Effect of Whey and Soy Protein Supplementation Combined With Resistance Training in Young Adults

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The purpose was to compare changes in lean tissue mass, strength, and myofibrillar protein catabolism resulting from combining whey protein or soy protein with resistance training. Twenty-seven untrained healthy subjects (18 female, 9 male) age 18 to 35 y were randomly assigned (double blind) to supplement with whey protein (W; 1.2 g/kg body mass whey protein + 0.3 g/kg body mass sucrose power, N = 9: 6 female, 3 male), soy protein (S; 1.2 g/kg body mass soy protein + 0.3 g/kg body mass sucrose powder, N = 9: 6 female, 3 male) or placebo (P; 1.2 g/kg body mass maltodextrine + 0.3 g/kg body mass sucrose powder, N = 9: 6 female, 3 male) for 6 wk. Before and after training, measurements were taken for lean tissue mass (dual energy X-ray absorptiometry), strength (1-RM for bench press and hack squat), and an indicator of myofibrillar protein catabolism (urinary 3-methylhistidine). Results showed that protein supplementation during resistance training, independent of source, increased lean tissue mass and strength over isocaloric placebo and resistance training (P < 0.05). We conclude that young adults who supplement with protein during a structured resistance training program experience minimal beneficial effects in lean tissue mass and strength.

Key Words: ergogenic aids, 3-methylhistidine, dual energy X-ray absorptiometry

Resistance training results in significant muscle protein turnover and the rate of muscle protein synthesis following exercise is elevated with oral consumption of amino acids (27). Given these findings, one would expect that an increase in amino acid availability through additional dietary protein during resistance training would increase muscle mass and strength. However, research is equivocal, with some showing a positive effect from protein supplementation (4, 6, 14, 21) while others report minimal or no benefits (7, 16).
Regarding dietary protein source, whey and soy protein have a high protein quality score and contain a relatively high proportion of essential amino acids (3, 15) necessary for muscle protein synthesis (27). Supplementing with whey protein (4, 6) and soy protein (6) during resistance training has been shown to have a positive effect on muscle mass in young adults. For example, young male adults who supplemented with whey protein (1.2 g · kg · d) during 6 wk of resistance training experienced significant gains in lean tissue mass over isocaloric placebo and resistance training (4). Furthermore, supplementing with whey protein or soy protein (33 g) during 9 wk of resistance training increased lean tissue mass over resistance training alone in young males, with no difference between protein sources (6). Although both protein sources appear beneficial, it is unknown whether advantages exist between whey protein and soy protein supplementation (1.2 g · kg · d) during resistance training in young men and women. The primary purpose of this study was to determine the effect of whey protein and soy protein supplementation during resistance training on muscle mass, strength, and myofibrillar protein catabolism during resistance training in young adults. Based on the findings of Phillips et al. (22) that milk-based proteins (i.e., whey, casein) stimulate greater amino acid uptake and net protein deposition over soy protein, we hypothesized that whey protein supplementation during resistance training would increase muscle mass over soy protein and resistance training alone. In addition, protein or amino acid supplementation increases the ratio of protein synthesis to degradation post-exercise (2). We therefore hypothesized that protein supplementation during resistance training, independent of source, would decrease myofibrillar protein catabolism over isocaloric placebo and resistance training.

**Methods**

**Subjects**

Twenty-seven subjects (18 female, 9 male) who were not participating in resistance type training volunteered for the study through newspaper advertisement. Subjects were required to fill out a Physical Activity Readiness Questionnaire (PAR-Q), which screens for health problems that may present a risk with performance of physical activity (25). Subjects that indicated a health problem were required to have medical approval before participating in the study. Subjects were free from other ergogenic aids for at least 12 wk prior to initial testing to eliminate any effects from other supplementation (5, 10). The study was approved by the University Ethics Review Board for Research in Human Subjects at St. Francis Xavier University and all subjects signed an informed consent prior to the study.

