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Protein Supplementation Does Not Enhance Recovery from Exercise-Induced Muscle Damage

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ABSTRACT

Starkoff BE, Lenz EK, Mattern CO, Too D, Byrne HK. Protein Supplementation Does Not Enhance Recovery from Exercise-Induced Muscle Damage. **JEPonline** 2020;23(1):99-112. This study determined whether the ingestion of a supplement containing carbohydrate and protein (CHO-PRO) improved recovery from exercise-induced muscle damage (EIMD) compared to an isocaloric carbohydrate (CHO) only control supplement, while controlling for diet. Twenty resistance-trained males (22.1 ± 3.9 yr, 176.0 ± 6.9 cm, 84.2 ± 17.6 kg) participated in a 10-day, double-blind, randomized trial. The subjects consumed a provided diet (61.0 ± 1.3% CHO, 26.3 ± 1.4% fat, 14.7 ± 0.8% protein) and daily supplement of 60 gm CHO or 40 g CHO + 20 gm protein for 8 days. On the 7th day, subjects completed an EIMD protocol. Creatine kinase and myoglobin were assessed prior to EIMD and at 12, 24, 48, and 72 hrs post exercise. Muscle soreness and lower body muscle force production were measured pre-EIMD and at 24, 48, and 72 hrs post-EIMD. Creatine kinase levels (U/L) were elevated at 12 (227.1 ± 18.5), 24 (216.1 ± 17), 48 (189.6 ± 18.3), and 72 (168.1 ± 18) hrs post-EIMD compared to baseline (121.4 ± 15.2). Myoglobin levels were elevated 12 hrs post-EIMD (60.4 ± 56.6 $ng \cdot mL^{-1}$) compared to baseline (8.8 ± 6.3 $ng \cdot mL^{-1}$). No significant differences in creatine kinase or myoglobin between CHO and CHO-PRO groups were found. Muscle soreness was increased at all time points post-EIMD, yet there were no significant differences between conditions. There were also no significant differences in lower body muscle performance between groups. Results indicate that CHO-PRO supplementation does not reduce muscle damage or soreness following EIMD compared to CHO supplementation.

Key Words: EIMD, Nutrition, Post-Exercise Recovery, Recovery

INTRODUCTION

Novel eccentric exercise can lead to exercise induced muscle damage (EIMD), which creates delayed onset muscle soreness (DOMS), increases perception of effort (13), and reduces exercise performance and the ability to train consistently (13,14,28). As a result, research looking at ways to reduce the magnitude of EIMD is warranted. One of the most commonly studied approaches to attenuate EIMD is dietary manipulation of carbohydrate (CHO) and protein (PRO) intake (2). Although CHO supplementation has been shown to replenish muscle glycogen levels and enhance endurance exercise performance (29), CHO supplementation alone has demonstrated little to no effect on attenuating EIMD (13,28). Several investigations have therefore studied the effects of co-ingestion of CHO and PRO (CHO-PRO). However, the literature is controversial regarding whether the simultaneous ingestion of CHO-PRO supplementation is useful in augmenting recovery from EIMD. Numerous studies support the notion that co-ingestion of CHO-PRO enhances recovery from EIMD (1,3,6,16,19,21,23-27,31) while several investigations found the consumption of CHO-PRO had no added benefit (4,10,32,33). The ongoing controversy may, in part, be due to the fact that the majority of these investigations (1,3,4,6,10,16,23-26,31) did not employ an isocaloric design. As a result, it is difficult to determine if the protective effects against EIMD are due to the PRO or the fact that the participants were provided additional calories in the CHO-PRO condition versus the CHO condition.

Of the aforementioned studies, four utilized an isocaloric design (19,21,27,33). In spite of isocaloric supplementation, the studies had varied outcomes. Romano-Ely et al. (21) provided moderately-trained men with either a CHO or a CHO-PRO supplement to consume every 15 min during and immediately after cycling to exhaustion (21). No differences in performance were observed, but the CHO-PRO group demonstrated attenuation of EIMD as evidenced by lower post-exercise creatine kinase (CK) and lactate dehydrogenase (LDH) levels compared to the CHO group. Skillen et al. (27) also measured post-exercise CK levels in trained cyclists following an exhaustive time trial exercise. Cyclists in this study consumed either a CHO or CHO-PRO supplement for 2 wks. Although measures of vertical jump, muscle soreness, fatigue, and mood state were not different between groups, the CHO-PRO group did show reduced CK levels post-exercise when compared to the CHO group. Pritchett et al. (19) examined cycling time to exhaustion at 85% of VO_2 max 15 to 18 hrs after high intensity interval cycling training. The subjects were supplemented with either CHO or CHO-PRO. The CHO-PRO group demonstrated an attenuation of the initial increase in CK, although there was no difference in CK 15 hrs post high-intensity training (19). Wojcik et al. (33) studied the effect of CHO, CHO-PRO, and a placebo on untrained men. Muscle glycogen was depleted by cycling for 12 hrs prior to performing 100 eccentric quadriceps contractions at 120% of 1-repitition maximum. The subjects ingested the supplement immediately post-eccentric exercise and again 2 hrs later. Although peak torque decreased at 24 hrs post-exercise in all groups, supplement composition had no influence on muscle inflammation.

