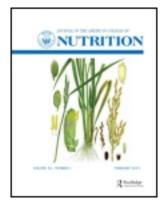
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Whey Protein Supplementation During Resistance Training Augments Lean Body Mass

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Whey Protein Supplementation During Resistance Training Augments Lean Body Mass

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Key words: whey, soy, protein, resistance training, lean body mass, body composition, amino acids, leucine

Compared to soy, whey protein is higher in leucine, absorbed quicker and results in a more pronounced increase in muscle protein synthesis.

Objective: To determine whether supplementation with whey promotes greater increases in muscle mass compared to soy or carbohydrate, we randomized non-resistance-trained men and women into groups who consumed daily isocaloric supplements containing carbohydrate (carb; n = 22), whey protein (whey; n = 19), or soy protein (soy; n = 22).

Methods: All subjects completed a supervised, whole-body periodized resistance training program consisting of 96 workouts (~9 months). Body composition was determined at baseline and after 3, 6, and 9 months. Plasma amino acid responses to resistance exercise followed by supplement ingestion were determined at baseline and 9 months.

Results: Daily protein intake (including the supplement) for carb, whey, and soy was 1.1, 1.4, and 1.4 g·kg body mass⁻¹, respectively. Lean body mass gains were significantly (p < 0.05) greater in whey (3.3 ± 1.5 kg) than carb (2.3 ± 1.7 kg) and soy (1.8 ± 1.6 kg). Fat mass decreased slightly but there were no differences between groups. Fasting concentrations of leucine were significantly elevated (20%) and postexercise plasma leucine increased more than 2-fold in whey. Fasting leucine concentrations were positively correlated with lean body mass responses.

Conclusions: Despite consuming similar calories and protein during resistance training, daily supplementation with whey was more effective than soy protein or isocaloric carbohydrate control treatment conditions in promoting gains in lean body mass. These results highlight the importance of protein quality as an important determinant of lean body mass responses to resistance training.

INTRODUCTION

Optimizing recovery after exercise is important for eliciting maximal training adaptations. A large body of literature exists on the role of protein and carbohydrate ingestion on measures of protein balance after resistance exercise [1]. Compared to the fasting state, a bout of resistance exercise has a positive impact on muscle protein synthesis, but in the absence of dietary protein intake, net protein balance remains negative [2], even if carbohydrate is ingested [3]. Moreover, there is now mounting evidence for distinct qualitative effects of protein sources on muscle anabolism [4–6].

Whey is distinguished from other protein sources due to its rapid digestion and high content of essential amino acids (EAAs), which are requisite for stimulating skeletal muscle protein synthesis [7]. Compared to soy protein, whey has 50% more branched-chain amino acids (BCAAs) leucine, isoleucine, and valine. BCAAs are essential amino acids that promote muscle protein synthesis and prevent muscle protein breakdown [8, 9], and they may offer protection from exercise-induced

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muscle soreness [10]. Whey is a rich source of leucine (>10%)that can directly activate contractile muscle protein synthesis alone through the mammalian target of the rapamycin (mTOR) signaling pathway [11]. Compared to soy, whey protein after resistance exercise results in greater concentrations of leucine in the blood and specific phosphorylation of mTOR and S6K1, demonstrating greater activation of molecular signaling related to protein synthesis [12]. In humans, ingestion of whey protein at rest [4, 5] or after resistance exercise [13, 14] stimulates muscle protein synthesis. In a direct comparison of protein sources consumed after a bout of resistance exercise, rates of muscle protein synthesis over a 3-hour postexercise period were \sim 2-fold higher after whey than casein, whereas soy was intermediate in between whey and casein [6]. Leucine values in the blood over the 3-hour period were 73% greater than soy values and 200% greater than casein values [6].

Acute increases in mTOR signaling and skeletal muscle protein synthesis should logically translate into chronic increases in lean body mass, yet few studies have rigorously tested this hypothesis. There is evidence that supplementation with whey protein alone [15] or a combination of whey and casein [16, 17] is more effective than a carbohydrate supplement at augmenting lean body mass responses to resistance training. In comparison to casein, ingestion of whey protein during resistance training has been shown to promote greater increases in lean body mass and reductions in body fat [18]. On the other hand, a recent study showed no difference between whey and soy protein with regard to lean body mass adaptations to resistance training, but the very short intervention (6 weeks) was likely too short to observe subtle differences in the anabolic potential of these 2 protein sources [19]. To our knowledge, no studies have compared protein sources (whey vs soy) with regard to lean body mass responses to chronic resistance training longer than 6 months. In this article, we report lean body mass, body composition, and amino acid responses to 9 months of resistance training in a group of healthy men and women randomly assigned to supplement with whey protein, soy protein, or carbohydrate. We hypothesized that with whole-body resistance training, whey protein supplementation would enhance leucine availability and gains in lean body mass.

MATERIALS AND METHODS

Experimental Approach

A prospective parallel 3-group study design was used to compare the effects of nutritional supplementation on body composition to resistance training. Healthy men and women were randomly assigned in a double-blind manner to supplement daily with whey protein (whey), soy protein (soy), or carbohydrate (carb). All subjects performed supervised resistance training. Body mass, body composition, and maximal strength were determined at baseline and after 32 (\sim 3 months), 64 (\sim 6 months), and 96 (\sim 9 months) workouts. An acute resistance exercise test followed by supplementation with whey, soy, or carbohydrate was performed at baseline and at 9 months to determine plasma amino acid response patterns.

Subject Recruitment and Retention

Subjects were men and women aged 18-35 years and not participating in a systematic, high-intensity resistance program within 1 year prior to enrollment. Subjects were permitted to participate in recreational sports or other activities but were not allowed to participate in outside intense training to avoid incompatibility with respect to muscular adaptations. Women were normally menstruating (cycle lengths 28-32 days). Exclusion criteria included the following: hypertension (systolic blood pressure [SBP] > 150 or diastolic blood pressure [DBP] > 95 mmHg), diabetes, use of tobacco products, use of cholesterollowering and blood pressure medications, change in body weight > 3 kg during the past 3 months, use of anti-inflammatory medication (aspirin, NSAIDs), alcohol consumption > 3 drinks/day or 21/week, pregnancy or intention to become pregnant or abnormal menstrual phase, initiation or change in hormonal birth control within last 3 months, allergy to whey or soy, and musculoskeletal injuries or physical limitations affecting ability to exercise.

More than 1200 subjects responded to recruitment efforts from March 2008 to January 2011 (see Fig. 1). Many individuals either failed to follow up or did not meet basic inclusion criteria. A total of 335 participants completed screening forms at an informational session and 169 subjects started baseline testing. Subjects who completed all baseline tests (n = 147) were matched according to sex, body mass, and body fat and then randomly assigned to the whey, soy, or carb group to ensure equal distribution of men and women of similar body composition in each group. Subjects were informed of the purpose and possible risks of the investigation prior to signing an informed consent document approved by the university's institutional review board.

