Effects of Supplement Timing and Resistance Exercise on Skeletal Muscle Hypertrophy

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ABSTRACT

CRIBB, P. J., and A. HAYES. Effects of Supplement Timing and Resistance Exercise on Skeletal Muscle Hypertrophy. *Med. Sci. Sports Exerc*., Vol. 38, No. 11, pp. 1918–1925, 2006. **Purpose:** Some studies report greater muscle hypertrophy during resistance exercise (RE) training from supplement timing (i.e., the strategic consumption of protein and carbohydrate before and/or after each workout). However, no studies have examined whether this strategy provides greater muscle hypertrophy or strength development compared with supplementation at other times during the day. The purpose of this study was to examine the effects of supplement timing compared with supplementation in the hours not close to the workout on muscle-fiber hypertrophy, strength, and body composition during a 10-wk RE program. **Methods:** In a single-blind, randomized protocol, resistance-trained males were matched for strength and placed into one of two groups; the PRE-POST group consumed a supplement (1 $g \cdot kg^{-1}$ body weight) containing protein/creatine/glucose immediately before and after RE. The MOR-EVE group consumed the same dose of the same supplement in the morning and late evening. All assessments were completed the week before and after 10 wk of structured, supervised RE training. Assessments included strength (1RM, three exercises), body composition (DEXA), and vastus lateralis muscle biopsies for determination of muscle fiber type (I, IIa, IIx), cross-sectional area (CSA), contractile protein, creatine (Cr), and glycogen content. **Results:** PRE-POST demonstrated a greater ($P < 0.05$) increase in lean body mass and 1RM strength in two of three assessments. The changes in body composition were supported by a greater ($P < 0.05$) increase in CSA of the type II fibers and contractile protein content. PRE-POST supplementation also resulted in higher muscle Cr and glycogen values after the training program ($P < 0.05$). **Conclusion:** Supplement timing represents a simple but effective strategy that enhances the adaptations desired from RE-training. **Key Words:** WHEY PROTEIN, CREATINE, CARBOHYDRATE, SUPPLEMENTATION, HISTOCHEMISTRY, LEAN BODY MASS

Oral supplementation with whole proteins or essential amino acids (EAA) immediately before and/or after resistance exercise (RE) is shown to promote a better anabolic response (i.e., a higher stimulation of protein synthesis and a positive net protein balance) compared with placebo treatments (27,28). In young adults, the presence of carbohydrate (glucose) seems to enhance this response (20). Therefore, it has been suggested that the consumption of a protein–carbohydrate supplement immediately before and after RE (i.e., supplement timing) may provide the ideal anabolic conditions for muscle growth (29). Muscle hypertrophy (1,12) or a trend for greater gains in lean body mass (LBM) (9,22) have been observed from the intake of nutrients (i.e., protein) close to RE. However, the participants in these studies were not permitted to consume any nutrients other than the designated supplement for up to 3 h before and after each workout. Therefore, the results can be attributed to the presence (or absence) of macronutrients, such as protein. However, because normal eating patterns were inhibited, these effects could not be attributed to supplementation *per se*. Additionally, no studies have examined whether this supplement-timing strategy may provide greater benefits in terms of muscle hypertrophy or strength development compared with the consumption of the same supplement at other times during the day.

Supplementation with creatine monohydrate (CrM) has been consistently shown to promote greater gains in LBM and strength during RE training compared with placebo-treated groups (17). These beneficial effects are thought to occur via the accumulation of Cr in skeletal muscle (15). The uptake of Cr by muscle seems to stimulate transcription factors that regulate contractile protein synthesis (30) and/or increase phosphocreatine (PCr) availability (15), which is thought to promote greater work capacity and strength improvements during training (23). Since 1993, more than 200 studies have examined the effects of CrM supplementation on exercise performance (23). However, comparatively few studies have provided insights on strategies that may increase CrM transport into muscle (8,24). For example, some research suggests that taking CrM in the hours surrounding RE may improve muscle hypertrophy (8), but no research has examined whether taking CrM at this time may result in greater accumulation within muscle or provide greater adaptations compared

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with supplementation at other times of the day (i.e., the hours not close to the workout). Additionally, no studies have quantified the extent of muscle Cr content alongside fiber-specific hypertrophy (i.e., type I, IIa, IIx) in response to CrM supplementation at different times of the day.

The aim of this study was to examine the effects of supplement timing during RE training with a CrM-containing protein/carbohydrate supplement in comparison with supplementation at times not close to the immediate pre- and postworkout periods. Unlike others that have examined the effects of strategic supplementation during RE, we wanted to examine the effects of supplementation in the presence of normal eating patterns. Based on the results from previous work in this area (1,8,9,12,20,22,27) it was hypothesized that supplement timing would provide greater chronic adaptations (i.e., greater increases in LBM, strength, and muscle-fiber hypertrophy) compared with supplementation in the hours not close to RE.