**Experimental Protocol**

The study used a double-blind repeated measures design in which every subject participated in resistance training and were randomized to receive whey protein, soy protein, or placebo for 6 wk. Prior to the first visit to the laboratory for initial testing and data collection, all subjects were instructed to refrain from physical activity for 48 h as it has been shown that muscle protein synthesis and degradation is elevated for 48 h post-exercise (23). The dependent variables measured before
and after 6 wk of protein supplementation and resistance training were: 1) lean tissue mass; 2) strength (squat and bench press one repetition maximum, 1-RM); and 3) urinary 3-methylhistidine excretion (an indicator of myofibrillar protein catabolism). In addition, subjects completed dietary records for 3 d during the first and final week of resistance training and supplementation to assess nutrient differences between groups.

Randomization and Supplementation

An individual, who was not involved in the study, was responsible for randomizing the subjects and coding the supplements to ensure all subjects and investigators remained blinded throughout the study. Entry and analysis of data was performed by analyzing coded groups. After matching subjects for age and body mass to minimize differences between groups, each subject was randomly assigned to supplement orally with whey protein (W; 1.2 g/kg body mass whey protein + 0.3 g/kg body mass sucrose powder), soy protein (S; 1.2 g/kg body mass soy protein + 0.3 g/kg body mass sucrose powder), or placebo (P; 1.2 g/kg body mass maltodextrine + 0.3 g/kg body mass sucrose powder), consumed in three equal doses (i.e., 0.5 g/kg body mass supplement powder dissolved in water before their training session, 0.5 g/kg body mass of supplement powder dissolved in water after their training session, and 0.5 g/kg body mass of supplement powder dissolved in water before going to bed). Subjects were instructed to consume the supplement shortly before and after (~30 min) each training session as it has been shown that the timing of protein ingestion is crucial for creating an anabolic environment for muscle growth, with ingestion before (1, 27) and after resistance training (1, 14) appearing optimal. On non-training days, subjects were instructed to consume the supplement in water in three equal doses. The supplements were mixed with sucrose powder to ensure that supplements and placebo were similar in energy content, taste, texture, and appearance. The protein dose of 1.2 g/kg body mass was chosen because it is an approximate amount shown to increase muscle mass during resistance training (4). Supplementation compliance was indirectly monitored by verbal communication and having subjects return empty supplement bags when picking up additional supplements.

Resistance Training

Prior to the start of the study, each subject familiarized themselves with the resistance training equipment by participating in three supervised resistance training sessions three times a week for 3 wk in the research weight training room located in the Department of Human Kinetics at St. Francis Xavier University. Familiarization with the resistance training equipment helped decrease the amount of learning (i.e., rapid improvement in the ability to perform a training exercise) which may contribute to the increase in strength during the initial stages of resistance training (11, 12). Subjects subsequently participated in 6 wk of resistance training combined with the protein or placebo supplements. All subjects followed the same high volume, periodized, free weight resistance training program for 6 wk. Training sessions were supervised as previous research has demonstrated greater gains compared to unsupervised training (20). The program consisted of 4 to 5 sets of
6 to 12 repetitions at 60 to 90% of 1-RM. Resistance training started on the first day of supplementation and consisted of a 4-d split routine involving whole body musculature. We have previously used this program successfully to increase muscle mass and strength (4). Briefly, day 1 involved chest and triceps musculature and included seven different exercises; day 2 involved back and biceps musculature and included six different exercises; day 3 involved leg, shoulder, and abdominal musculature included nine different exercises; and day 4 was a rest day. This program was broken into five blocks of two cycles for 8 d, for a total of 40 d. Training logs were completed by each subject and included the weight used for each exercise and the number of sets and repetitions performed. Total training volume/session over the 6 wk resistance training program was calculated by multiplying the weight used with the number of sets and repetitions for each exercise (9).

