Original Article

Impact of Protein Supplements on Muscle Recovery After Exercise-induced Muscle Soreness

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The intent of this study was to determine whether nutritional supplements [protein ($0.4\,g \cdot kg^{-1}$) *vs.* carbohydrate ($0.4\,g \cdot kg^{-1}$) *vs.* placebo] would affect muscle recovery differently after eccentric exercise-induced muscle soreness in untrained healthy young men ($n = 21$) aged 20–28 years. During this double-blind randomized block study design, each subject completed three, 3-day trials (separated by ≥ 2 weeks), identical except for treatment, with each subject serving as his own control. Trials began with a bout of right-leg eccentric exercise (Biodex), followed directly by treatment. At 0 (baseline), 24 and 48 hours, data were collected: creatine phosphokinase from pre-exercise blood samples, subjective muscle soreness questions, and strength tests (power, torque, work). ANOVA indicated that exercise caused mild muscle damage, as evidenced by an overall day effect ($p \leq 0.0001$) for muscle soreness, with the lowest median values (0–10 scale) on day 1 (0.7), increasing ($p \leq 0.0001$) on day 2 (3.2), and remaining elevated on day 3 (3.4). We also noted an overall day effect ($p \leq 0.0001$) for creatine phosphokinase, with the lowest median values on day 1 ($136\,U \cdot L^{-1}$), increasing ($p \leq 0.0001$) on day 2 ($235\,U \cdot L^{-1}$), and remaining elevated on day 3 ($189\,U \cdot L^{-1}$). ANOVA revealed no significant treatment effect on indicators of soreness or damage during recovery. Our results indicated that protein or carbohydrate supplement after exercise that caused mild muscle damage did not facilitate muscle recovery in adequately nourished, healthy young men. [*J Exerc Sci Fit* • Vol 8 • No 2 • 89–96 • 2010]

Keywords: creatine phosphokinase, eccentric exercise, muscle soreness

Introduction

Unaccustomed exercise typically causes muscle soreness, which usually begins within 24 hours and peaks within 48 hours after exercise. Eccentric exercise, which occurs when a skeletal muscle lengthens as it produces force, provides a common exercise mode to induce muscle damage. Common responses to exercise-induced muscle damage include increased muscle soreness (Newham et al. 1983b), decreased peak torque, decreased power output, decreased range of motion (Chapman et al. 2006; Paschalis et al. 2005b; Vincent & Vincent 1997), increased inflammatory response (Stupka et al. 2000), and increased creatine phosphokinase (CPK) (Nosaka et al. 2006; Paschalis et al. 2005b; Byrnes et al. 1985). Effective eccentric exercises include running downhill (Newham et al. 1986), stepping down (Newham et al. 1983a), and action of the elbow flexors (Chapman et al. 2006) or knee extensors (Paschalis et al. 2005b) on a dynamometer. The extent of muscle damage, however, depends on the sex (Pincivero et al. 2003; Stupka et al. 2000), training status (Vincent & Vincent 1997), and/or nutritional status of the individual.

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During exercise, amino acids are used to build and repair tissue as well as to supply energy, whereas carbohydrate is the primary energy source for muscle contraction. In the event that amino acids and/or carbohydrate may be limiting substrates during the course of exercise, optimal muscle protein synthesis and turnover, as well as muscle recovery, may be impeded (Bohe et al. 2003). Hence, nutrition supplements before or after eccentric exercise may significantly impact muscle damage and muscle recovery; specifically, a protein supplement has considerable potential to decrease muscle damage and soreness (Ohtani et al. 2006; Sugita et al. 2003). In previous studies (Tipton & Wolfe 2001, 1998), increases in whole body and muscle protein accretion were noted with ingestion of a protein supplement immediately following exercise. Studies reported that amino acid supplements given after exercise led to more rapid recovery from muscle fatigue, decreased plasma CPK activity, decreased muscle soreness, and lessened exercise-induced proteolysis (Ohtani et al. 2006; Flakoll et al. 2004; Nissen et al. 1996). Further research (Levenhagen et al. 2001) suggested that post-exercise protein ingestion was most beneficial to muscle protein accretion when consumed immediately after exercise versus several hours later. Researchers (Zehnder et al. 2004; Sherman et al. 1983) have also investigated whether carbohydrate supplements decrease muscle soreness because of increased energy availability or muscle glycogen repletion. However, most researchers found that carbohydrate intake did not affect CPK activity (Close et al. 2005), muscle soreness (Close et al. 2005; Nelson et al. 2004), and/or muscle strength (Nelson et al. 2004). Nevertheless, we thought it was important to compare a protein supplement with an energy-equivalent carbohydrate supplement in apparently well-nourished healthy men.