Dietary Protocol

Subjects were free living but were provided specific and regular dietary counseling by registered dietitians that focused on consuming adequate energy to prevent major loss or gain in body mass. There was a major emphasis on ensuring a standard protein intake of 1.0 to 1.2 g·kg body mass⁻¹ (not including supplementation), which is 25% to 50% above the recommended dietary allowance (RDA) but not so high to potentially confound subtle differences between protein sources. For the whey and soy groups, the addition of the daily protein supplement (~22 g·day⁻¹) increased protein intake to ~1.4 g·kg⁻¹. Energy needs for body mass maintenance were determined by resting metabolic rate testing using a Parvomedics TrueOne 2400

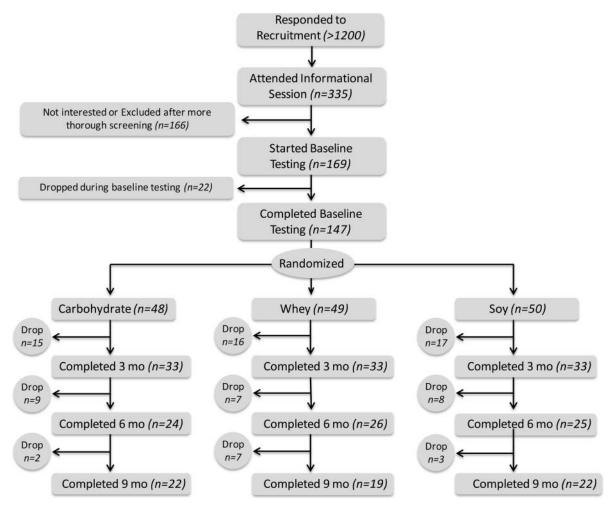


Fig. 1. Study flow diagram.

metabolic cart (Sandy, UT) with adjustments for activity. To assist in monitoring energy and protein intake, all subjects were provided with a handheld personal digital assistant loaded with a customized dietary program (Edward A. Greenwood, Inc., Brookline, MA). Subjects entered daily food and beverage consumption including amounts, time of day, and brands (when available) for 5 days every 6 weeks. The 5-day diet records were reviewed and goals were reinforced during one-on-one sessions with registered dietitians. A subject's food and beverage selections, with a focus on protein content of foods, were carefully reviewed by the dietitian during the counseling session for accuracy prior to transferring the information to a dedicated laboratory computer for analysis. Subjects were weighed weekly on a calibrated digital scale (Defender 5000, Ohaus, Florham Park, NJ). Subjects showing a trend for excessive weight gain or loss (± 2.5 kg over baseline weight) were flagged and discussed by the nutrition team at weekly meetings and provided specific guidance to adjust caloric and/or protein intakes. Dietary data were collapsed and reported as mean nutrient intakes at baseline and 3, 6, and 9 months.

Supplement Protocol

Using a double-blind protocol, the carbohydrate, whey, and soy powdered supplements were provided in identical individualized packets. They were isocaloric and isonitrogenous (whey and soy). Nutrient composition based on analysis of 5 packets each of carbohydrate (maltodextrin), whey protein concentrate, and soy isolate (isoflavone free) supplements by an independent laboratory (Medallion Labs, Minneapolis, MN) is shown in Table 1. Packets were given to subjects with instructions to mix the contents in 240 mL of water. Subjects were provided a 2week supply with instructions to consume the supplement in the morning with breakfast on nontraining days and immediately after exercise on training days. Compliance was assured by having subjects ingest supplements in the presence of study personnel on training days. Subjects also recorded the date and time of supplement ingestion on log sheets. Empty packets were returned and counted at the end of each 2-week period. In addition, all supplements were spiked with 200 mg of para-aminobenzoic acid (PABA). Unannounced urine samples were collected from each subject during a training session approximately one time

	Carbohydrate	Whey	Soy
Energy (kcal)	191	194	189
Carbohydrate (g)	45.2	22.5	24.5
Protein (g)	0.8	21.6	20.0
Fat (g)	0.8	1.9	1.3
Saturated fat (g)	0.3	1.0	0.4
Calcium (mg)	201	189	214
Iron (mg)	2.1	2.0	4.4
Phosphorus (mg)	44	114	213
Sodium (mg)	154	183	402
Magnesium (mg)	18	28	28
Potassium (mg)	55	164	78
Amino acid composition (mg)			
Alanine	_	1050	726
Arginine	_	667	1329
Aspartic acid	_	2029	1835
Glutamic acid	_	3519	3262
Glycine	_	409	674
Histidine	_	405	447
Isoleucine	_	1174	770
Leucine	_	2211	1372
Lysine	_	1918	1097
Phenylalanine	_	703	904
Proline	_	1181	810
Serine	_	1126	856
Threonine	_	1442	671
Tyrosine	_	652	651
Valine	_	1140	794

^aValues are per serving (packet). Subjects consumed one packet per day.

per month for analysis of the presence of this marker in the urine. Urine (~1 mL) was aliquoted into a storage tube, immediately frozen with liquid nitrogen, and stored at -80° C until analyzed for PABA using a colorimetric method described by Yamato and Kinoshita [20] with modifications. The intra-assay coefficient of variation was 7.9%. If PABA concentrations were 2.5-fold higher than baseline or >30 ug·ml⁻¹, this indicated that the subject was compliant with supplement ingestion on that day. Based on PABA, compliance with the supplement protocol was 82%.

Resistance Training Program

The whole-body resistance training program consisted of a flexible, progressive, nonlinear, periodized program characterized by within-week variation of the acute program variables as described by Kraemer and Fleck [21]. Unique to this study is that we wanted no workout to just be "going through the motions" and thus the terms *flexible* and *nonlinear*. If, for example, a subject could not perform the weight or repetitions needed for a "heavy" workout, the trainer then defaulted to one of the other workouts, typically a light workout. But throughout the weekly cycle, each subject had the same exposure to the same workouts, just not on the same day of the week. The resistance training program was designed to develop whole-body musculature and was implemented symmetrical to each joint, upper and lower body, and anterior and posterior body development. Four styles of workout were utilized: light (12-15 repetitions, short rest period of 60-90 seconds, lighter intensity); medium (8-10 repetitions, moderate intensity); heavy (3–6 repetitions, long rest periods of 2-3 minutes, high intensity); and power (wholebody exercises using 30%-45% of the estimated 1 repetition maximum [RM], 3-minute rest periods) for the major muscle group exercises as well as assistance exercises. Exercises used included squats (Smith Machine, Life Fitness, Schiller Park, IL; and free bar), hang cleans, bench presses, bicep curls, calf exercises, abdominal exercises, lat pull downs, lunges, upright rows, push presses, and weight plate lifts. Multiple sets (3-5) of each resistance exercise were performed. The program utilized free-weight (barbell and dumbbell), machine, and body weight exercises and limited use of plyometrics (i.e., vertical and horizontal medicine ball throws). Supervised training sessions took place in the weight room at the University of Connecticut with a goal of 1 trainer for every 2-3 subjects. Morning, noon, and evening training sessions were offered throughout the week to accommodate varying subject schedules and availability. The duration of sessions varied from 30 to 75 minutes depending on the type of workout performed. The program was divided into three 12-week mesocycles and continued until subjects accrued 96 workouts (\sim 9 months).

We used a designated contact person system whereby subjects were assigned to a member of the training staff after completing baseline testing. This person's responsibility was to schedule subjects for weekly training sessions, hold the subjects accountable for completing the scheduled sessions, and communicate any changes in the training schedule (i.e., inclement weather cancellation). Training sessions were offered throughout breaks from the school year. If short-term vacations or conflicts occurred during this time, subjects were given the sequence of workouts and trained on their own, which was validated by the supervising trainer.

