Journal of Strength and Conditioning Research, 2006, 20(3), 643–653

The Effects of Protein and Amino Acid Supplementation on Performance and Training Adaptations During Ten Weeks of Resistance Training

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Abstract. Kerksick, C.M., C.J. Rasmussen, S.L. Lancaster, B. Magu, P. Smith, C. Melton, M. Greenwood, A.L. Almada, C.P. Earnest, and R.B. Kreider. The effects of protein and amino acid supplementation on performance and training adaptations during ten weeks of resistance training. *J. Strength Cond. Res. 20(3):643–653. 2006.*—The purpose of this study was to examine the effects of whey protein supplementation on body composition, muscular strength, muscular endurance, and anaerobic capacity during 10 weeks of resistance training. Thirty-six resistance-trained males (31.0 ± 8.0 years, 179.1 ± 8.0 cm, 84.0 ± 12.9 kg, 17.8 ± 6.6%) followed a 4 days-per-week split body part resistance training program for 10 weeks. Three groups of supplements were randomly assigned, prior to the beginning of the exercise program, in a double-blind manner to all subjects: 48 g per day ($g \cdot d^{-1}$) carbohydrate placebo (P), 40 $g \cdot d^{-1}$ of whey protein + 8 $g \cdot d^{-1}$ of casein (WC), or 40 $g \cdot d^{-1}$ of whey protein + 3 $g \cdot d^{-1}$ branched-chain amino acids + 5 $g \cdot d^{-1}$ L-glutamine (WBG). At 0, 5, and 10 weeks, subjects were tested for fasting blood samples, body mass, body composition using dual-energy x-ray absorptiometry (DEXA), 1 repetition maximum (1RM) bench and leg press, 80% 1RM maximal repetitions to fatigue for bench press and leg press, and 30-second Wingate anaerobic capacity tests. No changes ($p > 0.05$) were noted in all groups for energy intake, training volume, blood parameters, and anaerobic capacity. WC experienced the greatest increases in DEXA lean mass (P = 0.0 ± 0.9; WC = 1.9 ± 0.6; WBG = −0.1 ± 0.3 kg, $p < 0.05$) and DEXA fat-free mass (P = 0.1 ± 1.0; WC = 1.8 ± 0.6; WBG = −0.1 ± 0.2 kg, $p < 0.05$). Significant increases in 1RM bench press and leg press were observed in all groups after 10 weeks. In this study, the combination of whey and casein protein promoted the greatest increases in fat-free mass after 10 weeks of heavy resistance training. Athletes, coaches, and nutritionists can use these findings to increase fat-free mass and to improve body composition during resistance training.

Key Words. casein, whey, branched-chain amino acids, glutamine, digestion speed

Introduction

Athletes involved in intense training have higher dietary protein needs than individuals who do not train (25, 33, 34, 40). Evidence exists to indicate that these types of athletes have protein needs that are one to two times that of the Recommended Daily Allowance (11, 33). Not all sources of protein are of the same quality, however. Protein sources that contain all of the essential amino acids are considered to be complete proteins, while those that do not contain all of the essential amino acids are considered to be incomplete (7). Protein sources with a higher concentration of the branched-chain amino acids (BCAAs) (e.g., leucine, isoleucine, valine) and the other essential amino acids are of a higher protein quality and are more effective at promoting protein synthesis (6). Recent improvements in the processing of proteins from food (e.g., soy protein, egg protein, casein, whey, etc.) in the form of nutritional supplements have resulted in high amounts of essential amino acids and low amounts of dietary fat.

In addition, intense training has been shown to decrease the availability of essential amino acids, which may slow the rate of tissue repair and growth (25). Athletes involved in intense training need to ingest enough high-quality protein and specific amino acids in their diet to maintain essential amino acid availability during training. Whey protein is a high-quality protein, which contains higher amounts of all the necessary amino acids when compared to other common food sources of protein (e.g., egg, soy, milk, etc.), and whey protein has been found to help promote protein synthesis and prevent protein degradation (11). Ingestion of whey protein has been found to cause a rapid transient increase in the plasma levels of amino acids, causing increased protein synthesis and little change in protein catabolism (5). On the other hand, casein is the other primary component of milk protein. Casein is considered to be a complete protein, but it does not have as high a concentration of the BCAAs and other amino acids as whey protein (7). Casein releases more slowly postprandially, which results in an attenuated release of its amino acids. Research indicates that this difference in protein digestion or speed of amino acid release results in differing levels of protein synthesis and breakdown (5). No studies, however, have been conducted to determine if the combination of whey plus casein protein would provide greater support, leading to a greater development of training adaptations during resistance training.

BCAA supplementation has been found to increase the availability and subsequent utilization of BCAA by the body (7, 25, 27, 28). BCAAs have been theorized to decrease catabolism during heavy training as well as to prevent increases in the free tryptophan to BCAA ratio

(7, 11, 25, 27), which has been associated with exercise-induced elevations in serotonin, central fatigue, and overtraining (7, 11, 24, 28, 30). BCAA supplementation prior to and during exercise has been purported to reduce catabolism and postpone central fatigue. Theoretically, BCAA supplementation during training may help improve training adaptations (37).

Glutamine is the most abundant amino acid found in skeletal muscle and plasma, and it comprises over 60% of the total free amino acid pool (19, 31, 39). Glutamine supplementation has been reported to enhance protein synthesis as well as improve immune function (7, 11, 27). Research on glutamine indicates it may help minimize the catabolic response by the body during times of heavy stress and trauma (e.g., surgery, burns, exercise, etc.). Glutamine supplementation during training has been theorized to promote muscle growth and decrease exercise-induced immunosuppression (20, 24, 25, 30, 36).

Individually, whey protein, BCAAs, and glutamine have been indicated to have anticatabolic and ergogenic properties. While different combinations of these purported ergogenic aids have been suggested, few have been investigated scientifically. The purpose of this study was to determine whether whey protein enhances training adaptations and whether adding BCAA and glutamine to whey protein promotes greater training adaptations in comparison to an isoenergetic amount of carbohydrate.

It was hypothesized that supplementation with 2 different formulations of protein supplementation would produce significantly greater training adaptations (strength and body composition) in the early stages of a resistance training protocol, in comparison to an isoenergetic placebo (37).

METHODS

Experimental Approach to the Problem

This study was conducted as a double-blind, placebo-controlled, randomized trial, with all subjects matched according to age and fat-free mass prior to beginning of the resistance training program. All subjects were tested at 0, 5, and 10 weeks to determine the changes in criterion variables. It was hypothesized a priori that protein supplementation would enhance training adaptations in comparison to placebo.