The intent of this study was to determine whether nutritional supplements (protein *vs.* carbohydrate *vs.* placebo) would affect muscle recovery differently after exercise-induced muscle soreness in untrained healthy young men. Our hypothesis was that a protein supplement would promote more rapid and greater muscle recovery after a single bout of exercise-induced muscle soreness compared with carbohydrate or placebo.

Methods

This double-blind crossover study was designed to compare the effects of protein, carbohydrate and placebo beverage supplements on muscle recovery using an indirect marker of muscle damage (CPK) and independent markers of muscle function (muscle soreness questionnaire, muscle strength test). Each subject served as his own control, and thus completed the identical protocol three times, consuming one of three supplements each time. The supplements were administered in random order, thus preventing a trial sequence effect. The supplements were composed of: (1) protein [whey protein ($0.4\,g \cdot kg^{-1}$ body weight) with cherry flavoring (Syntrax; SIO3 Inc., Cape Girardeau, MO, USA)] dissolved in 240 mL of water; or (2) carbohydrate [sugar ($0.4\,g \cdot kg^{-1}$ body weight) and cherry flavoring (Kraft Foods Global, Inc., Glenview, IL, USA)] dissolved in 240 mL of water; or (3) placebo [artificial sweetener ($0.0485\,g \cdot kg^{-1}$ body weight; McNeil Nutritionals, LLC, Ft. Washington, PA, USA) and cherry flavoring (Kraft Foods Global, Inc.)] dissolved in 240 mL of water. Protein and carbohydrate treatments were isocaloric. Each subject served as his own control.

Subject criteria and selection

The study design, purpose, and potential risks were explained to each potential subject, with an opportunity to ask questions. Prior to screening, each subject signed an informed consent document. The Human Subjects Institutional Review Board at Iowa State University approved the study (IRB ID#05-341). All portions of the study were carried out in the Fitness and Metabolism Unit (FMU) of the Nutrition and Wellness Research Center on the Iowa State University campus. Prior to enrolment in the study, each volunteer was screened to determine eligibility, and measurements were recorded for height, weight, blood pressure and heart rate. Height and weight were used to calculate the body mass index (BMI) for each subject. Volunteers were required to be non-smokers, untrained, 18–28-year-old males, with a BMI in the range of $18.0–29.9\,kg \cdot (m^2)^{-1}$. To further determine eligibility and screen for past or current health problems, each potential subject completed a medical history and a physical activity questionnaire. Phlebotomists collected blood samples by venepuncture from fasted (10 hours) participants between 6:00 a.m. and 8:30 a.m. Each participant had an initial blood draw for whole blood and serum samples, respectively, analyzed for a complete blood count with differential and a general chemistry panel (ChemScreen) by a certified clinical laboratory (Laboratory Corporation of America, Kansas City, MO, USA). We excluded volunteers with evidence of hypertension, liver disease, kidney disease, diabetes, asthma, and/or abnormal blood values. We included subjects who had maintained a relatively

constant degree of physical activity and excluded those who had recently increased their level of resistance training prior to participating in this study. Subjects were required to refrain from vitamin/mineral supplement and medication use during the study. We enrolled volunteers in the study who met all the inclusion criteria and none of the exclusion criteria.