**Lean Tissue Mass**

Lean tissue mass was assessed by dual energy X-ray absorptiometry (DXA) at the beginning of the study and following 6 wk of resistance training and supplementation. Whole-body DXA scans were performed using a Hologic QDR-1000 in array mode and analyzed (excluding the head region) using system software version 7.01. The same technician analyzed all DXA scans. Reproducibility was previously determined on 10 subjects, 1 wk apart, and measuring the coefficient of variation, defined as the square root of the between-test variance (standard deviation), divided by the combined (marginal) mean of the test results for days 1 and 2, multiplied by 100 (to produce a percentage). The coefficient of variation for lean tissue mass was 0.54%.

**Muscle Strength**

Squat and bench press strength were assessed using a one-repetition maximum (1-RM) standard testing procedure as previously described (4, 9) prior to and following supplementation and resistance training. These two exercises were chosen as an index of muscular strength because they involve the major muscle groups in the lower and upper body. To measure the 1-RM squat, a hack squat machine (Northern Lights Fitness Inc., Cornwall, Ontario) was used. Each subject positioned his/her feet approximately shoulder width apart inside the squat rack with head, shoulder, and back tightly secured against the seat apparatus. Subjects were instructed to lower themselves until an internal angle of 90° at the knee was achieved before returning to the upright position. A warm-up consisted of 10 repetitions of squat using a weight determined by each subject to be comfortable. Weight was then progressively increased for each subsequent 1-RM attempt with a 2 min rest interval. The 1-RM was usually reached in four to six trials, including the warm-up set.

For bench press, subjects were positioned on the bench with both feet flat on the floor. Subjects were not allowed to lift their buttocks off the bench or arch their backs during a lift. A complete repetition went from the top straight-arm position, down until the bar touched the chest, and then ended with the bar returning to the top straight-arm position. A warm-up consisted of 10 repetitions with a comfortable starting weight as determined by each subject. Weight was then progressively increased for each subsequent 1-RM attempt with a 2 min rest interval. The 1-RM was usually reached in four to six trials, including the warm-up set. The reproduc-
ibility of the squat and bench press was assessed by testing 1-RM strength on 12 subjects on two occasions, 1 wk apart. Coefficients of variation were 5.97% for squat and 1.89% for bench press.

**Urinary Analysis for 3-methylhistidine**

For measurement of 3-methylhistidine, an indicator of myofibrillar protein catabolism, urine was collected during the last 24 h of a 72 h meat-free diet immediately before the first resistance training session (i.e., day 1) and immediately following the last resistance training session (i.e., day 40). A meat-free diet was implemented because meat consumption increases urinary 3-methylhistidine values and may falsely represent an increase in myofibrillar protein turnover (18). Three days of a meat-free diet are required to return 3-methylhistidine levels to baseline (19). The designated urine collection procedure was to discard the first urination upon waking in the morning and then collect all urine samples for 24 h, including the first urination upon waking the following morning. Urine samples were brought to the researcher where the subject’s urine volume was recorded. Aliquots (200 µL) of each urine sample were drawn off from the 24 h collection and stored at –20 °C until analyzed. The concentration of 3-methylhistidine was determined by high-performance liquid chromatography by methods previously described (17). The daily amount of 3-methylhistidine excreted by each subject was determined by multiplying the concentration by the 24 h urine volume. This amount of 3-methylhistidine was then expressed relative to lean tissue mass (µmol/kg) (9). The intra-assay coefficient of variation from duplicate samples was 6.3%.

**Dietary Assessment**

Dietary intake was recorded for 3 d during the first and final week of supplementation and resistance training to assess whether there were differences in total energy and macronutrient composition between the protein and placebo treatment conditions. Dietary intake was not recorded immediately before and immediately following the study because habitual dietary intake immediately before and immediately following the study would be altered because of the 72 h meat-free diet required for determination of urinary 3-methylhistidine. Subjects used a 3 d food booklet to record what they ate for two weekdays and one weekend day. Subjects were instructed to record all food items, including portion sizes consumed for the three designated days. The Food Processor version 7.8 dietary analysis program (Nutrition Analysis & Fitness Software, ESHA Research, Salem, OR) was used to analyze the food records. Each food item was entered and the program provided total energy consumption on average over the 3 d as well as energy from carbohydrates, fats, and proteins individually.