The nonlinear periodization program had built-in accommodations that made it well suited for research in college-aged individuals. Weekly training schedules were randomized to prevent boredom. We used a flexible nonlinear program with the workouts optimized for each type of loading. If trainees did not reach workout loading goals (RM ranges), a replacement workout in the sequence was used and that workout was performed later in the training sequence. For example, if a subject was not able to perform a heavy workout with high quality within the first exercise sets, the trainer defaulted to a rest day or light day that a subject could complete with higher quality. This allowed for only high-quality workouts to be performed in each session. The use of flexible nonlinear resistance training uniquely allowed for optimal training and accommodation for sickness. injury, and normal fluctuations in performance capability that inevitably occurs over long periods of time. Yet the goals for each week were maintained with no differences in the workout stimuli between groups.

Testing Protocol

Prior to any testing, subjects were familiarized with the proper technique and sequence of all tests to be performed during the actual performance test visit. Height was determined in triplicate and averaged using a stadiometer (Seca, Hamburg, Germany). Body mass was measured to the nearest 0.1 kg using a calibrated digital scale. Body composition was assessed using dual-energy x-ray absorptiometry (Lunar Prodigy, Madison, WI). Performance testing consisted of 1 RM squat and bench press performed on a Smith Machine using proper technique and the same starting positions each time. Upon arrival, subjects performed a dynamic warm-up consisting of 5 minutes of cycle ergometer exercise followed by a series of dynamic stretches. Two warm-up sets of the specific exercise were completed at 50% estimated 1 RM (8-10 repetitions) and 80% estimated 1 RM (2-5 repetitions). For the squat, the parallel depth defined as the 90-degree relationship between the femur and the lower leg (i.e., knee at 90 degrees) for each subject was set with a plum line that the bottom of the thigh had to reach with each repetition in order to be counted as correct technique. Full range of motion was required for both the bench press and squat exercises. The testing protocol consisted of 3-5 attempts with the highest mass lifted with proper form recorded as the 1 RM.

On a separate day, at baseline and at 9 months only, an acute heavy resistance exercise test was performed to determine plasma amino responses to exercise. Subjects arrived at the laboratory following a 12-hour overnight fast and refraining from alcohol, caffeine, over-the-counter medications, and exercise for 24 hour. Upon arrival, hydration was confirmed by urine specific gravity (USG) with a handheld refractometer (model TS400, Reichert, Lincoln, IL). If USG >1.025, the subject drank water before retesting 30 minutes later. Thus, all subjects were hydrated prior to any testing protocol. An indwelling Teflon cannula was inserted into a superficial antecubital forearm vein. After resting quietly in the seated position for 10 minutes, a preexercise blood sample was obtained. Subjects then performed a dynamic warm-up followed by 6 sets of 10 repetitions of squat exercise on a Smith Machine with a 2-minute rest between sets. The load for the first set was set at 60% 1 RM and was subsequently adjusted based on volitional fatigue to attain a 10 RM on each set. Again, repetitions were counted as complete only if the participants reached a parallel position defined as the 90-degree relationship between the femur and the lower leg (i.e., knee at 90 degrees), which was again verified by a plum line that marked the individual's parallel position at the bottom of the squat. Upon completion of the sixth set, subjects consumed a full serving of the supplement corresponding to their group assignment. Additional blood samples were obtained at 15, 30, and 60 minutes postexercise while subjects remained in the seated position. Whole blood was collected into sodium heparin tubes, centrifuged (1500 \times g for 15 minutes at 4°C),

Plasma Amino Acid Analyses

Frozen heparinized plasma was thawed at room temperature and centrifuged at $10,000 \times g$ for 5 minutes to remove any particulate matter from the fluid. A 25- μ L aliquot of the supernatant was diluted 4-fold with a 1.25 μ M internal standard solution of 3,5-diiodotyrosine. The resultant mixture was then transferred to a 3 kDa molecular-weight cutoff filter (Pall Corporation, Port Washington, NY) and centrifuged for 10 minutes at 7500 g. The low-molecular-weight filtrate was then diluted 12.5-fold in the appropriate mobile phase and used for amino acid analysis. Amino acids were quantitated by ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) using an Acquity Ultra-Performance LC system (Waters, Milford, MA) coupled to a Waters Micromass Quattro Premier triple quadrupole instrument (Waters). The UPLC-MS/MS experimental parameters for this study were based on the instrument manufacturer's application notes and published LC-MS/MS methodologies [22-24] for underivatized amino acid analysis. Separation was achieved using an LC C18 bridged-ethyl hybrid column (1.7 μ m particles $\times 2.1 \times 50$ mm, Waters) with mobile phases of 0.1% (w/v) pentadecafluorooctanoic acid and 0.1% (v/v) formic acid in water/acetonitrile (v/v) solvent mixtures of 95.5%/0.5% (phase A) and 10%/90% (phase B) respectively. The sample components were eluted by increasing the percentage of solvent B to 2%, 5%, 20%, 40%, and 100% at 0.5, 0.8, 2, 4, and 4.5 minutes, respectively. The column was then equilibrated to 0% phase B over 2.5 minutes where it was held for an additional 6 minutes to recondition the column. All data acquisition and processing were performed using MassLynx 4.1 and QuanLynx software (Waters).

Statistical Analyses

Assuming 80% power at an α -level of 5%, we calculated that 17 participants were required to determine a 0.5 kg difference in lean body mass between groups (nQuery Advisor, Statistical Solutions, Saugus, MA). Because this was a biological efficacy study, only subjects who completed the required training sessions and were compliant with the supplement protocol (>90%) were analyzed. Means and standard measures of variation were calculated for outcome data, and distributions were examined for approximate normality and logarithmically adjusted if necessary as all data sets met the assumptions for linear statistics before analysis. Amino acid responses to exercise were summarized by area under the curve using the trapezoidal method. A linear model using a 3-way mixed factorial analysis of variance (ANOVA; i.e., (Carb, Whey, Soy) \times Time); i.e., baseline 3, 6, 9 months) was used to analyze these data. To determine differences among groups at baseline

Table 2. Subject Characteristics^a

	Carbohydrate	Whey	Soy
<i>n</i> (M/F)	13/9	13/6	11/11
Age (years)	22.3 ± 3.1	$22.8~\pm~3.7$	24.0 ± 2.9
Height (cm)	172.0 ± 8.7	171.8 ± 10.3	170.5 ± 2.9
Body mass (kg)	$72.4~\pm~14.9$	74.1 ± 15.7	$72.0~\pm~8.4$
Fat mass (kg)	$19.5~\pm~9.0$	19.4 ± 11.3	20.5 ± 11.3
Lean body mass (kg)	$49.8~\pm~9.8$	51.7 ± 10.7	48.5 ± 10.0
Body fat (%)	$26.4~\pm~8.7$	25.3 ± 12.0	27.3 ± 11.0
Squat 1 RM (kg)	71 ± 5	83 ± 6	60 ± 5
Bench press 1 RM (kg)	$45~\pm~5$	52 ± 5	$45~\pm~5$

M = male; F = female; RM = repetition maximum.

^aValues are mean \pm SD. No significant differences between groups.

and changes over specific intervals, a one-way ANOVA was used. When main effects were significant, Bonferroni corrections (or least significant difference [LSD] equivalent to a notype I error rate adjustment) were made for corresponding pairwise comparisons. Performance testing exhibited an ICCR of $p \ge 0.95$. Significance was set at $p \le 0.05$.

RESULTS

There were no significant differences between groups in physical characteristics at baseline (see Table 2).