Subjects

Thirty-six apparently healthy male (group whey protein plus BCAAs plus L-glutamine [WBG]: $n = 15$, group whey protein plus casein [WC]: $n = 10$, group carbohydrate placebo [P]: $n = 11$) subjects between the ages of 18 and 50 years volunteered to participate in this study. As a result of an insufficient number ($n = 8$ for all 3 groups) of female participants who responded to our recruitment efforts and completed the protocol, only the male participants have been reported. Subjects were informed with regard to the experimental procedures and they signed informed consent statements and completed medical history forms in adherence with the human subjects guidelines of The University of Memphis and the American College of Sports Medicine prior to any data collection. All subjects' descriptive characteristics are presented in Table 1.

TABLE 1. Subject characteristics.*

Variable	Mean ± *SD*
Age (y)	31.0 ± 8.0
Height (cm)	179.2 ± 8.0
Weight (kg)	84.0 ± 12.9
Current training ($h \cdot wk^{-1}$)	7.7 ± 3.6
Days training ($days \cdot wk^{-1}$)	4.5 ± 0.8
Years of training (y)	5.3 ± 4.4

* $N = 36$.

Entrance Criteria

In order to participate in this study, subjects had to (a) sign statements indicating they had no current or past use of anabolic steroids; (b) be experienced with resistance training (>1 year of training), such that they were currently training >3 hours per week with a program that included both the bench press and leg press or squat exercises; (c) refrain from participating in any nonleisure endurance training for greater than 20 minutes at a time (e.g., running, cycling, swimming, etc.) for the entire study; (d) have not ingested creatine, HMB, thermogenics, or any ergogenic levels of any nutritional supplements for an 8-week period and have not taken any nutritional supplements or nonprescription drugs during the study; (e) agree to follow a predetermined workout program; (f) not have any existing medical conditions that would compromise participation in the study; and (g) avoid any regular nutritional practices that might confound the results of the study (i.e., vegetarianism, caloric restriction, food allergies, etc.).

Familiarization and Testing Sessions

Subjects participated in 1 familiarization session and 3 identical testing sessions. During the familiarization session, informed consent statements were signed and medical and exercise history forms were completed. A general physical examination (e.g., heart rate, blood pressure, breath sounds, reviewing medical history form, etc.), according to American College of Sports Medicine risk stratification criteria for low-risk individuals, was then completed by a research nurse. Subjects completed practice trials of all strength testing and anaerobic capacity equipment before being provided specific instructions by National Strength and Conditioning Association–Certified Strength and Conditioning Specialist individuals with regard to all exercise techniques, proper recording of training data (i.e., lifts performed, repetitions, amount of weight lifted, etc.), and how to properly record their nutritional intake. Specifically, participants were required to record all food and liquid consumed over a 4-day period, which consisted of 3 weekdays and 1 weekend day. Investigators provided detailed instruction on portion sizes as well as general information regarding food preparation. The investigators also clarified any questions regarding methods of the study.

Approximately 1 week separated the familiarization session from the baseline testing session (T0) to allow time for subjects to complete the nutritional log. Presupplementation assessments throughout baseline testing included (a) a 4-day dietary record; (b) an 8-hour fasting venous blood sample; (c) measurement of body mass, total body water via bioelectrical impedance analysis, and body composition assessment using dual-energy x-ray absorptiometry (DEXA); and (d) 1 repetition maximum (1RM) strength tests on the bench press and leg press. (After each respective 1RM test, subjects completed a maximal repetitions to fatigue test with 80% of their predeter-

TABLE 2. Composition of supplement groups.*

Nutrient	Carbohydrate placebo	Whey protein + casein protein	Whey protein + BCAA + glutamine
Calories (kcals)	192	212	212
Protein (g)	0	48	48
Carbohydrate (g)	48	~2	~2
Fat (g)	0	~1.5	~1.4
Alanine (mg)	—	2.17	1.93
Arginine (mg)	—	1.57	1.27
Aspartic acid (mg)	—	5.46	4.90
Cysteine (mg)	—	0.94	0.91
Glutamic acid (mg)	—	7.84	6.16
Glycine (mg)	—	0.94	0.80
Histidine (mg)	—	1.20	0.96
Isoleucine (mg)	—	2.93	702.56
Leucine (mg)	—	5.37	1,504.64
Lysine (mg)	—	4.55	3.93
Methionine (mg)	—	1.17	0.94
Phenylalanine (mg)	—	1.83	1.42
Proline (mg)	—	3.34	2.51
Serine (mg)	—	2.96	2.50
Threonine (mg)	—	3.72	3.38
Tryptophan (mg)	—	0.82	0.72
Tyrosine (mg)	—	1.74	1.30
Valine (mg)	—	2.89	802.44
Glutamine (mg)	—	—	5000
Total BCAAs (mg)	—	11.19	3,009.64
Total EAAs (mg)	—	24.48	3,020.99

* BCAAs = branched-chain amino acids; EAAs = essential amino acids.

mined 1RM.); and (e) anaerobic capacity using a computerized 30-second Wingate testing system on a cycle ergometer.

Subjects were matched according to fat-free mass and age. In a double-blind and randomized manner, subjects were assigned to supplement their normal diet for 10 weeks with 1 of 3 supplement groups, which are provided in Table 2. The control group was a P, which contained 48 g of carbohydrate. The 2 experimental groups were protein-based supplements. The first of these 2 groups (WC) consisted of 40 g of whey protein and 8 g of casein protein. The second experimental group consisted of 40 g of whey protein plus 5 g of L-glutamine plus 3 g of the BCAAs. To control for any differences in energy intake or nitrogen intake and subsequent training adaptations as a result of supplementation, all groups were isocaloric, in addition to the 2 protein groups being isonitrogenous.

All supplements were in powder form and were of similar smell, consistency, and texture. In a blinded fashion, all supplements were packaged in individual foil packets and verified for macronutrient content as well as glutamine and BCAA content by Covance Laboratories (Madison, WI). Collecting and counting empty supplement packets verified subject compliance to supplementation protocol. Subjects were instructed to report to the research nurse at the end of each week of training to report the frequency or severity of any possible side effects as well as their compliance to the training and supplementation protocols.

The training program consisted of 4 workouts per week (2 upper-body and 2 lower-body workouts), which primarily utilized multijoint exercises that targeted the major muscle groups. Table 3 provides a layout of the exact training program.

All subjects were required to perform each exercise to the point that they reached muscular failure at the last repetition of each set (46). Subjects were instructed to rest for approximately 1 minute between sets and for 2 minutes between each exercise. All workouts were completed at each participant's own training facility, and all training compliance and supervision was verified by having a training partner, fitness instructor, or personal trainer sign off on each workout completed.