**Statistical Analyses**

A 3 (whey vs. soy vs. placebo) × 2 (pre vs. post) ANOVA with repeated measures on the second factor was used to determine whether there were any differences between the protein and placebo groups over time for the dependent variables of lean tissue mass, strength, 3-methylhistidine, and diet (energy and macronutrient contents). A one-factor ANOVA was used to determine differences in average train-
ing volume (kg \times \text{sets} \times \text{reps}) per session between the protein and placebo groups and to determine whether there were differences in baseline measurements between groups. Tukey’s post hoc tests were used to determine differences between group means. All results are expressed as means \pm standard deviation. The magnitude of the difference between significant means (i.e., effect size) was determined by \eta^2. Eta squared is a measure of the proportion of the total variance that is explained by the treatment effects. A \eta^2 value of 0.8 represents large differences, 0.5 represents medium differences, and 0.2 represents small differences (13). Statistical analyses were carried out using SPSS version 10.02 for Windows (SPSS, Inc., Chicago, IL). Statistical significance was set at \( P < 0.05 \).

Results

Of the original 31 subjects who volunteered, 27 completed the study. One subject from the W group withdrew because of shoulder and back pain and one subject in each group withdrew because of time constraints. Baseline characteristics of the 27 subjects who completed the study are: W group (age 24.0 \pm 6 y; weight 69.3 \pm 12 kg; height 170.5 \pm 15 cm; \( n = 9 \): 6 female, 3 male), S group (age 22.5 \pm 6 y; weight 71.8 \pm 15 kg; height 169.3 \pm 18 cm; \( n = 9 \): 6 female, 3 male) and P group (age 23.0 \pm 6 y; weight 69.3 \pm 12 kg; height 170.7 \pm 21 cm; \( n = 9 \): 6 female, 3 male). There were no differences between the protein and placebo groups for any of the baseline measurements. There were no differences in average training volume per session between the W, S, and P groups. The W group had a mean average volume of 16,397 \pm 3615 kg per training session, the S group had an average volume of 15,678 \pm 2994 kg per training session, while the P group had an average volume of 14,681 \pm 3366 kg per training session. Dietary intake did not differ significantly between groups and did not differ significantly over the course of training (Table 1). In addition, there were no differences between the protein and placebo groups over time for changes in bone mineral content and density and fat mass.

Table 1  Total Calories (kcal/d) and Macronutrient (g \cdot kg \cdot d) Content of W, S, and P Groups for 3 Days During the First and Final Week of Supplementation and Training

<table>
<thead>
<tr>
<th></th>
<th>W Week 1</th>
<th>W Week 6</th>
<th>S Week 1</th>
<th>S Week 6</th>
<th>P Week 1</th>
<th>P Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kilo-</td>
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<td>3129 \pm</td>
<td>2978 \pm</td>
<td>3015 \pm</td>
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<td>591</td>
<td>702</td>
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<td>960</td>
<td>387</td>
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<tr>
<td>Carbo-</td>
<td>6.2 \pm 3.3</td>
<td>5.9 \pm 3.9</td>
<td>5.7 \pm 5.4</td>
<td>5.7 \pm 3.3</td>
<td>6.1 \pm 3.3</td>
<td>6.4 \pm 3.9</td>
</tr>
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<td>hydrates</td>
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<tr>
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<td>1.3 \pm 1.1</td>
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<td>1.2 \pm 1.0</td>
<td>1.4 \pm 1.1</td>
<td>1.3 \pm 1.1</td>
</tr>
<tr>
<td>Protein</td>
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<td>1.9 \pm 1.3</td>
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<td>1.8 \pm 1.4</td>
<td>1.7 \pm 1.5</td>
<td>1.7 \pm 1.3</td>
</tr>
</tbody>
</table>