Protocol

We chose a protocol similar to that used in previously published studies (Paschalis et al. 2005b; Babul et al. 2003; Stupka et al. 2001). Bioelectrical impedance analysis was used to assess body composition (Quantum Bioelectrical Body Composition Analyzer; RJL Systems, Clinton Township, MI, USA), with three measurements recorded and averaged for each subject. During screening, each volunteer participated in an exercise test to measure leg flexion/extension muscle strength on the dynamometer (Biodex System 3 Isokinetic Dynamometer; Biodex Medical Systems, Shirley, NY, USA), and participated in a short training session that explained the future exercise protocol. Muscle strength indicators recorded during this baseline test and all subsequent exercise tests included: extension peak torque, extension maximum repetition total work, extension average power, extension average peak torque, flexion peak torque, flexion maximum repetition total work, flexion average power, and flexion average peak torque.

Subjects were instructed to maintain their normal diets before and during the study, but they were instructed to not exceed two alcoholic beverages per day, beginning 3 days before and lasting until the completion of the trial. Also, subjects were instructed to refrain from any unaccustomed exercise beginning 1 week before and lasting until the completion of the trial. Each participant completed three, 3-day trial periods. Beginning 3 days before the first visit to the FMU, each subject kept a written 24-hour dietary intake record. This record continued until the first day of the trial (time = 0 hours), and thus the record did not include the protein or carbohydrate supplement administered during the trial. Each subject also began recording his 24-hour physical activity record the day prior to the first day of the trial. This record continued until the end of each trial.

Each trial began with the fasted subjects arriving at the FMU in the early morning (0 hours). Researchers measured height, weight, blood pressure and heart rate, and a phlebotomist drew blood by venepuncture. Each subject recorded his initial muscle soreness, performed a short muscle strength test on the dynamometer identical to the screening test, and completed a level of exertion evaluation. The subjects self-reported their soreness using a continuous-range evaluation scale (0–10, with 0 representing "not at all sore" and 10 representing "extremely sore") (Chapman et al. 2006; Nosaka et al. 2006). Perceived level of exertion was assessed using a scale from 0 cm to 10 cm (with 0 representing "no exertion" and 10 representing "maximum exertion"). The purpose of this evaluation was to encourage subjects to exert maximal effort and for researchers to compare exertion levels between trials.

Each subject performed the eccentric exercise protocol intended to induce muscle damage, which consisted of 10 sets of 10 repetitions of eccentric knee extensions at 60 $\deg \cdot s^{-1}$ on a Biodex dynamometer. The range of motion was set from 90° to 180° and intensity was set relative to each subject's initial maximum strength test (concentric single repetition maximum). Subjects were encouraged to perform maximal voluntary effort through verbal encouragement, and they were asked to achieve a designated target as shown on the Biodex screen. A 1-minute break occurred between each interval to enable subjects to resist the torque of the Biodex; the entire protocol lasted about 15 minutes. After the first and last exercise intervals, each subject completed an evaluation of his level of exertion. Immediately following the exercise protocol, each subject drank the protein or carbohydrate or placebo beverage. He then completed another evaluation of his muscle soreness and provided another blood sample. Thirty minutes after exercising, each subject had his blood pressure and heart rate taken. He was then allowed to leave the FMU, but was asked to refrain from consuming any food until at least 45 minutes after the completion of exercise. This was to ensure comparability across subjects and to minimize the influence of potentially confounding factors. Water was provided ad libitum throughout the protocol.

Subjects returned to the FMU in a fasted state the following 2 days (24 and 48 hours), at approximately the same assigned time as the first day. Preliminary measurements of weight, blood pressure and heart rate were recorded, and a blood sample was taken. Each subject completed a muscle strength test, an evaluation of level of exertion, and an adverse events questionnaire. Muscle soreness evaluations for the right leg were completed before and after the strength test. At least 2 weeks after the first trial, each subject returned and repeated identical protocol, except that a different treatment beverage was consumed. At least 2 weeks later, each subject again returned, and ingested the third beverage option.