TABLE 3. Resistance training program.

Weeks	Monday, Thursday*†	Tuesday, Friday*†
1–4	Bench press, 3 × 10	Leg press, 3 × 10
	Chest flies, 3 × 10	Leg extensions, 3 × 10
	Lat pull, 3 × 10	Deadlift, 3 × 10
	Seated row, 3 × 10	Lunges, 3 × 10
	Shoulder press, 3 × 10	Lying leg curls, 3 × 10
	Shoulder shrugs, 3 × 10	Heel raises, 3 × 10
	Bicep curls, 3 × 10	Ab crunches, 3 × 25
	Tricep extensions, 3 × 10	
5–8	Bench press, 3 × 8	Leg press, 3 × 8
	Chest flies, 3 × 8	Leg extensions, 3 × 8
	Lat pull, 3 × 8	Deadlift, 3 × 8
	Seated row, 3 × 8	Lunges, 3 × 8
	Shoulder press, 3 × 8	Lying leg curls, 3 × 8
	Shoulder shrugs, 3 × 8	Heel raises, 3 × 8
	Bicep curls, 3 × 8	Ab crunches, 3 × 25
	Tricep extensions, 3 × 8	
9, 10	Bench press, 3 × 6	Leg press, 3 × 6
	Chest flies, 3 × 6	Leg extensions, 3 × 6
	Lat pull, 3 × 6	Deadlift, 3 × 6
	Seated row, 3 × 6	Lunges, 3 × 6
	Shoulder press, 3 × 6	Lying leg curls, 3 × 6
	Shoulder shrugs, 3 × 6	Heel raises, 3 × 6
	Bicep curls, 3 × 6	Ab crunches, 3 × 25
	Tricep extensions, 3 × 6	

* One minute rest between sets.
† Two minutes rest between exercises.

Subjects reported back to the lab after 5 (T1) and 10 weeks (T2) of training and supplementation for follow-up assessments. Prior to testing sessions, subjects were instructed to complete a 4-day dietary analysis during weeks 2, 5, 8, and 10 to ensure that dietary habits did not change throughout the study. All dietary logs and training logs were turned in, and subjects underwent follow-up testing that was identical to the baseline (presupplementation) testing session. Specifically, subjects donated an 8-hour fasting venous blood sample and had body mass, total body water, and body composition determined. Subjects then determined their 1RM to evaluate any changes in maximal strength and completed maximal repetitions to fatigue tests with 80% of their predetermined 1RM to determine any changes in local muscular endurance. Lastly, subjects completed a 30-second Wingate anaerobic capacity test.

Procedures

Supplements were prepared in powder form with identical texture, taste, and appearance and were independently packaged and labeled in single-serving foil packets for double-blind administration. Subjects were instructed to mix the supplement with water, juice, or milk and to ingest the solution as quickly as possible, but ideally within 2 hours, following their workouts on training days and in the morning (~9:00 AM) of nontraining days. Subjects were instructed to maintain their normal diet throughout the supplementation and training period. All dietary records were analyzed by the same individual, who had several years of experience entering dietary records using the ESHA Food Processor software version 7.8 (ESHA Research, Salem, OR). Unavailable foods were entered into the database from the manufacturer labels. The 4-day average of caloric intake, carbohydrate intake, protein intake, and fat intake was computed for later statistical analysis.

Subjects reported to the lab after avoiding strenuous exercise for 48 hours and fasting for 8 hours prior to each testing session. Subjects generally reported to the lab between 8:00 AM and 10:00 AM on the day of their baseline testing, and all follow-up tests were completed at similar times to help control for any changes in diurnal variation. Subjects then donated approximately 25 ml (4 teaspoons) of blood via venipuncture of a vein in the forearm using standard procedures. Two 10-ml serum separation vacutainers and one 5-ml anticoagulant tube containing K_3 vacutainer was inserted for blood collection using multiple sample phlebotomy techniques. Serum from the serum separation tube was centrifuged at 2,360 $\times$ g for 10 minutes using a Biofuge 17R centrifuge (Heraeus Inc., Osterode, Germany). Serum from both serum separation tubes was transferred into 3 microcentrifuge tubes and frozen at -80° C for subsequent analysis. Remaining serum was transferred from the serum separation tube and placed into a sterile collection tube. Serum and whole blood samples were refrigerated and sent to Quest Diagnostics Labs (Minneapolis, MN). A complete 31-panel clinical chemistry profile and various markers of muscle or protein breakdown (aspartate aminotransferase, alanine aminotransferase, creatine kinase, urea nitrogen, creatinine, and total protein) was run on serum samples using the Technicon DAX model 96-0147 automated chemistry analyzer (Technicon, Inc., Terry Town, NY) following standard clinical procedures. Whole blood cell counts with percent differentials were run on whole blood samples using a Coulter STKS automated analyzer using standard procedures (Coulter, Inc., Hialeah, FL). These analyzers were calibrated daily to controls according to manufacturer's recommendations and federal guidelines for clinical diagnostic laboratories. Test to test reliability of performing these assays ranged from 2–6% for individual assays, with an average variation of $\pm$3% (Quest Diagnostics). Samples were run in duplicate to verify results if the observed values were outside control values or clinical norms according to standard procedures.

Subject body weight was obtained using a calibrated Healthometer digital strain gauge electronic scale (Bridgeview, IL) with a precision of $\pm$0.02 kg. Total body water was estimated using a Valhalla Bioelectrical Impedance Analyzer (Valhalla Scientific, San Diego, CA) (45). Whole-body (excluding cranium) composition was estimated, following procedures described previously (21, 22), by certified investigators using a Hologic QDR-4500W DEXA using Hologic software (version 9.80C; Waltham, MA). This test evaluates body composition and body density by scanning the entire body with a low dose of radiation, a procedure that takes approximately 6 minutes. An analysis of the subject's fat mass, soft tissue (muscle) mass, and bone mass was provided and was used to determine body composition changes throughout the duration of the study. The DEXA scans regions of the body (right arm, left arm, trunk, right leg, left leg) to determine bone mass, fat mass, and lean mass within each region. The scanned bone, fat, and lean mass for each region are then subtotaled to determine whole-body (excluding cranium) values. Percent body fat was determined by dividing the amount of fat mass by the total scanned mass (bone mass, fat mass, and lean mass). DEXA has been found to be a highly reliable method of determining soft-tissue body composition and percent body fat for whole body and all respective regions determined (14, 18, 21, 22, 35).