Table 3	Daily	Dietary	Nutrient	Intake ^a
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There were no significant differences between groups at baseline in dietary intake (see Table 3). Energy intake remained constant over the intervention in all groups. We also met our goal of achieving an average protein intake between 1.0 and 1.2 g/kg body mass (not including supplements). As planned, the main dietary change was a significant increase in protein intake in the whey and soy groups and an increase in carbohydrate in the carb treatment group. Subjects in the whey and soy groups consumed $\sim 20 \text{ g} \cdot \text{day}^{-1}$ more protein than those in the carb group, corresponding to ~ 1.4 and $1.1 \text{ g} \cdot \text{kg}$ body mass⁻¹, respectively. Dietary fat was constant across groups and time, representing about 25%–30% of total energy.

Body mass and lean body mass significantly increased at 3 months and remained significantly higher than baseline at 6 and 9 months for all groups (see Table 4). The increase in body mass was not different between groups, but gains in lean body mass were significantly greater in the whey group at all testing points, whereas there were no differences between the carb and soy groups. After 9 months of training, all but 2 subjects in the whey group showed greater increases in lean body mass than the mean response for the soy group (see Fig. 2). The same pattern of a greater increase in lean body mass in the whey group was evident when looking at only men (carb = 2.6 ± 1.0 , whey = 3.6 ± 1.6 , soy = 2.6 ± 1.4 kg) or women (carb = 1.9 ± 1.0 , whey = 2.8 ± 1.2 , soy = 1.1 ± 1.6 kg). There were no significant main time or group effects for fat mass. There was a main effect

	Baseline	3 Months	6 Months	9 Months	ANOVA
Energy (kcal)					
Carb	1892 ± 115	1992 ± 80	2019 ± 66	2003 ± 76	T: $p = 0.701$
Whey	2111 ± 121	2116 ± 84	2129 ± 69	$2083~\pm~80$	G: $p = 0.434$
Soy	2032 ± 112	$2061~\pm~78$	1967 ± 64	2104 ± 74	$G \times T: p = 0.552$
Protein (g)					_
Carb	82.6 ± 6.0	77.2 ± 3.7	77.7 ± 3.4	76.6 ± 3.5	T: $p = 0.072$
Whey	92.5 ± 6.3	$101.3 \pm 3.9^{*}$	$99.3 \pm 3.6^{*}$	$102.8 \pm 3.7^{*}$	G: $p = 0.000$
Soy	86.2 ± 5.8	$99.1 \pm 3.6^{*}$	$94.5 \pm 3.3^{*}$	$97.3 \pm 3.4^{*}$	$G \times T: p = 0.014$
Protein (g/kg body mass)					*
Carb	1.14 ± 0.28	1.08 ± 0.12	1.08 ± 0.10	1.06 ± 0.13	T: $p = 0.272$
Whey	1.27 ± 0.41	1.38 ± 0.14	1.35 ± 0.22	1.39 ± 0.18	G: $p = 0.000$
Soy	1.27 ± 0.45	1.41 ± 0.23	1.32 ± 0.14	1.35 ± 0.13	$G \times T: p = 0.109$
Carbohydrate (g)					*
Carb	238 ± 15	270 ± 12	276 ± 13	275 ± 14	T: $p = 0.002$
Whey	275 ± 16	288 ± 12	303 ± 13	285 ± 15	G: $p = 0.314$
Soy	274 ± 15	278 ± 11	275 ± 12	307 ± 14	$G \times T: p = 0.074$
Fat (g)					-
Carb	62.2 ± 5.7	66.3 ± 3.9	69.2 ± 3.6	67.5 ± 3.9	T: $p = 0.108$
Whey	72.3 ± 6.0	65.3 ± 4.1	64.3 ± 3.8	62.5 ± 4.1	G: $p = 0.420$
Soy	66.7 ± 5.6	62.4 ± 3.8	57.4 ± 3.5	58.1 ± 3.8	$G \times T: p = 0.345$
Cholesterol (mg)					_
Carb	259 ± 33	234 ± 26	228 ± 24	294 ± 29	T: $p = 0.218$
Whey	253 ± 35	$227~\pm~28$	217 ± 25	239 ± 31	G: $p = 0.424$
Soy	226 ± 32	$208~\pm~26$	212 ± 24	207 ± 29	$G \times T: p = 0.682$

ANOVA = analysis of variance; G = main group effect; T = main time effect; G \times T = Group \times Time effect.

^aValues are mean \pm SD. Baseline nutrient intake was obtained from a 5-day diet record and each remaining time point (3, 6, 9 months) reflects the average of two 5-day diet records for each person. Data analyzed by 3 \times 4 ANOVA.

 $p^* < 0.05$ from corresponding baseline value.

Table 4. Change	in Body	Composition	Responses to	Resistance	Training ^a

	Δ 3 Months	Δ 6 Months	Δ 9 Months	<i>p</i> -Value (between groups)
Body mass (kg)				
Carb	2.2 ± 2.2	2.4 ± 1.9	1.8 ± 2.4	Δ 3 months = 0.083
Whey	2.8 ± 2.1	3.3 ± 2.7	3.1 ± 3.0	Δ 6 months = 0.444
Soy	1.4 ± 1.8	2.4 ± 3.1	2.2 ± 4.0	Δ 9 months = 0.448
Fat mass (kg)				
Carb	-0.2 ± 1.8	-0.2 ± 1.9	-0.5 ± 2.2	Δ 3 months = 0.311
Whey	-0.1 ± 1.5	-0.4 ± 2.4	-0.6 ± 2.7	Δ 6 months = 0.971
Soy	-0.7 ± 1.7	-0.2 ± 2.7	0.2 ± 4.1	Δ 9 months = 0.634
Lean body mass (kg)				
Carb	2.4 ± 1.4	2.6 ± 1.4	2.3 ± 1.7	Δ 3 months = 0.030
Whey	$3.1 \pm 1.5^{*}$	$3.5 \pm 1.3^{*}$	$3.3 \pm 1.5^{*}$	Δ 6 months = 0.056
Soy	1.9 ± 1.1	2.4 ± 1.7	1.8 ± 1.6	Δ 9 months = 0.016
Percentage body fat				
Carb	-0.9 ± 1.9	-1.0 ± 2.1	-1.2 ± 2.5	Δ 3 months = 0.367
Whey	-1.1 ± 1.4	-1.6 ± 2.4	-1.5 ± 2.6	Δ 6 months = 0.856
Soy	-1.5 ± 1.8	-1.4 ± 2.4	-0.6 ± 3.6	Δ 9 months = 0.634
Squat 1 RM (kg)				
Carb	23.7 ± 12.3	33.8 ± 14.4	43.7 ± 14.6	Δ 3 months = 0.722
Whey	20.4 ± 11.2	30.8 ± 14.3	35.8 ± 13.8	Δ 6 months = 0.759
Soy	22.4 ± 13.7	33.8 ± 14.2	39.8 ± 16.2	Δ 9 months = 0.279
Bench press 1 RM (kg)				
Carb	10.3 ± 7.1	13.9 ± 7.5	16.0 ± 8.1	Δ 3 months = 0.450
Whey	11.7 ± 6.7	12.2 ± 16.8	20.1 ± 2.3	Δ 6 months = 0.879
Soy	9.1 ± 5.3	13.3 ± 5.7	15.9 ± 1.4	Δ 9 months = 0.200

RM = repetition maximum.

 a Values are mean \pm SD. There were significant main time effects for body mass, lean body mass, percentage body fat, and maximal strength. In each case, values were significantly different compared to baseline but not different at 3, 6, and 9 months.

