Whey Protein Before and During Resistance Exercise Has No Effect on Muscle Mass and Strength in Untrained Young Adults

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Purpose: To determine the effects of whey protein before and during resistance exercise (RE) on body composition and strength in young adults. Methods: Participants were randomized to ingest whey protein (PRO; 0.3 g/kg protein; n = 9, 24.58 ± 1.8 yr, 88.3 ± 17.1 kg, 172.5 ± 8.0 cm) or placebo (PLA; 0.2 g/kg cornstarch maltodextrin + 0.1 g/kg sucrose; n = 8, 23.6 ± 4.4 yr, 82.6 ± 16.1 kg, 169.4 ± 9.2 cm) during RE (3 sets of 6–10 repetitions for 9 whole-body exercises), which was performed 4 d/wk for 8 wk. PRO and PLA were mixed with water (600 ml); 50% of the solution containing 0.15 g/kg of PRO or PLA was consumed immediately before the start of exercise, and ~1.9% of the remaining solution containing ~0.006 g/kg of PRO or PLA was consumed immediately after each training set. Before and after the study, measures were taken for lean-tissue mass (dual-energy X-ray absorptiometry), muscle size of the elbow and knee flexors and extensors and ankle dorsiflexors and plantar flexors (ultrasound), and muscle strength (1-repetition-maximum chest press). Results: There was a significant increase (p < .05) in muscle size of the knee extensors (PRO 0.6 ± 0.4 cm, PLA 0.1 ± 0.5 cm), knee flexors (PRO 0.4 ± 0.6 cm, PLA 0.5 ± 0.7 cm) and ankle plantar flexors (PRO 0.6 ± 0.7 cm, PLA 0.8 ± 1.4 cm) and chest-press strength (PRO 16.6 ± 11.1 kg, PLA 9.1 ± 14.6 kg) over time, with no differences between groups. Conclusion: The ingestion of whey protein immediately before the start of exercise and again after each training set has no effect on muscle mass and strength in untrained young adults.

Keywords: timing, dosing, protein source, bolus
start of resistance exercise and again after each training set for 8 weeks. Before the first visit to the laboratory for initial testing and data collection, participants were instructed to refrain from intense physical activity and alcohol for 24 hr, caffeine for 6 hr, and food and drink for 2 hr. The primary dependent variables measured before and after 8 weeks of supplementation and training were body composition (lean tissue, fat mass, bone mineral); muscle thickness of the muscle groups surrounding the elbow, knee, and ankle joints; and strength (chest-press 1-repetition maximum; 1-RM). In addition, participants completed dietary records for 3 days during the first and final weeks of the study to assess nutrient differences between groups. At the end of the intervention, participants were asked whether they perceived to have been on the whey protein or placebo.

Participants

Healthy young adults who were not engaged in structured resistance-exercise training for 6 weeks before the start of the study volunteered. Non-resistance-trained participants were chosen to potentially maximize the physiological adaptations from resistance exercise. It has been previously shown that untrained adults experience a 0.4-cm greater increase in knee-extensor muscle size from whey protein (0.3 g/kg) and resistance exercise (12 weeks) than with placebo (Candow, Chilibeck, Facci, Abeysekara, & Zello, 2006). With an effect size of .7 (expected minimum change from whey protein and resistance exercise), alpha level of .05, power of 80%, and correlation between the means of .9, 34 participants were required. Participants completed a baseline physical activity questionnaire where the number of times on average per week of strenuous (i.e., heart beats rapidly), moderate (i.e., not exhausting), and mild activities (i.e., minimal effort) were performed (Godin & Shephard, 1985). Participants also completed a Physical Activity Readiness Questionnaire, which assessed their readiness for participation in the resistance-exercise training program. Participants were excluded if they had consumed ergogenic aids (i.e., protein, creatine supplements) for ≤6 weeks before the start of the study, if they were vegetarians, if they were smokers, or if they had preexisting kidney or liver abnormalities. Participants were instructed not to change their diet or engage in additional physical activity that was not part of their normal daily routine. The study was approved by the university ethics review board at the University of Regina. Participants were informed of the risks and purposes of the study before their written consent was obtained.

