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Effects of Leucine and Whey Protein Supplementation During Eight Weeks of Unilateral Resistance Training

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[1]Department of Kinesiology, California State University, Fullerton, Fullerton, California 92834; [2]Department of Oral Biology, University of Nebraska Medical Center, College of Dentistry, Lincoln, Nebraska 68583; [3]Department of Nutrition and Health Sciences, Center for Youth Fitness and Sports Research, University of Nebraska–Lincoln, Lincoln, Nebraska 68588; [4]Department of Health and Exercise Science, University of Oklahoma, Norman, Oklahoma 73019; [5]Advanced Medical Imaging, Lincoln, Nebraska 68516.

Abstract. Coburn, J.W., D.J. Housh, T.J. Housh, M.H. Malek, T.W. Beck, J.T. Cramer, G.O. Johnson, and P.E. Donlin. Effects of leucine and whey protein supplementation during eight weeks of unilateral resistance training. *J. Strength Cond. Res. 20(2): 284–291. 2006.*—The purpose of this study was to determine the effects of resistance training in combination with a leucine and whey protein supplement or a carbohydrate placebo on strength and muscle cross-sectional area (CSA). Thirty-three men (mean age ± SD = 22.4 ± 2.4 years) were assigned to 1 of 3 groups: (1) supplementation group (SUPP), (2) placebo group (PL), or (3) control group (CON). The SUPP and PL performed unilateral training of the leg extensor muscles with the nondominant limb for 8 weeks. The strength of each limb, muscle CSA of the quadriceps femoris (QF), and body composition were assessed pretraining and posttraining. The results indicated significant increases in strength for both limbs in the SUPP but only the trained limb in the PL. The increase in strength for the trained limb of the SUPP was greater than that for the trained limb of the PL. There was no significant increase in strength for either limb in the CON. There were significant increases in the CSA of all muscles of the QF of the trained limb for the SUPP and PL, and of the vastus lateralis of the untrained limb for the SUPP. The increases in QF CSA did not differ between the SUPP and PL. No significant CSA changes were found for either limb in the CON. There were no significant changes in body composition for the SUPP, PL, or CON. The current findings suggest that leucine and whey protein supplementation may provide an ergogenic effect which enhances the acquisition of strength beyond that achieved with resistance training and a carbohydrate placebo.

Key Words. amino acids, muscular strength, quadriceps femoris, strength training, cross-training, cross-education

Introduction

Resistance training can have a dramatic effect on increasing muscular strength and hypertrophy. For hypertrophy to occur, muscle protein synthesis must exceed muscle protein catabolism and amino acid availability is a key factor in promoting net protein synthesis (2, 3, 35). Significant decreases in serum amino acids, particularly leucine and isoleucine, have been found following resistance training (24, 25). Supplementing the diet with leucine and other amino acids prevents this decline and increases amino acid availability to the muscles (26, 33). The ingestion of leucine and other essential amino acids (EAAs), which are amino acids that are essential in the diet because they cannot be synthesized and include isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, immediately following exercise can enhance these effects (5, 33). In addition, there is evidence that net protein synthesis is even greater when the EAAs are ingested immediately before resistance exercise (34). The combination of resistance training and EAA supplementation is a potent stimulator of protein synthesis (29, 32).

Whey protein is a rich source of EAAs, including the branched chain amino acids (BCAAs) leucine, isoleucine, and valine. Because it is a source of amino acids, it has been studied for its potential ergogenic effects in conjunction with resistance training. One recent study found that subjects performing resistance training while supplementing with whey protein only or whey protein plus creatine monohydrate had greater gains in lean tissue mass and leg extension strength than subjects performing resistance training and taking a placebo (7). Another study found that the combination of whey protein plus L-glutamine and BCAAs was more effective than whey protein alone at increasing fat-free body mass and improving exercise performance (bench press and leg press repetitions) following 10 weeks of resistance training (10).

Previous studies have also examined the effects of unilateral resistance training on strength in the untrained limb. A number of these studies have shown a cross-training effect for strength (9, 16–19, 27, 36, 37), while others have not (15, 31, 40, 41). No studies, however, have examined the effects of leucine and-or whey protein supplementation on adaptations in the untrained limb.

Given the results of recent studies (2, 3, 5, 7, 10, 24–26, 29, 32–35), it is possible that supplementing with a combination of leucine and whey protein, both before and immediately after resistance training sessions, may provide added benefits in terms of increasing muscular strength and size. The purpose of this study was to determine the effects of 8 weeks of unilateral leg extension resistance training in combination with a leucine and whey protein supplement or a carbohydrate placebo on strength and cross-sectional area of the muscles of the quadriceps femoris (QF)in the trained and untrained limbs.