Another confounding issue is the control of habitual dietary intake while the subjects are enrolled in these types of investigations. True dietary control requires the researchers to feed the subjects for the duration of the experiment, which is both laborious and expensive. Of the isocaloric supplementation studies mentioned above, various methods of recording daily caloric intake were employed with dietary records or recall being the most common (19,21).

Only the Wojcik et al. (33) investigation truly controlled for dietary intake by feeding the subjects a standard diet for 9 days.

The present study design attempts to control for habitual, as well as supplemental nutrition by providing all the food to the subjects for the duration of the study and by employing an isocaloric design for supplement provision. Therefore, the purpose of our study was to determine whether or not a CHO-PRO supplement improves recovery from EIMD when compared to an isocaloric CHO control supplement in resistance trained college males while controlling for diet. It was hypothesized that a CHO-PRO supplement would facilitate the subjects' recovery. Thus, the markers of fatigue would be lower and individuals consuming a CHO-PRO supplement would be able to demonstrate increased muscle performance relative to their peers consuming only the CHO supplementation.

METHODS

Subjects

A total of 20 healthy, active males participated in a resistance training program for at least 2 days·wk^{-1} for 3 months. The inclusion criteria required the subjects to be free of lower body orthopedic injury. In addition, during the study the subjects were required to avoid resistance training, consumption of anti-inflammatory medications, participation in holistic therapies (e.g., such as massage), and ingestion of nutritional supplements. The characteristics of the subjects are presented in Table 1.

Table 1. The Characteristics of the Subjects.

Variables	Mean ± SD
Age (yrs)	22.1 ± 3.9
Height (cm)	176.0 ± 6.9
Weight (kg)	84.2 ± 17.6

yrs = years, **cm** = centimeters, **kg** = kilograms

Procedures

Experimental Overview

This experiment utilized a 10-day, double-blinded, randomized, isocaloric design to examine recovery from EIMD using a CHO only supplement compared to a CHO-PRO supplement. Participants read and signed an informed consent document approved by the university's institutional review board before participating in the study.

A summary of the research timeline is provided in Figure 1. In brief, the subjects completed 7 visits over a 10-day timeframe. On day1/visit 1, they completed a health history questionnaire to be sure there were no pre-existing conditions (e.g., lower body orthopedic injury, nutritional allergies, or anti-inflammatory use) precluding their participation in the study. Once the subjects qualified for the study, their body weight and height were measured using a calibrated digital scale and stadiometer (Seca Model 769, Hamburg, Germany). Each subject was then randomly assigned to one of two nutritional conditions (CHO or CHO-PRO). A colleague who was not directly related to the data collection assigned the subjects to their

nutritional group, and provided the corresponding supplement. On day 2/visit 2, the subjects returned to the laboratory to pick up all of their food that was to be consumed from day 3 through the completion of the study. During days 3 through 10, the subjects consumed the food and followed their normal routine. On day 7/visit 3, the subjects underwent the following baseline assessments: (a) measures of CK and myoglobin in the blood; (b) subjective measure of muscle soreness using a pain scale with established reliability and validity; and (c) lower body muscle force performance using measures of both isokinetic strength and vertical jump height. Then the subjects performed an EIMD protocol previously used by Howatson et al. (12). Each assessment was performed again at 12, 24, 48, and 72 hrs after the EIMD protocol.

