

THE EFFECT OF A CARBOHYDRATE AND PROTEIN SUPPLEMENT ON RESISTANCE EXERCISE PERFORMANCE, HORMONAL RESPONSE, AND MUSCLE DAMAGE


JACOB J. BATY, HYONSON HWANG, ZHENGPING DING, JEFFREY R. BERNARD, BEI WANG, BONGAN KWON, AND JOHN L. IVY

Exercise Physiology and Metabolism Laboratory, Department of Kinesiology and Health Education, University of Texas, Austin, Texas 78712.

ABSTRACT. Baty, J.J., H. Hwang, Z. Ding, J.R. Bernard, B. Wang, B. Kwon, and J.L. Ivy. The effect of a carbohydrate and protein supplement on resistance exercise performance, hormonal response, and muscle damage. *J. Strength Cond. Res.* 21(2): 321–329. 2007.—The purpose of this study was to determine whether resistance exercise performance and postexercise muscle damage were altered when consuming a carbohydrate and protein beverage (CHO-PRO; 6.2% and 1.5% concentrations). Thirty-four male subjects (age: 21.5 ± 1.7 years; height: 177.3 ± 1.1 cm; weight: 77.2 ± 2.2 kg) completed 3 sets of 8 repetitions at their 8 repetition maximum to volitional fatigue. The exercise order consisted of the high pull, leg curl, standing overhead press, leg extension, lat pull-down, leg press, and bench press. In a double-blind, posttest-only control group design, subjects consumed 355 ml of either CHO-PRO or placebo (electrolyte and artificial sweetener beverage) 30 minutes prior to exercise, 177 ml immediately prior to exercise, 177 ml halfway through the exercise bout, and 355 ml immediately following the exercise bout. There were no significant differences between groups relative to exercise performance. Cortisol was significantly elevated in the placebo group compared to the CHO-PRO group at 24 hours postexercise. Insulin was significantly elevated immediately pre-exercise, after the fourth lift, immediately postexercise, 1 hour, and 6 hours postexercise in CHO-PRO compared to the placebo group. Myoglobin levels in the placebo group approached significance halfway through the exercise bout and at 1 hour postexercise ($p = 0.06$ and 0.07 , respectively) and were significantly elevated at 6 hours postexercise compared to the CHO-PRO group. Creatine kinase levels were significantly elevated in the placebo group at 24 hours postexercise compared to the CHO-PRO group. The CHO-PRO supplement did not improve performance during a resistance exercise bout, but appeared to reduce muscle damage, as evidenced by the responses of both myoglobin and creatine kinase. These results suggest the use of a CHO-PRO supplement during resistance training to reduce muscle damage and soreness.

KEY WORDS. cortisol, creatine kinase, insulin, muscle damage, myoglobin, weightlifting

INTRODUCTION

ome of the cornerstone experiments in endurance sport nutrition have demonstrated improved performance, whether as improved time-to-fatigue or in time trial settings, when consuming a carbohydrate and electrolyte supplement during the exercise bout (6, 12, 27). Recent work, however, suggests that these original nutritional guidelines may need to be adjusted. In the past five years, evidence has accumulated suggesting that endurance per-

formance can be further enhanced by consuming a carbohydrate and protein (CHO-PRO) supplement compared to a carbohydrate-only supplement (14, 28).

When examining previous literature on carbohydrate supplementation during resistance exercise, one finds mixed results, with some studies showing no effect on exercise performance (9) and others showing an improvement in performance (10, 11, 19). Supported by these latter studies in which performance was improved, along with the recent findings with endurance exercise that reported improved performance when subjects consumed a CHO-PRO supplement, we set out to examine whether consuming a CHO-PRO supplement during resistance exercise would improve performance. Specifically, the study examined whether supplementing with CHO-PRO before and during exercise would improve the amount of weight that could be lifted during an intense resistance exercise workout. The subjects performed 3 sets of 8 repetitions at their predetermined 8 repetition maximum (RM). The ability to lift more than 8 repetitions on the third set of any exercise was defined as an improvement in exercise performance.

Nutritional supplementation can result in an anabolic hormonal response to both endurance and resistance exercise (5, 17, 18, 21, 23, 33). Supplementing with carbohydrate and protein has been found to reduce the rise in cortisol and epinephrine (21, 23, 30), while elevating insulin during exercise (35) and insulin and growth hormone postexercise (5, 33). Supplementation has also been found to decrease muscle protein breakdown and stimulate muscle protein synthesis postexercise (2, 25, 26). Moreover, their effect on protein synthesis appears to be additive (20, 22). Based on the anabolic response promoted by CHO-PRO supplementation, it could be predicted that such supplementation would reduce muscle damage during an intense resistance exercise workout. Therefore, a second goal of this study was to examine the effect of CHO-PRO supplementation on muscle damage. As indicators of muscle damage, we selected to evaluate plasma myoglobin and creatine kinase (CK) concentrations.

We found consumption of a CHO-PRO supplement did not result in increased exercise performance during the resistance exercise workout compared to that of a placebo. However, during and postexercise, the subjects who consumed the CHO-PRO supplement did demonstrate significantly reduced muscle damage compared to the subjects who consumed the placebo.

TABLE 1. Physical characteristics of test groups.*

	Placebo subjects	CHO-PRO subjects
Age (yr)	21.3 ± 0.7	21.7 ± 2.8
Height (cm)	177.7 ± 1.4	176.9 ± 1.7
Weight (kg)	80 ± 4.2	74.6 ± 1.8
Lean body mass (kg)	60.5 ± 1.9	59.9 ± 1.5
Body fat (%)	23.0 ± 2.1	19.4 ± 1.8

* Values are mean ± SEM. CHO-PRO = carbohydrate and protein supplement.

METHODS

Experimental Approach to the Problem

To test our hypotheses regarding the effects of a CHO-PRO supplement on a bout of weight lifting, we recruited 36 untrained male subjects to participate in the study. After an adaptation phase of 3 weeks, the subjects were paired according to lean body mass. This was followed by the random assignment of each subject within a pair to either a placebo or CHO-PRO treatment group. The placebo group received an electrolyte and artificial sweetener solution and the CHO-PRO group received a 6.2% carbohydrate-1.5% protein solution (both provided by Pacific Health Laboratories Inc., Woodbridge, NJ). The CHO-PRO supplement was selected because it had previously been found to improve endurance performance and reduce muscle damage during endurance exercise (14, 28). Sixteen subjects received the placebo and 18 subjects received the CHO-PRO drink. Two subjects in the placebo group elected to discontinue the study. On the day of testing, we asked the subjects to complete three sets of each exercise at their 8RM. The first 2 sets consisted of 8 repetitions, and on the third set, the subjects were asked to complete as many repetitions as possible until volitional fatigue. The 2 major dependent variables involved in this study centered on the amount of weight lifted and the blood parameters.