Methods

Experimental Approach to the Problem

A randomized, double-blind design was used to compare the effectiveness of resistance training combined with

leucine and whey protein supplementation vs. a carbohydrate placebo on strength, muscle cross-sectional area (CSA), and body composition. There were no dietary restrictions during the course of this study, and subjects were encouraged to continue with their normal dietary habits. The design allowed for examination of the effects of adding either a leucine and whey protein supplement or a carbohydrate placebo to an existing diet when combined with resistance training. This is similar to the way the product would likely be used by consumers. The leucine-whey protein supplement and carbohydrate placebo were isocaloric to control for differences in added energy intake. A control group (CON) was used to determine the muscle size and strength changes that might occur over the same time period in subjects who neither performed resistance training nor ingested the supplement or placebo. A unilateral training design was used to allow for examination of cross-training effects. The examination of individual muscles of the QF, at different levels of the thigh, was done because previous research (15, 28) has shown that hypertrophy can differ from one muscle to another and from one level of the muscle to another. This information may be of interest to other researchers as well as those who use supplements such as the whey protein and leucine product examined.

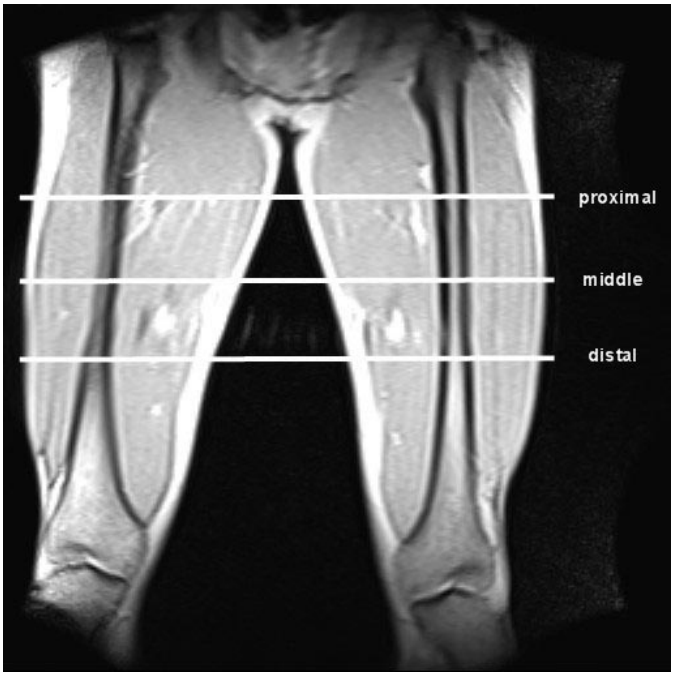

FIGURE 1. Coronal magnetic resonance imaging scan indicating the locations (levels) of the thighs where the axial scans were performed.

Subjects

Thirty-three men volunteered to be subjects for this investigation. All procedures were approved by the University Institutional Review Board for Human Subjects and the subjects signed informed consent prior to any testing. None of the subjects were taking medications or nutritional supplements that would interfere with the results of the study. Subjects were excluded if they had participated in a resistance training program for their legs in the 90 days preceding the beginning of this investigation. Using a double-blind design for the supplement (SUPP) and placebo groups (PL), the subjects were randomly assigned into 1 of 3 groups: (1) SUPP (n = 11; age 21.3 ± 2.0 years; body mass 77.2 ± 11.5 kg; height 181.3 ± 6.3 cm); (2) PL (n = 12; age 22.8 ± 2.8 years; body mass 82.0 ± 10.5 kg; height 180.8 ± 7.6 cm); or (3) CON (n = 10; age 23.2 ± 1.9 years; body mass 82.0 ± 9.7 kg; height 179.9 ± 6.1 cm).

Supplement Protocol

The SUPP received 20.0 g of whey protein and 6.2 g of leucine in 8 oz of water, the PL received 26.2 g of maltodextrin in 12 oz of water, and the CON received nothing. After an overnight fast, the subjects ingested the supplement (SUPP) or placebo (PL) 30 minutes prior to and immediately after each resistance training session. On nontraining days, the SUPP and PL ingested 1 dose of the supplement or placebo before breakfast. Other than supplements, there were no dietary restrictions during the course of this study, and subjects were encouraged to continue with their normal dietary habits.