Manufacturer and state-certified investigators performed all DEXA analysis, and quality control calibration procedures were performed on a spine phantom (Hologic X-CALIBER Model DPA/QDR-1; Hologic, Inc., Waltham, MA) prior to each testing session according to procedures previously described (1). Mean coefficients of variation in bone mineral content and bone mineral density measurements on the spine phantom ranged between 0.41–0.55% throughout the life of the unit. Subjects were positioned on the DEXA table using standardized methods for each test. Test-retest reliability studies performed on male athletes with this DEXA machine yielded mean deviation for total bone mineral content and total fat free and soft tissue mass of 0.31%, with a mean intra-class correlation of 0.985 (1).

After body composition analysis, subjects performed 1RMs and maximal repetitions to fatigue tests using 80% of their predetermined 1RM with both the bench press and leg press. A warm up of 2 sets of 10 repetitions at ~50% 1RM was followed by 3 to 5 progressive 1RM attempts with 2 minutes rest in between attempts using a standard 20-kg barbell and a standard bench found in many fitness facilities. Grip width was recorded and the weight plates used were standardized between trials. Subjects were required to maintain good lifting form (i.e., feet in contact with the floor, buttocks remaining in contact with bench, no bouncing of the bar off of the chest)

TABLE 4. Dietary intake for the whey protein + glutamine + branched-chain amino (WBG)-, whey protein + casein (WC)-, and carbohydrate placebo (P)-supplemented subjects given as means ± *SD*.

Variable	Group*	Week 0	Week 2	Week 5	Week 8	Week 10
Energy intake	WBG	38.8 ± 15.1	34.2 ± 8.3	31.6 ± 12.9	33.2 ± 13.1	36.3 ± 12.1
(kcal·kg^{-1}·d^{-1})	WC	30.8 ± 3.7	35.5 ± 8.9	36.1 ± 8.8	33.7 ± 10.4	32.4 ± 5.1
	P	39.8 ± 11.4	36.8 ± 10.9	33.4 ± 7.6	30.2 ± 5.3	29.2 ± 6.2
Carbohydrate	WBG	4.4 ± 2.0	3.7 ± 1.2	3.2 ± 1.6	3.7 ± 2.0	4.0 ± 1.9
intake	WC	3.3 ± 0.5	3.6 ± 1.1	3.7 ± 0.8	3.8 ± 1.4	3.8 ± 1.0
(g·kg^{-1}·d^{-1})	P	4.9 ± 1.4	4.8 ± 1.4	4.2 ± 1.0	3.9 ± 0.8	3.8 ± 0.8
Protein intake	WBG	2.3 ± 0.5†	2.1 ± 0.4†	2.1 ± 0.8†	2.0 ± 0.4†	2.1 ± 0.5†
(g·kg^{-1}·d^{-1})	WC	2.1 ± 0.3†	2.5 ± 0.7†	2.5 ± 0.9†	2.3 ± 0.8†	2.2 ± 0.6†
	P	1.6 ± 0.5	1.6 ± 0.7	1.7 ± 0.5	1.5 ± 0.4	1.4 ± 0.3
Fat intake	WBG	1.3 ± 0.7	1.2 ± 0.4	1.1 ± 0.6	1.2 ± 0.6	1.3 ± 0.6
(g·kg^{-1}·d^{-1})	WC	1.0 ± 0.3	1.2 ± 0.4	1.3 ± 0.4	1.0 ± 0.4	0.9 ± 0.3
	P	1.5 ± 0.5	1.2 ± 0.5	1.1 ± 0.4	1.0 ± 0.2	0.9 ± 0.3

* Group WBG: $n = 15$; Group WC: $n = 10$; Group P: $n = 11$.
† WBG, WC > P ($p < 0.05$).

during all lifts. Once bench press 1RM was determined, subjects were given 5 minutes to rest and then completed a maximal repetitions to fatigue test with 80% of their predetermined 1RM with the bench press. Subjects were then given 5 minutes of rest, and leg press 1RM was performed on an AMF hip sled (AMF, Jefferson, IA). Subjects were positioned flat on their back in an adjustable back and shoulder support, which was adjusted to allow each subject to be positioned with their thighs approximately 1–2 inches from their torso and their knees at an angle approximately equal to 90° with their feet comfortably positioned. Back and shoulder support, foot placement, and weight plates used were standardized between testing sessions. Subjects were required to maintain good lifting form (hands and forearms at their sides with lower back flat on the back pad). Subjects typically used 4 to 6 attempts to achieve their leg press 1RM while appropriately adjusting the weight, with 2 minutes of rest between attempts. Upon identifying the 1RM, 5 minutes of rest were given before subjects again completed maximal repetitions to fatigue test with 80% of their predetermined 1RM using the leg press. During all testing sessions, subjects were equally advised using standardized lifting criteria (12, 23, 46) and they were encouraged by the testers, who were all certified strength and conditioning specialists. Test-to-test reliability of performing these strength tests in our lab on resistance-trained subjects has yielded low mean coefficients of variation and high reliability for the bench press (1.9%, intraclass $r = 0.94$) and hip sled or leg press (0.7%, intraclass $r = 0.91$) (26).

Subjects completed a 30-second Wingate anaerobic capacity sprint test on a cycle ergometer. The sprint tests were performed on a computerized $CardiO_2$ cycle ergometer equipped with toe clips at a standardized work rate of 3.85 J·kg body mass^{-1}·revolution^{-1} ($ErgometR_x$ Corp., St. Paul, MN). Seat position and height were recorded and standardized between trials. The ergometer was connected via an RS232 parallel interface to a Dell 466/Le Optiplex computer (Dell Computer Corp., Austin, TX) using $ErgometR_x$ Cardioscribe and Exerscribe software ($ErgometR_x$ Corp.). Crank frequency was measured using a crystal referenced optic encoder with a precision range of 0–200 revolutions·min^{-1} and an accuracy of ±1 revolutions·min^{-1}. Pedal torque was determined by a calibrated strain gauge with a range of 0 to 2,000 W and an accuracy of ±1%. Data were collected and downloaded into the computer at 0.5-second intervals. Correlation coefficients of test-retest reliability in our lab for Wingate sprints tests is $r = 0.96 \pm 3$ (29).

Statistical Analyses

A priori power analysis revealed power values of 0.16, 0.78, and 1.03 for small (0.25), moderate (0.75), and large effect sizes (1.25), respectively, for the n size used in this study. These findings indicate that the n size used in the investigation was of an appropriate level to detect significant differences among groups. As a result of the insufficient number of female participants ($n = 8$) who volunteered and completed the protocol, statistical analysis was completed only on the 36 remaining male participants. All criterion-dependent variables were analyzed by separate univariate analysis of variance (ANOVA) with repeated measures. Dietary intake (caloric intake, carbohydrate, protein, and fat) were evaluated by individual 2-way (group × test) ANOVA using the SPSS for Windows statistical package (version 11.5; SPSS, Inc., Chicago, IL). Data were considered significantly different when $p \leq 0.05$. Post-hoc procedures were conducted when necessary using Tukey post-hoc procedures. Delta scores (post-pre values) were calculated on selected variables and analyzed by 1-way ANOVA for further interpretation of these data, which are presented graphically. Data are presented as means ± *SD*.