Analysis of dietary and physical activity records

An important goal of the study was to maintain similar circumstances throughout all trials to minimize potential confounding from various extraneous factors. Thus, each subject was asked to maintain a similar dietary intake and physical activity level for 3 days prior to each trial, as well as identical eccentric exertion during all three trials. All 24-hour diet records were analyzed using Nutritionist Pro (Axxya Systems, Stafford, TX, USA) nutrition analysis software. All 24-hour physical activity records were analyzed by quantifying subjects' daily activities (metabolic equivalents) as rest, light activity, moderate activity, or heavy activity. These category distinctions were based on standard published lists of various activities (Ainsworth et al. 1993).

Analysis of blood samples

We separated plasma from whole blood (centrifuged at room temperature for 10 minutes at 1,500*g*) and stored aliquots at –80°C until analyses. We determined plasma CPK activity in duplicate using a Creatine Kinase Reagent Set (Pointe Scientific, Inc., Canton, MI, USA), according to the manufacturer's instructions. The absorbance (measured at 340 nm) of the samples was determined on a Beckman spectrophotometer (Beckman Coulter, Inc., Fullerton, CA, USA) and compared to standards provided by the company. The spectrophotometer measured the rate of NADH formation, directly proportional to serum CPK activity (mediating conversion of creatinine phosphate to creatine).

Statistical analysis

Statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA), with results considered statistically significant at $p \leq 0.05$. Descriptive statistics included means (± standard deviation) for normally distributed data (height, weight, level of exertion, dietary intake, physical activity levels, and muscle strength values) and medians for data that were not normally distributed (CPK and muscle soreness). To determine whether day (0, 24 or 48 hours), trial number or treatment type exerted an effect on CPK or muscle soreness, ANOVA was performed. We used a mixed model, with fixed effects for day, trial and treatment, and incorporated a random effect for subject.

Results

Initial subject characteristics for the 21 healthy young males in their twenties are reported in Table 1. Based on BMI, none of the subjects were obese. At baseline, ANOVA revealed no statistical differences in subject characteristics/activities among the three treatment groups (protein, carbohydrate, placebo) (Table 2). The main indices of muscle damage after the eccentric exercise protocol were CPK ($U \cdot L^{-1}$) and subjective muscle soreness evaluations (0–10-cm scale), although we also assessed exercise measures of strength from the Biodex. It was clear that the eccentric exercise protocol caused the intended muscle damage (Figures 1 and 2). ANOVA for overall day effect revealed significant differences for CPK ($p \leq 0.0001$) and muscle soreness ($p \leq 0.0001$), as well as 6 of 8 muscle strength tests (p value ranged from 0.007 to 0.034), with smaller values for muscle soreness on day 1 compared with day 2 or day 3. Clearly, the eccentric exercise effectively achieved muscle damage. In examining the overall trial effect, we noted several

Table 1. Characteristics of the 21 subjects at baseline*

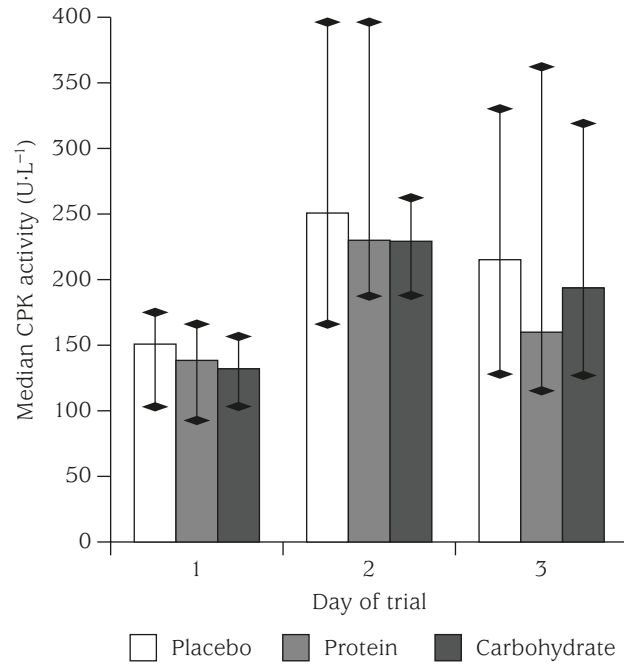
Age (yr)	23 ± 2 (20–28)
Weight (kg)	79.2 ± 10.1 (63.7–98.2)
Height (cm)	179.6 ± 5.4 (170.1–191.2)
Body mass index [$kg \cdot (m^2)^{-1}$]	24.6 ± 3.0 (19.8–29.9)
Lean body mass† (kg)	63.7 ± 5.3 (53.9–73.4)
Lean body mass (%)	80.1 ± 4.0 (72.0–88.5)
Fat mass† (kg)	16.2 ± 5.0 (8.4–27.1)
Fat mass (%)	19.9 ± 4.0 (11.5–28.0)

