International Journal of Sport Nutrition and Exercise Metabolism, 2003, **13**, 382-395

Effect of a Carbohydrate-Protein Supplement on Endurance Performance During Exercise of Varying Intensity

John L. Ivy, Peter T. Res, Robert C. Sprague, and Matthew O. Widzer

Increasing the plasma glucose and insulin concentrations during prolonged variable intensity exercise by supplementing with carbohydrate has been found to spare muscle glycogen and increase aerobic endurance. Furthermore, the addition of protein to a carbohydrate supplement will enhance the insulin response of a carbohydrate supplement. The purpose of the present study was to compare the effects of a carbohydrate and a carbohydrate-protein supplement on aerobic endurance performance. Nine trained cyclists exercised on 3 separate occasions at intensities that varied between 45% and 75% VO_{2max} for 3 h and then at 85% VO_{2max} until fatigued. Supplements (200 ml) were provided every 20 min and consisted of placebo, a 7.75% carbohydrate solution, and a 7.75% carbohydrate / 1.94% protein solution. Treatments were administered using a double-blind randomized design. Carbohydrate supplementation significantly increased time to exhaustion (carbohydrate 19.7 ± 4.6 min vs. placebo 12.7 ± 3.1 min), while the addition of protein enhanced the effect of the carbohydrate supplement (carbohydrate-protein 26.9 ± 4.5 min, $p < .05$). Blood glucose and plasma insulin levels were elevated above placebo during carbohydrate and carbohydrate-protein supplementation, but no differences were found between the carbohydrate and carbohydrate-protein treatments. In summary, we found that the addition of protein to a carbohydrate supplement enhanced aerobic endurance performance above that which occurred with carbohydrate alone, but the reason for this improvement in performance was not evident.

Key Words: glycogen, glucose, insulin, lactate, perceived exertion

Introduction

Adequate muscle glycogen stores are essential for optimum performance during prolonged exercise of moderate to moderately-high intensity (1, 14, 15, 21). Once the muscle glycogen stores are depleted, exercise must either be discontinued or the exercise intensity significantly reduced. Because of the importance of muscle glycogen during prolonged exercise, methods for increasing its concentration above normal prior to (4) and following exercise (5, 25, 30, 35) and reducing its rate of

The authors are with the Department of Kinesiology and Health Education at The University of Texas, Austin, TX 78712.

utilization during exercise (3, 19, 33, 34) have been extensively studied. Methods for reducing muscle glycogen utilization during prolonged cycling exercise have had limited success when the exercise is continuous and of a moderately high intensity (15). However, carbohydrate supplementation can limit the decline in muscle glycogen during prolonged low intensity (3, 33), intermittent (19), and variable intensity exercise (34). Possibly accounting for the differences in muscle glycogen responses among exercise protocols is the plasma insulin response to a carbohydrate supplement.

Carbohydrate supplementation during moderately high intensity exercise has a limited effect on the plasma insulin concentration (15, 20). In contrast, carbohydrate supplementation during low (2, 23, 33), intermittent (19), and variable intensity (34) exercise results in an increase in the plasma insulin concentration. In fact, exceptionally high plasma insulin levels have been reported following carbohydrate supplementation during variable intensity exercise and associated with a sparing of muscle glycogen and an increase in endurance performance (34).

The addition of protein to a carbohydrate supplement has been reported to potentiate the plasma insulin response of the supplement following a fast or prolonged aerobic exercise (28, 30, 35). Thus, it is possible that a carbohydrate-protein supplement may be more effective than a carbohydrate supplement for the sparing of muscle and possibly liver glycogen during variable intensity exercise (10). Because endurance performance and rate of muscle glycogen utilization are inversely related, we compared the effects of a carbohydrate-protein supplement with a carbohydrate supplement on endurance performance. We hypothesized that supplementing with carbohydrate-protein would be more beneficial during prolonged variable intensity exercise than supplementing with carbohydrate, in part, because of the potentiating effect of protein on the plasma insulin response. We found that the addition of protein to a carbohydrate supplement enhanced endurance performance but that this enhancement in performance could not be ascribed to a greater plasma insulin response.

Methods

Subjects

The subjects were 9 trained, male cyclists between 22 and 30 years of age (mean = 27.3 ± 1.3 yr). They weighed 69.6 ± 2.5 kg and had a VO_{2max} of 61.3 ± 2.4 ml/kg/min. A 10th subject with a low VO_{2max} (44.1 ml/kg/min) was eliminated from the study because he could not complete the exercise protocol. Prior to their volunteering, each subject was fully informed of the study and signed an informed consent form approved by The University of Texas.

Initially, the subjects reported to the laboratory for assessment of their VO_{2max} and for familiarization with the exercise protocol that would be used to determine their endurance capacity. VO_{2max} was determined on an electrically braked cycle ergometer (Ergometrics 800-S, Sensormedics, Biz, Germany) using a continuous, graded exercise protocol. The protocol consisted of a 4-min warm-up and then 2-min stages beginning at 200 W, with increasing workload of 50 W at each stage until 350 W. After 350 W, the workload was increased 25 W every minute. A respiratory exchange ratio greater than 1.10 and an increase in VO_2 less than 0.2 L/min over the previous workload were the criteria to ascertain that VO_{2max} was achieved. On a

separate day, subjects performed a practice trial, which simulated the experimental trials, with the exception that water was provided and there were no blood draws. Each subject kept a training log and diet record during the week prior to the start of the experimental trials. From these records, the daily physical activity pattern and diets of the subjects to be used during the subsequent weeks of experimental testing were established.