RESULTS

Nutritional Data

All nutritional data are represented relative to body weight in kilograms and are presented in Table 4. No significant differences ($p > 0.05$) among groups for total calories ($p = 0.822$; WBG: 34.8 ± 12.3 kcals·kg^{-1}·d^{-1}, WC: 33.7 ± 7.7, P: 33.9 ± 9.1), carbohydrates ($p = 0.057$; WBG: 3.8 ± 1.7 g·kg^{-1}·d^{-1}, WC: 3.6 ± 1.0, P: 4.3 ± 1.1), or fat ($p = 0.251$; WBG: 1.24 ± 0.6 g·kg^{-1}·d^{-1}, WC: 1.9 ± 0.4, P: 1.1 ± 0.4). A significant group difference ($p < 0.001$) for protein intake was found. Least significant difference post-hoc analysis revealed that the WBG (2.12 ± 0.5 g·kg^{-1}·d^{-1}, $p < 0.001$) and WC (2.33 ± 0.6 g·kg^{-1}·d^{-1}, $p < 0.001$) groups had higher protein intakes than did the P group (1.57 ± 0.5 g·kg^{-1}·d^{-1}). This finding is expected, as both protein groups supplemented their diets

TABLE 5. Body composition changes for the whey protein + glutamine + branched chain amino acids (WBG)-, whey protein + casein (WC)-, and carbohydrate placebo (P)-supplemented subjects given as means ± *SD*.

Variable	Group*	Week 0 (T0)	Week 5 (T1)	Week 10 (T2)	Significance	
Body mass (kg)	WBG	85.3 ± 14.8	85.1 ± 14.4	85.3 ± 14.6	Group	0.933
	WC	81.2 ± 12.7	82.8 ± 12.6	84.2 ± 12.2	Time	0.054
	P	85.1 ± 11.0	85.5 ± 11.3	85.3 ± 10.9	G × T	0.094
DEXA total scanned mass (kg)	WBG	80.4 ± 14.1	80.3 ± 14.2	80.5 ± 14.1	Group	0.935
	WC	77.5 ± 12.5	79.1 ± 12.5	79.6 ± 12.1	Time	0.032†
	P	80.2 ± 10.5	80.8 ± 10.8	80.5 ± 10.4	G × T	0.067
DEXA lean mass (kg)	WBG	62.7 ± 11.1	62.7 ± 11.1	62.6 ± 10.8	Group	0.944
	WC	61.3 ± 8.6	63.1 ± 8.2‡	63.2 ± 8.0‡	Time	0.051
	P	63.5 ± 8.2	64.2 ± 8.5	63.5 ± 7.3	G × T	0.026
DEXA fat mass (kg)	WBG	15.3 ± 7.5	15.3 ± 7.1	15.5 ± 7.0	Group	0.816
	WC	13.8 ± 6.6	13.6 ± 6.9	13.9 ± 6.6	Time	0.451
	P	14.2 ± 5.5	14.1 ± 5.7	14.4 ± 6.0	G × T	0.990
DEXA bone mineral content (kg)	WBG	2.38 ± 0.5	2.41 ± 0.6	2.39 ± 0.5	Group	0.749
	WC	2.45 ± 0.4	2.45 ± 0.4	2.47 ± 0.4	Time	0.055
	P	2.54 ± 0.5	2.55 ± 0.5	2.55 ± 0.6	G × T	0.967
DEXA fat-free mass (kg)	WBG	65.1 ± 11.5	65.1 ± 11.6	65.0 ± 11.3	Group	0.937
	WC	63.8 ± 8.9	65.5 ± 8.5‡	65.6 ± 8.3‡	Time	0.046†
	P	66.0 ± 8.8	66.8 ± 9.0	66.1 ± 7.8	G × T	0.026
DEXA % body fat (%)	WBG	18.8 ± 7.3	18.8 ± 6.9	19.0 ± 6.7	Group	0.756
	WC	17.3 ± 6.4	16.7 ± 6.5	17.1 ± 6.2	Time	0.974
	P	17.5 ± 6.1	17.2 ± 6.2	17.5 ± 6.3	G × T	0.734

* Group WBG: $n = 15$; Group WC: $n = 10$; Group P: $n = 11$.
† Significant main effect for time ($p < 0.05$).
‡ Greater increase over time for WC compared to P and WBG ($p < 0.05$).

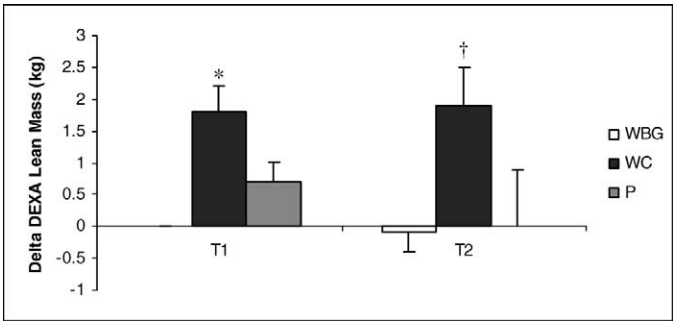

FIGURE 1. Delta value change in dual-energy x-ray absorptiometry (DEXA) lean mass (kg) at 0, 5, and 10 weeks. Data are mean ± *SD*. WBG = whey protein + branched-chain amino acids (BCAA) + glutamine group ($n = 15$); WC = whey protein + casein protein group ($n = 10$); P = carbohydrate placebo group ($n = 11$). * = Significant increase from T0 ($p < 0.05$); † = greater increase over time for WC compared to P and WBG ($p < 0.05$).

with an additional 48 g of protein, compared to no additional protein intake for the carbohydrate placebo group.

Training Volume

Total training volume (sets × reps × load) was calculated for all subjects. Statistical analysis revealed that no significant differences existed ($p > 0.05$) between any of the groups for both upper-body ($p = 0.125$; WBG: 401 ± 130 kg, WC: 503 ± 161 kg, P: 400 ± 115 kg) and lower-body ($p = 0.842$; WBG: 479 ± 185 kg, WC: 508 ± 121 kg, P: 471 ± 153 kg) total training volume.

Medical Monitoring

No significant clinical side effects, related or unrelated to the study, were reported to the research nurse by any subject throughout the entire course of the study. All subjects tolerated both the training and supplementation protocols without any problems.