*p < 0.05 from corresponding carb and soy values.

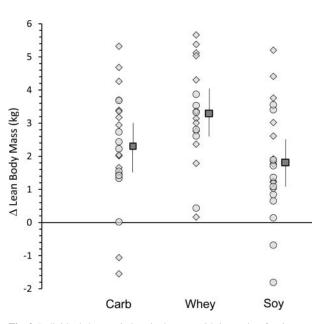


Fig. 2. Individual changes in lean body mass with 9 months of resistance training in subjects supplemented with carbohydrate (n = 22), whey protein (n = 19), or soy protein (n = 22). Diamonds = men, circles = women. Boxes represent mean \pm 95% confidence interval. Whey > carb and soy by one-way analysis of variance.

of time on percentage body fat as shown by a decrease after 3 months that remained lower over the entire study, but there were no group differences. There was a main effect of time on maximal bench press and squat strength as shown by a significant increase at 3 months that remained higher than baseline at 9 months. As expected with a resistance training program alone, the carb, whey, and soy groups all showed a significant and similar increase in maximal bench press (35%, 40%, and 36%, respectively) and squat (62%, 44%, and 65%, respectively).

Fasting concentrations of leucine were significantly increased (20%) in the whey group and remained unchanged in the carb and soy groups. Exercise-induced plasma amino acid patterns closely followed the amino acid composition of supplements consumed immediately after resistance exercise (see Table 5). Plasma leucine increased more than 2-fold 60 minutes after exercise in the whey group, whereas values were slightly increased in the soy group and decreased in the carb group (see Fig. 3). The same response patterns were observed in plasma BCAA and EAA, with the whey group showing a near doubling in concentration 60 minutes postexercise compared to a more moderate response in the soy group.

There were few significant correlations among plasma amino acids and changes in lean body mass with the exception of leucine. Several measures of leucine at different time points were positively correlated with changes in lean body mass; the

	Pre-exercise	15 Minutes	30 Minutes	60 Minutes	AUC	AUC ANOVA
Alanine				Pretraining		
Carb	212 ± 48	336 ± 61	342 ± 71	299 ± 79	$22,929 \pm 4112$	T: 0.501
Whey	212 ± 79	324 ± 84	375 ± 90	432 ± 112	$25,390 \pm 6107$	G: 0.090
Soy	$229~\pm~71$	$340~\pm~97$	$370~\pm~82$	$389~\pm~98$	$25,266 \pm 6113$	G × T: 0.664
]	Posttraining		
Carb	243 ± 50	359 ± 79	368 ± 82	344 ± 103	$25,177 \pm 5379$	
Whey	229 ± 85	352 ± 130	369 ± 137	440 ± 130	$26,243 \pm 7856$	
Soy	$224~\pm~54$	349 ± 91	$383~\pm~73$	$416~\pm~115$	$26,067 \pm 5439$	
Arginine				Pretraining		
Carb	61 ± 20	59 ± 22	56 ± 19	52 ± 20	4275 ± 1492	T: 0.026
Whey	57 ± 19	62 ± 19	74 ± 22	89 ± 23	5251 ± 1328	G: 0.020
Soy	52 ± 20	58 ± 19	76 ± 24	91 ± 26	5153 ± 1365	G × T: 0.025
,			1	Posttraining		
Carb	70 ± 23	68 ± 25	66 ± 20	62 ± 21	4995 ± 1613	
Whey	55 ± 20	58 ± 20	60 ± 20 67 ± 26	85 ± 24	4918 ± 1463	
Soy	65 ± 16	72 ± 25	94 ± 38	114 ± 45	$6426 \pm 2067^*$	
-	05 ± 10	12 ± 25			0420 ± 2007	
Asparagine	20 ± 2	24 + 22		Pretraining 33 ± 24	24(6 + 1264)	T. 0 109
Carb	29 ± 8	34 ± 22	35 ± 22	33 ± 24 53 ± 19	2466 ± 1264	T: 0.198
Whey	28 ± 9 31 ± 12	28 ± 8 32 ± 12	$38 \pm 12 \\ 43 \pm 24$	53 ± 19 51 ± 25	2660 ± 768 2911 ± 1287	G: 0.017 G × T: 0.477
Soy	51 ± 12	52 ± 12			2911 ± 1287	G X 1: 0.4//
				Posttraining		
Carb	38 ± 15	34 ± 14	35 ± 13	35 ± 16	2615 ± 1082	
Whey	34 ± 9	31 ± 8	38 ± 17	54 ± 23	2895 ± 904	
Soy	36 ± 13	38 ± 17	49 ± 23	60 ± 28	3383 ± 1377	
Citruline				Pretraining		
Carb	22 ± 7	18 ± 5	17 ± 4	14 ± 4	1340 ± 344	T: 0.824
Whey	20 ± 5	18 ± 5	19 ± 5	20 ± 6	$1429~\pm~356$	G: 0.037
Soy	20 ± 4	18 ± 5	18 ± 5	18 ± 5	1363 ± 345	$G \times T: 0.908$
]	Posttraining		
Carb	23 ± 6	20 ± 5	18 ± 4	16 ± 4	1449 ± 350	
Whey	22 ± 7	19 ± 6	19 ± 6	20 ± 6	1493 ± 406	
Soy	21 ± 6	18 ± 5	19 ± 5	20 ± 7	1449 ± 404	
Cysteine				Pretraining		
Carb	44 ± 10	43 ± 13	46 ± 13	45 ± 12	3333 ± 866	T: 0.116
Whey	49 ± 16	50 ± 18	58 ± 20	67 ± 31	4033 ± 1647	G: 0.002
Soy	44 ± 17	41 ± 18	45 ± 19	46 ± 18	3268 ± 1341	G × T: 0.371
2			1	Posttraining		
Carb	46 ± 14	45 ± 16	47 ± 12	49 ± 15	3488 ± 1040	
Whey	55 ± 21	52 ± 10	60 ± 26	77 ± 42	4487 ± 1753	
Soy	50 ± 19	47 ± 18	53 ± 21	54 ± 18	3800 ± 1394	
Glutamate				Pretraining		
Carb	58 ± 21	82 ± 33	83 ± 32	73 ± 26	5653 ± 1802	T: 0.283
Whey	58 ± 21 59 ± 27	82 ± 33 90 ± 39	33 ± 32 102 ± 45	105 ± 55	6776 ± 270	G:5 0.186
Soy	59 ± 27 62 ± 26	90 ± 39 81 ± 36	102 ± 43 90 ± 33	103 ± 33 84 ± 34	60776 ± 270 6055 ± 2234	$G \times T: 0.799$
309	02 ± 20	01 ± 50			0055 ± 2254	G × 1.0.777
a 1		04 1 07		Posttraining	550 () 1001	
Carb	56 ± 21	81 ± 27	79 ± 32	73 ± 33	5536 ± 1881	
Whey	67 ± 28	92 ± 33	100 ± 39	124 ± 62	6382 ± 2134	
Soy	53 ± 23	74 ± 27	83 ± 29	82 ± 38	5548 ± 2012	
Glutamine				Pretraining		
Carb	740 ± 190	730 ± 196	738 ± 201	707 ± 211	$54,734 \pm 14,147$	T: 0.103
Whey	760 ± 194	754 ± 190	809 ± 196	905 ± 245	$60,141 \pm 14,166$	G: 0.025
Soy	686 ± 184	677 ± 193	718 ± 183	759 ± 196	$53,692 \pm 13,819$	$G \times T: 0.551$
]	Posttraining		
Carb	$834~\pm~151$	821 ± 131	821 ± 169	806 ± 157	$61,556 \pm 10,737$	
	000 1 155	792 154	826 L 100	880 ± 272	$67,827 \pm 28,026$	
Whey Soy	829 ± 155 712 ± 140	$782 \pm 154 \\ 696 \pm 155$	$826 \pm 190 \\ 752 \pm 124$	330 ± 272	$55,583 \pm 10,202$	