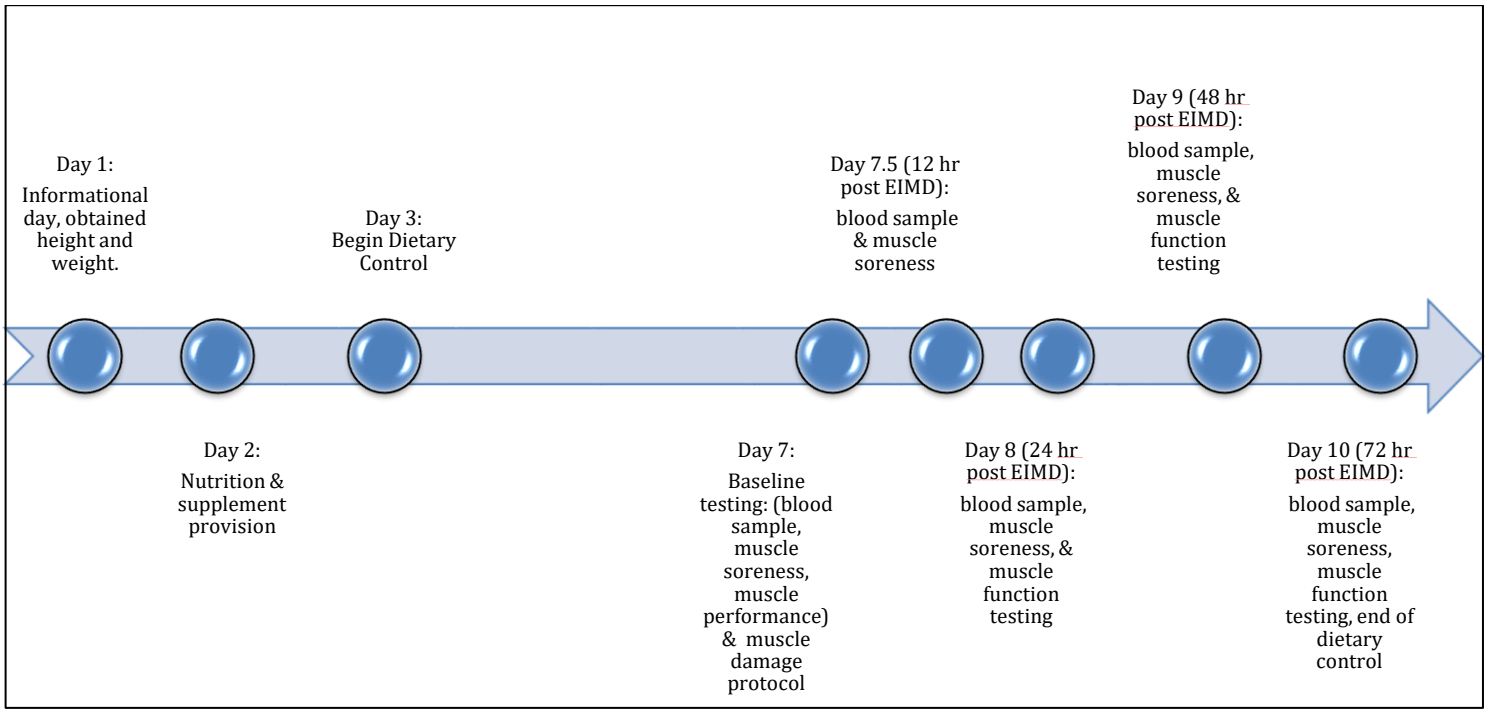

Figure 1. Experimental Timeline.

Nutritional Intervention

On day 1, the subjects completed a questionnaire that inquired about food preference and allergies. This was used to purchase food for the subjects to consume during the study. The caloric needs of the subjects were based on their estimated basal metabolic rate (BMR) using the Mifflin- St. Jeor equation [BMR = (10 x wt) + (6.25 x ht) - (5 X age) + 5] with the activity factor 1.45 (given that the subjects were active college students) (18). On study day 2, an 8-day supply of food was provided to each subject to begin consuming on day 3 through the completion of the study (day 10). The average macronutrient breakdown for the subjects totaled: (a) 61.0 ± 1.3% carbohydrate; (b) 26.3 ± 1.4% fat; and (c) 14.7 ± 0.8% protein, which were within the recommendations set forth in the 2015-2020 USDA's Dietary Guidelines for Americans. The subjects were informed that if they were still hungry after their allotted consumption of calories they were permitted to eat additional vegetables provided by the researchers until satisfied. The subjects' nutritional breakdown by supplement group is found in Table 2.

The subjects were randomly assigned to 1 of the 2 nutritional conditions. Primary researchers and subjects were blinded as to which supplementation product each subject received until the conclusion of the study. In addition to the provided food items, the subjects were given supplemental beverages containing either 60 gm of CHO (240 kcals) (CHO condition) or an isocaloric beverage with CHO and PRO in a 2:1 ratio containing 40 gm of CHO and 20 gm of PRO (240 kcals) (CHO-PRO condition). The beverages were split into two containers and the subjects were asked to consume one container in the morning (120 kcals) and another one in the evening (120 kcals), resulting in 240 kcals per day. On the day of muscle damage (day 7), the subjects were told to consume the beverage (120 kcals) 1 hr prior to undergoing the EIMD protocol, to consume an additional two beverages (240 kcals) immediately after the EIMD protocol, and another one (120 kcals) in the evening, resulting in 480 kcals of supplement consumed on the EIMD day.

Baseline Testing

Following a 2-hr fast, the subjects were asked to consume 120 kcals of their supplement 1 hr before the baseline testing / EIMD exercise session. Upon arrival to the laboratory, a blood sample was acquired, and lower body muscle performance was assessed and a baseline level of muscle soreness was determined. Finally, at the end of the visit the subjects completed the EIMD protocol.