Values are means \pm standard deviation. Data is based on the average for 1 d from 3 d food records and do not include the addition of supplement or placebo.
There was a significant group × time interaction for lean tissue mass ($P < 0.05$). Post hoc analysis indicated that all groups increased lean tissue mass with training ($P < 0.05$). After 6 wk of training, lean tissue mass was significantly greater in the W group (+2.5 kg or 4.7%) and S group (+1.7 kg or 3.1%) ($\eta^2 = 0.1$) compared to P (+0.3 kg or 0.5%), with no other differences (Figure 1).

There was a significant group × time interaction for squat and bench press 1-RM ($P < 0.05$). Post hoc analysis indicated that all groups increased strength with training ($P < 0.05$). After 6 wk of training, squat 1-RM was significantly greater in W group (+26.7 kg or 38.6%) and S group (+23.7 kg or 34%) ($\eta^2 = 0.9$) compared to the P group (+14.1 kg or 19.7%), with no other differences (Figure 2).

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**Figure 1** — Lean tissue mass before and after training with supplementation of whey protein (W), soy protein (S), and placebo (P). Values are means ± standard deviation. * Indicates all groups increased lean tissue mass with training ($P < 0.05$). ** Indicates W and S groups increased lean tissue mass more than P with training ($P < 0.05$).

**Figure 2** — Squat 1-RM strength before and after training with supplementation of whey protein (W), soy protein (S), and placebo (P). Values are means ± standard deviation. * Indicates all groups increased strength with training ($P < 0.05$). ** Indicates W and S groups increased strength more than P with training ($P < 0.05$).
press 1-RM was also significantly greater after training in the W group (+ 8.2 kg or 14%) and S group (+ 7.6 kg or 13.4%) ($\eta^2 = 0.6$) compared to the P group (+ 4 kg or 7.1%; Figure 3).

There was a significant increase in urinary 3-methylhistidine excretion with training ($P < 0.05$), with no differences between groups. The relative increases for 3-methylhistidine for the W, S, and P groups were 24%, 30%, and 42%, respectively (Figure 4).

**Figure 3** — Bench press 1-RM strength before and after training with supplementation of whey protein (W), soy protein (S), and placebo (P). Values are means ± standard deviation. * Indicates all groups increased strength with training ($P < 0.05$). ** Indicates W and S groups increased strength more than P with training ($P < 0.05$).

**Figure 4** — Urinary 3-methylhistididne ($\mu$mol/kg lean tissue mass) before and after training with supplementation of whey protein (W), soy protein (S), and placebo (P). Values are means ± standard deviation. *Significantly different from before training ($P < 0.05$).
Discussion

To our knowledge, this is the first study to compare the effect of whey protein and soy protein supplementation combined with resistance training in young men and women. Based on the findings of Phillips et al. (22) that observed greater amino acid uptake from milk protein compared to soy protein, we hypothesized that whey protein would be more effective compared to soy protein during resistance training. We also hypothesized that protein supplementation, independent of source, would be more effective than resistance training alone. Results showed that protein supplementation, independent of source, combined with resistance training increased lean tissue mass to a greater extent than resistance training alone. These results are in agreement with Brown et al. (6) who observed a significant increase in lean tissue mass in young male adults supplementing with whey protein and soy protein (33 g) during 9 wk of resistance training, with no change in the training-only group. Results across studies suggest that consuming additional dietary protein during resistance training, independent of source, may be responsible for the greater increase in lean tissue mass.

It was speculated that whey protein would lead to superior gains over soy protein. However, our findings did not support this hypothesis. One possible explanation for the lack of greater gains in the whey protein group was the duration of our resistance training program. Six weeks of resistance training was chosen based on our previous work in younger men (4). However, our previous study examined differences between whey protein during resistance training versus resistance training alone, with no comparison to soy protein. Other differences include subject population (young male athletes vs. sedentary male/female volunteers) and physical performance assessment (free weight squat vs. hack squat). In addition, Phillips et al. (22), observed a greater increase in lean tissue mass using dual energy X-ray absorptiometry in young healthy men after supplementing with milk protein compared to placebo during 12 wk of resistance training. There were no differences between the soy protein group and placebo, suggesting that a longer training intervention (i.e., ≥ 12 wk) may be needed to produce meaningful differences.