Subjects

Thirty-four male subjects who were either untrained to resistance training or had not participated in resistance training for a minimum of 6 months (age: 21.5 ± 1.7 years; height: 177.3 ± 1.1 cm; weight: 77.2 ± 2.2 kg) were recruited from the local community to participate in this study. The mean physical characteristics of each test group are displayed in Table 1. The study was limited to males to reduce variation in hormonal response to the resistance exercise. All subjects were explained the potential risks and benefits associated with participation in the experiment prior to signing an informed consent, approved by the Institutional Review Board at the University of Texas at Austin.

Procedures

Pre-Screening. Participants reported to the laboratory during the 2 weeks prior to the beginning of the training period to provide background information and health history, to sign consent forms, and to undergo a dual energy X-ray absorptometry (DEXA) body scan to assess body composition and lean body mass (LBM). Subjects were excluded from the study if they were on any medications that might influence their exercise performance. Subjects were also excluded from the study if they admitted to being on any nutritional or pharmaceutical supplements to enhance muscle mass, strength or athletic performance during the previous 6 months.

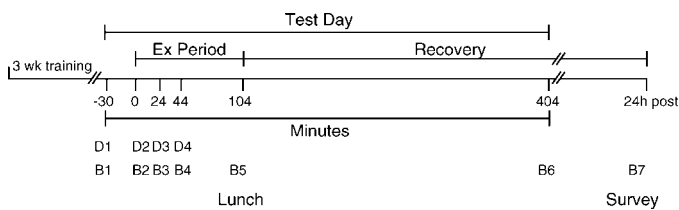


FIGURE 1. Illustration of experimental protocol timeline. D = times drinks (supplement or placebo) were provided; B = times blood samples were drawn.

Adaptation and Learning Phase. For 3 weeks, meeting 3 times per week (Monday, Wednesday, Friday, 6:30 AM), the participants underwent adaptive training sessions. Each session was instructed and supervised, with attention paid to proper lifting mechanics and form and appropriate weight selection. The 7 lifts included high pull, lat pull-down, standing overhead press, leg extension, leg curl, leg press, and bench press. These exercises were selected because they incorporated all the large muscle groups of the limbs and trunk, resulting in a major percentage of the total musculature of the body being stressed. The participants' progress was measured and resistance adjusted via 5RM testing every Friday during the 3 weeks of the adaptation phase.

Diet and Exercise Controls. Following the 3 weeks of adaptation, the participants did not meet the following Monday, to ensure a full and adequate rest and recovery. The subjects were asked to refrain from any resistance work of any kind for the 4 days prior to the testing day. Light aerobic activities were allowed, but complete rest was strongly encouraged, especially on the day prior to testing. Prior to the day of testing, the participants were asked to fast for 12 hours, and the last meal was a standardized meal (Ensure, Abbott Laboratories, Columbus, OH). This was done to minimize the effects on initial blood substrate concentrations and metabolic measurements.

Testing Day (see Figure 1). The testing day occurred on the Wednesday of the fourth week. The subjects arrived and an initial blood sample was drawn (30 minutes pre-exercise). Following this blood draw, the subjects drank 355 ml of their first drink and then rested quietly for 30 minutes. At this time, they received their second blood draw (0 minutes pre-exercise) and consumed 177 ml more of the drink. They then began the lifting session in pairs, in a staggered start, and supervised to insure that every group proceeded through each exercise in a similar sequence. The participants were instructed to lift one warm-up set of 8 repetitions at 80% of their 8RM, then 1 set of 8 repetitions at 100% of their estimated 8RM. For the third set, the resistance was the same as the second set (100% of 8RM), but the subjects were asked to lift as many repetitions until volitional fatigue. Lifts above 8 on the third set were interpreted as an increase in exercise performance. Each pair of participants was given 2 minutes total to complete each set and rest. Typically, each set lasted <20 seconds, followed by a brief time to adjust the partners' resistance, and then the partner completed his lifts in <20 seconds. Rest intervals between sets thus averaged ~100 seconds per individual. Blood was drawn following the fourth exercise (fourth lift) and the participants drank 177 ml of their drink at this point. Immediately postexercise (0 minutes postexercise), a fourth blood draw was taken and 355 ml of drink provided. The participants remained in the weight room,

resting quietly for 1 hour, after which time another blood draw was taken (1 hour postexercise), and a standardized lunch provided. The subjects were asked to refrain from eating until they returned to the laboratory 5 hours later for a sixth blood draw (6 hours postexercise), after which they could eat freely. A seventh blood draw and postexercise survey were taken 24 hours postexercise (24 hours postexercise). All drinks were provided in absolute volumes rather than as a percentage of body weight to keep the research design as practical as possible.

Measurements

Body Composition. Prior to the adaptation phase of the study, body composition was assessed using the Medical Systems Prodigy Model DEXA unit (General Electric, Madison, WI). This unit is a reliable measure of body composition and is valid to within 1–3% body fat compared to underwater weighing. The machine was calibrated using the calibration block provided by the company (General Electric) every morning prior to each subject being measured. The DEXA is a 3 compartment model design for assessing body composition, dividing the body into bone, fat, and fat-free mass. The total region percentage fat was used to assess the subjects' body fat level.

Weight-Lifting Capacity. The weightlifting equipment was produced by a variety of manufacturers. Eagle Fitness Systems by Cybex International (Midway, MA) manufactured the hamstring curls machine, leg extension cable machines, and the leg press sled. The latissimus pull-down machine was manufactured by Samson (Las Cruces, NM). Free weights were used for the bench press, high pull, and shoulder press.

Blood Analysis. Blood samples (5 ml) were placed in test tubes (Fischer Scientific, Pittsburgh, PA) cooled on ice, containing 0.2 ml of ethylenediaminetetraacetic anhydride solution (EDTA, 24mg·ml⁻¹, pH 7.4). From each blood draw, approximately 60 µl was removed to analyze blood glucose values using a Basic One Touch blood glucometer (Life Scan Inc., Milpitas, CA). 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). Calibration of the glucometer was performed with standards provided by Lifescan, Inc. Each EDTA sample was then separated into 3 chilled 12 × 75 ml tubes, and 0.5 ml was placed in tubes containing 1 ml of 10% perchloric acid (PCA). The sample placed in the PCA tube was then agitated to ensure that the red blood cells were completely ruptured. All collection tubes remained on ice for no longer than 1 hour after which they were centrifuged for 10 minutes at 3,000 rpm at 4° C. Following centrifugation, the plasma was transferred to new 12 × 75 tubes and immediately frozen at -180° C for later analysis. Lactate was determined by enzymatic analysis (13). Cortisol and insulin were determined using a competitive ¹²⁵I radioimmunoassay (MP Biomedicals, Costa Mesa, CA) in which the plasma hormone competes with its ¹²⁵I-labeled counterpart for a subsaturating concentration of antibody. The more ¹²⁵I-labeled hormone displaced from the antibody, the greater the plasma concentration. Following an incubation step, antigen-antibody complexes are isolated by centrifugation and radioactivity counted in a gamma counter (Beckman 5500; Beckman Bioanalytical Systems Group, Fullerton, CA). The percent coefficient of variation (%CV) was 4.22 for insulin and 5.33 for cortisol.