METHODS

Participants. Twenty-three recreational male bodybuilders met the requirements to commence this study. To qualify as participants the men had to (a) have no current or past history of anabolic steroid use, (b) have been training consistently (i.e., 3–5 $d \cdot wk^{-1}$) for the previous 6 months, (c) submit a detailed description of their current training program, (d) have not ingested any ergogenic supplement for 12 wk before the start of supplementation, and (e) agree not to ingest any other nutritional supplements or nonprescription drugs that might have affected muscle growth or the ability to train intensely during the study. All participants were informed of the potential risks of the investigation before signing an informed consent document approved by the human research ethics committee of Victoria University and the Department of Human Services, Victoria, Australia. All procedures conformed to National Health and Medical Research Council guidelines for the involvement of human subjects for research and conformed to the policy statement regarding the use of human subjects and written informed consent published by *Medicine & Science in Sports & Exercise*®.

Supplementation. After baseline testing, the participants were matched for maximal strength (1RM) in three weight-lifting exercises (see strength assessments) and then randomly assigned to one of two supplement groups. The PRE-POST group consumed their supplement just before commencing and straight after finishing their workout (four times per week for 10 wk). The MOR-EVE group consumed the same supplement in the morning before breakfast and late evening before sleep, each training day; these times were at least 5 h outside of the workout. All participants were prescribed 1 g of the supplement per kilogram of body weight (1 $g^{-1} \cdot kg^{-1}$ bw), to be consumed twice on training days only. The supplement contained (per 100 g), 40 g of protein (from whey isolate), 43 g of carbohydrate (glucose), < 0.5 g of fat, and 7 g of CrM and was provided by AST Sport Science (Golden, CO). This dose provided an 80-kg participant with 32 g of protein, 34.4 g of carbohydrate, < 0.4 g of fat, and a 5.6 g of CrM in each serving (a total of 1124 kJ). The chosen supplement dose was based on previously reported intakes of this population (18) and was similar to previous studies that had involved protein (1) or CrM (8) supplementation close to RE. The participants were instructed to maintain their habitual daily diet during the trial. That is, the MOR-EVE group consumed the supplement before breakfast, performed RE (for 1 hr) between 3 and 6 p.m., consumed their normal evening meal approximately 1–2 h after the workout, and then consumed their second supplement dose before sleep. The PRE-POST group ate and trained at similar times to the MOR-EVE group, but this group took their supplement servings immediately before and after each workout. The participants signed a consent form stipulating they would follow their habitual daily diet (as determined by dietary records), take the supplement only as prescribed, and not consume any other type of supplement that might affect body composition during the study. Participants were given approximately a 1-wk supply of the supplement at the start of each week and were asked to return the container before receiving the next week's supply, as an indicator of compliance to the dosing procedure.

Obviously, individuals in this study were not blinded to which group that they were in. However, the researcher and personal trainer involved in the trial were blinded to the groups. After baseline testing, each participant was handed a sealed envelope by an individual not involved in the study. The envelope contained a letter notifying the participant of his group allocation and instructions on how to consume the supplement doses. To maintain this blinded procedure, the participants were asked not to discuss their dosing protocol and to consume their supplement while not in the presence of others involved in the study. Ability to comply with this request was made very easy for the participants. Each was supplied with several identical opaque drink bottles in which they consumed water *ad libitum* during each workout; the MOR-EVE group mixed and consumed their supplement with water in one of these bottles at home, whereas participants in the PRE-POST group carried their supplement servings in dry bottles that were kept in lockers at the facility, consuming their supplements discreetly and away from others just before commencing each workout, and again as soon as the workout had been completed.

Before the study, the participants were shown how to record nutrient intake, and each participant was asked to submit three written dietary recordings (each recording consisted of 3 d) for the calculation of macronutrient and energy intake. Participants were asked to submit one of these recordings before the study, one in the first week, and another in the final week of the training/supplementation program. Macronutrient and energy intake was analyzed using Nutritionist PRO (First Data Bank, San Bruno, CA). The participants were weighed on a Seca 703 stainless-steel digital medical scale (Seca, Perth, WA) on each

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TABLE 1. Baseline characteristics.