*Data presented as mean ± standard deviation (range); †assessed using bioelectrical impedance analysis.

Table 2. Dietary intake of the 21 subjects at baseline according to treatment*†

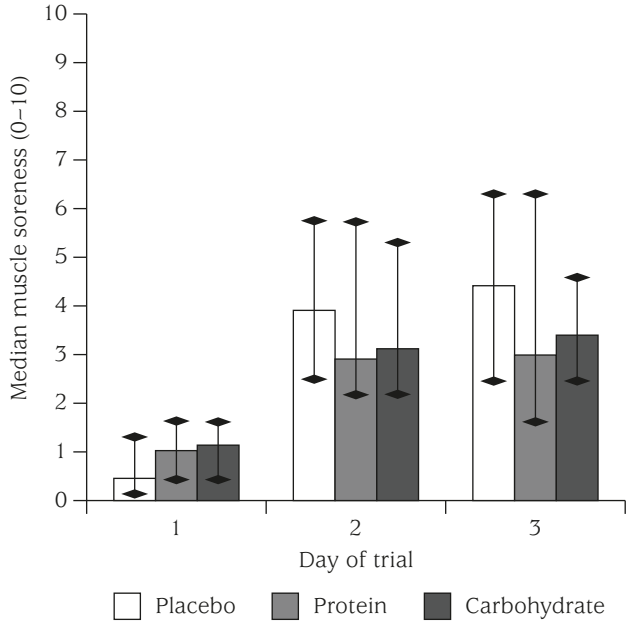
Potential confounding factors	Treatment group			Comparison among groups, p‡
	Placebo	Protein	Carbohydrate	
Energy ($kJ \cdot d^{-1}$)	7,934 ± 1,800	8,093 ± 1,775	8,273 ± 2,403	0.86
Protein ($g \cdot d^{-1}$)	78 ± 17	75 ± 17	83 ± 21	0.41
Carbohydrate ($g \cdot d^{-1}$)	236 ± 66	244 ± 56	248 ± 92	0.87
Fat ($g \cdot d^{-1}$)	73 ± 26	74 ± 36	81 ± 27	0.66

*Data presented as mean ± standard deviation; †dietary intake does not include protein or carbohydrate supplement intake; ‡no significant differences (ANOVA) in energy or macronutrient intake among treatment groups.



Day	Treatment*	Min	25th quartile	50th quartile	75th quartile	Max
1	Placebo	40	101	150	173	479
1	Protein	46	95	138	170	463
1	Carbohydrate	53	112	131	158	290
2	Placebo	103	159	251	391	924
2	Protein	86	184	229	394	836
2	Carbohydrate	111	180	228	258	1,172
3	Placebo	92	139	214	325	2,241
3	Protein	57	120	160	359	572
3	Carbohydrate	93	131	194	314	844

Fig. 1 Serum creatine phosphokinase (CPK) activity according to treatment. *There was no significant difference in CPK activity (ANOVA) among treatment groups.