Creatine kinase (CK) was determined by enzymatic analysis (Diagnostic Chemicals Limited, Charlottetown, Canada). The conversion of creatine phosphate plus adenosine diphosphate to creatine plus adenosine triphosphate by creatine kinase is linked to several enzyme reactions to produce nicotinamide adenine diphosphopyridine nucleotide-reduced form (NADPH). The rate of NADPH is a measure of creatine kinase activity. The rate of NADPH was measured on a Beckman DU640 spectrophotometer (Beckman Bioanalytical Systems Group) at 37° C. The %CV for CK was 2.3. Myoglobin was determined by ELISA (MP Biomedicals, Orangeburg, NY). The test is based on the principle of a solid phase enzyme-linked immunosorbent assay. Mouse monoclonal antimyoglobin antibody is used for solid phase immobilization. Once the myoglobin is immobilized, it is exposed to a second antimyoglobin antibody (goat) with horseradish peroxidase attached. After the myoglobin has reacted with both antibodies, free enzyme-linked antibody is removed by washing and the bound myoglobin is exposed to tetramethyl-benzidine. This reacts with the horseradish peroxidase to form a blue color. The addition of a stop-solution changes the color to yellow. The concentration of the myoglobin is directly proportional to the color intensity. Absorbance is measured spectrophotometrically at 450 nm. All assays were measured in duplicate except creatine kinase, which was measured in triplicate.

Survey

At 24 hours postexercise, the subjects were asked to complete a survey questionnaire that was produced internally by the investigators. The survey was designed to discern some psychological aspects of the study, such as whether the subjects perceived a performance-enhancement from the supplement and whether they had an accurate impression about what supplement they received, as well as some physiological aspects such as the degree of fatigue at the end of the weight-lifting session and the extent of muscle soreness at the time of filling out the survey. For most questions, the answers were either nominal or ordinal and ranged from 1 to 5.

Statistical Analyses

The study was a double-blind, posttest-only control group design, to test for main and interaction effects (treatment × time). The experimental design was a between-within mixed model design (2 × 7), in which the between-subjects factor was the drink (CHO-PRO or placebo) and the within-subjects factors was timing (blood draws 1–7). Across the time points, the 2 groups differed in the type of drink provided. Statistical significance was set at $p \leq 0.05$. All results are presented as means ± standard error of the mean (*SEM*).

RESULTS

To assess the effect of CHO-PRO supplementation on exercise performance, we provided subjects with a CHO-PRO supplement or placebo before and during exercise on the testing day and asked them to lift to volitional fatigue on the third set of 3 sets, each performed at the subjects' 8RM. We found that there were no differences in performance between the groups throughout the exercise bout. Exercise performance was similar whether expressed as total weight lifted per exercise (kg) or weight lifted scaled per LBM multiplied by the number of repetitions completed (kg lifted·kg⁻¹ LBM × repetitions) (Table 2).

Results for blood glucose indicated a significant group

TABLE 2. Weight lifted for each exercise during the third set for each treatment group.*

	Exercises							Total
	High pull	Leg curl	Shoulder press	Leg extension	Lat pull-down	Leg press	Bench press	
Weight lifted (kg)								
CHO-PRO	58.8 ± 2.4	48.7 ± 2.0	39.3 ± 2.0	62.0 ± 2.4	65.6 ± 2.1	198.9 ± 9.1	61.0 ± 3.3	534.4 ± 18.9
Placebo	62.5 ± 2.0	46.3 ± 2.4	39.9 ± 2.2	58.7 ± 2.1	67.5 ± 1.7	217.2 ± 13.1	63.5 ± 3.5	555.5 ± 21.8
Weight lifted·kg ⁻¹ LBM × Repetitions								
CHO-PRO	9.2 ± 0.7	6.7 ± 0.4	6.2 ± 0.5	11.6 ± 0.4	11.0 ± 0.6	40.1 ± 0.7	8.4 ± 1.0	93.2 ± 4.0
Placebo	9.8 ± 0.9	7.1 ± 0.6	6.5 ± 0.7	11.0 ± 0.5	10.4 ± 0.6	40.5 ± 0.5	7.1 ± 0.8	92.3 ± 5.5

* Values are mean ± SEM. Weight lifted·kg⁻¹ LBM × repetitions is the weight lifted divided by body mass multiplied by the number of repetitions performed during the third set of an exercise. CHO-PRO = carbohydrate and protein supplement.

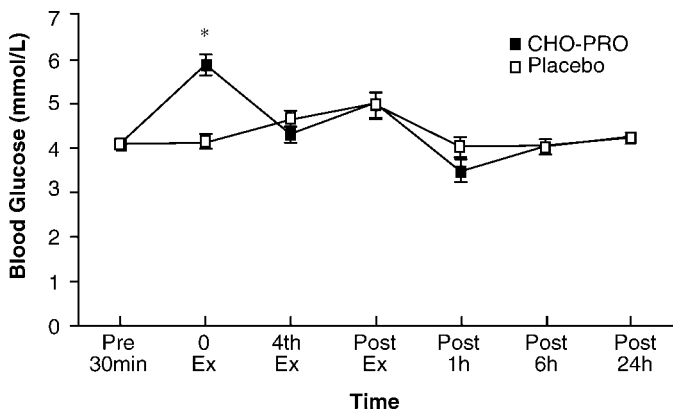


FIGURE 2. Blood glucose levels 30 minutes before exercise (Pre 30min), immediately before exercise (0 Ex), after completion of the fourth exercise (4th Ex), immediately post exercise (Post Ex), and at times postexercise as indicated for subjects receiving a placebo or carbohydrate and protein (CHO-PRO) supplement. Group means ± SEM are presented at each time point. * Represents significant difference ($p \leq 0.05$) between treatment groups at the corresponding time point.

× time interaction and a significant main effect for time. However, there was not a significant main effect for group $F(1, 30) = 0.85, p > 0.10$. Post-hoc comparisons revealed that statistical differences in glucose between treatments were found only immediately pre-exercise, with the CHO-PRO treatment significantly elevated compared to the placebo group (Figure 2). In both groups the blood glucose level rose slightly during exercise, and fell to below baseline levels by 1 hour postexercise. The blood glucose levels returned to baseline levels by 6 hours postexercise.