Testing

The dynamic constant external resistance (DCER) leg extension strength of each limb was tested by determining each subject's unilateral 1 repetition maximum (1RM). A Body-Solid plate-loaded leg extension machine (Model CEC340; Forest Park, IL) was used for all strength testing and training. Each subject sat with his torso against the backrest and was instructed to hold tightly to the handles at the sides of the device. The backrest was adjusted to align the anatomical axes of the knees with the mechanical axis of the machine. Shin pads, attached to the machine's lever arm, were placed against the subject's legs. The shin pads were a fixed distance from the axis of rotation of the lever arm and thus not adjustable. Positioning, however, was consistent for each subject across all tests.

Determination of 1RM DCER strength involved the application of progressively heavier loads until the subject could not lift the resistance through the full range of motion (approximately 1.57 rad) according to methods suggested by Kraemer and Fry (20). If necessary, subsequent trials were performed with lighter loads until the 1RM was determined within 2.27 kg. Subjects had 2-minute rest between trials. The order of testing of the limbs was randomized for the pretraining testing session and was maintained for the posttraining session. The intraclass reliability coefficient (ICC) for DCER measurements was R = 0.97.

The SUPP, PL, and CON underwent pretraining and posttraining magnetic resonance imaging (MRI) (Philips Polaris 1.0-T scanner; Bathel, WA) to determine CSA of each muscle of the QF (vastus lateralis [VL], vastus intermedius [VI], vastus medialis [VM], and rectus femoris [RF]) of both thighs at 3 locations (levels). Coronal scans of the thighs were used to determine the length of the femur from superior border of the head to inferior border of the medial condyle (Figure 1). Three axial scans were then taken at approximately 33%, 50%, and 67% (proximal, middle, and distal levels, respectively) of this distance (Figure 2). Posttesting for muscle CSA was determined within 48 to 96 hours following the last resistance training session.

Repetition time and echo time were set at 620 and

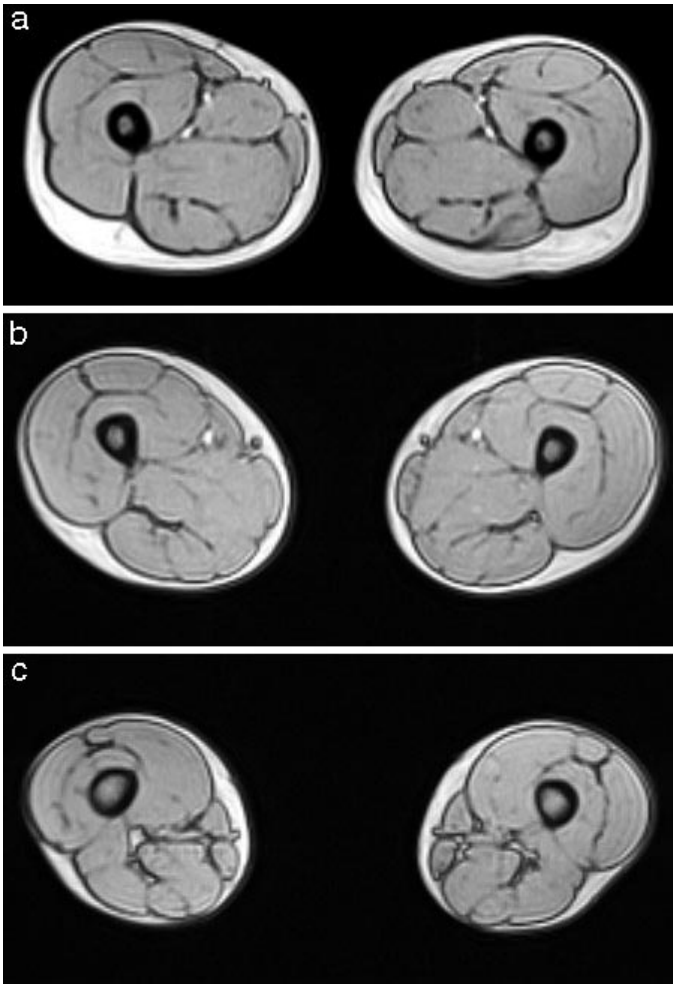

FIGURE 2. Axial magnetic resonance imaging scans at the (a) proximal, (b) middle, and (c) distal levels.