Experimental Protocol

On three separate occasions, the subjects cycled to fatigue. Subjects were considered fatigued if they could no longer maintain the required exercise intensity for 15 s continuously, or dropped below the required exercise intensity for the third time.

During each exercise session, the subjects were administered, using a double-blind, randomized, counter-balanced design, 200 ml of a flavored aspartame-sweetened placebo supplement, a 7.75% liquid carbohydrate supplement, or a 7.75% carbohydrate / 1.94% protein supplement (Accelerade, PacificHealth Laboratories Inc.). All supplements contained the same amount of electrolytes and vitamins. Details of each supplement are presented in Table 1. Supplements were provided immediately before the start of exercise and every 20 min thereafter until the exercise intensity was increased to 85% VO_{2max}. Thereafter, no supplements were provided. The 3 experimental trials were spaced a minimum of 7 days apart. The subjects were required to perform standardized workouts on the 2 days before each trial. Diets were voluntarily controlled during the days preceding each trial based on the dietary records of the subjects. The subjects were instructed to eat the same

Table 1 Comparison of Ingredients in Each Supplement

Ingredients (per 100 ml)	Supplements		
	PLA	CHO	CHO-PRO
Carbohydrate (g)	0	7.75	7.75
Fat (g)	0	0.30	0.30
Protein (g)	0	0	1.94
Cholesterol (mg)	0	3.2	3.2
Sodium (mg)	56.8	56.8	56.8
Potassium (mg)	21.8	21.8	21.8
Calcium (mg)	17.7	17.7	17.7
Magnesium (mg)	35.3	35.3	35.3
Vitamin C (mg)	35.3	35.3	35.3
Vitamin E (mg)	17.7	17.7	17.7

Note. Supplements in volumes of 200 ml were provided immediately prior to exercise and every 20 min thereafter until the exercise intensity was increased to 85% VO_{2max}. PLA: placebo, CHO: carbohydrate, CHO-PRO: carbohydrate-protein.

meals the 2 days before each trial and record their food consumption. This information was turned in prior to each experimental trial.

On the day of a trial, the subjects reported to the laboratory 30 min before the start of exercise having fasted for 12 hours. They were weighed and fitted with a heart rate monitor (Polar Beat, Polar Electro Oy, Finland), and a 20-gauge catheter inserted into a large forearm vein and kept patent with sterile saline. Once the catheter was in place the subjects mounted the bicycle ergometer. After remaining seated on the ergometer for 2 min, a resting blood sample was drawn, heart rate recorded and then the cycling exercise started.

The exercise protocol, a modification of that used by Brouns (11), is illustrated in Figure 1. Cycling started with a 30-min warm-up at 45% VO_{2max} and was followed by cycling 6 times for 8 min at 75% VO_{2max} alternated with cycling 6 times for 8 min at 45% VO_{2max}. Next, the subjects cycled for 3 min at 75% VO_{2max} and for 3 min at 45% VO_{2max}. This sequence was then repeated 9 times. Subjects then cycled at 85% VO_{2max} until fatigue. The subjects performed their respective trials at the same time of the day and at the same ambient temperature (19–21 °C). Air was constantly circulated over the subjects via two floor fans. In addition, all timing devices were

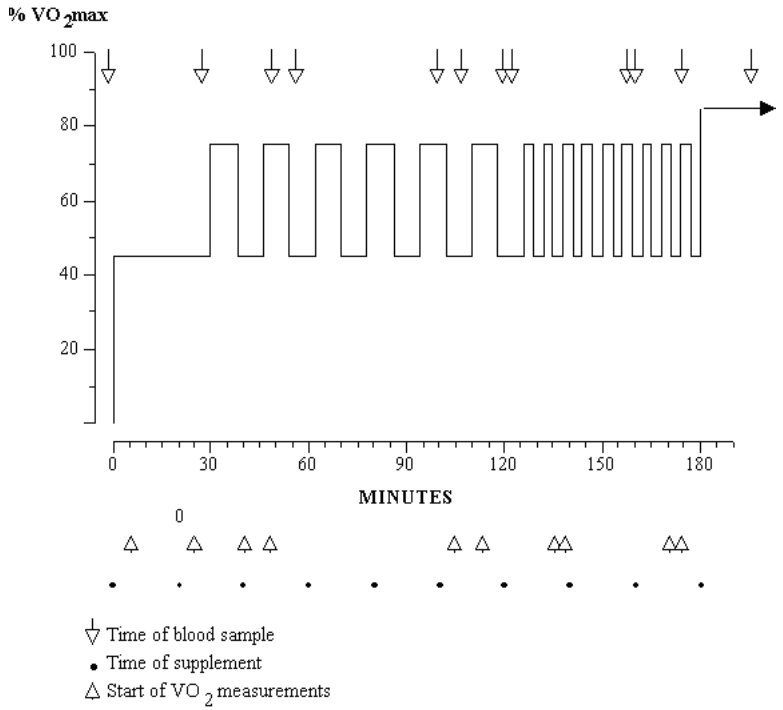

Figure 1 — Diagram of experimental protocol indicating work rates and times for blood samples, respiratory gas analyses, and time of supplementation.

hidden from the subjects prior to the start of exercise, and no feedback on performance was provided to the subjects until the study was completed.