Characteristics	PRE-POST (N = 8)	MOR-EVE (N = 9)	P
Age (yr)	21 ± 3	24 ± 4	0.21
Training age (yr)	3 ± 2	3 ± 2	0.32
Height (cm)	178 ± 5	178 ± 2	0.81
Body mass (kg)	82 ± 9	78 ± 5	0.32
Lean mass (kg)	69 ± 6	65 ± 6	0.14
Fat mass (kg)	12 ± 4	13 ± 4	0.70
CSA type I (μm^2)	3206 ± 389	2887 ± 382	0.11
CSA type IIa (μm^2)	4604 ± 590	4491 ± 584	0.69
CSA type IIx (μm^2)	4507 ± 613	4360 ± 594	0.62
1RM bench (kg)	127 ± 22	121 ± 14	0.51
1RM deadlift (kg)	153 ± 18	142 ± 19	0.25
1RM squat (kg)	148 ± 24	148 ± 26	0.46

Values are mean ± SD.

occasion that the recordings were obtained. Energy intake is expressed in kilocalories per kilogram of body weight per day; protein and carbohydrate are expressed in grams per kilogram of body weight per day.

Resistance-training protocol. Questionnaires demonstrated that the participants had been training consistently (i.e., 3–5 $d \cdot wk^{-1}$) for at least 6 months before expressing interest in this investigation. However, to ensure the participants were trained and to minimize the impact of a new program on strength and hypertrophy adaptations, the men underwent a structured training program (similar to the one used in this study) for 8–12 wk before commencing this trial. The 10-wk RE program used in the study (Max-OT™, AST Sport Science, Golden, CO) has been described elsewhere (10). Subjects began this program the week immediately after baseline assessments. In brief, the program was designed specifically to increase strength and muscle size. It consisted of high-intensity (overload) workouts using mostly compound exercises with free weights. Training intensity for the program was determined using repetition maximums (RM) from strength tests. Once a designated RM was reached, the participants were encouraged by the trainer to increase the weight used. This progressive overload program was divided into three phases, preparatory (70–75% 1RM), overload phase 1 (80–85% 1RM), and overload phase 2 (90–95% 1RM). Qualified personnel supervised each participant on a one-to-one basis during every workout. Aside from the personal training each participant received during the 10-wk program, they also kept training diaries to record exercises, sets, repetitions performed, and the weight used throughout the program; these were viewed by the trainer on a weekly basis. The following assessments occurred in the week before and after the 10-wk RE program.

Experimental protocols. Strength assessments consisted of the maximal weight that could be lifted once (1RM) in three weight-training exercises: barbell bench press, deadlift, and squat. A recognized 1RM testing protocol and exercise execution guidelines were followed, as has been previously documented (2). Briefly, the participant's maximal lift was determined within no more than five single attempts after three progressively heavier warm-up sets. Participants were required to successfully lift each weight before attempting a heavier weight. Each exercise was completed before the next attempt and in the same order. Reproducibility for these tests was determined on two separate occasions that provided a CV ranging from 0.5 to 5%.

Body composition. Lean mass (total fat-free mass), fat mass, and body fat percentage were determined using a Hologic QDR-4500 dual-energy x-ray absorptiometer (DEXA) with the Hologic version V 7, REV F software (Waltham, MA). Whole-body scans were performed on the same apparatus by the same licensed operator. Quality-control calibration and scanning procedures were performed as previously described (10). Participants were scanned at the same time of the day, that is, in the morning in a fasted state. For longitudinal studies in which relatively small changes in body composition are to be detected, whole-body scanning with this instrument has been shown to be accurate and reliable (CV 0.8–2.8%) (21).

Muscle analyses. Muscle biopsies for determination of muscle fiber type, cross-sectional area (CSA), contractile protein content, and metabolite concentrations were taken approximately 30 min after a leg workout that was completed on the Monday of the first and last week of the RE program. Muscle biopsies (100–450 mg) were taken by the same medical practitioner, using the percutaneous needle technique (5-mm diameter), with suction to ensure adequate sample size (13), at a similar depth in the vastus lateralis muscle. On the day of the procedure, the participants were asked to consume their supplement once before the biopsy; either in the morning (MOR-EVE) or just before training (PRE-POST). A small part of the muscle sample was immediately frozen for metabolite analysis. The remaining tissue was mounted using OCT medium and snap frozen in isopentane precooled in liquid nitrogen and stored at −80°C for histochemical analysis.

Histochemical analysis of muscle fibers was performed by staining for ATPase to classify muscle fiber types I, IIa, and IIx based on the stability of their ATPase activity. A preincubation medium of pH 4.54 along with the standard preincubations of 4.3 and 4.6 were used to enable a clear differentiation of the type II subgroups as described by Dubowitz (11). The biopsy samples were serially sectioned (12 μm thick) on a cryostat microtome at −20°C. Baseline and endpoint cross-sections were assayed simultaneously. A loaded image of the stained cross-sections was analyzed using Analytical Imaging Station (AIS) software (Imaging Research Inc. Ontario, Canada)

TABLE 2. Dietary analyses.