4.47 ms, respectively. All MRIs were transferred to a personal computer for CSA measurement of the individual quadriceps muscles of both thighs using DicomWorks v1.3.5 software. Two of the investigators, who were unaware of group membership or time of testing performed all CSA measurements. Intratester reliability and intertester objectivity of the CSA measurements were determined by measuring images from 10 randomly selected subjects on 2 occasions, separated by 72 hours. Each investigator had an ICC of $R > 0.98$ and a *SEM* of ≤2.0% of the mean of the CSA measurements. In addition, there were no significant ($p > 0.05$) differences between the mean CSA values for test vs. retest measurements for either of the investigators. The ICC for the intertester objectivity comparison was $R = 0.983$, with an *SEM* of 2.4% of the mean. Furthermore, there was no significant ($p > 0.05$) difference between the mean CSA values from the 2 investigators.

Body composition was assessed pretraining and posttraining. The subjects were instructed to avoid exercise for at least 12 hours prior to testing, and each subject indicated that he was normally hydrated and in a postabsorptive state (at least 4 hours) upon arrival to the laboratory. Body weight was determined to the nearest 0.11 kg using a state certified physicians scale. Body density (BD) was assessed from underwater weighing (UWW) with correction for residual lung volume (RV) using the oxygen dilution method of Wilmore (39). RV was determined on land with the subject seated in a position similar to that assumed during UWW. The average of similar scores (within 0.1 L) from 2 or 3 trials was used as the representative RV. Underwater weight was measured in a submersion tank in which a nylon swing seat was suspended from a 10-kg Salter scale (REGO Designs & Patents, model #230). The average of the 2 or 3 highest weights from 6 to 10 trials was used as the representative underwater weight. Percent body fat (% fat) was calculated using the formula of Brozek et al. (6), with fat-free mass (FFM) and fat mass (FM) derived mathematically. Previous test-retest reliability data for UWW from our laboratory indicated that for 16 young men measured 24–72 hours apart, the ICC was $R = 0.98$ with an *SEM* of 0.9% fat.

Training

Each member of the SUPP and PL performed 8 weeks of unilateral DCER training of the leg extensor muscles on the same leg extension machine used for the DCER testing. The training was performed with the nondominant limb (based on kicking preference) 3 times per week. Each training session consisted of 2 warm-up sets with progressively heavier loads followed by 3 to 5 sets of 6 repetitions at 80% of the DCER 1RM. The subjects performed 3 sets the first week of training, 4 sets the second week, and 5 sets during weeks 3–8. The trained limb was tested for DCER 1RM before the third training session of every week to adjust the training load for the following week.

Statistical Analyses

DCER 1RM strength data were analyzed using a 3-way (limb [trained, untrained] × time [pretraining, posttraining] × group [SUPP, PL, CON]) mixed factorial analysis of variance (ANOVA) utilizing the SPSS 12.0 for Windows software package (SPSS Inc., Chicago, IL). Limb and time were treated as within subjects factors, while group was used as a between subjects factor. The muscle CSA data were analyzed using a 5-way (muscle [VL, VI, VM, RF] × level [proximal, middle, distal] × limb [trained, untrained] × time [pretraining, posttraining] × group [SUPP, PL, CON]) mixed factorial ANOVA. Muscle, level, limb, and time were used as "within subjects" factors and group was used as a "between subjects" factor. Separate 2-way (group [SUPP, PL, CON] × time (pretraining, posttraining]) mixed factorial ANOVAs were used to analyze the body weight (BW) and body composition variables (% fat, FM, FFM). Group was used as a "between subjects" factor and time was used as a "within subjects" factor. Tukey post hoc tests were used, and an alpha of $p \leq 0.05$ was considered significant for all comparisons. Based on previous studies (16, 17) a priori analyses were used to determine sample sizes that yielded power values of 0.90 or greater for the muscle CSA and strength data.

RESULTS

Strength

The results indicated significant increases in 1RM DCER strength for both the trained and untrained limbs in the SUPP, but increases for the trained limb only in the PL (Table 1). The increase in 1RM DCER strength of the

TABLE 1. 1 repetition maximum dynamic constant external resistance strength in kg (mean ± *SEM*).

	Trained limb			Untrained limb		
	Pre	Post	% Change	Pre	Post	% Change
SUPP group	48.6 ± 1.9	63.3 ± 3.4	30.3*†	48.1 ± 2.9	55.0 ± 2.6	14.5*
PL group	51.4 ± 3.7	62.9 ± 4.3	22.4*	53.9 ± 3.4	55.4 ± 3.1	2.8
CON group	49.7 ± 3.5	51.5 ± 3.2	3.6	54.4 ± 5.2	56.9 ± 4.4	4.6

* Significant change from pre to post, $p < 0.05$.
† Significantly greater change in the supplement group than the placebo group, $p < 0.05$.

trained limb for the SUPP was greater than that for the PL. The CON did not change in strength for either limb during the 8-week study.