Table 5. Resistance Exercise-Induced Plasma Amino Acid Responses^a

(Continued on next page)

Table 5. Resistance Exercise-Induced Plasma Amino Acid Responses^a (Continued)

Glycine Carb						
				Pretraining		
	184 ± 72	$177~\pm~78$	175 ± 74	163 ± 70	$13,114 \pm 5427$	T: 0.570
Whey	175 ± 56	164 ± 59	176 ± 49	199 ± 69	$13,266 \pm 4084$	G: 0.865
Soy	159 ± 67	$148~\pm~62$	164 ± 76	178 ± 82	$12,068 \pm 5226$	G × T: 0.955
	100 1 50	175 50	17() ()	Posttraining	12 222 + 4202	
Carb	188 ± 56	175 ± 59	176 ± 62	174 ± 59	$13,332 \pm 4293$	
Whey	181 ± 67	166 ± 64	180 ± 78	195 ± 82	$13,409 \pm 5295$	
Soy	158 ± 46	147 ± 43	158 ± 46	181 ± 62	$11,955 \pm 3510$	
Histidine				Pretraining		
Carb	105 ± 42	120 ± 46	110 ± 47	99 ± 41	8237 ± 3115	T: 0.188
Whey	117 ± 38	148 ± 55	140 ± 44	128 ± 37	9905 ± 3060	G: 0.616
Soy	98 ± 33	131 ± 60	112 ± 36	110 ± 30	8596 ± 2914	$G \times T: 0.902$
	100 20	116 - 40	106 - 26	Posttraining	70(0 + 2071	
Carb	100 ± 39	116 ± 42	106 ± 36	106 ± 43	7968 ± 2971	
Whey	118 ± 42	149 ± 67	128 ± 51	122 ± 38	9819 ± 3585	
Soy	101 ± 29	124 ± 49	116 ± 39	111 ± 29	8580 ± 2580	
Isoleucine	~~ · · -	.	.	Pretraining	2052	m
Carb	57 ± 17	55 ± 19	55 ± 19	42 ± 19	3953 ± 1213	T: 0.000
Whey	59 ± 10	71 ± 19	116 ± 56	162 ± 51	7522 ± 2202	G: 0.402#
Soy	58 ± 15	61 ± 17	83 ± 34	96 ± 32	5550 ± 1583	$G \times T: 0.907$
	60 1 17	<pre><0 25</pre>	52 1 14	Posttraining	1006 1 1005	
Carb	60 ± 17	60 ± 25	52 ± 14	42 ± 14	4026 ± 1295	
Whey	68 ± 20	73 ± 20	113 ± 64	172 ± 72	7786 ± 2887	
Soy	60 ± 12	64 ± 17	83 ± 27	97 ± 30	5664 ± 1367	
Leucine				Pretraining		
Carb	110 ± 34	106 ± 39	103 ± 31	80 ± 28	7544 ± 2256	T: 0.000
Whey	108 ± 21	125 ± 29	182 ± 71	265 ± 88	$12,505 \pm 3305$	G: 0.074#
Soy	106 ± 25	111 ± 26	142 ± 51	157 ± 48	9798 ± 2392	$G \times T: 0.157$
Cert	115 26	110 29	102 25	Posttraining 85 ± 30	7520 1 2451	
Carb	$115 \pm 36 \\ 130 \pm 36$	$110 \pm 38 \\ 142 \pm 35$	$103 \pm 35 \\ 199 \pm 89$	85 ± 30 290 ± 97	7538 ± 2451 $13,973 \pm 3986$	
Whey Soy	130 ± 30 110 ± 20	142 ± 33 119 ± 36	199 ± 89 146 ± 45	290 ± 97 162 ± 49	$10,058 \pm 2517$	
-	110 ± 20	119 ± 50	140 ± 45		10,058 ± 2517	
Methionine	11 4	10 2	11 ± 4	Pretraining	901 946	T. 0.000
Carb	11 ± 4	10 ± 3		10 ± 4	801 ± 246	T: 0.000
Whey	$\begin{array}{c} 12 \pm 4 \\ 12 \pm 4 \end{array}$	12 ± 3 12 ± 3	16 ± 4	$\begin{array}{c} 18 \ \pm \ 4 \\ 14 \ \pm \ 4 \end{array}$	$1077 \pm 260 \\ 950 \pm 268$	G: 0.001#
Soy	12 ± 4	12 ± 3	13 ± 4		950 ± 208	$G \times T: 0.557$
Carb	12 ± 3	12 ± 3	11 ± 2	Posttraining 11 ± 4	867 ± 189	
Whey	12 ± 3 14 ± 4	12 ± 3 14 ± 4	$\frac{11 \pm 2}{17 \pm 4}$	11 ± 4 20 ± 4	1200 ± 259	
Soy	14 ± 4 13 ± 3	14 ± 4 13 ± 3	17 ± 4 16 ± 4	16 ± 3	1200 ± 239 1089 ± 216	
Ornithine	10 1 0	10 1 0	10 ± 1	Pretraining	1007 1 210	
Carb	68 ± 28	58 ± 18	60 ± 20	54 ± 17	4489 ± 1419	T: 0.406
Whey	60 ± 20 62 ± 26	63 ± 21	$\begin{array}{c} 00 \pm 20 \\ 70 \pm 22 \end{array}$	78 ± 23	5089 ± 1596	G: 0.252
Soy	62 ± 20 62 ± 20	61 ± 17	70 ± 22 70 ± 18	78 ± 25 79 ± 25	5069 ± 1390 5064 ± 1360	$G \times T: 0.850$
509	02 1 20	01 ± 17	70 ± 10	Posttraining	5001 ± 1500	G X 1. 0.050
Carb	63 ± 27	57 ± 24	57 ± 25	55 ± 24	4320 ± 1831	
Whey	56 ± 18	57 ± 21 59 ± 17	61 ± 18	71 ± 23	4606 ± 1348	
Soy	65 ± 33	62 ± 30	66 ± 34	68 ± 26	4875 ± 2158	
Phenylalanine				Pretraining		
Carb	41 ± 13	40 ± 10	42 ± 19	37 ± 20	3012 ± 904	T: 0.003
Whey	41 ± 13 45 ± 12	40 ± 10 49 ± 17	42 ± 19 55 ± 18	62 ± 16	3012 ± 904 3822 ± 911	G: 0.001#
Soy	43 ± 12 42 ± 18	49 ± 17 45 ± 17	53 ± 18 53 ± 20	$\frac{62}{58} \pm 22$	3708 ± 1370	$G \times T: 0.094$
50,	12 - 10	10 11 11	55 <u>1</u> 20		5700 ± 1570	G / 1. 0.0)-
Carb	51 ± 17	48 ± 15	47 ± 14	Posttraining 44 ± 16	3545 ± 1077	
Whey	51 ± 17 52 ± 15	48 ± 13 51 ± 13	47 ± 14 53 ± 15	44 ± 10 61 ± 16	3343 ± 1077 4046 ± 922	
Soy	52 ± 13 55 ± 17	51 ± 13 59 ± 20	53 ± 13 71 ± 26	77 ± 24	4040 ± 922 4901 ± 1455	