Variable	PRE-POST	MOR-EVE
Energy ($kcal \cdot kg^{-1} \cdot d^{-1}$)		
Before	43.7 ± 6.6	44.4 ± 4.8
Week 1	44.1 ± 6.9	42.9 ± 4.1
Week 10	42.8 ± 6.6	42.2 ± 2.8
Carbohydrate ($g \cdot kg^{-1} \cdot d^{-1}$)		
Before	4.88 ± 1.3	4.63 ± 0.7
Week 1	4.86 ± 1.0	4.62 ± 0.8
Week 10	4.79 ± 0.9	4.50 ± 0.7
Protein ($g \cdot kg^{-1} \cdot d^{-1}$)		
Before	1.84 ± 0.4	2.08 ± 0.4
Week 1	1.91 ± 0.4	2.17 ± 0.3
Week 10	1.92 ± 0.4	2.11 ± 0.3

No differences between groups at any time point (mean ± SD).

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TABLE 3. Body mass, composition, and 1RM strength.

	PRE-POST (N = 8)		MOR-EVE (N = 9)		P
Variable	Baseline	Endpoint	Baseline	Endpoint	Group × Time
Body mass (kg)	81.8 ± 3.2	84.3 ± 3.2*	78.2 ± 1.8	79.6 ± 1.7	0.015
Lean mass (kg)	69.5 ± 2.3	72.3 ± 2.3*	65.2 ± 1.5	66.7 ± 1.5	0.002
Fat mass (kg)	12.1 ± 1.5	11.9 ± 1.4	12.9 ± 1.2	13.0 ± 1.3	0.114
% body fat	13.7 ± 1.4	12.6 ± 1.3*	15.7 ± 1.4	15.7 ± 1.5	0.001
1RM squat (kg)	144.4 ± 8.2	164.8 ± 8.6*	138.3 ± 8.5	154.4 ± 7.9	0.049
1RM bench press (kg)	126.9 ± 6.9	139.1 ± 6.8*	121.9 ± 4.7	130.9 ± 4.5	0.023
1RM dead lift (kg)	149.7 ± 6.5	168.1 ± 7.7	141.9 ± 6.4	156.6 ± 6.5	0.1

* Greater change compared with MOR-EVE ($P < 0.05$) (mean ± SE).

interfaced to a Zeiss microscope. Fiber-type percentages and CSA were determined from sections containing a mean of 210 (range 130–400) fibers. To assess reproducibility, all samples were measured twice on two separate occasions for percentage of total fiber area and mean area of fibers. The intraassay CV were 1.5 and 1.3%, respectively. For metabolite quantitation, 3 g of muscle tissue was freeze-dried, powdered, and then extracted in 0.5 M perchloric acid/1 mM EDTA and neutralized using 2 M $KHCO_3$. The samples were analyzed in triplicate for PCr, Cr, and glycogen using fluorimetric techniques as described by Harris et al. (15). All concentrations are expressed as millimoles per kilogram dry weight. Intraassay coefficients of variation were determined for each triplicate for all subjects and resulted in coefficients of 2.85, 4.45, 3.86, and 5.05% for ATP, PCr, Cr, and glycogen, respectively. Approximately 5 mg of muscle was used to determine contractile protein content, extracted as previously described (3). Protein concentrations were assessed in triplicate using a Bradford Protein Assay (Bio-Rad Protein Assay, Bio-Rad Laboratories, Hercules, CA) with BSA standards and spectrophotometric detection at 595 nm (5). The values obtained are in accordance with the results of others who have used this procedure to measure changes in muscle contractile protein content (3). To assess reliability, all samples were run twice on two separate occasions; the two runs resulted in a CV of 3.7%.

Statistics. Subject characteristics and dietary analyses are reported as means ± SD. All other values are reported as means ± SE. Statistical evaluation of the data was accomplished by two-way repeated-measures analysis of variance (ANOVA) with group (supplement) and time (training) as the factors using SPSS statistical analysis software (SPSS v 11.0; Chicago, IL). Where significant main effects were identified by ANOVA, *post hoc* analysis (Bonferroni-corrected Student's t test) was performed to locate differences. Simple regression was used to determine significant relationships among the deltas for selected variables. A P value of less than 0.05 was designated to indicate statistical significance.

RESULTS

Starting characteristics of groups. Six participants did not complete the trial for reasons unrelated to the study. All other participants attended all training sessions and completed all assessments. Therefore, baseline characteristics from 17 individuals (N = 8 PRE-POST; N = 9 MOR-EVE) is presented in Table 1. There were no differences between the groups in any variables at the start of the study ($P > 0.05$).