Muscle CSA

The results indicated that the SUPP, PL, and CON responded differently to 8 weeks of DCER training (Table 2). In the SUPP group, there were significant increases in the CSA of all muscles (VL, VI, VM, and RF) of the quadriceps femoris of the trained limb, at all levels (proximal, middle, and distal). For the untrained limb, however, only the proximal level of the VL increased in CSA. In the PL group, there were significant increases in CSA of all muscles (VL, VI, VM, and RF) of the quadriceps femoris for the trained limb, at all levels (proximal, middle, and distal). There were no changes, however, in muscle CSA for the untrained limb. The increases in CSA for the trained limb did not differ between the SUPP and PL. In the CON group, there were no changes in CSA for any of the muscles (VL, VI, VM, or RF) or levels (proximal, middle, or distal) of either limb during the 8 weeks of the study.

Body Composition

The results indicated that there were no significant training-induced changes in BW, % fat, FFM, or FM for the SUPP, PL, or CON (Table 3).

DISCUSSION

Strength

In the present study, 1RM DCER strength increased significantly in the trained limb for the SUPP (30.4%) and PL (22.4%), but not the CON. Furthermore, the increase in 1RM DCER strength of the trained limb for the SUPP was significantly greater than that of the PL. Thus, the leucine and whey protein supplementation combined with resistance training resulted in a greater mean increase in leg extension strength in the trained limb than did the combination of resistance training plus ingestion of a carbohydrate placebo.

Previous studies that have examined the effects of whey protein and-or leucine supplementation on muscular strength have yielded conflicting results (1, 7, 8, 30, 38). For example, Burke et al. (7) randomly assigned subjects to either a whey protein (WP) (1.2 $g \cdot kg^{-1} \cdot day^{-1}$), whey protein plus creatine monohydrate group (1.2 $g \cdot kg^{-1} \cdot day^{-1}$ and 0.1 $g \cdot kg^{-1} \cdot day^{-1}$, respectively) or PL (1.2 g. $kg^{-1.}$ day^{-1} of maltodextrin) for 12 weeks of resistance training. The results indicated that the subjects who supplemented with whey protein only had greater gains in leg extension peak torque than those in the PL. In addition, the subjects who ingested whey protein plus creatine demonstrated greater gains in bench press strength than those in the WP or PL. Other studies, however, have not shown beneficial effects from whey protein and-or leucine supplementation compared to resistance training alone (1, 8, 30, 38). Antonio et al. (1) assigned previously untrained women ($n = 21$) to either a placebo (cellulose) or an EAA (average daily dose of 18.3 g of EAAs in pill form with 1.83 g of leucine per 10 g of EAA) group. The subjects performed resistance exercise and aerobic training 3 times per week for 6 weeks. There were no significant changes in muscular strength for either group following the training period. Williams et al. (38) found that an amino acid plus glucose supplement (containing 11% leucine) was no more effective than a placebo (0.5 g dried milk powder, artificial sweetener, water, lemon flavoring, and coloring) for increasing isometric, isokinetic, or 1RM strength in subjects who performed leg extension training for 10 weeks. Ratamess et al. (30) found equivalent increases in 1RM squat and bench press strength after the second, third, and fourth weeks of a 4-week training program in group taking amino acids (0.4 g·kg body $weight^{-1}$, with 27.2 g of leucine per 100 g of amino acids) or a PL taking a placebo (powdered cellulose). It was found, however, that the amino acid supplementation prevented the decrement in performance seen in the PL during the initial phase of this overreaching program, which was designed to overwork subjects and then produce a rebound in strength and power performance (30). Chromiak et al. (8) found that the consumption of a postexercise supplement containing whey protein (13 g per serving), amino acids (including 0.53 g of leucine per serving), creatine, and carbohydrate combined with 10 weeks of resistance training did not promote greater gains in muscular strength than a carbohydrate-only drink combined with resistance training. The results of these studies indicated that supplementation with creatine, whey protein, leucine, essential amino acids, and carbohydrates, or combinations of these ingredients has been shown in some, but not all cases, to result in greater increases in strength than resistance training alone. The reason for the lack of consistent findings among studies may be due to factors such as the training volume and-or intensity, training experience of subjects, amount and combination of ingredients in the supplements, and the timing of supplement ingestion.