For the plasma insulin response, a significant group × time interaction, and significant main effects for time and for group were found. Follow-up post-hoc comparisons revealed that insulin levels in the placebo group remained at baseline throughout the study. Insulin levels in the CHO-PRO group were elevated throughout the study, with the exception of 30 minutes pre-exercise (Figure 3). The peak insulin levels in the CHO-PRO group occurred immediately pre-exercise at a mean value of $50.55 \pm 5.58 \mu\text{U}\cdot\text{ml}^{-1}$. These elevated insulin levels in the CHO-PRO group were statistically different immediately pre-exercise, after the fourth lift, immediately postexercise, 1 hour postexercise, and at 6 hours postexercise compared to the placebo group.

There were no differences in blood lactate between the groups throughout the testing session as determined by follow-up post-hoc comparisons (Figure 4). Lactate levels rose sharply during exercise and immediately postexercise

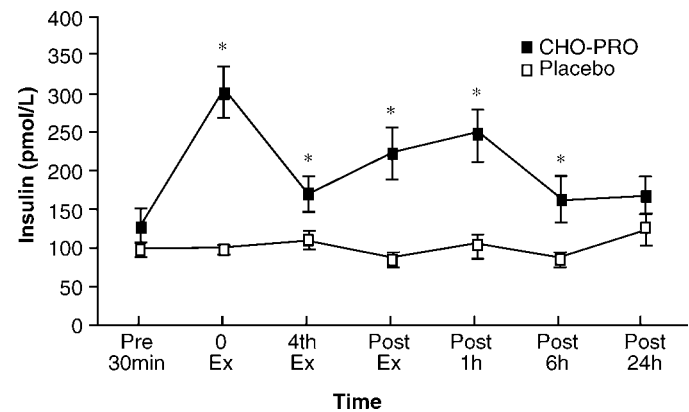


FIGURE 3. Plasma insulin levels for subjects receiving a placebo or carbohydrate and protein (CHO-PRO) supplement at the time points indicated for Figure 1. Group means ± SEM are presented at each time point. * Represents significant difference ($p \leq 0.05$) between treatment groups at the corresponding time point.

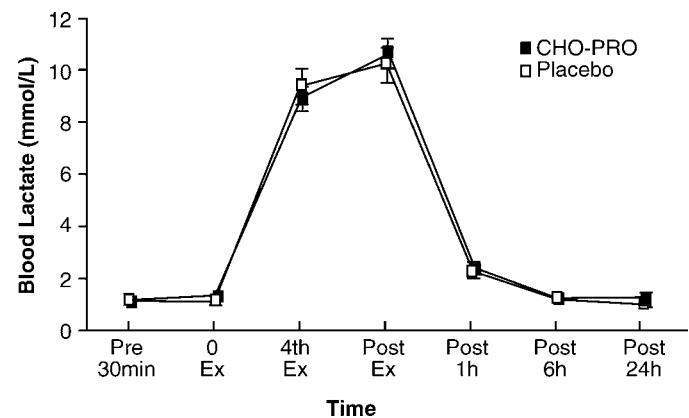


FIGURE 4. Blood lactate levels for subjects receiving a placebo or carbohydrate and protein (CHO-PRO) supplement at the time points indicated for figure 1. Group means ± SEM are presented at each time point.

cise averaged $10.18 \pm 0.69 \text{ mMol}\cdot\text{L}^{-1}$ for the placebo group and $10.63 \pm 0.58 \text{ mMol}\cdot\text{L}^{-1}$ for the CHO-PRO group. Lactate then returned to baseline by one hour post-exercise.

While plasma cortisol levels were elevated in both groups throughout the exercise bout, there were no significant differences found between the groups during this time (Figure 5). Cortisol levels then returned to baseline by 6 hours postexercise in both groups. However, cortisol

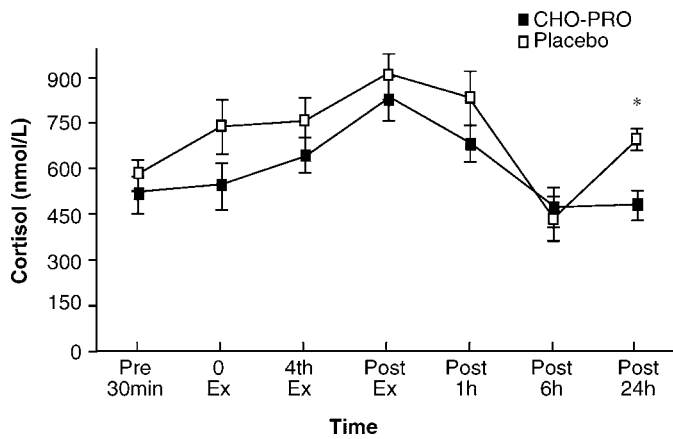


FIGURE 5. Plasma cortisol levels for subjects receiving a placebo or carbohydrate and protein (CHO-PRO) supplement at the time points indicated for figure 1. Group means \pm SEM are presented at each time point. * Represents significant difference ($p \leq 0.01$) between treatment groups at the corresponding time point.

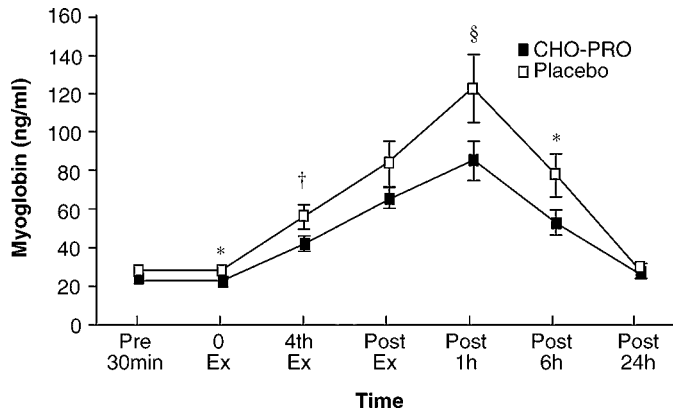


FIGURE 6. Plasma myoglobin levels for subjects receiving a placebo or carbohydrate and protein (CHO-PRO) supplement at the time points indicated for figure 1. Group means \pm SEM are presented at each time point. * Represents significant difference ($p \leq 0.05$) between treatment groups at the corresponding time point. † $p < 0.06$. § $p < 0.07$.

levels rose again at 24 hours postexercise, resulting in significantly higher blood cortisol levels in the placebo group.