Dietary analyses. Table 2 shows the average of 3-d written dietary recalls for energy ($kcal \cdot kg^{-1} \cdot d^{-1}$) and for carbohydrate and protein ($g \cdot kg^{-1} \cdot d^{-1}$) of the groups before and in the first and last weeks of the training program. The data do not include supplementation. No differences were identified between the groups or across time with regard to energy, protein, or carbohydrate intake ($P > 0.05$).

Body composition. Table 3 presents body mass, composition, and 1RM strength data. A group × time interaction ($P < 0.05$) was detected for body mass and LBM; the PRE-POST group demonstrated a greater gain in body mass and LBM (*post hoc* $P < 0.05$) compared with the MOR-EVE group (Fig. 1). The PRE-POST group also demonstrated a decrease in body fat percentage compared with the MOR-EVE group (group × time: $P < 0.05$; *post hoc* $P < 0.05$).

Strength. Both groups demonstrated an increase in strength in the barbell squat, bench press, and deadlift after the program (time: $P < 0.01$), and a group × time interaction ($P < 0.05$) was identified in the barbell squat and bench press. Compared with the MOR-EVE group, the PRE-POST group demonstrated greater gains in 1RM strength in these exercises (*post hoc* $P < 0.05$) (Fig. 2).

Muscle characteristics. Table 4 presents fiber-type proportions (percentage), CSA, and contractile protein content of vastus lateralis biopsy samples. No differences

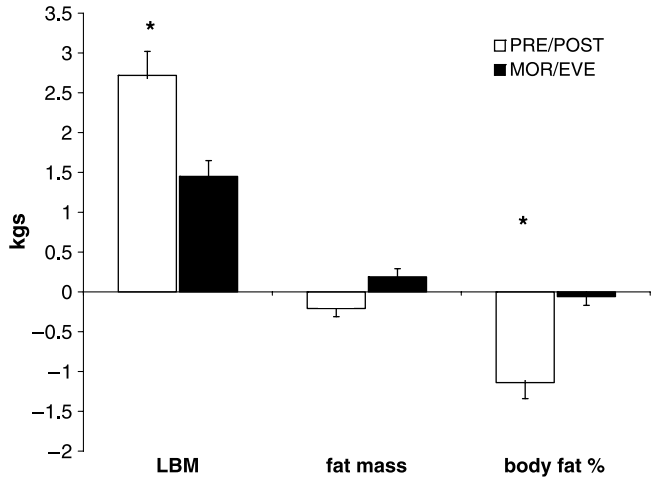

FIGURE 1—Body composition changes. * Greater change compared with MOR-EVE ($P < 0.05$).

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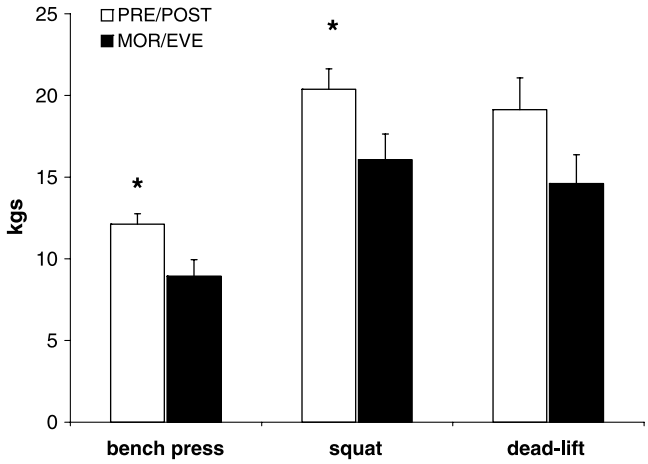

FIGURE 2—1RM strength changes. * Greater change compared with MOR-EVE ($P < 0.05$).

were identified between the groups or across time with regard to fiber proportions. A group × time interaction ($P < 0.05$) was observed in the CSA of the type IIa and IIx fibers; the PRE-POST group demonstrated greater increases in CSA of these fiber types (*post hoc* $P < 0.05$) (Fig. 3) and in contractile protein content (group × time $P < 0.01$; *post hoc* $P < 0.05$) compared with the MOR-EVE group.

Table 5 presents energy metabolite and glycogen data (mmol·kg^{-1} dry weight) obtained from vastus lateralis biopsy samples. A group × time interaction ($P < 0.01$) was detected for both PCr and total Cr. The PRE-POST group demonstrated higher PCr and total Cr concentrations compared with the MOR-EVE group after the program (*post hoc* $P < 0.05$). The PRE-POST group also demonstrated higher muscle glycogen concentrations compared with the MOR-EVE group after the program (group × time $P < 0.01$; *post hoc* $P < 0.01$).