Previous studies using unilateral DCER training have shown significant increases in 1RM strength in the untrained as well as the trained limb following concentric only (16, 19, 37), eccentric only (17, 18, 36), or concentric plus eccentric (9, 27) resistance training. Other studies, however, have not demonstrated a cross-training effect as a result of unilateral resistance training (15, 31, 40, 41). In the present study, significant increases in 1RM DCER strength in the untrained limb occurred in the SUPP

Table 2. Quadriceps femoris cross-sectional area (cm^2) (mean ± *SEM*).

	Proximal level			Middle level			Distal level		
	Pre	Post	% Change	Pre	Post	% Change	Pre	Post	% Change
SUPP									
Nondominant (trained) limb									
VL	32.64 ± 1.31	34.72 ± 1.66	6.38*	30.95 ± 1.58	33.39 ± 1.63	7.88*	16.18 ± 0.98	17.12 ± 1.00	5.84*
VI	21.26 ± 0.90	22.70 ± 1.16	6.76*	22.67 ± 1.08	23.44 ± 1.06	3.40*	17.26 ± 0.72	18.51 ± 0.81	7.20*
VM	10.11 ± 0.61	10.92 ± 0.65	8.00*	19.02 ± 0.95	19.44 ± 0.92	2.23*	25.50 ± 1.56	27.61 ± 1.35	8.27*
RF	14.87 ± 0.63	15.49 ± 0.72	4.17*	8.69 ± 0.88	9.56 ± 0.84	10.08*	1.95 ± 0.29	2.29 ± 0.24	17.50*
Dominant (untrained) limb									
VL	33.44 ± 1.52	34.49 ± 1.59	3.16*	32.58 ± 1.79	32.00 ± 2.08	−1.78	16.97 ± 1.00	16.89 ± 1.20	−0.47
VI	22.78 ± 1.16	23.38 ± 1.40	2.66	24.29 ± 1.34	23.25 ± 1.21	−4.27	18.27 ± 0.80	18.34 ± 0.89	0.39
VM	9.58 ± 0.51	10.11 ± 0.62	5.50	18.43 ± 0.91	18.45 ± 0.89	0.10	26.29 ± 1.42	26.53 ± 1.59	0.91
RF	14.93 ± 0.75	14.99 ± 0.71	0.46	8.78 ± 0.76	8.83 ± 0.85	0.59	2.12 ± 0.28	1.94 ± 0.26	−8.33
PL									
Nondominant (trained) limb									
VL	33.15 ± 1.04	34.42 ± 1.31	3.81*	33.07 ± 1.21	34.20 ± 1.23	3.44*	15.98 ± 1.27	16.83 ± 1.45	5.34*
VI	22.10 ± 0.89	23.61 ± 0.63	6.85*	25.89 ± 1.24	26.86 ± 1.42	3.73*	19.38 ± 0.95	20.13 ± 0.82	3.84*
VM	10.38 ± 0.41	11.28 ± 0.37	8.69*	18.43 ± 0.68	20.02 ± 0.91	8.62*	27.25 ± 1.13	27.52 ± 1.07	0.97*
RF	15.72 ± 0.99	16.09 ± 1.06	2.39*	10.43 ± 0.82	10.63 ± 0.94	1.94*	2.51 ± 0.24	2.65 ± 0.31	5.30*
Dominant (untrained) limb									
VL	34.46 ± 1.38	34.94 ± 1.25	1.40	33.62 ± 1.01	33.43 ± 1.00	−0.56	17.24 ± 1.30	17.47 ± 1.30	1.33
VI	23.27 ± 1.13	23.49 ± 0.69	0.96	26.30 ± 1.56	25.69 ± 1.36	−2.33	19.23 ± 0.89	19.53 ± 0.81	1.56
VM	10.12 ± 0.29	10.17 ± 0.29	0.46	18.09 ± 0.63	18.80 ± 0.78	3.94	27.58 ± 1.24	27.18 ± 1.15	−1.48
RF	15.73 ± 1.00	15.18 ± 0.93	−3.55	10.15 ± 0.88	9.84 ± 0.75	−3.08	2.29 ± 0.27	2.27 ± 0.29	−0.69
CON									
Nondominant limb									
VL	36.65 ± 1.72	36.52 ± 1.80	−0.36	34.43 ± 2.57	33.66 ± 2.82	−2.24	18.08 ± 1.76	17.09 ± 1.75	−5.49
VI	23.83 ± 1.24	23.51 ± 1.36	−1.34	25.66 ± 1.66	25.57 ± 1.44	−0.35	19.14 ± 1.32	18.29 ± 1.27	−4.44
VM	10.31 ± 1.10	10.78 ± 0.79	4.58	21.20 ± 1.12	20.69 ± 1.23	−2.42	28.72 ± 1.70	29.58 ± 1.94	3.01
RF	16.89 ± 1.17	16.44 ± 1.31	−2.66	10.15 ± 0.89	9.81 ± 0.86	−3.35	2.38 ± 0.29	1.88 ± 0.12	−21.22
Dominant limb									
VL	38.03 ± 1.87	38.43 ± 1.87	1.07	34.84 ± 2.43	34.37 ± 2.17	−1.33	17.15 ± 1.44	16.34 ± 1.36	−4.76
VI	24.56 ± 1.27	23.89 ± 1.31	−2.73	25.32 ± 1.82	25.48 ± 1.71	0.63	18.83 ± 1.43	17.97 ± 1.03	−4.59
VM	10.07 ± 0.97	10.75 ± 0.68	6.74	20.45 ± 0.78	19.85 ± 0.88	−2.94	26.85 ± 1.25	27.40 ± 1.52	2.05
RF	16.23 ± 1.26	16.36 ± 1.20	0.81	9.85 ± 0.94	9.54 ± 0.85	−3.17	2.13 ± 0.27	1.66 ± 0.15	−22.30

