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## Exercise Nutrition

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### **CARBOHYDRATE-PROTEIN DRINK IMPROVES TIME TO EXHAUSTION AFTER RECOVERY FROM ENDURANCE EXERCISE.**

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#### ABSTRACT

ERIC S. NILES, TONY LACHOWETZ, JOHN GARFI, WILLIAM SULLIVAN, JOHN C. SMITH, BRIAN P. LEYH, AND SAMUEL A. HEADLEY. **Carbohydrate-Protein Drink Improves Time to Exhaustion After Recovery from Endurance Exercise.** *JEPonline*, 2001 4(1):45-52. Ten endurance trained males were studied to investigate the ergogenic effects of isocaloric carbohydrate (CHO, 152.7 g) and carbohydrate-protein (CHO-PRO, 112 g CHO with 40.7 g PRO) drinks ingested after a glycogen lowering diet and exercise bout. Treatments were administered in a double-blind and counterbalanced fashion. After a glycogen lowering diet and run, two dosages of a drink were administered with a 60 min interval between dosages. The CHO-PRO trial resulted in higher serum insulin levels (60.84 vs 30.1  $\mu$ U/ml) 90 min into recovery than the CHO only trial ( $p < 0.05$ ). Furthermore, the time to run to exhaustion was longer during the CHO-PRO trial ( $540.7 \pm 91.56$  sec) than the CHO only trial ( $446.1 \pm 97.09$  sec,  $p < 0.05$ ). In conclusion, a CHO-PRO drink following glycogen depleting exercise may facilitate a greater rate of muscle glycogen resynthesis than a CHO only beverage, hasten the recovery process, and improve exercise endurance during a second bout of exercise performed on the same day.

**Key words:** Serum Insulin, Anaerobic Threshold, Ergogenic.

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## INTRODUCTION

Pre-exercise intramuscular glycogen levels determine the time to exhaustion during endurance events of moderate intensity (1,2). If endurance athletes are required to complete multiple events within the same day, it would be to their advantage to adopt practices designed to hasten the resynthesis of intramuscular glycogen. Fallowfield and Williams (3) demonstrated that by increasing the daily consumption of carbohydrate (CHO) to 8.8 g/kg body wt, restoration of intramuscular glycogen stores occurred within 22.5 hr following a bout of carbohydrate depleting endurance exercise. Ivy et al. (4) found that following endurance exercise subjects achieved rapid glycogen repletion after consuming CHO immediately as opposed to waiting several hours post-exercise. Blom et al. (5) studied varying dosages and types of simple sugars on post-exercise glycogen synthesis and found no significant difference in glycogen synthesis after either a 0.7 or a 1.4 g/kg body wt dosage of glucose.

Though CHO is definitely of prime importance to the endurance athlete, proteins (PRO) are now considered to be of greater importance than previously thought. For example, Nuttall et al. (6) reported that a PRO dose in combination with glucose elicited a higher insulin release than seen with either glucose or PRO alone and this resulted in the lowering of plasma glucose levels in a group of type 2 diabetics. In addition, Zawadzki et al. (7) studied nine male cyclists to determine if the ingestion of both CHO and PRO (CHO-PRO) after prolonged exercise would result in a higher insulin response compared to the ingestion of CHO alone. In addition, muscle biopsies were performed to quantify the rate of muscle glycogen resynthesis. The rate of glycogen storage during the CHO-PRO treatment was found to be 38% higher than during the trial in which only CHO was consumed. However, the results of this study have been questioned since the CHO-PRO combination provided the athletes with 43% more energy than the CHO only trial (8). Furthermore, there was no assessment of subsequent exercise performance following the ingestion of the two different beverages.

The present study was designed to compare the effects of a CHO-PRO drink versus an isocaloric drink containing only CHO on the recovery process following CHO depleting exercise. It was hypothesized that during the CHO-PRO trial, there would be a greater insulin release and a greater run time to exhaustion than during the CHO trial.

## METHODS

### Subjects

Ten male runners ranging in age from 24-34 years, with a minimum peak oxygen uptake ( $VO_{2peak}$ ) of 50 mL/kg/min and a percentage body fat below 15%, volunteered to participate in this study. All subjects gave their informed consent to participate in this study which was approved by the Institutional Review Board of the College.