For the plasma myoglobin response, a significant group \times time interaction, a significant main effect for time, and a significant main effect for group were found. There was a significant treatment effect present in the myoglobin response, with the placebo group elevated above the CHO-PRO group. Pre-exercise myoglobin levels were slightly elevated in the placebo group, with significant differences between the placebo and CHO-PRO groups occurring immediately pre-exercise (Figure 6). At the halfway mark of the exercise bout, the placebo group was elevated above the CHO-PRO group ($p = 0.06$). One hour postexercise myoglobin levels were 25% higher in the placebo group compared to the CHO-PRO group ($p = 0.07$). The placebo group had significantly elevated myoglobin levels compared to the CHO-PRO group at 6 hours postexercise ($p < 0.05$), but by 24 hours postexercise, the myoglobin levels were no longer significantly different and were back to baseline values.

For both groups, the general trend was for CK to rise

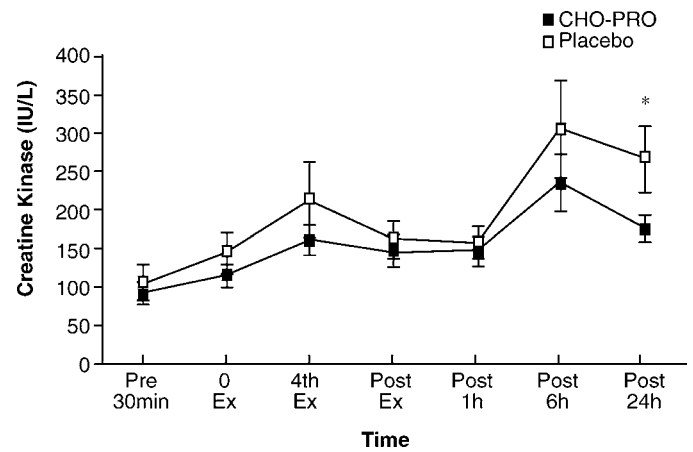


FIGURE 7. Plasma creatine kinase levels for subjects receiving a placebo or carbohydrate and protein (CHO-PRO) supplement at the time points indicated for figure 1. Group means \pm SEM are presented at each time point. * Represents significant difference ($p \leq 0.05$) between treatment groups at the corresponding time point.

from 30 minutes pre-exercise through 6 hours postexercise, after which the levels fell slightly (Figure 7). There was a dramatic increase in CK levels at 6 hours postexercise in both groups. While CK levels in the placebo group were elevated for the length of the study above those in the CHO-PRO group, these differences reached significance only at 24 hours postexercise.

Survey Data

The survey data showed significant differences between groups to one of the questions, and there was evidence of trends in other questions (Table 3). When asked, “Did the drink affect your performance?” there were significant differences in how the groups replied. A 1 value was coded, “Improved Performance”, a 2 was, “No Change to Performance”, and a 3 was, “Decreased Performance”. Significantly more subjects ($n = 10$) in the CHO-PRO group thought that the drink improved their performance compared to placebo subjects ($n = 3$). When asked, “How did you feel upon completing the workout?” with a 1 value being “Strong” and a 6 value being “Very Weak, Sick, Nauseous”, the CHO-PRO group tended to report being less tired immediately postexercise. Subjects in the CHO-PRO group answered on average 2.9, compared to 3.7 for the placebo group. The last 3 questions were asked only to those subjects who participated in the second session of the study. When asked, “Do you presently feel sore, 24 hours after exercise?”, 90% of the CHO-PRO subjects reported being less than or moderately sore, while only 56% of the subjects fed the placebo reported being less than or moderately sore. To the question regarding which drink they thought they received, 3 of the placebo subjects thought that they had received the placebo, 5 subjects were unsure, and 1 thought that he had received the CHO-PRO supplement. Six of the subjects within the CHO-PRO supplement group believed that they had received the CHO-PRO supplement, 3 reported that they were unsure what they received, and 1 reported that he thought he had received the placebo. When asked how certain they were of their answer to which treatment they received, both groups averaged 3.2, where a 3 was coded “Moderately Certain” and a 4 was coded “Unsure”.

TABLE 3. Responses to 24-hour postexercise survey regarding feelings upon completion of the workout.

	Placebo		CHO-PRO	
	Number of scores	Percentage	Number of scores	Percentage
How did you feel upon completing the workout?				
1 (Strong)	1	6.25%	1	5.88%
2 (Strong, but tired)	4	25.00%	8	47.06%
3 (Tired)	5	31.25%	4	23.53%
4 (Weak)	0	0.00%	1	5.88%
5 (Very weak)	1	6.25%	1	5.88%
6 (Very weak, sick, nauseous)	5	31.25%	2	11.76%
Do you presently feel sore, 24 hours after exercise?				
1 (Not sore at all; fresh)	1	11.11%	0	0.00%
2 (Slightly sore; fairly fresh)	1	11.11%	4	40.00%
3 (Moderately sore)	3	33.33%	5	50.00%
4 (Sore)	4	44.44%	0	0.00%
5 (Very sore; ouch)	0	0.00%	1	10.00%
Did the drink affect your performance?				
1 (Improved performance)	3	18.75%	10	58.82%
2 (No change to performance)	13	81.25%	5	29.41%
3 (Decreased performance)	0	0.00%	2	11.76%

* N = 33 for the first and third questions and n = 19 for the second question. $p < 0.05$ between two treatments. CHO-PRO = carbohydrate and protein supplement.

DISCUSSION

The purpose of this study was to examine the effects of consuming a CHO-PRO supplement compared to placebo prior to, during, and following an acute bout of resistance training on exercise performance and muscle damage. By measuring both hormonal and muscle damage indices postexercise, we hoped to elucidate some of the potential mechanisms for differences seen in the muscle damage responses. We found that a CHO-PRO supplement provided prior to, during, and following an acute bout of resistance training had no effect on exercise performance, but did appear to significantly reduce muscle damage compared to consumption of a placebo at those same times.

In all formats that exercise performance was expressed, whether as total weight lifted or total weight lifted scaled per kg LBM times the number of repetitions, the performances between the groups were remarkably similar. To help explain why no benefit to exercise performance was seen with supplementation, we relied on the review article by Haff et al. (10). They analyzed the confounding results from all of the studies involving CHO-only supplementation and work. There were two factors that Haff et al. (10) highlighted as necessary for a CHO-only supplement to increase exercise performance: (a) the work bout had to exceed 50 minutes in duration, and (b) the exercises had to focus on one main group of muscles, in order to sufficiently deplete muscle glycogen stores. While the exercise bout in our study did last longer than 50 minutes (subjects completed the sequence of lifts in ~60 minutes), the exercises selected in our study incorporated the entire body, not relying solely on one muscle group. While this protocol may not have limited glycogen depletion in selected fiber types (31), it would limit the amount of time each muscle group was used during exercise and thereby limit the influence of substrate availability on performance.