DISCUSSION

The major finding of this study was that after 10 wk of training, supplementation before and after each workout resulted in significantly greater improvements in 1RM strength and body composition (i.e., increase in LBM and decrease in body fat percentage) compared with a matched group who had consumed the same supplement at times outside of the pre- and postworkout time frames. A significantly greater muscle hypertrophy response from supplement timing was evident at three different levels. That is, the PRE-POST group demonstrated significantly greater increases in LBM, hypertrophy of the type-IIa and IIx fibers, and contractile protein. This is an important finding in that this is the first investigation to confirm improvements in body composition via RE training and dietary supplementation with hypertrophy responses at the cellular (i.e., fiber-specific hypertrophy) and subcellular levels (i.e., contractile protein content). Although these results support our hypothesis, it is the design of this study that makes these findings particularly relevant to a wide sector of the population.

Acute-response investigations have shown that supplementation with protein (or EAA) before and/or after RE will enhance the anabolic response by increasing muscle protein–synthesis rates, decreasing protein degradation and providing a higher net protein balance (27,28). The majority of data from longitudinal studies generally support the theory that protein supplementation before and/or after RE will enhance the chronic adaptations desired from training (i.e., muscle hypertrophy and strength). (1,9,12,22). However, the assessment conditions used in these studies may also mean the that results have less relevance in a real-world setting. That is, strength athletes and others who desire increases in strength and muscle mass from RE would not usually abstain from consuming protein for 3 h before and after exercise. A novel aspect of the present study is that the beneficial effects of supplement timing on strength and muscle hypertrophy were obtained when participants followed normal eating patterns.

Unlike previous studies that have assessed the chronic effects of supplementation close to RE (1,9,12,22), in the present study the participants consumed their habitual daily diet during the trial. The MOR-EVE group consumed the supplement before breakfast, performed RE (for 1 h) between 3 and 6 p.m., consumed their normal meal approximately 1–2 h after the workout, and then consumed their second supplement dose before sleep. The PRE-POST group ate and trained at similar times to the MOR/EVE group but took their supplement servings just before and straight after each workout. Both groups consumed their regular meals, but not other supplements that might have affected muscle growth during the trial. The use of bodybuilders in this trial was particularly advantageous because these athletes characteristically consume a protein-rich diet in regimented (frequent) meal patterns (18). For this reason, and because we observed the participants train

TABLE 4. Muscle-fiber proportion, CSA, and contractile protein.

	PRE-POST ($N = 8$)		MOR-EVE ($N = 9$)		P
Variable	Baseline	Endpoint	Baseline	Endpoint	Group × Time
Type I (%)	45 ± 0.01	43 ± 0.01	44 ± 0.01	45 ± 0.01	0.216
Type IIa (%)	41 ± 0.02	44 ± 0.02	44 ± 0.01	44 ± 0.01	0.481
Type IIx (%)	14 ± 0.01	13 ± 0.01	12 ± 0.01	12 ± 0.01	0.480
CSA (μm^2) type I	3206 ± 138	3632 ± 126	2887 ± 127	3217 ± 104	0.28
CSA (μm^2) type IIa	4604 ± 209	5757 ± 207*	4491 ± 195	5255 ± 160	0.006
CSA (μm^2) type IIx	4507 ± 217	5647 ± 221*	4360 ± 198	5135 ± 169	0.01
Contractile protein (mg·g^{-1})	61.2 ± 2.0	91.5 ± 1.9*	67.0 ± 2.6	84.6 ± 2.9	0.001

* Greater change compared with MOR-EVE ($P < 0.05$) (mean ± SE).

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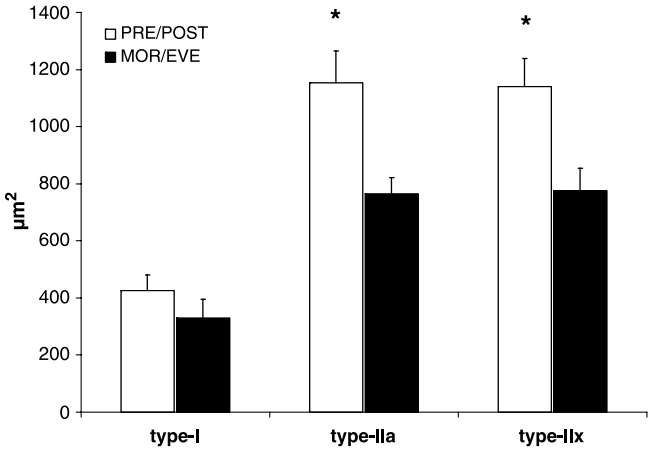

FIGURE 3—Changes in CSA (fiber types I, IIa, and IIx). * Greater change compared with MOR-EVE ($P < 0.05$).

for 8–12 wk before the start of the study, we were confident that those selected would maintain their normal diets during the trial. Aside from the results obtained, analyses of the nutrition diaries (Table 2) suggest that the participants did maintain their normal eating patterns during the trial period. For these reasons, it can be confidently suggested that the beneficial effects of supplement timing on muscle and strength development reported in this study cannot be attributed simply to the presence or absence of certain macronutrients in the hours surrounding RE. The adaptations observed from supplement timing primarily reflect a specific interactive effect between high-intensity muscle contraction and the presence of an abundance of nutritional material (i.e., EAA, Cr, and carbohydrates).