### Research Design

This was a repeated measures, double-blind and counter-balanced study. To avoid systematic order effects, subjects were randomly assigned to the various treatment conditions (CHO vs. CHO-PRO).

### Testing Procedures

#### *Preliminary tests*

All subjects had their  $VO_{2peak}$  determined via open circuit spirometry using a SensorMedics metabolic cart, (Model 2900, Yorba Linda, CA). A standard running protocol was used (9). Tests were terminated when at least two of the following criteria were met: a plateau in oxygen uptake with increasing work rate, a peak heart rate within 10 beats of the age predicted maximum, and an  $RER > 1.15$  (10).

During the test of  $\text{VO}_2\text{peak}$ , blood samples were collected (via capillary puncture from a finger) at baseline and during the last 30 s of each stage. All samples were analyzed by a 1500 YSI lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH) which was calibrated according to manufacturer's guidelines. The individual anaerobic threshold (IAT) was determined by using the log-log transformation as described by Beaver, Wasserman, and Whipp (11).

### ***Practice run***

Within a week following the  $\text{VO}_2\text{peak}$  test, a practice treadmill run was performed. This was done to familiarize the subjects with the protocol that they would use during the performance run. Once the IAT was achieved, the corresponding  $\text{VO}_2$  (IAT $\text{VO}_2$ ) was determined and a value corresponding to 10% above the IAT $\text{VO}_2$  was then calculated and served as the target  $\text{VO}_2$  value which the subject used during the final performance running test. This intensity was chosen to help ensure the predominant use of glycogen as a fuel.

### ***Diet manipulation***

To facilitate a reduction of intramuscular glycogen, subjects were instructed to follow a prescribed diet over a 48 hr period prior to the exercise tests. The exchange system was used to plan all of the diets. Diet recording sheets and exchange lists were issued to all subjects. All recorded diet logs were analyzed for the following: total calories, FAT, CHO, and PRO contents using Nutritionist III (N-Squared Computing, Salem, OR). The goal during this period was to have the subjects consume CHO, PRO, and fats in the following respective proportions 48 hrs prior to the test: 40, 40, and 20 %. Twenty-four hours prior to the test the subjects aimed to achieve a diet containing 35% CHO, 45% PRO and 20% FAT.

### ***Pre-testing exercise record***

Subjects were instructed to record the duration and intensity of exercise sessions during the 48 hrs prior to the treatment sessions. Seven subjects ran on the days prior to testing and were instructed to keep their heart rates below 70% of their age-predicted maximum values. Three subjects did not exercise the day prior to testing. All subjects then replicated the same exercise or rest prior to the second treatment session.

### ***Treatment sessions***

Each subject came to the laboratory after a 12-hr overnight fast, and proceeded to have height, weight, and skinfold measures taken. Skinfold sites consisted of the abdomen, supra iliac, triceps, and thigh (12). The glycogen depletion run was then administered. During the depletion run, subjects were given a total of 1 L of water administered in 200 mL volumes with the first drink given immediately prior to the start of exercise and the remainder given at approximately 12 min intervals. The room temperature was 24 °C during all sessions. The exercise intensity was set in accordance with Muoio et al. (13) at 80 % of  $\text{VO}_2\text{peak}$  for the first 30 min of the run. The intensity was then dropped to 70-75% of  $\text{VO}_2\text{peak}$  for the remainder of the run. Gas analysis was taken for the first 10-12 min until a steady state  $\text{VO}_2$  was attained, after which the mask was removed for subject comfort. At 30 min the mask was attached to monitor the drop in intensity. The mask was left on for 5-7 min until  $\text{VO}_2$  values leveled, after which the mask was removed for the remainder of the run.

A finger stick blood sample to determine plasma glucose levels was obtained at 40 min into the run and immediately analyzed. A Reflotron glucose analyzer (Boehringer Mannheim, Germany) was used for immediate glucose values. During the first treatment session, the depletion run lasted 45 min for all subjects. During the second treatment session, the run was terminated when the plasma glucose levels were within 5 mg/100 mL of the first session. Approximately one week later, during the second depletion run the mean running time was  $50.35 \pm 0.23$  min. Those subjects running longer than 45 min during the second testing session had a blood sample taken at 2.5 min intervals throughout the subsequent 5-10 minutes until glucose values were within the desired range, at which time the run was terminated.