Respiratory Gas Samples, Heart Rate, and Tissue Collection

To verify that the subjects were working at the proper intensity, and to determine caloric expenditure and carbohydrate oxidation rates, respiratory gas samples were collected during the last 3 min of predetermined exercise stages (See Figure 1). The subjects breathed through a Hans-Rudolph two-way, non-rebreathing valve, while inspired volumes and expired gases, sampled from a mixing chamber, were continuously analyzed with an automated system (Max-1, Physio-Dyne Instruments Corp., Quogue, NY, USA). Outputs from these instruments were directed to a laboratory computer for calculation of VO_2 and respiratory exchange ratio (R). Heart rates were monitored, and rating of perceived exhaustion (RPE) based on the Borg scale (9) was recorded at the end of respiratory gas sampling (Figure 1).

Blood samples (4 ml) were drawn from a forearm catheter immediately prior to exercise, at the end of predetermined exercise stages, and at fatigue (Figure 1). Two drops of blood were immediately used for the determination of blood glucose using a glucometer (One Touch Basic, Lifescan Inc., Milpitas, CA, USA). The validity and reliability of the glucometer were verified prior to its use in the study by comparing values obtained with the glucometer with those from a YSI 23A glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA). Calibration of the glucometer was performed with standards provided by Lifescan Inc. Once blood glucose was determined, 2 ml of blood were transferred to a tube containing EDTA (24 mg/ml, pH 7.4), and 1 ml was transferred to a tube containing 2 ml of 10% perchloric acid (PCA). The plasma samples were analyzed for insulin using a radioimmunoassay kit (Linco, St. Louis, MO, USA), and for free fatty acids (FFA) by enzymatic analysis (26). The PCA extracts were analyzed for lactate by enzymatic analysis according to Hohorst (22).

Statistical Analysis

Performance data were analyzed by a one-way analysis of variance for repeated measures. All other data were analyzed using a two-way analysis of variance (treatment × time) for repeated measures. Significant differences between means were determined by Least Squares Means analysis. SuperANOVA (Abacus Concepts Inc., Berkeley, CA, USA) was used for all statistical tests. Differences were considered significant at $p < .05$.

Results

Supplementation had a significant effect on performance (Figure 2). The carbohydrate treatment resulted in a significant increase in time to exhaustion when compared to placebo. Moreover, the carbohydrate-protein treatment resulted in a significant increase in time to exhaustion when compared to the carbohydrate treatment. The increase in performance was 55% when comparing carbohydrate with placebo and 36% when comparing carbohydrate-protein with carbohydrate.

Rating of perceived exertion gradually rose during the course of exercise. However, there were no differences in ratings among the three treatments. At fatigue, the average rating of perceived exertion was 19 ± 1 (Table 2).

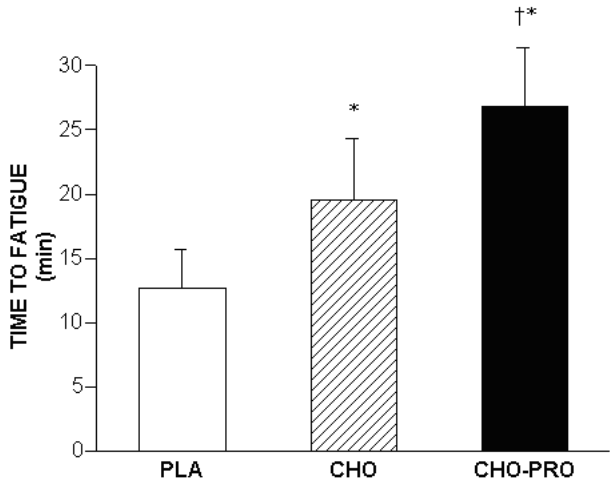

Figure 2 — Time to fatigue for the placebo (PLA), carbohydrate (CHO), and carbohydrate-protein (CHO-PRO) treatments. The subjects cycled at 85% VO_{2max} following 180 min of variable intensity exercise. Time to fatigue represents the amount of time the subjects could cycle at 85% VO_{2max}. *Significantly different than PLA; †significantly different than CHO.

Energy expenditure (Table 3) and heart rate (Table 2) during exercise were the same for the three treatments. However, the respiratory exchange ratio (Table 3) was significantly lower during the placebo treatment compared to the carbohydrate and carbohydrate-protein treatments from 30 min of exercise to fatigue. In addition, carbohydrate oxidation was significantly reduced, and fat oxidation significantly increased during the placebo treatment compared to the carbohydrate and carbohydrate-protein treatments (Table 3). There were no differences in respiratory exchange ratio, carbohydrate oxidation, or fat oxidation between the carbohydrate and carbohydrate-protein treatments.

Blood glucose was significantly higher during the carbohydrate and carbohydrate-protein treatments compared to the placebo treatment (Figure 3A). Differences occurred at 110 min of exercise and from 135 min of exercise to fatigue. Mean blood glucose was not different between carbohydrate and carbohydrate-protein treatments.

