes within individuals (2) and should be acceptable for within-subject comparisons of food consumption. The subject's diet records showed that there were no significant differences in calorie, protein, or carbohydrate content of their diets during the CHO and CHOPA treatment periods. Although intake of vitamins C and E was not evaluated, the amount provided by CHOPA was considerably higher (approximately 10-fold greater than the RDA) than one would expect to see in subjects not taking supplements outside of the study. Moreover, if antioxidants attenuate CK, LDH, and muscle soreness following exhaustive cycling, and if the subjects' diets included higher than usual amounts of these nutrients, there would have been a reduced likelihood of finding a difference between the treatments. Thus, the combination of exercise and dietary control measures, as well as the intake assessment data, suggest that these potentially confounding factors did not differ between the treatments.

There are a number of design factors that should be considered for future research. First, more valid comparisons could be made if three types of beverages (i.e., CHOPA, isocaloric CHO, and isocarbohydrate–CHO) were simultaneously studied. This design would help clarify whether the benefits of carbohydrate–protein beverages are due to the additional calories or somehow attributable to the unique properties of protein. Secondly, to determine whether adding protein to carbohydrate drinks attenuates fatigue, these beverages should be studied in conditions where carbohydrate intake and absorption are maximized. In the present study, the subjects tolerated the 9.3% carbohydrate solution, a concentration that is above the general recommendations. Considering that carbohydrate availability is a primary limiting factor in prolonged exercise, maximal capacity for carbohydrate absorption needs to be further addressed. If additional carbohydrate can be reasonably tolerated, a greater amount of energy can be provided during exercise. Furthermore, because protein is absorbed by a separate mechanism in the digestive tract, discovering the upper limit of carbohydrate absorption and then adding protein may prove to be an effective way to maximize performance. Finally, the effect of CHOPA-type beverages on muscle damage should be compared against a control trial that includes exercise to exhaustion without the aid of supplements and against one supplement containing only protein and another supplement containing only antioxidants. A control trial would be of particular value if the protein or antioxidants provided a small but significant benefit that could not be detected when statistically compared with the opposing supplement or CHOPA. The effects of these nutrients on muscle damage should also be evaluated with direct measures of damage (e.g., muscle biopsies or MRI) as well as biochemical markers specific to oxidative stress. Given the high variability of CK and muscle soreness observed in this and other studies (17,23), measures of muscle damage that are more direct and specific may provide better evidence of whether the benefits of CHOPA beverages are attributable to the protein, antioxidants, or a combination of both.

In conclusion, data from this study add to the growing body of evidence indicating that CHOPA beverages

consumed during and after exhaustive exercise may attenuate muscle damage. Also, because time to fatigue was the same between the isocaloric treatments, these data suggest that protein may serve as an important energy substrate when given in combination with CHO beverages

during exercise. These results further corroborate data from previous studies showing that performance benefits observed with carbohydrate and protein sports beverages may be due to a carbohydrate-sparing effect related to the oxidation of the additional protein calories.

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