Supplementation

Participants were randomized to ingest whey protein (PRO: 0.3 g/kg of protein isolate containing 0.15 g/kg of essential amino acids [see Table 1]; n = 9, 5 male, 4 female, 24.58 ± 1.8 years, 88.3 ± 17.1 kg, 172.5 ± 8.0 cm) or placebo (PLA; 0.2 g/kg cornstarch maltodextrin + 0.1 g/kg sucrose; n = 8, 4 male, 5 female, 23.6 ± 4.4 years, 82.6 ± 16.1 kg, 169.4 ± 9.2 cm) during resistance exercise (three sets of 6–10 repetitions of nine whole-body exercise) that was performed 4 days/week for 8 weeks. Each training session lasted approximately 90 min. The whey protein dose of 0.3 g/kg (~26 g) contained 0.15 g/kg (~13 g) of essential amino acids, necessary for the synthesis of skeletal-muscle proteins (Borsheim, Tipton, Wolf, & Wolfe, 2002). Protein and placebo were mixed with water (600 ml), and 50% of the solution, containing 0.15 g/kg of protein or placebo, was consumed immediately before the start of exercise to stimulate gastric emptying (Beelen et al., 2008). After preexercise ingestion, participants consumed ~1.9% (12 ml) of the remaining solution containing ~0.006 g/kg (0.5 g) of protein or placebo immediately after each set, as the ingestion of protein after muscle contractions is important for stimulating the rates of muscle protein synthesis (Phillips et al., 2005). Protein and placebo were consumed in a plastic shaker bottle with gradations (ml) on the side to ensure that 1.9% (12 ml) of the solution was consumed after each set. They were identical in energy content, color, taste, texture, volume, and appearance. Participants were instructed to refrain from food or drink for 1 hr before and after each training session so that a valid estimate of the effects of whey protein before and during exercise could be made. Water was permitted ad libitum. Compliance with the supplementation protocol was assessed by verbal communication with the participants on a weekly basis.

Table 1 Amino Acid Composition of Whey Protein Isolate

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Quantity (mg/kg)</th>
</tr>
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<tbody>
<tr>
<td>Leucine</td>
<td>32.1</td>
</tr>
<tr>
<td>Valine</td>
<td>16.8</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>18.6</td>
</tr>
<tr>
<td>Lysine</td>
<td>33.3</td>
</tr>
<tr>
<td>Methionine</td>
<td>6.6</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>8.4</td>
</tr>
<tr>
<td>Threonine</td>
<td>19.5</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>5.1</td>
</tr>
<tr>
<td>Alanine</td>
<td>15.3</td>
</tr>
<tr>
<td>Arginine</td>
<td>6.3</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>31.5</td>
</tr>
<tr>
<td>Cysteine</td>
<td>7.2</td>
</tr>
<tr>
<td>Glutamine</td>
<td>51.3</td>
</tr>
<tr>
<td>Glycine</td>
<td>4.8</td>
</tr>
<tr>
<td>Histidine</td>
<td>4.8</td>
</tr>
<tr>
<td>Proline</td>
<td>16.5</td>
</tr>
<tr>
<td>Serine</td>
<td>13.2</td>
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<tr>
<td>Tyrosine</td>
<td>8.4</td>
</tr>
</tbody>
</table>
Resistance-Exercise Program

Before the start of the study, participants were shown how to properly use the resistance-exercise training equipment. They were instructed to perform a 5-min aerobic warm-up (i.e., stationary cycle, elliptical trainer, treadmill) before engaging in unsupervised resistance exercise. Participants trained 4 days/week for 8 weeks and performed three sets of 6–10 repetitions to muscle fatigue, with 2-min rests between sets, for each exercise at an intensity corresponding to their 10-RM for each exercise. We have previously shown that \( \leq 8 \) weeks of structured resistance training is sufficient to increase muscle mass and strength in young untrained adults (Candow, Chilibeck, Burke, Mueller, & Lewis, 2011). Resistance exercises included leg press, chest press, lat pull-down, shoulder press, leg (knee) extension, leg curl (knee flexion), triceps extension, biceps curl, and calf press. Resistance was increased by 2–5 kg once a participant could complete three sets of 10 repetitions to muscle fatigue for an exercise. Once the resistance was increased, the participant maintained this load and performed a minimum of six repetitions and progressed until 10 repetitions were completed per set. Participants maintained daily training logs from which exercise compliance and training volume (Weight \( \times \) Sets \( \times \) Repetitions) were determined.