### ***Supplementation Period***

Following the depletion run, the subject was given the appropriate supplement drink for that session. Each drink contained the following nutrients: the CHO-PRO drink contained 112.0 g of dextrose and maltodextrin, and 40.7 g of protein consisting of milk and whey protein isolate mixture. The CHO drink contained 152.7 g of CHO in the same ratio of dextrose and maltodextrin.

Sixty min after the initial dosage, another serving of the same supplement was administered. To avoid any error in lactose contribution to caloric count, all supplements were ingested with water. The volume of each dosage totaled 600 mL. Two subjects had difficulty in ingesting the second dosage on both treatment days. Therefore a sample of 31.0 mL of the mixed drink was eliminated for these two subjects. The final test during the treatment session was a running performance test.

#### **Blood collection and analysis**

During the recovery period after the depletion run, blood was drawn via venipuncture, at the following two time periods: immediately after the depletion run (0 min), prior to receiving the first drink, and at the 90 min mark which was 30 min after the second ingestion of the drink. These time periods were chosen from the findings of Zawadzki et al. (7) who demonstrated that with a similar supplement as the product in the current, insulin peaked at roughly 30 min after the second dosage.

Blood samples of 8.3 mL were collected, left at room temp for 5 min, and then refrigerated for 30 min before centrifugation at 1500 rev/min for 10 min. The serum was removed and transferred to separate tubes and frozen at  $-20^{\circ}\text{C}$  for later analysis of insulin and glucose. Serum insulin assays were performed using an automated enzyme immunoassay method (Boehringer Mannheim ES300, Roche/Boehringer-Mannheim, Indianapolis, IN). Intra- and inter-assay coefficients of variation were 4.8 and 5.8 % respectively at 10 ng/dL, and 4.4 % at 60 ng/dL. Serum glucose was measured using a standard (glucose oxidase) automated laboratory method (Hitach 911, Roche/Boehringer-Mannheim, Indianapolis, IN).

#### **Running performance test (post-treatment)**

The purpose of the running test was to determine whether the CHO- PRO drink allowed subjects to run longer at the  $\text{VO}_2$  corresponding to 10 % above the IAT. The work rate at the IAT is suggested to be a good indicator of the racing potential for a distance runner (14).

A warm-up period of 5 minutes was allowed, as was the case with the  $\text{VO}_2$ peak test, after which the treadmill speed and grade were increased to bring the oxygen consumption values into the desired range. The timing of the performance run clock started when the  $\text{VO}_2$  corresponding to 10 % above the IAT was reached. Apart from the 5 min warm-up, 2 min were allowed for this process to occur. A range of  $\pm 3$  % was allowed due to difficulty in maintaining steady state oxygen consumption. The criterion for termination of the current test was the duration (min) when subjects voluntarily stopped. Verbal encouragement was given of equal measure for all subjects under both treatment sessions. The test was then terminated and a cool-down period was initiated for several minutes.

#### **Statistical Analysis**

To determine if significant differences existed between group means across time at 0, and 90 min post exercise for the two blood values (insulin, and glucose), two  $2 \times 2$  (drinks vs. time) factorial repeated measures ANOVA's were computed. Any significant interaction was then followed by a dependent t-test to show where the difference was located. To determine if any significant mean difference existed between the run time to exhaustion trials, a paired t-test was computed. All data were analyzed using the SPSS Statistical Package for the Social Sciences (15). The alpha level was 0.05, and data are presented as Mean $\pm$ SD.

## **RESULTS**

Ten endurance trained subjects completed this study. Their physical characteristics are presented in Table 1. The subjects who participated in this study were young (age,  $27 \pm 2.5$  years), aerobically trained ( $\text{VO}_2$ peak,  $59 \pm 5.5$  ml/kg/min) and lean (body fat,  $10.2 \pm 4.4$  %). As shown in Table 2, their dietary intake was similar during the 48 hours prior to each testing session.

**Insulin**

A significant interaction between drink and time with respect to serum insulin levels was found ( $p=0.007$ ). Using a repeated measures t-test, no significant difference was found ( $p=0.955$ ) in insulin values at baseline. However, at 90 minutes, the insulin levels were higher in the CHO-PRO than CHO trial ( $p=0.015$ ) (Figure 1).