Plasma insulin responses were similar to the blood glucose responses (Figure 3B). Both carbohydrate and carbohydrate-protein treatments resulted in mean insulin levels greater than placebo. Differences occurred between the carbohydrate-protein treatment and placebo from 30 min of exercise to 135 min of exercise and also at 177 min of exercise. The carbohydrate treatment was significantly different than placebo at 30 min of exercise and from 62 min of exercise to 177 min of exercise. There was no difference between treatments at exhaustion. In addition, there were no differences between the carbohydrate and carbohydrate-protein treatments at any time during exercise.

There was no difference in plasma FFA levels between the carbohydrate and carbohydrate-protein treatments (Figure 4A). However, FFA levels for the carbo-

Table 2 Perceived Exertion and Heart Rate During Prolonged Continuous Variable-Intensity Exercise

Treatments	Minutes								
	10	30	46	54	110	118	141	177	Fatigue
	Perceived Exertion (scale 6–20)								
PLA	9.2 ± 0.6	9.7 ± 0.3	10.3 ± 0.4	13.1 ± 0.4	11.6 ± 0.6	14.2 ± 0.6	14.9 ± 0.6	15.8 ± 0.7	18.9 ± 0.2
CHO	9.4 ± 0.4	10.3 ± 0.4	10.4 ± 0.4	13.4 ± 0.3	11.0 ± 0.4	13.9 ± 0.4	14.1 ± 1.5	15.1 ± 0.6	19.2 ± 0.3
CHO-PRO	9.7 ± 0.4	9.9 ± 0.3	10.4 ± 0.5	13.3 ± 0.3	11.3 ± 0.5	14.1 ± 0.5	14.6 ± 0.6	15.3 ± 0.8	19.4 ± 0.2
	Heart Rate (bts/min)								
PLA	117 ± 3	118 ± 3	121 ± 3	155 ± 3	122 ± 3	154 ± 3	154 ± 3	159 ± 3	171 ± 3
CHO	115 ± 3	117 ± 3	123 ± 3	156 ± 4	125 ± 3	157 ± 4	158 ± 3	159 ± 3	177 ± 3
CHO-PRO	119 ± 2	117 ± 2	119 ± 2	152 ± 3	122 ± 3	156 ± 3	155 ± 3	161 ± 2	181 ± 3

Note. Values are means ± *SE*. PLA: placebo; CHO: carbohydrate treatment; CHO-PRO: carbohydrate-protein treatment.

Table 3 Energy Expenditure, Respiratory Exchange Ratio, and Carbohydrate and Fat Oxidation During Prolonged Continuous Variable-Intensity Exercise

Treatments	Minutes									
	5	25	41	49	105	113	135	138	171	174
					Energy expenditure (kcal/min)					
PLA	9.5±0.3	9.5±0.3	9.7±0.3	15.7±0.6	9.8±0.5	15.5±0.6	11.0±0.3	14.5±0.6	11.0+0.3	14.5±0.6
CHO	9.6±0.4	9.6±0.4	9.7±0.3	15.6±0.6	9.8±0.4	15.7±0.5	11.0±0.4	14.8±0.6	11.4±0.3	15.0±0.4
CHO-PRO	9.5±0.4	9.5±0.3	9.6±0.3	15.4±0.6	9.8±0.4	15.7±0.6	10.9±0.4	14.7±0.6	11.2±0.4	14.7±0.6
					Respiratory exchange ratio ($V^{\cdot} CO_2 / V^{\cdot} O_2$					
PLA	0.90±0.01	0.89±0.01*	0.86±0.01*	0.94±0.01*	0.83±0.01*	0.90±0.01*	0.87±0.01*	0.86±0.01*	0.86±0.01*	0.85±0.01*
CHO	0.91±0.01	0.92±0.01	0.90±0.01	0.97±0.01	0.89±0.01	0.95±0.01	0.91±0.02	0.92±0.01	0.90±0.01	0.90±0.01
CHO-PRO	0.92±0.01	0.92±0.01	0.89±0.01	0.96±0.01	0.88±0.01	0.94±0.01	0.92±0.01	0.91±0.01	0.90±0.01	0.90±0.01
					Fat oxidation (g/min)					
PLA	0.36±0.03	0.40±0.03	0.49±0.04*	0.35±0.05	0.60±0.03*	0.54±0.05*	0.53±0.03*	0.74±0.04*	0.57±0.04*	0.76±0.04*
CHO	0.31±0.03	0.30±0.02	0.36±0.02	0.21±0.04	0.40±0.03	0.33±0.05	0.37±0.04	0.46±0.04	0.43±0.04	0.56±0.04
CHO-PRO	0.31±0.03	0.31±0.04	0.38±0.02	0.24±0.05	0.44±0.03	0.38±0.04	0.32±0.03	0.49±0.03	0.42±0.04	0.55±0.03
					CHO Oxidation (g/min)					
PLA	1.48±0.04	1.40±0.06*	1.22±0.05*	3.02±0.12	1.01±0.04*	2.58±0.11*	1.47±0.08*	1.82±0.10*	1.37±0.08*	1.79±0.90*
CHO	1.64±0.07	1.64±0.08	1.53±0.04	3.35±0.12	1.46±0.05	3.10±0.11	1.84±0.09	2.54±0.16	1.80±0.08	2.48±0.15
CHO-PRO	1.63±0.06	1.62±0.07	1.44±0.06	3.19±0.10	1.37±0.06	2.95±0.13	1.89±0.09	2.45±0.14	1.78±0.07	2.31±0.13

Note. Values are means ± *SE*. PLA: placebo; CHO: carbohydrate treatment; CHO-PRO: carbohydrate-protein treatment. *PLA significantly different than CHO and CHO-PRO.