Blood Borne Measurements

Blood samples (~1 mL) were obtained from a finger-stick using a sterile technique at the following time intervals: (a) baseline (pre-muscle damage); and (b) at 12, 24, 48, and 72 hrs (post-muscle damage). The blood sample was centrifuged for 10 min, after which the serum fraction was stored at -80° Celsius.

Serum CK concentration was measured via the enzyme coupled reaction assay technique using an Enzychrom™ Creatine Kinase Assay Kit (ECPK-100) (BioAssay Systems, Hayward, CA). Analyses were performed in duplicate using an ELx800 microplate absorbance reader and an ELx50 strip washer (BioTek Instruments, Winooski, Vermont).

Serum myoglobin concentration was measured via the enzyme –linked immunosorbent assay (ELISA) technique using a Calibiotech Myogloin ELISA kit (Calbiotech Inc. (CBI), Spring Valley, CA). Analyses were performed in duplicate using an ELx800 microplate absorbance reader and an ELx50 strip washer (BioTek Instruments, Winooski, Vermont).

Assessment of Lower Body Muscle Performance

The subjects were familiarized with the assessment of isokinetic muscle torque, power, and work for leg extension and leg flexion using the Biodex System 2 Isokinetic Dynamometer (Biodex Medical Systems, Shirley, NY). Prior to each use, the isokinetic dynamometer was calibrated according to the manufacturer's specifications. At baseline (pre-muscle damage) and at 24, 48, and 72 hrs (post-muscle damage), all the subjects completed a warm-up set of 6 sub-maximal repetitions performed at an angular velocity of 90 $deg \cdot sec^{-1}$ followed by 1 min of rest. Then, each subject performed 1 set of 5 maximal repetitions at an angular velocity of 90 $deg \cdot sec^{-1}$. After a 2-min rest period, another warm-up set of 6 sub-maximal repetitions was performed at an angular velocity of 180 $deg \cdot sec^{-1}$, followed by one additional minute of rest. Finally, 1 set of 15 maximal effort repetitions were performed at 180 $deg \cdot sec^{-1}$ of which the equipment recorded the muscle force exerted during exercise and the following output data

were recorded from each exercise session: (a) peak torque; (b) total work; and (c) absolute power.

Assessment of muscular power using the Vertec vertical jump tester (Sports Imports, Hilliard, OH) was conducted at baseline (pre-muscle damage) and at 24, 48, and 72 hrs (post-muscle damage). Before performing the actual jumps, the standing reach height of each subject was determined by having the subject stand underneath the Vertec while fully extending one arm upward. This height was used each time the subject returned for testing. Next, a total of three jumps were performed in the following manner. A counter movement jump, where from a standing position each subject descended quickly (to a knee flexion of approximately 90°) and then performed a maximal vertical jump, hitting the vanes of the Vertec with the dominant arm at the top of the jump. Each of the three jumps was performed with a 60 sec rest period between jumps. The highest value from each assessment day was recorded and used for data analysis.

Assessment of Muscle Soreness

The subjects self-reported their level of muscle soreness at baseline (pre-muscle damage) and at 24, 48, and 72 hrs (post-muscle damage) by placing a vertical mark on the muscle soreness scale (17) next to the descriptor that best indicated their level of discomfort.

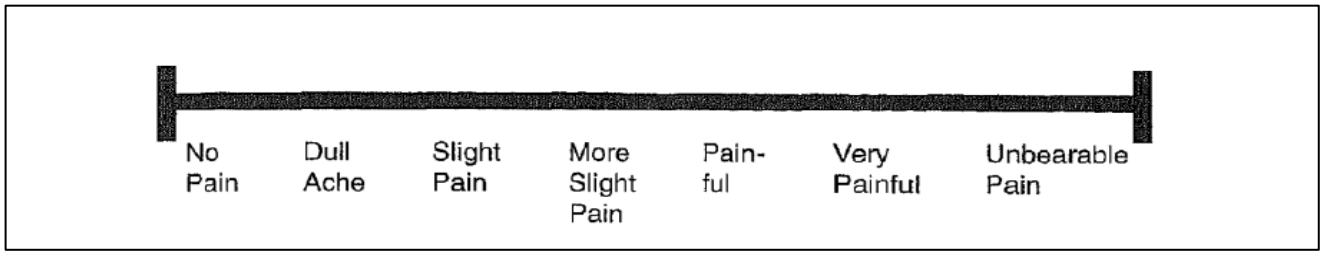

Figure 2. Muscle Soreness Scale.

Exercise Induced Muscle Damage (EIMD) Protocol

The subjects performed a previously established exercise protocol (12) designed to elicit EIMD. They performed 100 drop jumps from a 0.6-m box. They jumped from the box to the ground, performing 90° of knee flexion upon landing, and then immediately jumped up as high as they could. There were 10 sec of rest between each jump and 2 min of rest between each set of 20 jumps.