**Table 1: Physical Characteristics of Subjects.**

Parameter	Mean±SD	Range
Age (yrs)	27.4±2.5	24-34.0
Height (cm)	172.3±5.2	168-180.3
Weight (kg)	72.8±3.5	66-85.5
Body fat (%)	10.2±4.4	8.4-14.8
VO <sub>2peak</sub> (mL/kg/min)	59.4±5.5	51-66.5
IAT (% VO <sub>2peak</sub> )	78.1±6.7	73-83.2
Max HR at IAT (%)	87.7±3.3	83-91.2

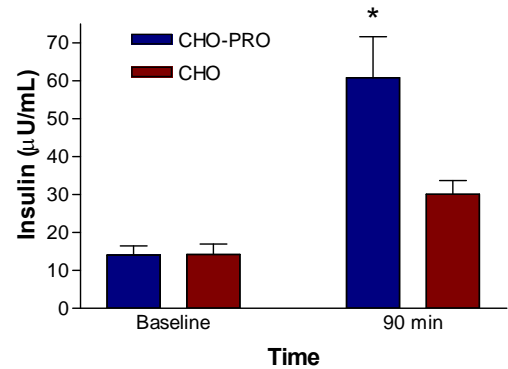


Figure 1: Serum insulin levels at baseline (min 0) and 90 min into recovery. \*= $p<0.05$  from CHO.

**Table 2: Percent of daily caloric intake for energy yielding nutrients during the 24 and 48 hours preceding the treatment sessions of both trials (Mean±SD).**

Nutrient	Time	CHO-PRO	CHO
FAT	48 hrs	26.7±1.3	26.6±1.1
	24 hrs	30.5±1.7	28.5±1.9
CHO	48 hrs	41.2±1.2	40.2±1.8
	24 hrs	34.3±1.1	35.6±1.0
PRO	48 hrs	32.1±1.1	33.2±1.9
	24 hrs	35.2±1.5	35.9±1.5
TOTAL Kcals	48 hrs	2994.3±33.8	2945.2±29.8
	24 hrs	2745.4±20.6	2755.0±27.0

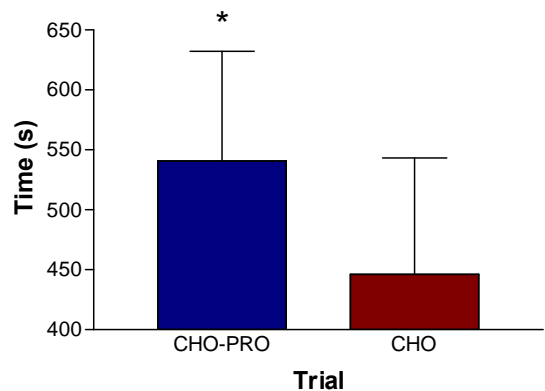


Figure 2: Mean run times to exhaustion during the CHO and CHO-PRO trials. \*= $p<0.05$  from CHO.

**Glucose**

Mean blood glucose concentrations across the two time periods (0 and 90 min) were 95.1±17.3 and 114.9±21.95 mg/dL for CHO, and 89.6±15.2 and 98.8±39.2 mg/dL for CHO-PRO, respectively. These values were not different between trials ( $p=0.152$ ). Furthermore, there was neither a time effect or a drink x time interaction.

**Table 3: Supplement dose comparisons between studies**

Study	CHO*	CHO:PRO:FAT*	↑ Insulin
Tarnopolsky et al. (1997)	1	0.75:0.1:0.02	NO
Roy & Tarnopolsky (1998)	1	0.66:0.2:0.05	NO
Niles et al.	2.09	1.54: 0.56	Yes
Zawadski et al. (1992)	1.53	1.53 :0.56	Yes

\* data are g/kg.

### **Running performance test**

The run time to exhaustion was longer during the CHO-PRO than CHO trial (Figure 2), and represented a 21.2% increase in time to exhaustion.

## **DISCUSSION**

The current study was designed to compare the effects of a CHO-PRO drink versus an isocaloric drink containing only CHO on blood parameters and endurance performance during the recovery period following CHO depleting exercise. We found that the administration of a CHO-PRO drink resulted in higher insulin values and a longer running time to exhaustion compared with a trial in which the subjects consumed an isocaloric CHO beverage. Under both conditions, plasma glucose levels were well maintained and did not differ between treatments though there was a trend towards a lower value in the CHO-PRO trial.