Body Composition and Muscle Size

Whole-body lean-tissue mass, fat mass, and bone mineral content were assessed using dual-energy X-ray absorptiometry (DXA; Hologic Wi System, Christie Group, MB, Canada), as previously described (Candow, Chilibeck, et al., 2006). The coefficient of variation for lean tissue was .54%.

Elbow- and knee-flexor and -extensor and ankle-dorsiflexor and plantar-flexor muscle thickness were measured using B-mode ultrasound (Aloka SSD-500, Tokyo, Japan). Detailed procedures for assessing muscle thickness have been previously described (Candow & Chilibeck, 2005). The same researcher performed the baseline and posttesting assessments. We have previously used muscle ultrasound to assess changes in muscle size from dietary protein supplementation (Candow, Chilibeck, et al., 2006). The coefficients of variation for muscle-thickness measurements were 2.6% (elbow flexors), 1.7% (elbow extensors), 3.1% (knee flexors), 0.9% (knee extensors), 2.1% (ankle plantar flexors; 7), and 4.0% (ankle dorsiflexors).

Muscle Strength

Chest-press strength was assessed using a 1-RM standard testing procedure (Candow, Burke, Smith-Palmer, & Burke, 2006). After a 5-min aerobic warm-up, participants performed two warm-up sets of 5–10 repetitions using a light weight. Two minutes after the warm-up sets, weight was progressively increased for each subsequent 1-RM attempt, with 2-min rest intervals between trials. The 1-RM was reached in four to seven trials, independent of the two warm-up sets. The coefficient of variation for chest-press strength was 3.1%.

Dietary Assessment

Dietary intake was recorded during the first and final 8 weeks of the study to assess potential differences in total energy and macronutrient composition between the PRO and PLA groups. Participants used a 3-day dietary recall booklet to record what they ate for 2 weekdays and 1 weekend day. They were shown how to properly fill out the booklet and instructed to record all food items, including portion sizes, consumed for the 3 designated days. The Interactive Healthy Eating Index (Center for Nutrition Policy and Promotion, United States Department of Agriculture; http://www.usda.gov/cnpp) was used to analyze food records. Each food item was entered, and the program provided total energy consumption on average over the 3 days, as well as energy from proteins, fats, and carbohydrates individually.

Statistical Analysis

A 2 (PRO vs. PLA) \( \times \) 2 (pre- and posttest periods) ANOVA with repeated measures on the second factor was used to determine differences between groups over time for each of the dependent variables of lean-tissue mass, muscle thickness, strength, fat mass, and bone mineral content. A 2 (PRO vs. PLA) \( \times \) 2 (Week 1 vs. Week 8) ANOVA with repeated measures on the second factor was used to determine differences in diet and training volume for each exercise over time. All results are expressed as \( M \pm SD \). Statistical analyses were carried out using SPSS version 18.0 for Windows XP (SPSS, Chicago, IL). Significance was set at \( p < .05 \).

Results

Of the initial 29 participants who volunteered, 17 completed the study and 12 participants withdrew due to time constraints. Participant characteristics and physical activity performed at baseline for those who completed the study are shown in Table 2. There were no differences in any baseline characteristics between groups. There was a significant decrease (\( p < .05 \)) in total energy (kcal), carbohydrate, and protein intake over time, with no change for fat intake (Table 3). Participants performed all training sessions and consumed the whey protein and placebo as instructed. Six participants did not know whether they were ingesting protein or placebo, 7 (2 PRO, 5 PLA) correctly guessed which supplement they were on, and 4 participants in PRO incorrectly guessed they were consuming placebo. No adverse effects were reported from the resistance-exercise training program, protein, or placebo. We originally intended to assess leg-press 1-RM strength, but due to problems with the leg-press machine, this was not achievable.
There was a significant increase \((p < .05)\) in muscle size of the knee extensors (PRO 0.6 ± 0.4 cm, PLA 0.1 ± 0.5 cm), knee flexors (PRO 0.4 ± 0.6 cm, PLA 0.5 ± 0.7 cm), and ankle plantar flexors (PRO 0.6 ± 0.7 cm, PLA 0.8 ± 1.4 cm; Table 4) and in chest-press strength (PRO 16.6 ± 11.1 kg, PLA 9.1 ± 14.6 kg; Figure 1) over time, with no differences between groups.