SUPP = supplement group; VL = vastus lateralis, VI = vastus intermedius, VM = vastus medialis, RF = rectus femoris; PL = placebo group; CON = control group.
* Significant change from pre to post, $p < 0.05$.

TABLE 3. Body composition (mean ± *SEM*).

	Group	Pre	Post	% Change
Body weight (kg)	SUPP	77.2 ± 3.5	77.8 ± 3.8	0.8
	PL	82.1 ± 3.0	81.9 ± 3.3	−0.2
	CON	82.0 ± 3.1	81.8 ± 3.1	−0.3
Percent fat	SUPP	16.4 ± 1.6	16.5 ± 1.8	0.9
	PL	16.6 ± 1.5	17.3 ± 1.9	4.1
	CON	17.7 ± 2.6	17.3 ± 2.5	−2.1
Fat free mass (kg)	SUPP	64.4 ± 2.7	64.7 ± 2.8	0.5
	PL	68.2 ± 2.5	67.4 ± 2.4	−1.3
	CON	67.3 ± 2.6	67.4 ± 2.5	0.1
Fat mass (kg)	SUPP	12.8 ± 1.7	13.1 ± 2.0	2.4
	PL	13.8 ± 1.5	14.5 ± 1.9	5.4
	CON	14.7 ± 2.3	14.4 ± 2.3	−2.1

There were no significant ($p > 0.05$) increases from pre to post for any group. SUPP = supplement group; PL = placebo group; CON = control group.

(14.6%), but not the PL. The cross-training effect has been attributed to (a) the diffusion of motor impulses to the untrained side of the body (13), (b) contraction of the musculature on the untrained side of the body to maintain balance and assume the proper position for unilateral exercise (13), (c) neural activity in the contralateral motor cortex (22), and-or (d) some unspecified spinal mechanism (14). Thus, it is generally accepted that the cross-training effect results from neural adaptations and not muscle hypertrophy. The increase in strength in the untrained limb for the SUPP but not PL, however, suggested that the supplement may have, in some way, accentuated the effects of the training in the untrained limb. Interestingly, the present findings for muscle CSA suggested that the supplement may have contributed to a hypertrophic effect in the untrained limb of the SUPP.

Muscle CSA

In the present study, the SUPP and PL exhibited significant increases in the CSA of all muscles and levels of the trained limbs (mean = 7.31% per muscle per level for SUPP and 4.58% per muscle per level for PL). The results of the present study were comparable to those of other investigations that found increases in muscle CSA from 3.3% to 34.0% following 8 to 12 weeks of DCER (11, 16), isokinetic (15, 23, 28), or variable resistance (12) leg extension training. In addition, Godard et al. (11) found that 12 weeks of leg extension training in conjunction with the postexercise consumption of an amino acid supplement (including 2.24 g of leucine) and carbohydrates (dextrose, sucrose, and fructose) led to a significant increase (7.0%) in thigh muscle CSA in older men (>65 y). The increase in thigh muscle CSA, however, was not greater than that for a control group which performed the same resistance training program, but did not ingest the supplement.