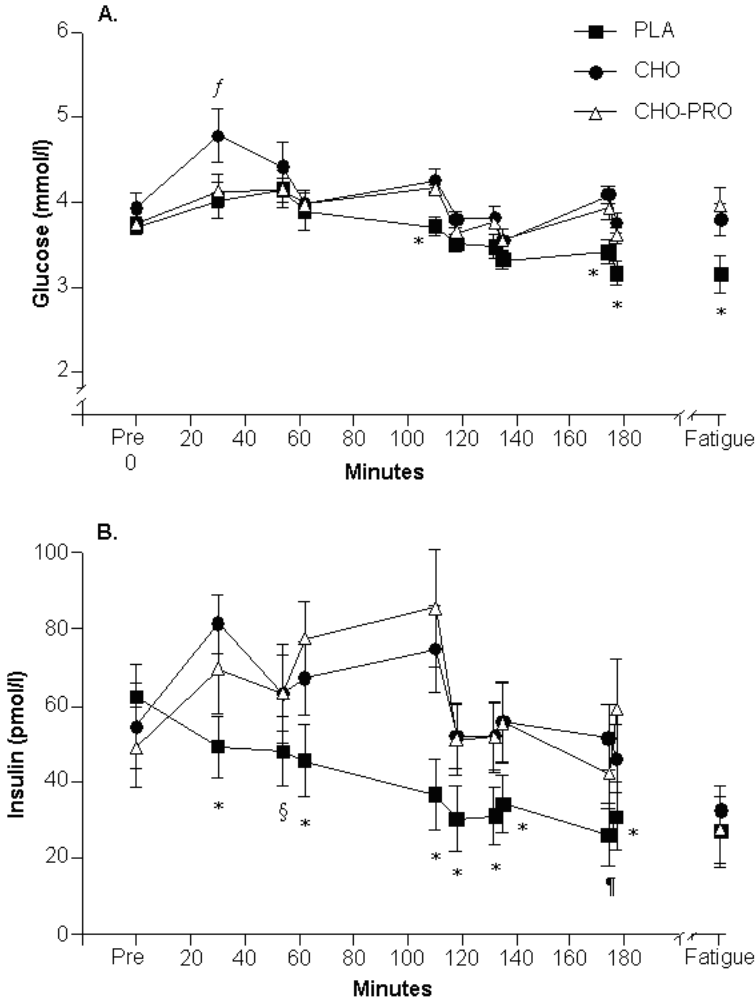

Figure 3 — Blood glucose (a) and plasma insulin (b) concentrations during prolonged continuous variable-intensity exercise. PLA, placebo treatment; CHO, carbohydrate treatment; CHO-PRO carbohydrate-protein treatment. *PLA significantly different than CHO and CHO-PRO; ¶PLA significantly different than CHO; §PLA significantly different than CHO-PRO; and *f* CHO significantly different than PLA and CHO-PRO.

hydrate and carbohydrate-protein treatments were significantly lower than the placebo treatment from 110 min of exercise to fatigue.

Prior to exercise, blood lactate averaged 1.03 ± 0.01, 1.24 ± 0.01, and 1.12 ± 0.01 mmol/L for the placebo, carbohydrate and carbohydrate-protein treatments, respectively (Figure 4B). Blood lactate was not different between treatments until fatigue. At this time, blood lactate averaged 4.95 ± 1.1 mmol/L for the placebo treatment, 5.67 ± 0.9 mmol/L for the carbohydrate treatment, and 6.22 ± 0.8 mmol/