Statistical Analyses

All the data are reported as mean ± standard deviation (SD) unless otherwise stated. The alpha level was set at $P \leq 0.05$. A repeated measures ANOVA with time and nutritional condition as within-subjects factors was used to compare each dependent variable (CK, myoglobin, muscle soreness, all measures of lower body performance) for the CHO versus the CHO-PRO conditions. A paired *t*-test was utilized when significant differences were found with time. For statistical power at 80% with $P \leq 0.05$, *post-hoc* results (means ± SD) were used to determine the minimum sample size.

RESULTS

Dietary Data

The subjects consumed 61.0 ± 1.3% carbohydrate, 26.3 ± 1.4% fat, and 14.7 ± 0.8% protein from the food that was provided. Table 2 illustrates how the macronutrient percentages shifted significantly once the isocaloric supplement was combined with the diet, resulting in the CHO group consuming a higher percentage of CHO and a lower percentage of PRO compared to the CHO-PRO group.

Table 2. Macronutrient Breakdown.

	CHO (n = 10)	**CHO-PRO** (n = 10)
CHO (%)	64.63 ± 1.41*	61.29 ± 1.08
Fat (%)	23.95 ± 1.25	24.36 ± 1.34
PRO (%)	13.34 ± 0.50	16.27 ± 0.91*

Values presented are means ± SD. **CHO** = Carbohydrate, **PRO** = Protein. *Indicates significant (P<0.05) differences in macronutrient intake with addition of supplement.

Blood Borne Measurements

Creatine kinase levels (U/L) (Figure 3) were significantly higher at 12 hrs (CHO = 216.4 ± 57.17; CHO-PRO = 235.9 ± 68.38), 24 hrs (CHO = 202.3 ± 65.46; CHO-PRO = 193.1 ± 75.41), and 72 hrs (CHO = 165.2 ± 78.93; CHO-PRO = 170.8 ± 69.47) post-EIMD when compared to baseline (CHO = 114.7 ± 68.01; CHO-PRO = 129 ± 50.96) in both nutritional conditions. However, there were no significant differences (P>0.05) found between the CHO and CHO-PRO groups at any time points.

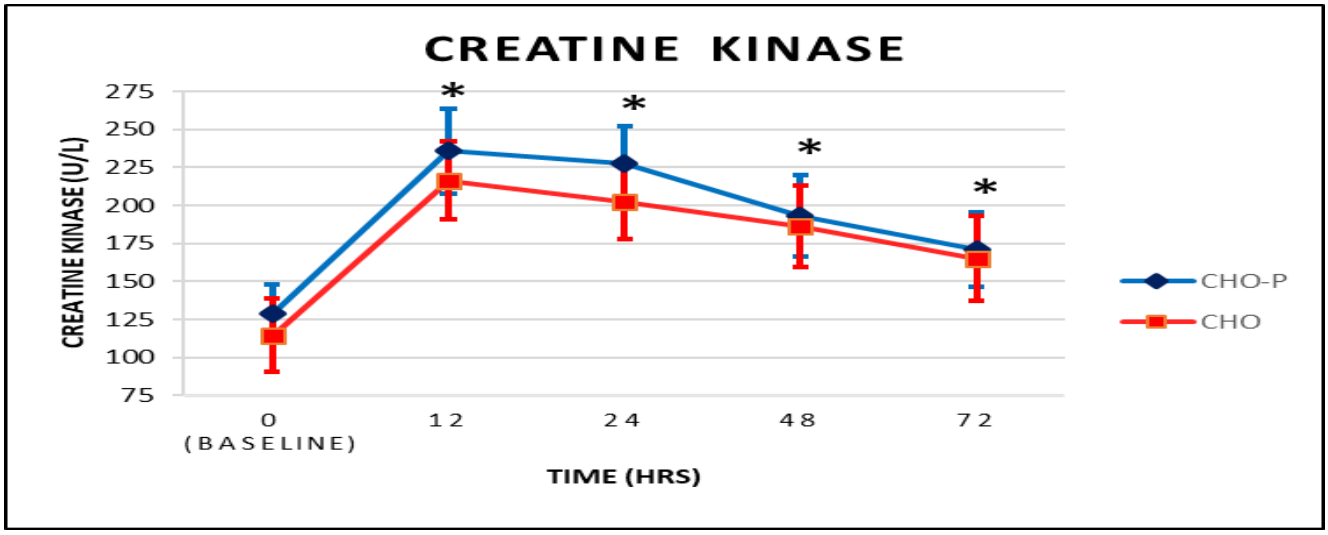

Figure 3. Creatine Kinase Response to Exercise Induced Muscle Damage. Values presented are means ± SE. *Indicates significant difference (P<0.05) from baseline (time 0).