Body Composition

Table 5 presents body composition, body mass, and bone density for all 3 groups. Significant increases across time for all 3 groups were seen for DEXA total scanned mass, DEXA fat-free mass, and DEXA lean mass. Significant interactions and subsequent post-hoc analysis of the body composition data revealed that the WC group was the only group that showed significant increases ($p < 0.05$) during the 2 follow-up testing sessions (T1 and T2) compared to the initial testing session (T0) for body mass, DEXA total scanned mass, DEXA fat-free mass, and DEXA lean mass. Delta values were graphed to highlight the changes in DEXA lean mass and DEXA fat-free mass and can be seen in Figures 1 and 2, respectively. No other significant differences were seen between any other body composition or bone mineral variables.

Strength Measures

Table 6 presents the strength measures for bench press 1RM, bench press repetitions to fatigue at 80% 1RM, leg press 1RM, and leg press repetitions to fatigue at 80% 1RM for all 3 groups. Significant increases over time, with no group differences, were found in bench press 1RM, leg press 1RM, and leg press lifting volume, indicating a positive adaptation to the resistance training program. Delta values were graphed to highlight the changes in bench press 1RM and leg press 1RM and can be seen in Figures 3 and 4, respectively. No other significant differences were found.

Anaerobic Capacity

Table 6 presents all Wingate anaerobic capacity data (e.g., peak power, total work, and fatigue index). No sig-

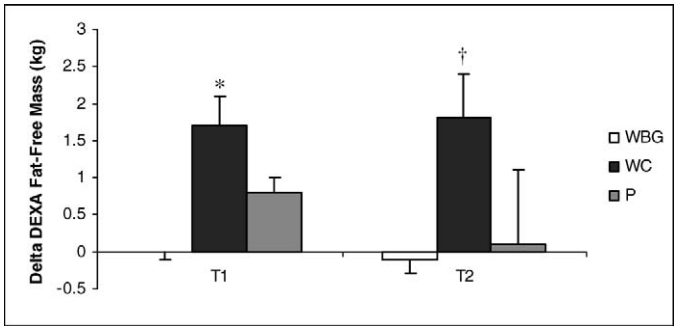

FIGURE 2. Delta value change in dual-energy x-ray absorptiometry (DEXA) fat-free mass (kg) at 0, 5, and 10 weeks. Data are mean ± *SD*. WBG = whey protein + branched-chain amino acids (BCAA) + glutamine group (n = 15); WC = whey protein + casein protein group (n = 10); P = carbohydrate placebo group (n = 11). * = Significant increase from T0 ($p < 0.05$); † = greater increase over time for WC compared to P and WBG ($p < 0.05$).

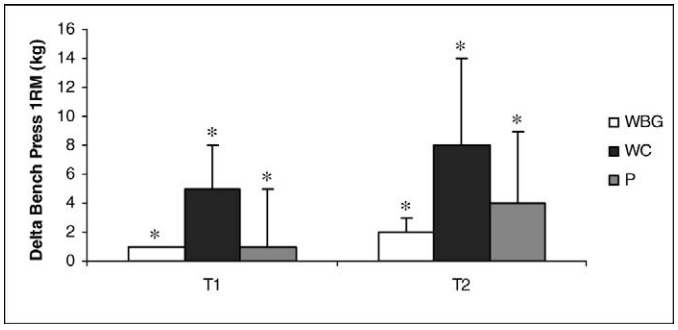

FIGURE 3. Delta value change in bench press 1 repetition maximum (1RM) (kg) at 0, 5, and 10 weeks. Data are mean ± *SD*. WBG = whey protein + branched-chain amino acids (BCAA) + glutamine group (n = 15); WC = whey protein + casein protein group (n = 10); P = carbohydrate placebo group (n = 11). * = Significant increase from T0 ($p < 0.05$).

nificant time or interaction effects ($p > 0.05$) were noted for any of the anaerobic capacity variables.

Hematological Analysis

No significant differences were observed between groups in total protein, albumin, globulin, glucose, electrolytes, liver enzymes, lipid profiles, total bilirubin, hemoglobin, hematocrit, red blood cells, or white blood cells. Table 7 presents selected markers of catabolism (creatinine, blood urea nitrogen, total protein, and uric acid) and muscle and liver enzymes (alanine aminotransferase, aspartate aminotransferase, creatine kinase, lactate dehydrogenase, and gamma-glutamyl transferase). A significant main effect for time (p = 0.006) was seen for uric acid; in regard to this variable, all groups were found to decrease across time. No significant differences were observed among groups with regard to these variables. A few hematological variables did significantly change (p = 0.041, basophil %; p = 0.021, mean corpuscle volume; and p = 0.038, red cell dimension width). However, differences among groups were minimal, not clinically significant, and within clinically accepted normative values (10).

DISCUSSION

This study evaluated whether 2 different forms of protein supplementation (whey protein with casein or whey protein with BCAA and glutamine) would elicit greater changes in clinical blood panels, body composition, strength changes, or anaerobic capacity in subjects participating in a 4 d·wk^{-1}, 10-week, total-body resistance training program in comparison to subjects who ingested an isoenergetic amount of carbohydrate. All of the tests

TABLE 6. Maximal strength (1 repetition maximum [1RM]), lifting volume (reps × weight [kg]) and Wingate anaerobic capacity changes for the whey protein + glutamine + branched-chain amino acids (WBG)-, whey protein + casein (WC)-, and carbohydrate placebo (P)-supplemented subjects given as means ± *SD*.

Variable	Group*	Week 0 (T0)	Week 5 (T1)	Week 10 (T2)	Significance	
Bench press 1RM (kg)	WBG	106 ± 30	107 ± 30	108 ± 29	Group	0.889
	WC	99 ± 22	104 ± 19	107 ± 16	Time	0.005†
	P	101 ± 25	102 ± 21	105 ± 20	G × T	0.258
Bench press volume (reps·kg)	WBG	735 ± 214	862 ± 314	816 ± 306	Group	0.335
	WC	669 ± 268	719 ± 231	683 ± 182	Time	0.785
	P	734 ± 128	681 ± 229	665 ± 189	G × T	0.166
Leg press 1RM (kg)	WBG	204 ± 51	222 ± 60	217 ± 52	Group	0.525
	WC	185 ± 47	196 ± 39	198 ± 37	Time	0.004†
	P	199 ± 56	211 ± 32	205 ± 37	G × T	0.722
Leg press volume (reps·kg)	WBG	2,862 ± 959	3,518 ± 1,422	3,182 ± 1,253	Group	0.802
	WC	3,115 ± 1,107	3,574 ± 1,775	3,756 ± 1,426	Time	0.003†
	P	3,189 ± 1,223	3,386 ± 1,209	3,660 ± 1,069	G × T	0.669
Peak power (W)	WBG	904 ± 244	915 ± 213	846 ± 134	Group	0.769
	WC	883 ± 102	900 ± 98	937 ± 122	Time	0.885
	P	925 ± 192	948 ± 167	917 ± 106	G × T	0.278
Total work (J)	WBG	237 ± 48	222 ± 55	220 ± 48	Group	0.950
	WC	212 ± 42	226 ± 39	226 ± 50	Time	0.935
	P	221 ± 55	232 ± 51	227 ± 46	G × T	0.124
Fatigue index (%)	WBG	64.3 ± 11.3	65.8 ± 10.8	63.6 ± 13.5	Group	0.856
	WC	66.5 ± 12.8	63.1 ± 11.6	64.6 ± 11.4	Time	0.834
	P	67.7 ± 11.3	63.5 ± 13.4	69.2 ± 17.4	G × T	0.777