A unique finding of the present study was the significant increase in the CSA of the VL at the proximal level of the untrained limb for the SUPP (6.44%), but not the PL. This suggests that the leucine and whey protein supplementation may have contributed to the increases in muscle CSA and strength in the untrained limb of the SUPP. The potential hypertrophic effect in the untrained limb of the SUPP, however, was observed for only one muscle (VL) at one level (proximal). Therefore, future studies should examine whether this increase in muscle CSA in the untrained limb can be replicated.

Another interesting finding of the present study was that the increased rate of strength gain for the SUPP compared to the PL was not accompanied by a greater rate of muscle hypertrophy. This finding may have been due to the effects of leucine and BCAA supplementation on the central nervous system. Previous research has indicated that BCAA supplementation, including leucine, can enhance both mental and physical performance associated with endurance training (4). Endurance training can reduce plasma levels of leucine and other BCAAs, increasing the ratio of tryptophan to BCAAs in the brain, indirectly leading to an increased concentration of serotonin in the brain (4). This exercise-induced increase in serotonin has been hypothesized to promote fatigue, decrease muscle power output, and depress motor neuron excitability, the so-called central fatigue hypothesis (21). Mero et al. (26) found that strength and speed training led to decreased serum amino acid levels, but that leucine supplementation (50 mg·kg body weight^{-1}·day^{-1}) prevented a decrease in serum leucine levels. In the present study, it is possible that leucine and whey protein supplementation prevented an exercise-induced decrease in BCAA concentrations, and therefore prevented development of central fatigue. This potential ergogenic effect of leucine and whey protein supplementation could promote increases in strength independent of changes in muscle hypertrophy.

Body Composition

In the present study, there were no significant training-induced changes in BW, % fat, FFM, or FM for the SUPP, PL, or CON. Previous research examining the effects of whey protein and-or amino acid supplements, including leucine, have demonstrated conflicting findings regarding body composition changes when combined with resistance training (1, 7, 8). Antonio et al. (1) found no change in BW, FFM, or FM in untrained females assigned to either an EEA group or PL following resistance and aerobic training performed 3 days per week for 6 weeks. Chromiak et al. (8) found comparable increases in FFM and decreases in % fat following 10 weeks of resistance training in subjects who consumed a supplement containing whey protein, amino acids, creatine, and carbohydrates or a placebo. Burke et al. (7) compared the effects of whey protein, whey protein plus creatine monohydrate, or a placebo (maltodextrin) on FFM during 6 weeks of resistance

training. It was found that the whey protein plus creatine monohydrate group increased FFM more than the WP or PL and that FFM increased more for the WP than the PL. In the present study, muscle CSA increased significantly for all muscles of the QF, at all levels of the trained limb, for both the SUPP and PL. The changes in muscle CSA, however, were primarily localized to the trained limb because of the unilateral training, and, therefore, may not have been sufficient to result in an increase in total body FFM.

In summary, the results of the present study indicated that the leucine and whey protein supplementation enhanced the acquisition of strength in the trained and untrained limbs of the SUPP beyond that demonstrated by the PL with resistance training plus a carbohydrate placebo. Furthermore, the resistance training in combination with leucine and whey protein supplementation resulted in an increase in muscle CSA for the VL at the proximal level of the untrained limb in the SUPP. There were no changes in muscle CSA of the untrained limb, however, for the PL. Further research is needed to determine if the increase in muscle CSA in the untrained limb for the SUPP can be replicated, and to determine the specific mechanism(s) by which resistance training, in conjunction with leucine and whey protein supplementation, enhances gains in strength and muscle CSA in the trained and untrained limbs, when compared to resistance training plus carbohydrate supplementation. A limitation of the present study, however, is that no dietary analyses were conducted to determine protein intakes before or after administration of the leucine and whey protein or carbohydrate supplements. It is possible that the leucine and whey protein supplement was administered to a group that was protein deficient compared to the PL, even though the subjects were randomly assigned to the groups. Therefore, it is recommended that future studies equate experimental groups for protein intake before supplementation, then use a matching and random assignment process to assign subjects to groups.

PRACTICAL APPLICATIONS

In the present study, dietary supplementation with leucine and whey protein provided an ergogenic effect that enhanced the acquisition of strength beyond that achieved with a carbohydrate placebo. Coaches and athletes who want to maximize gains in strength and muscle hypertrophy may wish to consider the use of a leucine and whey protein supplementation regimen.

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