Myoglobin levels (ng·mL^{-1}) (Figure 4) were significantly higher at 12 hrs post-EIMD (CHO = 60.3 ± 45.1; CHO-PRO = 60.3 ± 75.8) when compared to baseline (CHO = 9.4 ± 7.09; CHO-PRO = 7.6 ± 5.18), but by 24 hrs myoglobin levels were no longer significantly different from baseline. Additionally, there were no significant differences (P>0.05) found between the CHO and CHO-PRO groups at any time points.

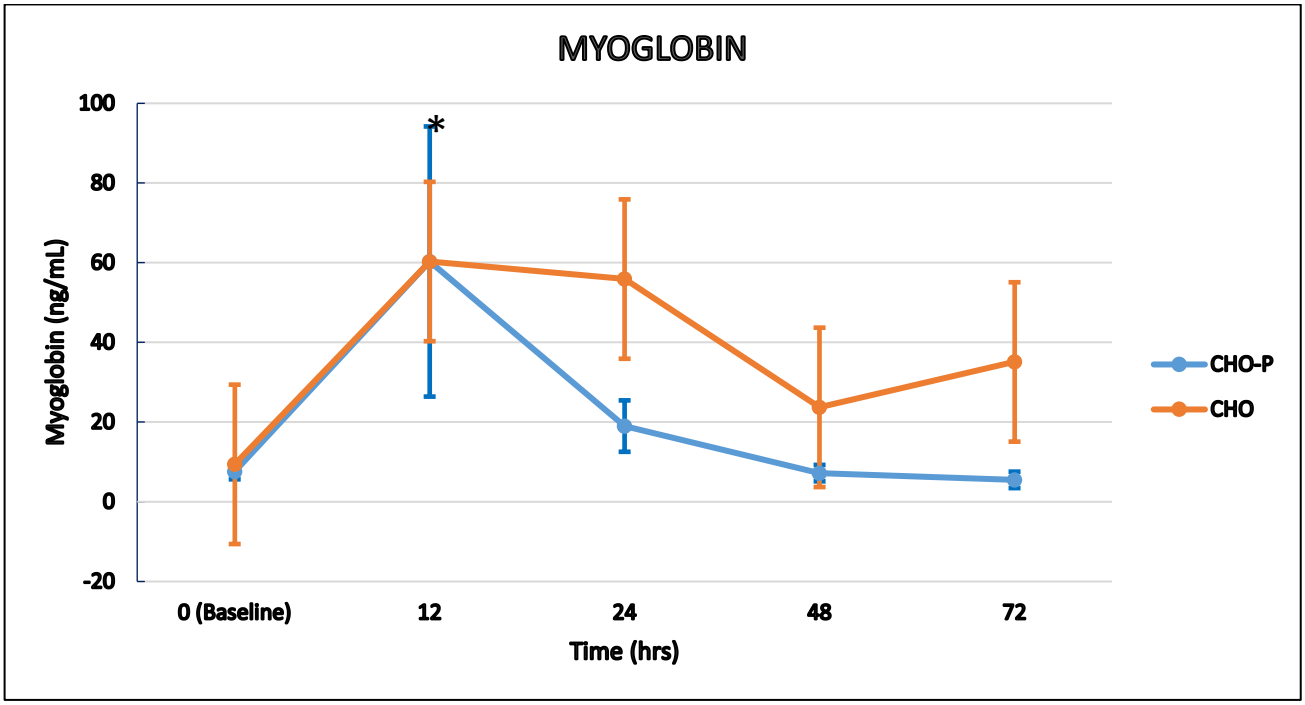

Figure 4. Myoglobin Response to Exercise Induced Muscle Damage. Values presented are means ± SE. *Indicates significant difference (P<0.05) from baseline (time 0).

Muscle Soreness

All subjects reported "no pain" on the muscle soreness scale prior to the EIMD protocol. After the EIMD session, all subjects reported an increase in muscle soreness of various levels (dull ache to very painful) at various time points (12 hrs to 72 hrs post EIMD). This suggests that muscle damage and the expected DOMS were evident in all subjects.

Lower Body Performance Measurements

Biodex

Two speeds (90 and 180 deg-sec^{-1}), two movements (flexion and extension), and two limb conditions (dominant and non-dominant) were examined for peak torque, total work, and absolute power. In comparisons between CHO and CHO-PRO, there were no significant differences in performance at the two speeds, movements, and limb conditions. There were no interactions between nutritional groups and time.

Vertical Jump

Vertical jump height (Figure 5) was not significantly different between CHO and CHO-PRO conditions at any time point, nor did it differ between baseline and any other time points.