The presence of EAA is shown to increase the acute stimulation of protein synthesis in muscle during RE and to provide a higher positive net protein balance over a 24-h assessment period (27). The presence of CHO (glucose) may enhance this anabolic stimulus, probably by increasing plasma insulin concentrations (which also serve to increase protein-synthesis rates when EAA are present) (20) or by reducing myofibrillar protein breakdown after RE (25). The major source of nitrogen in the supplement used in this trial was whey isolate, a protein that is rich in EAA, particularly the branch-chain amino acids (BCAA) (20–26 g·100 g^{-1} protein) (6). Whey protein generally has rapid absorption kinetics and stimulates a high rate of muscle protein synthesis in a similar fashion to oral doses of free-form EAA (27,28). Supplementation with EAA before and after RE results in higher stimulation of muscle protein synthesis and net gain in protein over a 24-h period (27). In particular, supplementation with BCAA during RE is shown to result in greater phosphorylation (activation) of $p70^{S6k}$ in skeletal muscles, a key (rate limiting) kinase in the signaling network controlling protein synthesis through translational initiation (16). Therefore, the beneficial effects of supplement timing on muscle hypertrophy may be (at least partly) attributed to the abundance of EAA and glucose during high-intensity muscle contraction. Supplementation with CrM is consistently shown to augment LBM and strength development during RE (23). During RE training, the addition of CrM to whey protein is shown to result in a greater gain in lean mass compared with whey protein or CHO alone (7). Therefore, CrM most likely contributed to the improvements in strength and hypertrophy observed in both groups in this study. However, an interesting finding from this study was that the PRE-POST group demonstrated significantly higher muscle Cr concentrations (both PCr and total Cr) after the trial. Before this investigation, no studies had examined the effects of CrM supplementation at different times during the day on muscle Cr concentrations and skeletal muscle morphology during RE training.

Improvements in muscular performance during high-intensity contractions are associated with ATP resynthesis as a consequence of increased PCr availability in muscle via CrM supplementation (15,17). Increasing the availability of PCr via supplementation is not only thought to enhance cellular bioenergetics of the phosphagen system but also the shuttling of high-energy phosphates between the mitochondria and cytosol to increase the availability of energy for contractile protein synthesis (4). Supplementation is taken up by muscle, where it appears to stimulate transcription factors that regulate the synthesis of contractile proteins (30). Enhanced cellular bioenergetics and/or greater expression of hypertrophy-related genes are just two possible explanations for hypertrophy responses observed in this study from CrM supplementation. However, it is less clear why the PRE-POST group demonstrated higher muscle Cr values after the study. A substantial amount of research demonstrates that CrM supplementation enables muscle to perform at a higher capacity during RE (23) and generally promotes greater muscle hypertrophy during RE (17). However, relatively few studies have examined dosing strategies that may increase the amount of exogenous Cr transported into muscle. CrM supplementation after submaximal exercise promotes muscle Cr uptake. Supplementation after RE is shown to increase the muscle girth and thickness of the limb (right or left arm) that is exercised (8). Based on the results obtained from the present study, along with findings by others (8,23), it could be suggested that supplement timing promotes more efficient Cr accumulation

TABLE 5. Muscle metabolites and glycogen.

	PRE-POST ($N = 8$)		MOR-EVE ($N = 9$)		P
	Baseline	Endpoint	Baseline	Endpoint	Group × Time
PCr (mmol·kg^{-1} d.w.)	78.1 ± 1.5	91.2 ± 1.4*	79.7 ± 2.6	81.6 ± 2.7	0.01
Total Cr (PCr + Cr) (mmol·kg^{-1} d.w.)	123.0 ± 2.3	153.2 ± 1.5*	129.0 ± 3.9	138.2 ± 3.8	0.001
Glycogen (mmol·kg^{-1} d.w.)	235.1 ± 12.4	294.0 ± 8.0*	234.0 ± 4.3	232.9 ± 2.8	0.001

* Greater increase compared with MOR-EVE ($P < 0.05$) (mean ± SE).