While selected muscle fiber type glycogen depletion could have limited performance, we believe fatigue was most likely due to the built up of local metabolites such as lactic acid and the depletion of high-energy phosphates. Supporting evidence for this assertion comes from

the peak lactate values, which were achieved immediately post-exercise. For the CHO-PRO group, peak lactate values were 10.63 ± 0.58 mmol·L⁻¹, and for the placebo group the peak lactate response was 10.18 ± 0.69 mmol·L⁻¹. Given the design of our study, with the rather short rest periods of ~1.5 minutes between sets, it is highly likely that local lactate concentrations rose with each sequential set within an exercise. By the third set of each exercise, the concentration of lactic acid and possibly depletion of creatine phosphate stores within muscle would have prevented the subjects from completing more repetitions.

As exercise performance was not different between groups, any difference found in muscle damage between groups would likely be due to an intervention effect and would not have been confounded by increased work in one group. The major finding of our study was that muscle damage as indicated by muscle enzyme release was significantly reduced postexercise when a CHO-PRO supplement was consumed prior to, during, and immediately postexercise compared to placebo. Subjects who received the CHO-PRO supplement as opposed to the placebo, reported less muscle soreness, also suggesting reduced muscle damage. We used the 24-hour postexercise survey to assess muscle soreness in the subjects. Muscle soreness is known to typically reach its peak between 24 and 48 hours postexercise. While there were no significant differences between the groups, 90% of the CHO-PRO subjects reported being less than or moderately sore, while within the placebo group, only 56% of the subjects reported being less than or moderately sore, and the remaining 44% reported being sore or very sore. Again, while our survey results did not reach significance, they were similar to the results seen by Flakoll et al. (7) in that supplementing with CHO-PRO reduces reported muscle soreness compared to placebo.

To sufficiently quantify muscle damage we used both plasma myoglobin and creatine kinase (CK) levels as indices of muscle damage, due to their different time courses in muscle release. Myoglobin, with a smaller molecular mass of 17 kDa, is both released quicker from damaged muscle and disappears more readily (through renal ex-

cretion) than creatine kinase. As it is smaller, myoglobin has a more direct route into the microvascular endothelium. Previous studies have reported that myoglobin can appear in the blood as early as 1 hour posteccentric exercise (24, 29).

Myoglobin was significantly elevated in the placebo group immediately prior to exercise, and while it was statistically significant, it was not physiologically significant, and this initial discrepancy did not influence later differences observed in the study. As myoglobin is a faster appearing index of muscle damage, it was not surprising that elevations approaching significance in myoglobin ($p = 0.06$ and 0.07 , respectively) were observed half-way through the exercise bout (fourth exercise) and at 1 hour postexercise in the subjects receiving placebo compared to those receiving the CHO-PRO. Significance was reached at 6 hours postexercise, with the placebo subjects having elevated myoglobin over the subjects receiving the CHO-PRO, but this difference was eliminated by 24 hours postexercise. These differences carried over to an overall significant treatment effect in the myoglobin response to exercise, with those subjects receiving the placebo having elevated myoglobin compared to those subjects receiving the CHO-PRO supplement.

Comparatively, creatine kinase has difficulty entering the microvascular endothelium directly because of its larger molecular mass of 80 kDa. Instead, creatine kinase is thought to be picked up by the lymphatic system before entering the bloodstream. Due to this longer route, creatine kinase has a longer latency period before appearing in the bloodstream, typically 24–48 hours posteccentric exercise, with peak levels occurring 96–120 hours posteccentric exercise (24, 29).

As creatine kinase is much slower to appear in the bloodstream, differences between the groups were not expected before the final blood draw at 24 hours postexercise. At this time, the CK levels were 151% higher in the placebo-fed subjects than in the CHO-PRO-fed subjects ($p < 0.05$).

Elevated CK levels 24 hours postexercise have been postulated to be caused by elevated cortisol (16, 18). In a study by Kraemer et al. (16), which compared resistance loads and rest intervals in 6 different resistance exercise protocols, they were able to find a correlation of 0.84 between the peak cortisol response 5 minutes postexercise and the peak CK concentration 24 hours postexercise. A similar relationship was found in a second study (18), where they had subjects perform 4 sets of 10 repetitions at the subjects' 10RM of both upper and lower body exercises. This protocol was repeated over 3 consecutive days; the largest cortisol response occurred postexercise on the first day, and the largest CK response was seen immediately postexercise on the second day. Cortisol is a strong catabolic hormone, and Kraemer et al. (16, 18) postulated that it was the catabolic effects of cortisol, which caused the large muscle damage, resulting in high CK in the bloodstream the following day.

Similar to the two studies by Kraemer et al. (16, 18), cortisol levels were elevated at 0 and 1 hour postexercise, as well as during exercise, in our study. While the levels were above baseline at these time points, the elevation was not significantly different between the two treatment groups. The only time that cortisol was significantly different between treatment groups was at 24 hours postexercise, when the placebo group was significantly elevated over the CHO-PRO group. The creatine kinase response mirrored the cortisol response; there were no sig-

nificant differences in CK levels between the groups until 24 hours postexercise, when the placebo group had significantly elevated CK levels compared to the CHO-PRO group. With no significant differences between groups immediately postexercise and significant elevations in CK at 24 hours postexercise, it is difficult to argue that the potentially greater muscle damage in the placebo group was due to the catabolic effects of cortisol in our study. We believe that the significant elevation in cortisol at 24 hours postexercise in the placebo group, which was accompanied with a significant elevation in CK at the same time point, was in response to the elevated muscle damage and not its cause. The finding that myoglobin was elevated during placebo when there were no differences in cortisol would also suggest cortisol was not responsible for muscle damage. Most likely, muscle damage was due to mechanical stress, resulting in the tearing of individual muscle fibers and increased muscle degradation.

However, significant differences between treatment groups for insulin were seen during and following exercise. Insulin was significantly elevated immediately preexercise, after the fourth lift, and 0, 1, and 6 hours postexercise in the CHO-PRO group compared to the placebo group. We would argue that it is potentially the anabolic effects of insulin that are responsible for the differences in muscle damage seen between the 2 groups postexercise.

Research indicates that insulin acts both through increasing rates of protein synthesis and by decreasing rates of protein breakdown to reduce muscle damage following resistance exercise (1, 3, 26). Biolo et al. (3) have shown that insulin is strongly anabolic postexercise and can reduce muscle protein breakdown. Prior to exercise, the researchers started infusion of several tracer amino acids, and then had the subjects complete a bout of resistance exercise. Following the exercise bout, they infused insulin for 3 hours. Insulin infusion significantly reduced protein breakdown. Separate findings by Gelfand et al. (8) regarding the action of insulin support these findings. They infused insulin for 2 hours in resting subjects and found a significant reduction in the rate of protein breakdown.