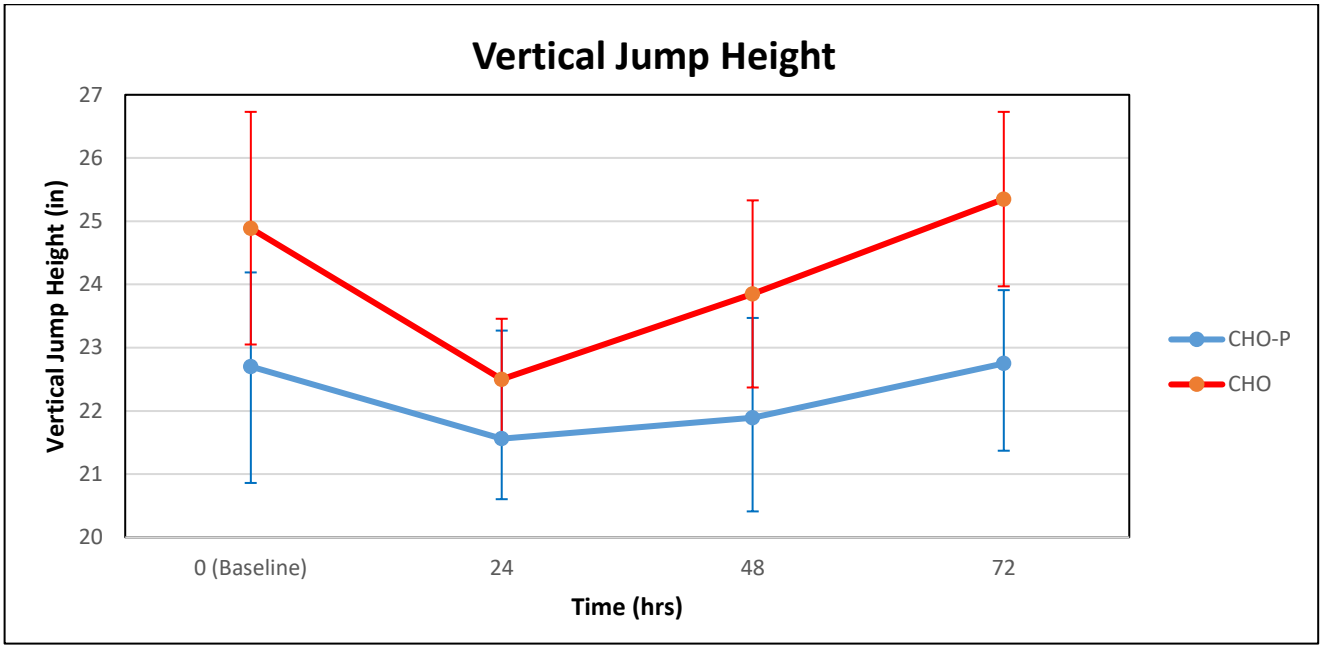

Figure 5. Vertical Jump Height Response to Exercise Induced Muscle Damage. Values presented as means ± SE.

DISCUSSION

In order to decrease the damaging effects of EIMD on protein synthesis and exercise performance, proper nutrition following exercise is warranted. Previous work examining the ingestion of either CHO or CHO-PRO supplementation on recovery from strenuous, eccentric exercise has demonstrated inconsistent findings due to differences in study design, subjects, indicators of muscle damage, and nutritional control. Therefore, the present study intended to examine differences in force production following EIMD between individuals consuming a commercially available isocaloric matched CHO versus a CHO-PRO supplementation. The primary findings in the present study demonstrate that EIMD did occur, with no significant differences in the extent of damage between the CHO group and the CHO-PRO group following EIMD. Similarly, no significant differences in lower body performance between the groups were identified.

Nutrition

Very few studies centered on this topic have used an isocaloric approach (5-7,11,19-21,27) and even fewer of these investigations have controlled the habitual diet of their subjects. In contrast, the current study was designed to carefully control the nutrition in their subjects Thus, the subjects provided diets similar in macronutrient composition throughout the study (Table 2), and they were provided isocaloric supplements. Despite this rigorous nutritional control, our results indicate that the isocaloric CHO-PRO supplementation may not benefit exercise performance following EIMD when compared to CHO supplementation.

Although carbohydrate ingestion after exercise has been shown to improve net PRO balance by attenuating exercise-induced muscle PRO breakdown (22), it is controversial whether

additional PRO in the diet provides a performance advantage. It has been suggested that supplementing with PRO prior to exercise provides a rapid supply of amino acids to the muscle during acute stages of recovery following exercise (34). It is also thought that the consumption of PRO before or concurrent with exercise may stimulate PRO synthesis during the exercise session, thus resulting in elevated muscle PRO synthesis for an extended period of time (34).