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within muscle and, therefore, greater strength gains and muscle hypertrophy during RE training. However, this aspect was not examined directly. Based on the results obtained, further investigations are warranted to examine dose responses and the extent of Cr accumulation during RE, and to fully elucidate the contributions of both CrM and whey protein to chronic adaptations during training.

Another novel finding was that the PRE-POST group finished the study with significantly higher muscle glycogen concentrations (Table 5). Muscle glycogen is considered a major contributor of energy production during RE (14). A single bout of high-intensity RE can result in a significant reduction (up to 40%) in muscle glycogen, particularly in type II muscle fibers (26). Because the type II fibers are responsible for maximum force production, low glycogen levels in these fibers have been associated with compromised performance during RE (14,29). The consumption of CHO before and after RE is presumed to spare muscle glycogen stores as well as offer an ergogenic benefit such as increased work capacity during subsequent workouts (14). For these reasons, it has been suggested that the consumption of CHO before and after RE may promote more efficient recovery between bouts, thereby enhancing the development of strength and hypertrophy during RE training (14,29). In the present study, the pre- and posttraining biopsies in this trial were taken 30 min after the completion of a leg workout performed on Monday of the first and last weeks of training. On both occasions, the groups were instructed to consume the first dose of their supplement in the prescribed manner. That is, the PRE-POST group consumed their PRO-CHO dose before the workout. Therefore, it could be suggested that the higher glycogen and Cr values detected in the PRE-POST group were simply attributable to increased availability of CHO and Cr from the supplementation on the day of the biopsy. However, if this were the case, then values from weeks 1 and 10 both should have been higher in the PRE-POST group. The data presented in Table 5 show that they clearly were not. The muscle samples taken in the first week showed no significant differences between the groups in glycogen or Cr, whereas the PRE-POST group showed significantly higher glycogen and Cr values in the samples obtained in week 10. Ingestion of CrM with CHO after exercise has previously been shown to stimulate glycogen repletion more than consuming CHO alone (24). Therefore, it could be suggested that PRE-POST supplement timing not only promoted more efficient CrM accumulation within muscle, but that this strategy may have also promoted more efficient muscle glycogen restoration during the RE program. In turn, these benefits may have enabled greater work capacity during subsequent workouts, thus helping to promote greater strength improvements and muscle hypertrophy. Although work capacity was not assessed, the significantly greater hypertrophy responses (in three of three assessments) and 1RM strength improvements (two of three assessments) demonstrated by the PRE-POST group after the program support this theory. When the metabolite results are considered alongside the morphology data, it is reasonable to suggest that the strategic consumption of nutrients such as whey protein, CHO, and CrM close to the workout creates a favorable environment that results in better muscle strength and hypertrophy development during RE training.

Aside from skeletal muscle morphology, the improvements in 1RM strength observed in this trial must also be attributed to the benefits of personalized coaching/supervision. Although the participants in our study were experienced participants, none had ever received personal training by a qualified instructor (personal training only occurred during the 10-wk trial, not during the training program before the study). Personalized instruction of the participants was a major strength of this study because this level of supervision is shown to provide better control of workout intensity and greater strength improvements during training (19). This level of supervision was important to our hypothesis because it ensured the best chance of enhanced physiological adaptations from supplement timing. This is based on the premise that those taking the supplement close to RE would obtain a greater anabolic response from each workout and progress at a faster rate. It is important to remember that the instructor was blinded to the supplement groups, yet the PRE-POST group demonstrated significantly greater gains in 1RM strength (in two of three assessments) and greater muscle hypertrophy responses (in three of three assessments), thus supporting the hypothesis presented.

In conclusion, although there has been a sound theoretical basis for expecting a beneficial effect from supplement timing, this is the first study to clearly demonstrate that this strategy results in greater strength and body composition improvements (i.e., a gain in lean mass and a decrease in body fat percentage) as well as muscle hypertrophy, compared with supplementation at times outside of the workout period. Unlike previous work that has examined chronic adaptations from nutrient consumption close to RE, a significantly greater muscle hypertrophy response from supplement timing was evident at three different levels (i.e., a greater increase in LBM, hypertrophy of the type IIa and IIx fibers, and contractile protein accrual). Additionally, these results were obtained with participants maintaining their normal eating patterns throughout the program. Therefore, we conclude that supplement timing represents a simple but effective strategy to enhance the adaptations that are desired from RE training. Clearly, this strategy would be of benefit to most healthy adults who perform RE to improve functional strength and body composition. However, this protocol may also have important implications for populations that require improvements in strength and body composition but that have a reduced capacity for exercise, such as the frail elderly, cardiac rehabilitation patients, or others living with conditions that compromise health, such as HIV, cancer, and the various muscular dystrophies.

The lead investigator is a consultant to AST Sports Science. The results of the present study do not constitute endorsement of the product by the authors or ACSM.

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