After 1 week of training, the volume of exercise performed was similar between groups (PRO 2,622 ± 1,561 kg, PLA 2,712 ± 701 kg). There was a significant increase in the volume of training performed for all exercises over time \((p < .05)\), with a greater increase in PRO than PLA for the leg press (PRO 716 ± 170 kg, PLA 329 ± 298 kg), leg extension (PRO 304 ± 116 kg, PLA 39 ± 98 kg), shoulder press (PRO 185 ± 78 kg, PLA 39 ± 41 kg), biceps curl (PRO 107 ± 45 kg, PLA 30 ± 23 kg), and calf raise (PRO 679 ± 349 kg, PLA 121 ± 189 kg; \(p < .05\)). The change in training volume was similar between groups for the chest press (PRO 158 ± 64 kg, PLA 90 ± 62 kg), lat pull-down (PRO 138 ± 106 kg, PLA 68 ± 33 kg), leg curl (PRO 198 ± 90 kg, PLA 50 ± 142 kg), and triceps extension (PRO 178 ± 67 kg, PLA 90 ± 41 kg).

There were no changes over time for body mass (PRO pre 88.3 ± 17.1 kg, post 87.3 ± 15.8 kg; PLA pre 72.5 ± 14.7 kg, post 72.4 ± 14.1 kg), lean-tissue mass (PRO pre 48.8 ± 9.1 kg, post 48.4 ± 9.1 kg; PLA pre 43.3 ± 9.4 kg, post 43 ± 9.4 kg; Figure 2), fat mass (PRO pre 30.3 ± 7.9 kg, post 30.2 ± 11.9 kg; PLA pre 20.7 ± 9.0 kg, post 21.4 ± 8.7 kg), or bone mineral content (PRO pre 2,078.3 ± 504.8 mg, post 2,072.6 ± 489.7 mg; PLA pre 1,964.3 ± 306.5 mg, post 1,967.8 ± 324.1 mg) or muscle size of the elbow flexors (PRO pre 3.1 ± 0.6 cm,
post 3.0 ± 0.6 cm; PLA pre 3.0 ± 0.8 cm, post 2.9 ± 0.8 cm), elbow extensors (PRO pre 3.9 ± 1.1 cm, post 4.1 ± 0.7 cm; PLA pre 3.5 ± 0.4 cm, post 3.7 ± 0.7 cm), and ankle dorsiflexors (PRO pre 3.6 ± 0.7 cm, post 3.4 ± 0.7 cm; PLA pre 3.3 ± 0.3 cm, post 3.7 ± 0.5 cm).

**Discussion**

To our knowledge, this is the first study to examine the effects of whey protein immediately before the start of resistance exercise and again after each training set in young adults. Contrary to our hypothesis, whey protein had no greater effect on muscle mass or strength than did placebo. Previous research has shown that whey and casein proteins stimulate the rates of muscle protein synthesis (Beelen et al., 2008; Koopman et al., 2007; Pennings et al., 2011; Tipton et al., 2007), and while it is difficult to compare results across studies, our lack of findings from whey protein may be influenced by the quantity of protein consumed after each set, the pattern of protein ingestion (i.e., small, frequent doses vs. bolus), and lack of carbohydrate. For example, using muscle biopsies and stable isotope-labeled amino acid tracers, Beelen et al. showed that the coingestion of casein protein (0.15 g/kg) and carbohydrate immediately before the start of exercise and then again every 15 min during an acute bout of resistance exercise increased the rates of muscle protein synthesis and improved whole-body net protein balance in young adults. We assessed the effects of whey protein (0.15 g/kg) immediately before the start of exercise and after each training set (0.006 g/kg) for 8 weeks. Therefore, it is possible that the substantially lower amount of whey protein consumed after each set (0.006 g/kg) than the amount of casein (0.15 g/kg) administered in the Beelen et al. study was too low to produce significant muscle hypertrophy, as assessed with DXA and muscle ultrasound, with repeated training sessions. Protein ingestion did, however, increase the amount of work performed over 8 weeks of training for five of the nine exercises (i.e., greater training response), and with the increase in muscle size of some (knee extensors, knee flexors, ankle plantar flexors) but not all muscle groups (elbow flexors, elbow extensors, ankle dorsiflexors) from resistance exercise, perhaps a longer training period (>8 weeks) with a larger sample using more sensitive and sophisticated techniques (i.e., magnetic resonance imaging, computed tomography) is required to observe significant changes in muscle mass from whey protein.
during training. Ultrasound measured changes in the size of muscle groups directly targeted by the exercises in our training program but does not differentiate between contractile and noncontractile tissue (i.e., intramuscular adipose tissue, connective tissue). Measurements of lean-tissue mass from DXA include other areas of the body, which could have influenced our ability to detect small differences in muscle accretion between groups over time.