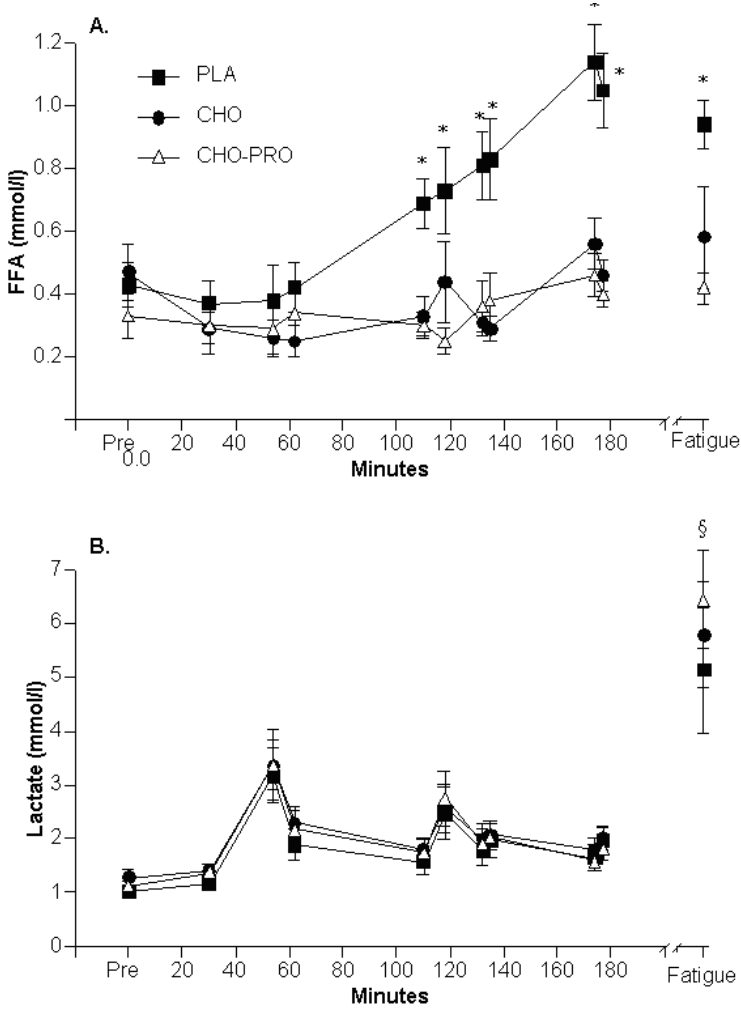

Figure 4 — Plasma free fatty acids (a) and blood lactate (b) concentrations during prolonged continuous variable-intensity exercise. PLA, placebo treatment; CHO, carbohydrate treatment; CHO-PRO, carbohydrate-protein treatment. §PLA significantly different than CHO-PRO.

L for the carbohydrate-protein treatment. The blood lactate during the carbohydrate-protein treatment was significantly greater than during the placebo treatment.

Discussion

While carbohydrate supplementation has not been found to spare muscle glycogen during steady-state moderate-intensity cycling exercise (15), it has been found to be effective during low intensity (33), intermittent (19), and variable intensity (34)

cycling exercise. Moreover, muscle glycogen sparing corresponds with an improved endurance performance (34). The sparing of muscle glycogen following carbohydrate supplementation appears to be related to a high plasma insulin response (34). The addition of protein to a carbohydrate supplement typically enhances the plasma insulin response of the supplement (28, 30, 35). Therefore, we hypothesized that during variable intensity exercise, a supplement composed of carbohydrate and protein would elicit a greater plasma insulin response and have a greater effect on the sparing of muscle glycogen and endurance performance than a carbohydrate supplement alone.

We found that carbohydrate supplementation improved endurance performance. However, we also found that there was an additional improvement in performance when protein was combined with carbohydrate. Compared to placebo, carbohydrate supplementation improved performance for all but 2 subjects, while only 1 subject did not show an improvement with carbohydrate-protein supplementation. Moreover, only 2 subjects did not improve their performance when receiving the carbohydrate-protein supplement when compared with the carbohydrate supplement. Of these 2 subjects, 1 subject had the same performance times for both treatments while the other subject performed better on the carbohydrate supplement.

Both carbohydrate and carbohydrate-protein supplementation increased the plasma insulin concentration during exercise above that produced by placebo. However, the insulin responses of the carbohydrate and carbohydrate-protein treatments did not differ. While the elevated plasma insulin response with carbohydrate and carbohydrate-protein supplementation may have contributed to an improved endurance performance by sparing muscle glycogen, this does not appear to account for the difference in performance observed between these two treatments.

It has been observed that carbohydrate supplementation delays the onset of fatigue during steady state moderate intensity exercise by preventing hypoglycemia and maintaining carbohydrate oxidation rather than by sparing or reducing the rate of muscle glycogen utilization (15). In the present study, the blood glucose concentration at fatigue was 3.15 ± 0.21 mmol/L with the placebo treatment and 3.79 ± 019 mmol/L and 3.96 ± 0.2 mmol/L with the carbohydrate and carbohydrate-protein treatments, respectively. However, it is unlikely that the improvement in performance with the carbohydrate and carbohydrate-protein treatments was simply due to a better maintenance of blood glucose. It has been suggested that blood glucose cannot fully support the carbohydrate requirements of exercise at intensities exceeding 75% VO_{2max} when the muscle glycogen stores are depleted (13). We found that after 180 min of variable intensity exercise, the subjects were only capable of cycling ~13 min at 85% VO_{2max} with the placebo treatment. In contrast, with the carbohydrate and carbohydrate-protein treatments, the subjects were able to cycle for ~20 and 27 min at 85% VO_{2max}, respectively. Because blood glucose is incapable of supporting the carbohydrate requirements needed to sustain exercise at intensities greater than 75% VO_{2max} (13), it is unlikely that the carbohydrate and carbohydrate-protein treatments would have been capable of significantly extending time to fatigue without adequate muscle glycogen stores. Thus, the observed increase in time to fatigue at this extreme exercise intensity would suggest that muscle glycogen was spared during both the carbohydrate and carbohydrate-protein treatments.

Although the addition of protein to the carbohydrate supplement improved endurance performance, the mechanism was not apparent. We had predicted that the

addition of protein would result in a greater sparing of muscle glycogen than carbohydrate alone, thus providing a greater glycogen reserve during the performance phase of the exercise session. This hypothesis is now questionable for several reasons, however. First, the insulin responses to the carbohydrate and carbohydrate-protein supplements were not different. Thus, there is no known reason for glycogen to be spared or synthesized at a greater rate with the carbohydrate-protein treatment as compared to carbohydrate treatment. Second, measurements of carbohydrate and fat oxidation were similar between these two treatments, which would also suggest that muscle glycogen utilization was similar. Conversely, we have recently observed a faster rate of muscle glycogen storage immediately post exercise when provided a carbohydrate-protein supplement, as compared with a carbohydrate supplement, despite similar plasma insulin responses for each supplement (24). This observation raises the possibility that the addition of protein to a carbohydrate supplement may facilitate muscle glycogen storage by a process not yet identified.