The addition of the PRO to the CHO appears to have enhanced the release of insulin. Insulin is known to facilitate the uptake of glucose into skeletal muscle via the translocation of GLUT-4 transporters from intracellular sites to the sarcolemma of muscle cells (16,8). Insulin may also have activated glycogen synthase and thereby further increased the rate of glycogen resynthesis (7) above which could be explained by the glucose provision alone or the prior exercise-induced muscle glycogen depletion (17).

Recently, there have been inconsistent reports in the literature regarding how the ingestion of PRO can increase the insulin response to a carbohydrate load, and also increase the rate of post-exercise glycogen synthesis (7,8,18). Our findings are in agreement with those of Zawadzki et al.(7), yet differ to others (8,18). These differences may be attributed to the dosage and composition of the supplement used in the different studies.

The studies in which there was no enhanced insulin response when PRO was added to a CHO based supplement used a lower relative dose of both PRO and CHO (Table 3). The dosage used in this study was very similar to the one used by Zawadzki et al. (7). However, in this study the total energy intake was similar between the two treatments. The findings of Zawadzki et al. (7) have been questioned since the CHO-PRO supplement contained 43% more calories than the CHO only supplement (8). Nevertheless, when this criticism was addressed, as was the case in this study, the addition of PRO still enhanced the release of insulin.

The amount of PRO used in this study (40.7 g) has previously been shown to lead to the release of insulin whether it is combined with CHO or if administered separately (6). Some researchers have found that the minimum threshold for PRO ingestion to effectively enhance insulin release is 8 g (19). Therefore, some studies in which there was no enhanced insulin release with the addition of the PRO, may have used a dose that was below the threshold required to elicit insulin release. For example, based upon the body weights of the subjects reported in the study conducted by Tarnopolsky et al. (8), the average PRO consumption for males was 7.3 g and 6.1 g for the females. However, the 8 g threshold for PRO does not totally explain the discrepancies between the studies since another research group gave their subjects approximately 20 g of protein with 57 g of CHO and did not observe an enhanced insulin release (18).

Another possible explanation for the differences between research on this topic relates to the addition of a small amount of fat to the CHO-PRO mixture. Fat is known to inhibit gastric emptying and lead to a reduction in plasma glucose levels (17). However, there is no evidence that the co-ingestion of fat with CHO blunts the release of insulin (17). Therefore, the presence of fat in the CHO-PRO mixture does not explain the differences between our findings and those of others who have not seen the enhanced insulin release with the addition of PRO.

Having discussed a number of possible reasons for the contrasting findings in the studies cited, the major cause for these differences may relate to the magnitude of the dose used. As can be seen in Table 3, those studies

which have observed higher insulin levels with the addition of PRO to a CHO supplement used substantially higher levels of both PRO and CHO. In the present study, peak serum insulin levels of 60.84  $\mu\text{U}/\text{mL}$  were found 30 min after the second dosage of the CHO-PRO drink. As has been previously stated, this higher insulin concentration probably enhanced glycogen synthase activity. Although the intramuscular measures of glycogen were not directly assessed, others have shown an increased rate of glycogen resynthesis following the administration of a similar beverage, under similar conditions (7). Therefore, we believe that following the administration of the CHO-PRO drink, the intramuscular concentration of glycogen may have been higher than when the beverage that only contained CHO was ingested.

Exercise time to exhaustion is known to be directly related to intramuscular glycogen content (1,2). After the CHO-PRO drink was ingested a significantly longer run time to exhaustion (21%) was observed compared to after the CHO drink. The intensity used for the performance run was set at a  $\text{VO}_2$  level of  $10 \pm 3.0\%$  above the IAT. Within this intensity range, intramuscular glycogen is thought to be the primary fuel for muscular activity (20). Therefore, since blood glucose was similar between trials and the run time to exhaustion was longer following the CHO-PRO trial, the assumption may be made that the greater hyperinsulinemia of the CHO-PRO trial stimulated a more rapid post exercise muscle glycogen synthesis and a larger muscle glycogen store prior to the performance run.

In conclusion, the recovery process of muscle glycogen seems to be accelerated when a drink which contains adequate amounts of both CHO and PRO is consumed compared to an isocaloric drink which only contains CHO. This nutritional strategy may be critical for athletes who need to engage in multiple events or training sessions during the course of a day. Further studies are needed to determine the threshold (i.e., minimum) combined dose of PRO and CHO required to optimize post-exercise muscle glycogen synthesis.

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