	Pre-exercise	15 Minutes	30 Minutes	60 Minutes	AUC	AUC ANOVA
Proline			F	Pretraining		
Carb	$191~\pm~72$	199 ± 67	$217~\pm~79$	$202~\pm~75$	$15,255 \pm 5331$	T: 0.097
Whey	193 ± 66	$213~\pm~67$	$261~\pm~65$	335 ± 85	$18,581 \pm 4636$	G: 0.248
Soy	$175~\pm~52$	$187~\pm~49$	$227~\pm~53$	$255~\pm~64$	$15,765 \pm 3874$	G × T: 0.192
			Р	osttraining		
Carb	219 ± 65	221 ± 63	$237~\pm~68$	236 ± 68	$17,133 \pm 4657$	
Whey	201 ± 61	211 ± 57	253 ± 77	340 ± 108	$18,532 \pm 4926$	
Soy	$170~\pm~48$	186 ± 48	$224~\pm~44$	$261~\pm~62$	$15,701 \pm 3296$	
Serine			F	Pretraining		
Carb	82 ± 34	78 ± 37	78 ± 34	71 ± 32	5793 ± 2535	T: 0.649
Whey	77 ± 39	73 ± 36	95 ± 56	123 ± 68	6770 ± 3489	G: 0.166
Soy	74 ± 40	71 ± 38	87 ± 55	94 ± 50	6070 ± 3401	G × T: 0.469
·			Р	osttraining		
Carb	89 ± 32	82 ± 31	81 ± 27	80 ± 28	6123 ± 2129	
Whey	79 ± 41	74 ± 39	94 ± 68	124 ± 72	6830 ± 3907	
Soy	83 ± 39	84 ± 44	101 ± 48	115 ± 58	7134 ± 338	
Taurine			F	Pretraining		
Carb	12 ± 4	14 ± 4	13 ± 4	13 ± 4	1047 ± 368	T: 0.396
Whey	12 ± 4 12 ± 3	14 ± 4 14 ± 4	13 ± 4 14 ± 4	13 ± 4 14 ± 4	1047 ± 300 1005 ± 226	G: 0.000
Soy	12 ± 3 11 ± 3	14 ± 4 13 ± 5	14 ± 4 12 ± 5	14 ± 4 11 ± 4	902 ± 308	$G \times T: 0.533$
509	11 ± 5	10 ± 0		osttraining	902 ± 900	G X 1.0.555
Carb	15 ± 6	17 ± 6	17 ± 7	17 ± 6	1236 ± 389	
Whey	15 ± 6 16 ± 6	17 ± 6 17 ± 6	17 ± 7 20 ± 8	17 ± 0 19 ± 8	1250 ± 589 1365 ± 480	
Soy	10 ± 0 15 ± 5	17 ± 0 16 ± 5	$\begin{array}{c} 20 \pm 3 \\ 17 \pm 6 \end{array}$	19 ± 8 17 ± 5	1303 ± 430 1233 ± 374	
Threonine	10 ± 0	10 ± 0		Pretraining	1200 - 071	
Carb	80 ± 47	81 ± 50	83 ± 48	77 ± 46	6057 ± 3521	T: 0.204
Whey	80 ± 47 90 ± 56	90 ± 56	118 ± 78	166 ± 112	8504 ± 5300	G: 0.025
Soy	90 ± 30 84 ± 67	90 ± 50 81 ± 63	118 ± 78 102 ± 93	100 ± 112 112 ± 99	7051 ± 5992	$G \times T: 0.495$
30y	04 ± 07	01 ± 05			1051 ± 5772	G × 1. 0.495
Carb	04 + 52	95 50	90 ± 50	osttraining 91 \pm 47	6707 2711	
Carb	94 ± 53	$85 \pm 52 \\ 103 \pm 64$			6707 ± 3711	
Whey	$113 \pm 66 \\ 89 \pm 50$	103 ± 64 86 ± 51	$137 \pm 98 \\ 104 \pm 58$	$187 \pm 127 \\ 121 \pm 80$	9896 ± 6185 7423 ± 4317	
Soy	89 ± 50	80 ± 51			7425 ± 4517	
Tryptophan				Pretraining		
Carb	34 ± 11	30 ± 9	35 ± 14	34 ± 15	2496 ± 723	T: 0.000
Whey	32 ± 12	37 ± 7	45 ± 10	63 ± 12	3269 ± 601	G: 0.000#
Soy	39 ± 16	38 ± 14	42 ± 15	47 ± 17	3073 ± 1124	$G \times T: 0.158$
				osttraining		
Carb	38 ± 16	34 ± 13	35 ± 13	37 ± 17	2675 ± 1036	
Whey	45 ± 14	47 ± 13	54 ± 15	71 ± 16	4017 ± 963	
Soy	45 ± 16	47 ± 15	54 ± 16	61 ± 17	3851 ± 1080	
Valine			F	Pretraining		
Carb	230 ± 135	230 ± 144	233 ± 153	195 ± 114	$16,802 \pm 10,220$	T: 0.322
Whey	212 ± 68	230 ± 130	270 ± 164	360 ± 160	$19,811 \pm 9661$	G: 0.018
Soy	$212~\pm~106$	201 ± 70	234 ± 83	252 ± 83	$16,748 \pm 6023$	$G \times T: 0.362$
			Р	osttraining		
Carb	253 ± 159	254 ± 167	$241~\pm~158$	214 ± 132	$18,130 \pm 11,564$	
Whey	$251~\pm~81$	255 ± 93	313 ± 196	388 ± 220	$22,373 \pm 10,886$	
Soy	$211~\pm~52$	$212~\pm~56$	$240~\pm~54$	$257~\pm~67$	$17,196 \pm 3896$	

Table 5. Resistance Exercise-Induced Plasma Amino Acid Responses^a (Continued)

 $AUC = area under the curve; ANOVA = analysis of variance; T = main time effect; G = main group effect; T \times G = Time \times Group effect.$

^aValues are mean \pm SD. Subjects performed squat (6 sets, 10 repetitions) followed immediately by consumption of carbohydrate, whey, or soy supplements. AUC data analyzed by 2 \times 3 ANOVA.

*Indicates significant difference (p < 0.05) from all other values.

#Indicates that whey and soy were significantly different (p < 0.05) from carb.