A second possible explanation for the difference in performance between the carbohydrate and carbohydrate-protein treatments is the central fatigue hypothesis. During exercise, plasma branch-chain amino acids (BCAA) decrease (6, 7), and there is an unloading of tryptophan from albumin due to a rise in plasma free fatty acids (17). Because tryptophan and BCAA compete for the same transporter across the blood-brain barrier, the increase in the ratio of plasma free tryptophan to BCAA enhances brain uptake of tryptophan. Tryptophan is a precursor to serotonin, which lowers brain activity and possibly induces central fatigue (16). Several studies have suggested that the addition of BCAA during exercise will improve endurance exercise performance (8, 12, 27); however, this finding is not universal (6, 29, 31). In fact, the inclusion of BCAA in a carbohydrate supplement was found not to benefit endurance exercise performance (6, 29). In addition, van Hall et al. (29) reported that supplementing with tryptophan during moderate intensity exercise had no adverse effect on endurance exercise capacity.

A third possible explanation for the improved endurance performance during carbohydrate-protein supplementation is that the addition of protein could provide precursors for the anaplerotic reactions required to maintain Krebs cycle intermediates in skeletal muscle. At the onset of exercise, there is a rapid expansion of Krebs cycle intermediates, but, as exercise persists, the concentration of these intermediates progressively decline—some, such as 2-oxoglutarate and oxaloacetate, to critically low levels (18). It has been proposed that fatigue during prolonged exercise may result from the depletion of Krebs cycle intermediates and thus the inability of the mitochondria to sustain aerobic energy production (32). Although carbohydrate supplementation is thought to provide some assistance with this process, carbohydrate supplementation may not be as efficient as providing the appropriate amino acids.

In summary, we verified that providing carbohydrate supplementation during prolonged aerobic exercise would improve endurance performance. More importantly, we found that the addition of protein to a carbohydrate supplement provided an additional ergogenic effect. Endurance performance was increased by an additional 36% with the addition of protein. The reason for this improvement is equivocal at this time but could be related to the sparing or more efficient use of muscle glycogen, the maintenance of plasma amino acid levels as they relate to central fatigue, or to anapleroic reactions and the retention of Krebs cycle intermediates. Further research will be required to address this question.

References

1. Ahlborg, B., J. Bergström, L.G. Ekelund, and E. Hultman. Muscle glycogen and muscle electrolytes during prolonged physical exercise. *Acta Physiol. Scand.* 70:129-142, 1967.
2. Ahlborg, G., and P. Felig. Influence of glucose ingestion on fuel-hormone response during prolonged exercise. *J. Appl. Physiol.* 41:683-688, 1976.
3. Bagby, G.J., H.J. Green, S. Katsuta, and P.D. Gollnick. Glycogen depletion in exercising rats infused with glucose, lactate, or pyruvate. *J. Appl. Physiol.* 45:425-429, 1978.
4. Bergström, J., L. Hermansen, E. Hultman, and B. Saltin. Diet, muscle glycogen and physical performance. *Acta Physiol. Scand.* 71:140-150, 1967.
5. Blom, P.C.S., A.T. Høstmark, O. Vaage, K.R. Kardal, and S. Mæhlum. Effect of different post-exercise sugar diets on the rate of muscle glycogen synthesis. *Med. Sci. Sports Exerc.* 19:491-496, 1987.
6. Blomstrand, E., S. Andersson, P. Hassmen, B. Ekblom, and E.A. Newsholme. Effect of branched-chained amino acid and carbohydrate supplementation on the exercise-induced change in plasma and muscle concentration of amino acids in human subjects. *Acta Physiol. Scand.* 153:87-96, 1995.
7. Blomstrand, E., F. Celsing, and E.A. Newsholme. Changes in plasma concentrations of aromatic and branch-chain amino acids during sustained exercise in man and their possible role in fatigue. *Acta Physiol. Scand.* 133:115-121, 1988.
8. Blomstrand, E., P. Hassmen, B. Ekblom, and E.A. Newsholme. Administration of branched-chain amino acids during sustained exercise-effects on performance and on plasma concentration of some amino acids. *Eur. J. Appl. Physiol*. 63:83-88, 1991.
9. Borg, G. Simple ratings method for estimation of perceived exertion. In: *Physical Work and Effort*, G. Borg (Ed.). New York: Pergamon, 1975, p. 39-46.
10. Botwell, J.L., K. Gelly, M.L. Jackman, A. Patel, M. Simeoni, and M.J. Rennie. Effect of oral glutamine on whole body carbohydrate storage during recovery from exhaustive exercise. *J. Appl. Physiol.* 86:1770-1777, 1999.
11. Brouns, F.J.P.H. *Food and Fluid Related Aspects in Highly Trained Athletes*. Haarlem, The Netherlands: Uitgeverij De Vrieseborch, 1988.
12. Calders, P., J-L. Pannier, D.K. Matthys, and E.M. Lacroix. Pre-exercise branched-chain amino acid administration increases endurance performance in rats. *Med. Sci. Sports Exerc.* 29:1182-1186, 1997.
13. Coggan, A.R. and E.F. Coyle. Effect of carbohydrate feedings during high-intensity exercise. *J. Appl. Physiol.* 65:1703-1709, 1988.
14. Costill, D.L., R. Bowers, G. Branam, and K. Sparks. Muscle glycogen utilization during prolonged exercise on successive days. *J. Appl. Physiol.* 31:834-838, 1971.
15. Coyle, E.F., A.R. Coggan, M.K. Hemmert, and J.L. Ivy. Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J. Appl. Physiol.* 61:165-172, 1986.
16. Davis, J.M., and S.P. Bailey. Possible mechanisms of central nervous system fatigue during exercise. *Med. Sci. Sports Exerc*. 29:45-57, 1997.
17. Davis, J.M., S.P. Bailey, J.A. Woods, F.J. Galiano, M.T. Hamilton, and W.P. Bartoli. Effects of carbohydrate feedings on plasma free tryptophan and branched-chain amino acids during prolonged cycling. *Eur. J. Appl. Physiol.* 65:513-519, 1992.
18. Gibala, M.J., M.A. Tarnopolosky, and T.E. Graham. Ticarboxylic acid cycle intermediates I human muscle at rest and during prolonged cycling. *Am. J. Physiol.* 272:E239-E244, 1997.
19. Hargreaves, M., D.L. Costill, A. Coggan, W.J. Fink, and I. Nishibata. Effect of carbohydrate feedings on muscle glycogen utilization and exercise performance. *Med. Sci. Sports Exerc.* 16:219-222, 1984.
20. Hargreaves, M., and C.A. Briggs. Effect of carbohydrate ingestion on exercise metabolism. *J. Appl. Physiol.* 65:1553-1555, 1988.