The present study provided the subjects with supplementation 4 days prior to and 4 days post EIMD. Also, on the day of EIMD, the subjects consumed an extra dose of supplementation prior to and following the exercise protocol. However, in spite of the supplementation protocol, there were no significant differences in the subjects' performance variables. Lastly, a habitual diet consisting of the recommended amounts of CHO (6 to 12 $g \cdot kg^{-1} \cdot day^{-1}$) and PRO (1.2 to 2.0 $g \cdot kg^{-1} \cdot day^{-1}$) may provide adequate nutrition for recovery from EIMD, which would eliminate the need for supplementation (30). The results of the current study may support this position in so much that providing the subjects with protein supplementation did not offer added protection against EIMD.

Muscle Damage

The indicators of muscle damage, including creatine kinase and myoglobin, increased significantly following the muscle damage protocol. However, even though both variables were significantly greater post EIMD compared to the baseline values, there were no statistically significant differences in the extent of muscle damage between the CHO group and the CHO-PRO group. Additionally, both groups self-reported muscle soreness to be greater at all time points following EIMD compared to the baseline values. These results indicate that the protocol elicited muscle damage. But, the isocaloric CHO-PRO supplementation did not attenuate the extent of muscle damage over time when compared to the CHO group.

When examining the impact of the CHO-PRO supplementation on endurance athletes, Hansen et al. (11) found no significant difference in the extent of muscle damage between the CHO-PRO group and the isocaloric CHO group in elite cyclists. Their study was similar to the present investigation in the macronutrient breakdown of the diet that was fed to the subjects throughout the duration of the present study. However, the subjects were only provided the supplement once per day, immediately post training. Perhaps, the advanced endurance training status of the subjects and the timing of supplement intake contributed to the lack of difference between the groups.

However, it is important to point out that other researchers reported that the addition of PRO attenuated EIMD markers in trained cyclists. For example, Romano-Ely et al. (21) found that cyclists consuming CHO-PRO supplementation demonstrated lower levels of muscle damage compared to the CHO group. Their study differed from the present study in that the diets were self-reported and monitored with a 24-hr diet recall before each protocol day. In addition, the subjects consumed their supplements during and post exercise. The current study provided food and supplementation for the subjects prior to, during, and following EIMD. Although the calorie content and macronutrient distribution of supplements were similar to the present study, the difference in results may be attributed to the dietary intake of each subject over the duration of the study. Similarly, Skillen et al. (27) identified that the addition of PRO to a CHO supplement reduced muscle damage. When compared to the

present study, they provided nutrition for the subjects prior to EIMD, yet this took the form of only one standardized breakfast and dinner immediately prior to their EIMD protocol.

Performance Variables

With the addition of a pure whey PRO isolate supplement including branch chain amino acids (BCAAs) in the CHO-PRO group, we would expect improved muscle PRO synthesis and, subsequently, greater performance on anaerobic tests following EIMD compared to the CHO group. However, there were no significant differences in the subjects' isokinetic force or jump height between the groups following EIMD. The non-significant differences between the supplementation groups on performance variables are similar to the current research (7,9,15) that examines the effects of specifically BCAAs on recovery from muscle damage. Our study used whey PRO isolate to potentially enhance muscle protein synthesis, in addition to the BCAAs in the supplement, yet the subjects in the CHO-PRO group did not demonstrate greater force production compared to the subjects in the CHO group. Again, this may be due to the achievement of recommended daily amounts of PRO through the provided diet that potentially negated the impact of added PRO in the supplements.

In contrast to our findings, other studies also utilizing PRO identified greater improvements in recovery and performance following muscle damage in the CHO-PRO group compared to the CHO group (5,6,8,16,19,21,24-27,31). The differences in these findings may be a result of the training status of the subjects. The subjects in the present study had extensive experience with resistance training, while other studies examined aerobic athletes or recreational athletes who may have had less resistance training experience. Hence, it is likely that the subjects from the other studies were more likely to experience greater EIMD and subsequent muscle PRO synthesis (5,8,16,19,21,24-27,31).

Limitations in this Study

The current study is not without its limitations. Despite providing the subjects with food throughout the study period, it was assumed that they would only consume what was provided and that they were honest in following the protocol. The subjects were resistance trained college aged men. Thus, the results cannot be generalized across other populations. To assist in future studies on the topic, researchers could examine differences in PRO supplement dosages, timing of PRO ingestion before or after EIMD, training status, and/or chronic EIMD exercises compared to acute EIMD exercises to mimic real life training.

CONCLUSIONS

This study examined differences in the extent of EIMD and effect of EIMD on performance variables following CHO or CHO-PRO supplementation. In addition, our study controlled for diet, providing the subjects with nutrition prior to, during, and following EIMD. Our findings indicate that CHO or CHO-PRO supplements are similar in terms of recovery from EIMD. The results do, however, extend the current literature by creating a study design that controlled dietary intake and utilized an isocaloric supplement. Depending on the quality of the regularly consumed diet, there may not be a need for additional PRO consumption before and after an exercise bout that causes EIMD.

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