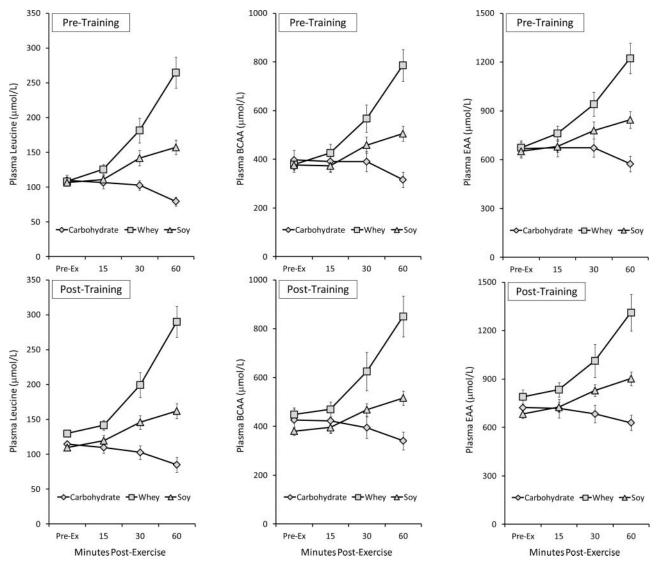


Fig. 3. Plasma leucine, branched-chain amino acid, and essential amino acid responses to a resistance exercise workout (6 sets of 10 repetitions) before and after 9 months of training.

most significant was fasting plasma leucine at 9 months versus changes in lean body mass (see Fig. 4). Although there was considerable overlap in fasting plasma leucine levels among individuals in the different groups, several subjects in the carb and soy groups had fasting values less than 100 μ mol · L⁻¹, whereas only one subject in the whey group fell below this arbitrary threshold. Within the group of individuals with fasting plasma leucine < 100 μ mol · L⁻¹, it is interesting to note than no one showed an increase in lean body mass greater than 4 kg (see Fig. 4).

DISCUSSION

Despite consistent research documenting the transient anabolic action of whey protein postexercise, there is a lack of research linking acute responses to chronic adaptations to training. This study was unique in that it (1) was the longest resistance training study to date investigating protein supplementation (9 months), (2) was the first to directly compare 2 isocaloric protein sources as well as a carbohydrate control, (3) involved a supervised resistance training program that included whole-body exercises, and (4) controlled protein intake throughout the intervention. Our results indicate that protein quality is an important determinant of lean body mass responses to resistance training in the context of untrained individuals consuming protein at levels slightly greater than the RDA but well within the normal range of protein intake for this population. There was a superior effect of whey protein supplementation accumulation of lean body mass with effects evident after 3 months and sustained throughout 9 months of training. Isocaloric supplementation with soy protein or carbohydrate was less effective in promoting gains in lean body mass. Whey supplementation was associated with higher fasting and exercise-induced elevations in plasma leucine, which

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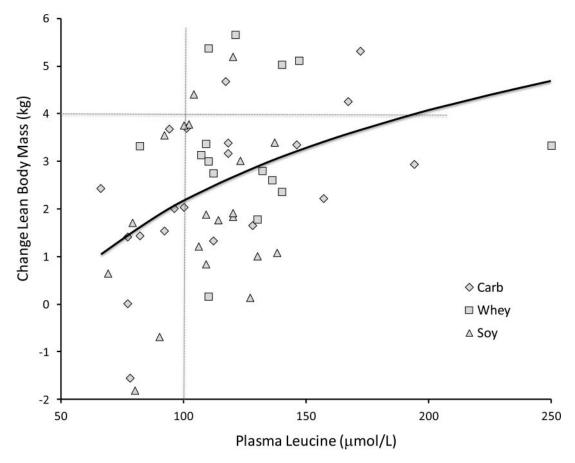


Fig. 4. Scatterplot of fasting plasma leucine at 9 months versus change in lean body mass from baseline to 9 months. Correlation coefficient (R^2) = 0.17, p < 0.005.

may account, in part, for greater anabolic effects in skeletal muscle.

A growing number of resistance training interventions have investigated the effects of protein supplementation, but few have involved long-term supervision of training and control of diet over periods longer than 3 months. A recent meta-analysis examined whether protein supplementation augments muscle mass with prolonged resistance training [25]. A total of 22 studies ranging from 6 to 24 weeks were analyzed. Carbohydrate was the placebo in most studies, and the source of protein was milk protein (whey or some combination of whey with other protein). Compared to placebo, protein supplementation resulted in an additional 0.7 kg gain in lean body mass over 3 months. This is the same additional gain in lean body mass observed in the present study for the whey and carb groups at 3 months.

Lean body mass increased after 3 months and plateaued at 6 and 9 months, indicating that the majority of gains in muscle mass can be achieved after 36 workouts in non-strengthtrained recreationally active young adults. The greater gains in lean body mass observed in the whey group did not translate into greater gains in maximal strength. Maximal strength is only partially related to muscle size, and it is not uncommon for resistance training-induced adaptations in muscle mass and maximal strength to be disconnected [26]. Nevertheless, the change in lean body mass at 3 months was significantly associated with the change in maximal squat and bench press at 3 months (r = 0.40 and 0.48, respectively). However, other variables such as neural adaptations have a greater contribution and are likely not impacted by whey supplementation. In addition, the varied exercises performed during training likely resulted in muscle mass gains that were distributed over the whole-body musculature, which would lessen the likelihood of manifesting in greater strength in a specific movement.

There was no difference in lean body mass response between the carb and soy groups, indicating that simply increasing protein intake from 1.1 to 1.4 g \cdot kg body mass⁻¹ is not adequate to optimize muscle gains to resistance training. This highlights the importance of protein quality over quantity within this range of protein intakes. There was no difference in fat mass responses between groups, but it is worth noting that fat mass did decrease, resulting in a significant decrease in percentage body fat for all groups. Thus, the gains in body mass for all groups, and the greater gains in the whey group, were attributed to gains in lean body mass. The lack of increase in fat mass, and an actual slight decline, is important from health and aesthetic perspectives.

The mechanism(s) underlying the greater adaptive response to whey in lean body mass could be related to the greater availability of leucine. Leucine, and more generally BCAA, are welldocumented fuels for muscle, but they are also potent signals activating an anabolic/anticatabolic program in skeletal muscle [27]. Ingestion of whey is clearly more effective than ingestion of soy protein at elevating circulating levels of BCAA, as well as total EAA postexercise (see Fig. 3). Peak concentrations of leucine were elevated more than 2-fold postexercise, and this has been shown to be of sufficient magnitude to elicit a robust increase in net muscle protein synthesis [28]. The cumulative effect of these daily anabolic surges, although transient, may be important to optimize gains in muscle mass. The 0.7 kg greater increase in lean body mass over the first 12 weeks would translate into an additional incorporation of only $1-2 \text{ g} \cdot \text{day}^{-1}$ of amino acids into skeletal muscle. Whey protein also increased fasting leucine levels on average 20%. This modest increase in basal leucine availability is surely a much weaker anabolic signal than the transient postprandial surges, but the total exposure of skeletal muscle to slightly elevated leucine over a 24-hour period could have a role in the adaptive response to resistance training. Interestingly, it was the fasting plasma leucine concentration that was most highly correlated with gains in lean body mass (see Fig. 4).

We do not know whether the protocol of whey ingestion used in this study (i.e., 22 g consumed immediately postexercise) is optimal. Previous studies have shown that 20 g of egg protein produced a maximal increase in muscle protein synthesis with no further increase with ingestion of 40 g in young individuals [29]. Better nitrogen retention was observed when resistance-trained men consumed whey protein in doses of 10– 20 g per serving postexercise compared to larger doses given in bolus [30]. In regards to timing, there is evidence that preexercise ingestion may stimulate muscle protein synthesis to a greater degree than ingestion after resistance exercise [31], but this study used free amino acids. A follow-up study by this group showed that ingestion of 20 g of whey protein before or after resistance exercise resulted in the same increase in muscle protein synthesis [13].

CONCLUSIONS

In conclusion, daily supplementation with ~ 20 g whey protein during resistance training is an effective strategy for augmenting gains in lean body mass in young, healthy, untrained men and women consuming protein levels slightly above the RDA. Gains in lean body mass occurred in the context of stable or small decreases in fat mass. These results point to protein quality as an important determinant of the adaptive response to whole-body resistance training.

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