Recent evidence indicates that the ingestion pattern of whey protein may influence the rates of muscle protein synthesis. In comparing the effects of a single dose (i.e., bolus) of whey protein (25 g) with that of smaller, more frequent doses (i.e., 2.5 g) every 20 min postexercise (eight sets of 8–10 repetitions for leg extension) in young adults (n = 8), West et al. (2011) found that the bolus ingestion of whey protein elevated blood essential amino acids, phosphorylation of anabolic signaling proteins involved in translation initiation, and the rates of muscle protein synthesis more than smaller doses of whey protein. Therefore, our whey-protein ingestion protocol (i.e., small, frequent doses after training sets) may have limited our ability to produce significant effects over time. Furthermore, resistance exercise increases the sensitivity of muscle protein synthesis rates to dietary amino acid provision for 3 days postexercise (Burd et al., 2011; Miller et al., 2005), which likely negated our ability to detect significant differences between groups over time, as stimulation of muscle protein synthesis is short-lived compared with the postexercise stimulation of protein synthesis.

Regarding the potential additive effects of protein and carbohydrate together, Beelen et al. (2008) found a greater effect on the rates of muscle protein synthesis from the coingestion of casein protein (0.15 g/kg or 11 g) and carbohydrate (0.15 g/kg) over carbohydrate alone immediately before the start of exercise and again every 15 min during a 2-hr bout of resistance exercise (i.e., approximately 88 g of protein consumed during exercise) in young adults. We have previously shown that the coingestion of whey protein (0.3 g/kg or 26 g) and carbohydrate (0.09 g/kg) immediately before resistance exercise training sessions for 12 weeks increased knee-extensor muscle size compared with carbohydrate alone (placebo) in healthy older adults (Candow, Chilibeck, et al., 2006), and in young adults, whey protein (1.2 g/kg or 84 g) and carbohydrate (0.3 g/kg) consumed during structured resistance training for 6 weeks increased whole-body lean-tissue mass and strength over carbohydrate placebo (Candow, Burke, et al., 2006). The combination of carbohydrate (50 g) and whey protein (25 g) immediately after an acute bout of knee-extension resistance exercise (four sets of 8–12 repetitions) increased the phosphorylation of protein kinase B (Akt) but had no greater effect on the rates of muscle protein synthesis than whey protein alone (Staples et al., 2011). Mechanistically, the combination of amino acids/protein and carbohydrate may stimulate mTOR signaling properties (Koopman et al., 2007; Staples et al., 2011) and muscle protein synthesis (Beelen et al., 2008; Kimball, Farrell, & Jefferson, 2002) and attenuate muscle protein catabolism during and after exercise (Koopman et al., 2004). Results across studies suggest that protein (≥25 g) enhances the machinery for muscle protein synthesis postexercise, and the coingestion of protein and carbohydrate may improve muscle accretion over time during a resistance-exercise training program.

Results from this study indicate that ingesting whey protein before and during resistance-exercise training sessions has no effect on muscle mass or strength in a small-sample population of young, untrained adults. Future research should compare the effects of bolus ingestion of whey protein before, during, and after acute and longer term resistance exercise in young and older adults.

References