21. Hermansen, L., E. Hultman, and B. Saltin. Muscle glycogen during prolonged severe exercise. *Acta Physiol. Scand.* 71:129-139, 1967.
22. Hohorst, H.J. Determination of L-lactate with LDH and DPN. In: *Methods of Enzymatic Analysis*, H.U. Bergmeyer (Ed.). New York: Academic Press, 1965, pp. 266-270.
23. Ivy, J.L., M.V. Dover, L.G. Goodyear, W.M. Sherman, S. Farrell, and H. Williams. Endurance improved by ingestion of a glucose polymer supplement. *Med. Sci. Sports Exerc.* 15:466-471, 1983.
24. Ivy, J.L., H.W. Goforth, Jr., B.M. Damon, T.R. McCauley, E.C. Parsons, and T.B. Price. Early post-exercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. *J. Appl. Physiol.* 93:1337-1344, 2002.
25. Ivy, J.L., A. Katz, C.L. Cutler, W.M. Sherman, and E.F. Coyle. Muscle glycogen synthesis after exercise: effect of time of carbohydrate ingestion. *J. Appl. Physiol.* 64:1480-1485, 1988.
26. Miles, J., R. Glasscock, J. Aikens, J. Gerich, and M. Haymond. A microfluorometric method for the determination of free fatty acids in plasma. *J. Lip. Res.* 24:96-99, 1983.
27. Mittleman, K.D., M.R. Ricci, and S.P. Bailey. Branched-chain amino acids prolong exercise during heat stress in men and women. *Med. Sci. Sport Exerc.* 30:83-91, 1998.
28. Spiller, G.A., C.D. Jensen, T.S. Pattison, C.S. Chuck, J.H. Whittam, and J. Scala. Effect of protein dose on serum glucose and insulin response to sugars. *Am. J. Clin. Nutr.* 46:474-480, 1987.
29. Van Hall, G., J.S.H. Raaymakers, W.H.M. Saris, and A.J.M. Wagenmakers. Ingestion of branched-chain amino acids and tryptophan during sustained exercise in man: failure to affect performance. *J. Physiol*. 486:789-794, 1995.
30. van Loon, L.J.C., W.H.S. Saris, M. Kruijshoop, and A.J.M. Wagenmakers. Maximising post-exercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid and protein hydrolysate mixtures. *Am. J. Clin. Nutr.* 72:106-111, 2000.
31. Varnier, M., G.P. Leese, J. Thompson, and M.J. Rennie. Stimulatory effect of glutamine on glycogen accumulation in human skeletal muscle. *Am. J. Physiol.* 269:E309-E315, 1995.
32. Wagenmakers, A.J.M., J.H. Coakley, and R.H.T. Edwards. Metabolism of branch-chain amino acids and ammonia during exercise: clues from McArdle's disease. *Int. J. Sports Med.* 11:S101-S113, 1990.
33. Yaspelkis, B.B., III, and J.L. Ivy. Effect of carbohydrate supplements and water on exercise metabolism in the heat. *J. Appl. Physiol.* 71:680-687, 1991.
34. Yaspelkis, B.B., III, J.G. Patterson, P.A. Anderla, Z. Ding, and J.L. Ivy. Carbohydrate supplementation spares muscle glycogen during variable intensity exercise. *J. Appl. Physiol.* 75:1477-1485, 1993.
35. Zawadzki, K.M., B.B. Yaspelkis, III, and J.L. Ivy. Carbohydrate-protein supplement increases the rate of muscle glycogen storage post exercise. *J. Appl. Physiol.* 72:1854-1859, 1992.

Acknowledgments

We wish to thank Zhenping Ding for his excellent technical assistance. The study was supported by a research grant from PacificHealth Laboratories Inc., Woodbridge, NJ.