By reducing the amount of protein degradation resulting from resistance exercise and limiting muscle damage, it would be possible to limit the amount of efflux out of the muscle of both myoglobin and CK. If insulin also increased the rate of protein synthesis, this could enhance the rate of tissue repair and limit the amount of CK leakage, but this process would probably be too slow acting to affect the response of myoglobin, given its small size and quick time response compared to CK.

It is also possible to increase skeletal muscle protein synthesis independent of increasing plasma insulin by providing appropriate amino acids following exercise. Biolo et al. (2) infused amino acids following a resistance exercise bout and reported a large increase in protein synthesis compared to rest. In a study by Tipton et al. (32) consuming 40g of essential amino acids (EAAs) post-resistance exercise training nonsignificantly increased protein synthesis, but did significantly increase net protein balance compared to a placebo feeding, and Borsheim et al. (4) found that feeding subjects 6 g of EAAs at 1 and 2 hours postresistance exercise significantly increased protein synthesis and net protein balance. Moreover, Miller et al. (22) found significant increases in protein synthesis when subjects were fed $0.087 \text{ g}\cdot\text{kg}^{-1}$ of EAA following a bout of resistance exercise.

Reports of the largest increases in protein synthesis come from studies that incorporate both the elevation of insulin along with provision of amino acids. In the above study by Miller et al. (22), increased synthesis rates when fed amino acids only was not as robust as when supplementing with a mixture of carbohydrate and essential amino acids. In fact, protein synthesis rates were additive for the carbohydrate and amino acids supplement compared with the carbohydrate-only and amino acids-only supplements. Levenhagen et al. (20) and Koopman et al. (15) observed similar results. Following a 60-minute bout of cycling exercise, Levenhagen et al. (20) provided subjects with placebo, a CHO supplement, or a CHO-PRO supplement. Leg protein synthesis rates following CHO-PRO supplementation improved 600% compared with placebo and 400% compared with CHO-only supplementation. Koopman et al. (15) were able to demonstrate a significantly reduced whole body protein breakdown and a significantly increased whole body protein synthesis by feeding subjects a CHO-PRO or CHO-PRO-leucine supplement in comparison to providing a CHO-only supplement following a resistance exercise bout. Furthermore, van Loon et al. (34) found a significant negative correlation between the insulin response and plasma levels of select amino acids after subjects cycled for ~90 minutes of interval-based activity and were fed CHO-only or CHO-PRO supplements postexercise. The greater the insulin response to the feedings, the greater the protein synthesis response, as evidenced from the decrease in plasma amino acids.

The major findings of this study were that providing a CHO-PRO supplement before and during a resistance exercise session does not increase work capacity, but does appear to significantly reduce muscle damage, as evidenced by responses of both myoglobin and creatine kinase. The muscle damage could potentially have been reduced by suppressing the cortisol response to exercise, but probably was more influenced by the CHO-PRO supplement-induced elevation in plasma insulin during exercise and the first 6 hours of recovery. It is possible that an elevation in plasma amino acids could have stimulated protein synthesis and thereby reduced muscle damage as well.

PRACTICAL APPLICATIONS

It is the goal of competitive athletes to train at high-intensity exercise bouts as frequently as possible, to maximize and optimize both the training stress and the adaptation response. By consuming a CHO-PRO supplement similar to the one used in our study at a similar schedule during exercise, athletes can significantly reduce the amount of muscle damage produced in a given resistance exercise bout. By minimizing the amount of muscle damage created, athletes should be able to reduce the length of the recovery phase following exercise, and allow the athlete to participate in the next high-intensity exercise bout in a shorter period of time. Reducing muscle damage, enhancing the recovery process and reducing stress of exercise are also important considerations for individuals just starting an exercise training program or who regularly engage in resistance training. Therefore, a recommendation for CHO-PRO supplementation can be made for novice lifters, and individuals who are actively engaged in a resistance training program. One should, however, be cognizant of the amount of carbohydrate being consumed around their workout and factor this amount into his or her overall dietary plan.