Results of a greater increase in lean tissue mass and strength from protein supplementation support previous findings in young men (4). Resistance training causes an increase in protein synthesis and protein breakdown (26). Consequently, the net balance between protein synthesis and protein breakdown improves after resistance training, but in the post-absorptive state, remains negative (27). Amino acid intake is known to be a potent stimulator of protein synthesis. Amino acid ingestion before and following exercise increases protein synthesis and attenuates protein breakdown (27, 28). The increase in amino acid availability from protein supplementation could potentially increase translational efficiency (i.e., the process by which messenger RNA directs the amino acid sequence of a growing polypeptide during protein synthesis). Increased translational efficiency, in the presence of increased amino acid availability, may explain the greater increase in lean tissue mass during resistance training (8).

Recent evidence suggests that the timing of protein intake during resistance training is important for creating an anabolic environment for muscle growth (27). Protein versus carbohydrate supplementation before and after resistance training sessions for 14 wk resulted in a significant increase in muscle cross-sectional area
of the vastus lateralis in young males (1). Young adults who ingested an amino acid solution immediately before a bout of acute heavy resistance training experienced an increase in muscle protein synthesis (27). The authors suggest that the greater rate of net muscle protein synthesis to consuming the essential amino acids immediately before rather than after exercise was due to increased amino acid delivery to muscle from exercise-increased blood flow. In the present study, subjects were instructed to consume 0.5 g/kg body mass of protein/sucrose powder before (~ 30 min) and 0.5 g/kg body mass of protein/sucrose powder after (~ 30 min) their resistance training sessions. Since habitual dietary protein intake was well above (~ 1.8 g · kg body mass · d) the recommended level of 0.8 g · kg body mass · d, the timing of amino acid delivery from protein supplementation before and after resistance training sessions was likely important.

Three-methylhistidine is an amino acid located primarily in skeletal muscle from the post-translation modification of specific histidine residues in myofibrillar proteins (18). During myofibrillar protein catabolism, the released 3-methylhistidine is neither re-utilized for protein synthesis nor metabolized oxidatively but, instead, is quantitatively excreted in the urine (18); therefore, it serves as a useful indicator of myofibrillar protein catabolism (24). Resistance training results in significant myofibrillar protein turnover. Protein or amino acid supplementation increases the ratio of protein synthesis to degradation post-exercise (2). Therefore, protein supplementation during resistance training should theoretically attenuate the rise in urinary 3-methylhistidine induced by resistance training alone. However, results showed no effect from protein supplementation on urinary 3-methylhistidine excretion over time. These findings are in agreement with Campbell et al. (7) who also found no effect from protein supplementation (0.8 to 1.6 g · kg · d) during 12 wk of resistance training on urinary 3-methylhistidine levels in healthy older adults. For the present study, the greater increase in lean tissue mass, with no change in 3-methylhistidine, suggests protein supplementation increased muscle protein synthesis with no effect on protein breakdown.

In conclusion, whey protein and soy protein supplementation combined with 6 wk of resistance training had beneficial effects on lean tissue mass and strength over isocaloric placebo and resistance training. Future research with a larger sample size and longer training period should assess translational efficiency to determine the mechanism of any ergogenic response from amino acids. In addition, examining the effect that changes in body composition, physical functional ability, physical activity, and food intake patterns has on protein kinetics in males and females is needed.

Acknowledgments

This study was funded in part by the Natural Sciences and Engineering Research Council (NSERC), and the University Council for Research (UCR) at St. Francis Xavier University, Antigonish, Nova Scotia.

References