* Group WBG: n = 15; Group WC: n = 10; Group P: n = 11.
† Significant main effect for time ($p < 0.05$).

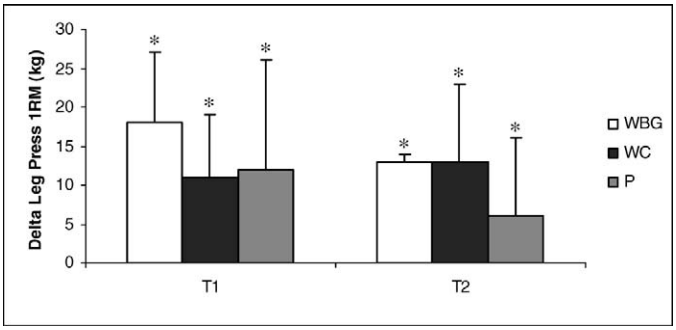

FIGURE 4. Delta value change in leg press 1 repetition maximum (1RM) (kg) at 0, 5, and 10 weeks. Data are mean ± *SD*. WBG = whey protein + branched-chain amino acids (BCAA) + glutamine group ($n = 15$); WC = whey protein + casein protein group ($n = 10$); P = carbohydrate placebo group ($n = 11$). * = Significant increase from T0 ($p < 0.05$).

employed were widely used for these purposes (12, 13, 18, 21). The major findings from this study were that significant changes in body composition variables (DEXA total scanned mass, DEXA lean mass, and DEXA fat-free mass) were significantly increased in the whey protein with casein group compared to the other 2 groups. Further, significant increases across time in all groups for bench press 1RM, leg press 1RM, and leg press volume indicate that the resistance training protocol utilized was an effective program (Table 3) in terms of stimulating increases in strength in previously resistance-trained athletes. In this regard, it is possible that because of the unique layout (split-body program) of the resistance training protocol, an effective stimulus was provided, which resulted in the physiological improvements seen in this investigation.

It was hypothesized that supplementation with 2 different formulations of protein supplementation would produce significantly greater training adaptations (strength and body composition) through greater prevention of catabolism and an improved anabolic response (37). Results from the present study provide only partial support of this hypothesis. In this regard, these findings support the hypothesis that protein supplementation during training may improve training adaptations, in comparison to ingesting an isoenergetic amount of carbohydrate (32, 40, 41). It is important to note, however, that the nutritional intervention provided (essentially 40 g of protein) in this investigation did not have to be in the form of a nutritional supplement but could instead be attained through greater ingestion of traditional foods. Although the authors do not disagree with this notion, the convenience of supplements, their comparative expense, and the lack of concomitant dietary fat (especially saturated fat) continue to make protein supplementation an important consideration. Additionally, the results of the present study provide further evidence that different types of protein may have varying effects on training adaptations (25, 33, 40). The present findings contrast with the results of Colker and colleagues (8), who reported that inclusion of 5 g of glutamine and 3 g of BCAAs to 40 g of

TABLE 7. Selected markers of catabolism and muscle and liver enzymes for the whey protein + glutamine + branched-chain amino acids (WBG)-, whey protein + casein (WC)-, and carbohydrate placebo (P)-supplemented subjects given as mean ± *SD*.

Variable	Group*	Week 0 (T0)	Week 5 (T1)	Week 10 (T2)	Significance	
Creatine kinase ($U \cdot L^{-1}$)	WBG	262 ± 256	269 ± 333	247 ± 149	Group	0.937
	WC	247 ± 119	215 ± 107	247 ± 155	Time	0.807
	P	213 ± 122	258 ± 231	255 ± 238	G × T	0.794
Lactate dehydrogenase ($U \cdot L^{-1}$)	WBG	144 ± 18	146 ± 29	146 ± 14	Group	0.165
	WC	175 ± 63	149 ± 21	160 ± 21	Time	0.488
	P	156 ± 33	142 ± 31	154 ± 32	G × T	0.652
Aspartate aminotransferase ($U \cdot L^{-1}$)	WBG	24.9 ± 7.9	24.1 ± 9.4	23.6 ± 6.8	Group	0.342
	WC	41.4 ± 7.6	23.6 ± 5.0	27.1 ± 7.7	Time	0.156
	P	25.7 ± 7.3	24.4 ± 7.8	24.7 ± 5.8	G × T	0.293
Alanine aminotransferase ($U \cdot L^{-1}$)	WBG	23.4 ± 10.1	19.4 ± 6.0	21.3 ± 7.6	Group	0.461
	WC	27.7 ± 17.3	22.1 ± 6.8	27.9 ± 7.9	Time	0.177
	P	28.1 ± 19.9	23.0 ± 12.0	23.4 ± 9.3	G × T	0.508
Total protein ($g \cdot dL^{-1}$)	WBG	7.14 ± 0.38	7.09 ± 0.46	7.26 ± 0.43	Group	0.562
	WC	7.30 ± 0.56	7.13 ± 0.55	7.24 ± 0.52	Time	0.754
	P	7.40 ± 0.53	7.18 ± 0.38	7.35 ± 0.55	G × T	0.096
GGT ($U \cdot L^{-1}$)	WBG	25.1 ± 10.5	22.5 ± 7.5	24.2 ± 11.4	Group	0.756
	WC	25.1 ± 8.3	26.8 ± 12.3	29.7 ± 16.2	Time	0.578
	P	32.1 ± 24.7	26.8 ± 12.2	29.6 ± 19.3	G × T	0.062
Creatinine ($mg \cdot dL^{-1}$)	WBG	1.12 ± 0.19	1.19 ± 0.12	1.14 ± 0.15	Group	0.504
	WC	1.18 ± 0.10	1.17 ± 0.08	1.22 ± 0.14	Time	0.100
	P	1.10 ± 0.18	1.14 ± 0.14	1.15 ± 0.14	G × T	0.871
BUN ($mg \cdot dL^{-1}$)	WBG	16.11 ± 5.0	20.46 ± 6.6	17.27 ± 4.5	Group	0.309
	WC	16.64 ± 4.6	17.90 ± 5.0	17.65 ± 3.4	Time	0.258
	P	15.00 ± 3.7	16.17 ± 3.8	15.09 ± 3.9	G × T	0.776
BUN : creatinine ratio	WBG	14.81 ± 4.8	17.53 ± 5.8	15.20 ± 3.6	Group	0.382
	WC	14.36 ± 4.8	15.45 ± 4.5	14.75 ± 3.8	Time	0.980
	P	13.81 ± 3.12	14.45 ± 4.1	13.09 ± 3.8	G × T	0.753
Uric acid ($mg \cdot dL^{-1}$)	WBG	4.97 ± 1.40	4.79 ± 1.33†	4.61 ± 1.22†	Group	0.543
	WC	5.54 ± 1.40	5.12 ± 0.99†	4.99 ± 1.40†	Time	0.006
	P	5.15 ± 1.60	4.72 ± 1.02†	4.40 ± 0.77†	G × T	0.678