REFERENCES

- BIOLO, G., R.Y.D. FLEMING, AND R.R. WOLFE. Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. *J. Clin. Invest.* 95:811–819. 1995.
- BIOLOG, G., K.D. TIPTON, S. KLEIN, AND R.R. WOLFE. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am. J. Physiol.* 273:E122–E129. 1997.
- BIOLO, G., B.D. WILLIAMS, R.Y.D. FLEMING, AND R.R. WOLFE. Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise. *Diabetes* 48:949–957. 1999.
- BORSHEIM, E., K.D. TIPTON, S.E. WOLF, AND R.R. WOLFE. Essential amino acids and muscle protein recovery from resistance exercise. *Am. J. Physiol.* 283:E648–E657. 2002.
- CHANDLER, R.M., H.K. BYRNE, J.G. PATTERSON, AND J.L. IVY. Dietary supplements affect the anabolic hormones after weight-training exercise. *J. Appl. Physiol.* 76:839–845. 1994.
- COYLE, E.F., A.R. COGGAN, M.K. HEMMERT, AND J.L. IVY. Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrates. *J. Appl. Physiol.* 61:165–172. 1986.
- FLAKOLL, P.J., T. JUDY, K. FLINN, C. CARR, AND S. FLINN. Postexercise protein supplementation improves health and muscle soreness during basic military training in marine recruits. *J. Appl. Physiol.* 96:951–956. 2004.
- GELFAND, R.A., AND E.J. BARRETT. Effect of physiological hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man. *J. Clin. Invest.* 80:1–6. 1987.
- HAFF, G.G., A.J. KOCH, J.A. POTTEIGER, K.E. KUPHAL, L.M. MAGEE, S.B. GREEN, AND J.J. JAKICIC. Carbohydrate supplementation attenuates muscle glycogen loss during acute bouts of resistance exercise. *Int. J. Sport Nutri. Exerc. Metab.* 10:326–339. 2000.
- HAFF, G.G., M.J. LEHMKUHL, L.B. MCCOY, AND M.R. STONE. Carbohydrate Supplementation and Resistance Training. *J. Strength Cond. Res.* 17:187–196. 2003.
- HAFF, G.G., C.A. SCHROEDER, A.J. KOCH, K.R. KUPHAL, M.J. COMEAU, AND J.A. POTTEIGER. The effects of supplemental carbohydrate ingestion on intermittent isokinetic leg exercise. *J. Sports Med. Phys. Fit.* 41:216–222. 2001.
- 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.
- HOHORST, H.J. Determination of L-lactate with LDH and DPN. In: *Methods of Enzymatic Analysis*. H.U. Bergmeyer, ed. New York: Academic, 1965. pp. 266–270.
- IVY, J.L. P.T. RES, R.C. SPRAGUE, AND M.O. WIDZER. Effect of a carbohydrate-protein supplement on endurance performance during exercise of varying intensity. *Int. J. Sports Nutr. Exerc. Metab.* 13:382–395. 2003.
- KOOPMAN, R. A.J.M. WAGENMAKERS, R.J.F. MANDERS, A.H.G. ZORENC, J.M.B. SENDEN, M. GORSELINK, H.A. KEIZER, AND L.J.C. VAN LOON. Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects. *Am. J. Physiol.* 288:E645–E653. 2005.
- KRAEMER, W.J., J.E. DZIADOS, L.J. MARCHITELLI, S.E. GORDON, E.A. HARMAN, R. MELLO, F.J. FLECK, P.N. FRYKMAN, AND N.T. TRIPLETT. Effects of different heavy-resistance exercise protocols on plasma beta-endorphin concentrations. *J. Appl. Physiol.* 74:450–459. 1993.
- KRAEMER, W.J., N.A. RATAMESS, J.S. VOLEK, K. HAKKINEN, M.R. RUBIN, D. N. FRENCH, A.L. GOMEZ, M.R. MCGUIGAN, T.P. SCHEETT, R.U. NEWTON, B.A. SPIERING, M. IZQUIERDO, AND F.S. DIQUARDI. The effects of amino acid supplementation on hormonal responses to resistance training overreaching. *Metabolism* 55:282–291. 2006.
- KRAEMER, W.J., J.S. VOLEK, J.A. BUSH, M. PUTUKIAN, AND W.J. SEBASTIANELLI. Hormonal responses to consecutive days of heavy-resistance exercise with or without nutritional supplementation. *Am. Physiol. Soc.* 85:1544–1555. 1998.
- LAMBER, C.P., M.G. FLYNN, J.B. BOONE, T.J. MICHAUD, AND J. RODRIGUEZ-ZAYAS. Effects of carbohydrate feeding on multiple-bout resistance exercise. *J. Appl. Sports Sci. Res.* 5:192–197. 1991.
- LEVENHAGEN, D.K., C. CARR, M.G. CARLSON, D.J. MARON, M.J. BOREL, AND P.J. FLAKOLL. Postexercise protein intake enhances whole-body and leg protein accretion in humans. *Med. Sci. Sports Exerc.* 34:828–837. 2002.
- LI, T.L., AND M. GLEESON. The effects of carbohydrate supplementation during the second of two prolonged cycling bouts on immunoenocrine responses. *Eur. J. Appl. Physiol.* 95:391–399. 2005.
- MILLER, S.L., K.D. TIPTON, D.L. CHINKES, S.E. WOLF, AND R.R. WOLFE. Independent and Combined Effects of Amino Acids and Glucose after Resistance Exercise. *Med. Sci. Sports Exerc.* 35:449–455. 2003.
- NIEMAN, D.C., J.M. DAVIS, D.A. HENSON, S.I. GROSS, C.L. DUMKE, A.C. UTTER, D.M. VINCI, J.A. CARSON, A. BROWN, S.R. MCANULTY, L.S. MCANULTY, AND N.T. TRIPLETT. Muscle cytokine mRNA changes after 2.5 h of cycling: Influence of carbohydrate. *Med. Sci. Sports Exerc.* 37:1283–1290. 2005.

24. NOSAKA, K. P.M. CLARKSON, AND F.S. APPLE. Time course of serum protein changes after strenuous exercise of the forearm flexors. *J. Lab. Clin. Med.* 119:183–188. 1992.
25. RASMUSSEN, B.B., K.D. TIPTON, S.L. MILLER, S.E. WOLF, R.R. WOLFE. An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. *J. Appl. Phys.* 88:386–392. 2000.
26. ROY, B.D., M.A. TARNOPOLSKY, J.D. MACDOUGALL, J. FOWLES, AND K.E. YARASHESKI. Effect of glucose supplement timing on protein metabolism after resistance training. *J. Appl. Physiol.* 82:1882–1888. 1997.
27. SALTIN, B., AND J. KARLSSON. Muscle glycogen utilization during work of different intensities. In: *Muscle Metabolism During Exercise*. New York: Plenum Press, 1971. p. 289.
28. SAUNDERS, M.J., M.D. KANE, AND M.K. TODD. Effects of a carbohydrate-protein beverage on cycling endurance and muscle damage. *Med. Sci. Sports Exerc.* 36:1233–1238. 2004.
29. SAYERS, S.P., AND P.M. CLARKSON. Short-term immobilization after eccentric exercise. Part II: Creatine kinase and myoglobin. *Med. Sci. Sports Exerc.* 35:762–768. 2003.
30. SCHARHAG, J., T. MEYER, M. AURACHER, H.H. GABRIEL, AND W. KINDERMAN. Effects of graded carbohydrate supplementation on the immune response in cycling. *Med. Sci Sports Exerc.* 38:286–292. 2006.
31. TESCH, P.A., L.L. PLOUTZ-SNYDER, L. YSTRÖM, M.J. CASTRO, AND G.A. DUDLEY. Skeletal muscle glycogen loss evoked by resistance exercise. *J. Strength Cond. Res.* 12:67–73. 1998.
32. TIPTON, K.D., A.A. FERRANDO, S.M. PHILLIPS, D. DOYLE, AND R.R. WOLFE. Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am. J. Physiol.* 276:E628–E634. 1999.
33. THYFAULT, J.P., M.J. CARPER, S.R. RICHMOND, M.W. HULVER, AND J.A. POTTEIGER. Effects of liquid carbohydrate ingestion on markers of anabolism following high-intensity resistance exercise. *J. Strength Cond. Res.* 18:174–179. 2004.
34. VAN LOON, L.J.C., M. KRUIJSHOOP, H. VERHAGEN, W.H.M. SARIS, AND A.J.M. WAGENMAKERS. Ingestion of protein hydrolysate and amino acid-carbohydrate mixtures increases postexercise plasma insulin responses in men. *J. Nutr.* 130:2508–2513. 2000.
35. YAPSPALKIS, 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.

Acknowledgments

We would like to thank the many subjects that participated in this study and the technical help of Kim Beckwith and Lynn Cialdella. This research was funded by a grant from Pacific Health Laboratories, Inc. Bongan Kwon, Ph.D. was a visiting scholar from The Korean National University of Physical Education, Seoul, Korea

Address correspondence to Dr. John L. Ivy, johnivy@mail.utexas.edu.