Day	Treatment*	Min	25th quartile	50th quartile	75th quartile	Max
1	Placebo	0	0.2	0.4	1.3	4.6
1	Protein	0	0.4	1.0	1.6	2.3
1	Carbohydrate	0	0.4	1.1	1.6	2.7
2	Placebo	1.2	2.4	3.9	5.8	8.0
2	Protein	0.9	2.3	2.9	5.6	7.0
2	Carbohydrate	0.8	2.0	3.1	5.4	9.3
3	Placebo	1.0	2.7	4.4	6.2	8.7
3	Protein	0	1.8	3.0	6.3	9.9
3	Carbohydrate	1.7	2.3	3.4	4.8	8.7

Fig. 2 Muscle soreness evaluation (0–10-cm scale) according to treatment. *There was no significant difference in muscle soreness (ANOVA) among treatment groups.

significant differences among trials. ANOVA for overall trial effect showed significant differences for muscle soreness ($p \leq 0.0001$) and 6 of 8 exercise muscle strength tests (p value ranged from ≤ 0.0001 to 0.017). Specifically, greater muscle damage occurred during trial 1 than during trial 2 or 3, as evidenced by statistically significant differences in muscle soreness values between trials 1 and 2 ($p \leq 0.0001$) and between trials 2 and 3 ($p = 0.0002$), as well as greater CPK values ($p = 0.039$) and several muscle strength indices in trial 2 compared with trial 1. We analyzed CPK activity and muscle soreness evaluation by treatment type, as depicted graphically (Figures 1 and 2). However, examining the overall treatment effect, we documented no significant difference as indicated by either CPK or muscle soreness values. Thus, the eccentric exercise protocol produced significant muscle damage with a documented trial effect, but treatment type had no effect on muscle soreness or recovery.

Discussion

Contrary to other studies (Ohtani et al. 2006; Sugita et al. 2003), the results of this study indicated no significant effect of treatment on muscle damage, soreness or recovery after moderate eccentric exercise (Figures 1 and 2). However, similar to previous studies (Chapman et al. 2006; Paschalis et al. 2005a; Vincent & Vincent 1997; Newham et al. 1983a, the eccentric exercise protocol successfully induced muscle damage, as evidenced by increased CPK activity and perceived muscle soreness values from day 1, above that of sequential trials on days 2 and 3. Also, not surprisingly and similar to other studies (Stupka et al. 2001; Byrnes et al. 1985), we documented a trial effect, with significantly greater muscle damage during and after trial 1 compared with trial 2 or 3. This protective effect due to repeated exercise exposure is well documented, and undoubtedly, the 2-week minimum recovery time we required between trials was not sufficiently long to overcome the protective effect. Perhaps as a control, we might have used the non-dominant leg.

Although several studies (Ohtani et al. 2006; Flakoll et al. 2004; Sugita et al. 2003; Wojcik et al. 2001) indicated that protein and/or carbohydrate supplements after eccentric exercise decreased muscle damage compared with placebo, some authors (Williams 1999; Kreider et al. 1993) remain skeptical that protein and/or carbohydrate supplements cause increased muscle recovery. Evidence for a lack of effect on muscle recovery has been demonstrated recently in female participants who underwent exercise-induced muscle injury and then consumed a carbohydrate or carbohydrate/protein beverage immediately after eccentric exercise (Green et al. 2008). Thus, our results concur with this study in females.

A few possible factors may explain why our data did not indicate significantly decreased muscle damage after protein and/or carbohydrate supplement intake. Perhaps the main reason we did not document a treatment effect was because our exercise protocol induced mild-to-moderate muscle damage. Other studies using alternative eccentric exercise protocols created more severe muscle damage, indicated by CPK activity as high as 1,500 $U \cdot L^{-1}$ from downhill walking (Newham et al. 1986) or as high as 34,500 $U \cdot L^{-1}$ from eccentric stepping (Newham et al. 1983a). Our aim was to determine whether a protein supplement would increase muscle recovery subsequent to a less extreme degree of muscle damage, to simulate a more realistic and applicable situation. Thus, we chose to use a less damage-producing protocol similar to published studies (Paschalis et al. 2005b; Babul et al. 2003; Stupka et al. 2001).