* Group WBG: $n = 15$; Group WC: $n = 10$; Group P: $n = 11$.

whey protein promoted greater gains in lean mass after 10 weeks of resistance training. However, these researchers did not employ a control group, and the protein groups studied were not isonitrogenous. Consequently, it is difficult to draw conclusions from this study, and it is also difficult to make direct comparisons between investigations.

In the current study, significant improvements in both lean mass and fat-free mass were found for the whey protein with casein group, as determined by DEXA, compared to the other 2 groups. While this finding was unexpected at the time the study was conducted, it is not surprising upon consideration of some recent findings. It has been well documented that whey and casein possess different patterns of amino acid release, which has been shown to greatly affect the extracellular amino acid concentrations and the resulting levels of protein synthesis and protein breakdown (2, 5–7, 11). In this regard, Bohe and colleagues (3, 4) have concluded that the uptake ability by the muscle for amino acids may have an upper limit, and they have also concluded that the stimulation of protein synthesis may be largely a result of the extracellular concentration of amino acids, which provides additional evidence to explain the greater increases in body composition seen by the whey protein with casein group. In contrast to this hypothesis, Tipton and colleagues used acute 20-g doses of whey and casein protein after a single bout of intense resistance training and concluded that there was no difference in protein balance between the groups (43). Although their study did support the notion of different patterns of amino acid release, this difference did not result in a significant difference in the amount of muscle hypertrophy (43) after 1 acute bout of resistance exercise. Nonetheless, results from previous studies and the current study continue to provide evidence that a combination of whey and casein protein may be an effective means to promote improvements in body composition.

Interestingly, the present study did not support some previous findings that laid the theoretical groundwork for the inclusion of glutamine and BCAAs in protein supplements. Many studies have cited glutamine as having anticatabolic properties, but these studies often used higher doses (>20 g·d^{-1}) (16, 17, 38) and clinically catabolic subjects (9, 15), compared to those used in the current study. It is possible that the daily glutamine needs of resistance-trained athletes might be more than those provided in this study or that the amino acid levels of glutamine naturally present in the intact forms of whey and casein protein provided enough glutamine so that the additional provision in the WBG group provided no further effect (44). In this regard, whey and casein proteins are high-quality, complete protein sources with naturally occurring high levels of the essential amino acids and glutamine. The average dose of essential amino acids for either the WC or WBG group provided approximately 23–26.5 g of these amino acids (7). In support of this suggestion, Tipton and colleagues (44) have exhibited that ingestion of either 40 g of only essential amino acids or 40 g of a mixed amino acid solution that contained 21.4 g of the essential amino acids 1 hour after a standard resistance exercise bout resulted in a similar 2.5-fold increase in amino acid concentrations and a 70% increase in protein synthesis. They concluded that no further benefit was seen from the additional ~19 g of essential amino acids from the solution containing 40 g of only essential amino acids (44). In the present study, the 2 protein groups used contained slightly more amino acids than were used in the Tipton study, indicating that the additional glutamine and branched-chain amino acids in the WBG group might have provided no further benefit.

Additionally, in the past several years timing of nutrients has become a highly researched area (2, 6, 42, 47). In the present study subjects were asked (although there was no way of verifying that they had done so) to ingest their supplements after each resistance training workout and in the mornings of nontraining days. Consequently, the subjects in this study may not have been taking full advantage of the postexercise time period, known to be highly anabolic in nature (2, 6, 42, 47). However, much more research needs to be done to make specific conclusions with regard to timing of nutrient administration, the magnitude of impact on training adaptations, and how these factors relate to the findings from the present study.

In conclusion, supplementing the diet with a protein supplement containing 40 g·d^{-1} of whey and 8 g·d^{-1} of casein while resistance training improves the training adaptations seen in comparison to subjects who ingest a carbohydrate placebo or a similar protein group. It is important to note that these findings apply for a 10-week time period and reflect the initial physiological response of completing this type of resistance training program while supplementing with protein. Moreover, the 2 types of protein formulations did seem to have varying effects on body composition variables. These findings indicate that the potential ergogenic value of protein supplementation during the early phases of training may vary depending on the specific amino acid composition of the supplement. More research is needed to examine the effects of different types of supplementation as well as of the timing of amino acid and protein supplementation on training adaptations.

PRACTICAL APPLICATIONS

The popularity of protein supplementation has increased exponentially in the last decade. As a result, many products are currently available that are marketed to both competitive and recreational athletes as a means to improve training adaptations or performance. Consequently, coaches, athletes, trainers, players, and parents often have questions with regard to the safety and efficacy of many of these products. This study provides important information and will help to answer the questions of these individuals with regard to the physiological consequences of using protein supplements. Results of this study indicate that protein supplementation is effective at promoting increases in fat-free mass and muscle endurance during the initial stages of a resistance training program, and a combination of whey protein and casein protein appears to be more effective at promoting gains in fat-free mass.

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Acknowledgments

We would like to thank the subjects who participated in this study and the laboratory assistants in the Exercise and Sport Nutrition Laboratory, who assisted with data collection and analysis. This study was funded by a research grant from Royal Numico. Investigators in the Exercise and Sport Nutrition Laboratory independently collected, analyzed, and interpreted the results from this study and have no financial interests in the results of this study. Presentation of results in this study does not constitute endorsement of the supplements investigated by Baylor University, the authors, or the National Strength and Conditioning Association.

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