Another possible explanation for the lack of treatment effect may have been the small dose of protein (0.4 g protein $\cdot$ kg^{-1} body mass) provided to the apparently well-nourished subjects in this study. Although their reported energy [~7,934 $kJ \cdot d^{-1}$ (1,896 kcal)] intake (based on three, 24-hour diet records) was low, it is unlikely that our participants' diets were lacking in either energy or protein (78–83 $g \cdot d^{-1}$), since they did not lose weight during the study. These values were likely an underestimate of energy intake, given that study participants typically underreport dietary intake (Jonnalagadda et al. 2000). One would expect that in well-nourished individuals, the extracellular pool of amino acids would not be particularly limiting, except perhaps after a long-term fast (Bohe et al. 2003). Hence, a relatively small amount of supplemental protein may not be sufficient to exert an effect on extracellular amino acid concentrations and thus influence intracellular protein synthesis subsequent to moderate muscle damage. The dose administered in research studies (Williams et al. 2003; Wojcik et al. 2001) varies greatly, but it is commonly ~1.0 g protein $\cdot$ kg^{-1} body mass. However, studies often administer much larger doses. One study (Nosaka et al. 2006) compared the effect of ingesting various doses of a protein supplement (with one dose being 3.6 g) on exercise-induced muscle damage. Before and after endurance exercise using the elbow flexors, subjects ingested a total of either two (7.2 g) or 10 (36 g) doses of protein supplement. The subjects who consumed only two protein doses versus placebo reported no significant difference in CPK activity or muscle soreness, but those who consumed 10 protein doses versus placebo showed significantly lower CPK activity and muscle soreness. Clearly, the greater but not lesser amount of protein in supplement form exerted a significant effect on muscle recovery after exercise. Our study was performed before the Nosaka et al. (2006) study was published.

Our study had several limitations in that the participants were self-selected, 20–28-year-old, well-nourished, healthy male volunteers. Hence, the results are not applicable to the male population as a whole, and conclusions from the data cannot be generalized to females. Another limitation was that the subjects were free-living men, and thus we did not control the subjects' physical activity or dietary intake. Although subjects were instructed to refrain from physical activity for 1 week before each trial, their actual physical activity was not prescribed or quantified. Thus, their usual physical

activity may have had an undeterminable effect on muscle soreness and CPK activity. Although each subject was instructed to repeat his 3-day recorded dietary intake from the first trial during the second and third trials, this did not necessarily occur in practice in the majority of the subjects. Also, the exercise bouts were performed on a Biodex machine, which uses a person's own resistance to determine his level of power output. Subjects were strongly encouraged to work as hard as they were able and we provided visual targets as goals on the Biodex screen, but the work performed from subject to subject undoubtedly varied. However, because each subject served as his own control, this should not have confounded the overall results, as long as each subject performed similarly from trial to trial. Indeed, from the researchers' perspective, subjects performed similarly from trial to trial. In addition, we evaluated a relatively small number ($n = 21$) of subjects, although similar studies (Paschalis et al. 2005a; Nelson et al. 2004) indicated a response to treatment, with subject numbers ranging from 5 to 20. However, a trial with a greater number of subjects would provide greater statistical power for detecting small changes, which may not have been detectable in our study because of inherent interindividual variability and relatively small treatment doses. Nevertheless, this trial mimicked real-life situations, which is one of this study's strengths.

In conclusion, a protein or carbohydrate supplement consumed after moderate eccentric exercise did not significantly affect muscle damage or recovery, likely because muscle damage was mild and the supplement dose was relatively low ($0.4\,g \cdot kg^{-1}$ body weight). Further research with subjects who are marginally nourished or with older subjects is warranted to determine whether larger doses of supplement may promote recovery in the face of greater exercise-induced muscle damage.

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