Muscle Performance, Size, and Safety Responses After Eight Weeks of Resistance Training and Protein Supplementation: A Randomized, Double-Blinded, Placebo-Controlled Clinical Trial

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Abstract

Herda, AA, Herda, TJ, Costa, PB, Ryan, ED, Stout, JR, and Cramer, JT. Muscle performance, size, and safety responses after eight weeks of resistance training and protein supplementation: A randomized, double-blinded, placebo-controlled clinical trial. J Strength Cond Res 27(11): 3091–3100, 2013—The purpose of this study was to examine the effects of 2 different types of protein supplementation on thigh muscle cross-sectional area (CSA), blood markers, muscular strength, endurance, and body composition after 8 weeks of low- or moderate-volume resistance training in healthy, recreationally trained, college-aged men. One hundred and six men were randomized into 5 groups: low-volume resistance training with bioenhanced whey protein (BWP_LV; n = 22), moderate-volume resistance training with BWP (BWP_MV; n = 20), moderate-volume resistance training with standard whey protein (SWP_MV; n = 22), moderate-volume resistance training with a placebo (PLA; n = 21), or moderate-volume resistance training with no supplementation (CON; n = 21). Except for CON, all groups consumed 1 shake before and after each exercise session and one each on the nontraining day. The BWP_LV, BWP_MV, and SWP_MV groups received approximately 20 g of whey protein per shake, whereas the BWP groups received 5 g of additional polyethylene glycosylated (PEG) leucine. Resistance training sessions were performed 3 times per week for 8 weeks. There were no interactions (p > 0.05) for muscle strength and endurance variables, body composition, muscle CSA, and safety blood markers, but the main effects for training were observed (p ≤ 0.05). However, the Albumin:Globulin ratio for SWP_MV was lower (p = 0.037) than BWP_LV and BWP_MV. Relative protein intake (PROREL) indicated a significant interaction (p < 0.001) with no differences across groups at pre; however, BWP_LV, BWP_MV, and SWP_MV had a greater intake than did PLA or CON at post (p < 0.001). This study indicated that 8 weeks of resistance training improved muscle performance and size similarly among groups regardless of supplementation.

Key Words: strength training, muscle cross-sectional area, leucine, training volume, body composition, whey protein

Introduction

Many studies over the past 15 years have demonstrated acute improvements in muscle protein synthesis (MPS) by resistance training (12,46), protein consumption (50), and a combination of resistance training and protein supplementation (55,62). The benefits of protein supplementation have been attributed to enhancing MPS, or at minimum, reducing muscle protein breakdown at rest (3,64) and after an acute bout of resistance exercise (6,54,55). These studies have led to a marked improvement in the scientific understanding of how resistance training and protein supplementation improve MPS, and as a result, practical recommendations have been made regarding the timing of protein supplementation relative to resistance training (55). For instance, it is generally recommended that protein is consumed before and after resistance training—within
Resistance Training and Protein Supplementation

a critical window of 1 hour postexercise (17,53,55). Furthermore, a knowledge regarding the quantity and quality of the proteins consumed has also been refined by these studies (39). For example, the culmination of results has suggested that 20 g of whey protein (containing ~6 g of essential amino acids), with an emphasis on the amino acid, leucine, is an optimal amount of protein consumption to stimulate the critical mammalian target of rapamycin pathway for MPS (3,20,40,51,64).

Although these general recommendations are not always reported as being advantageous (57), these acute studies have been important contributions and must consistently be validated by experimentally examining the chronic adaptations of resistance training and protein supplementation. Furthermore, consumers often question the safety of protein supplementation in regard to negative effects on the kidneys because of previous literature (8,37,38) and media coverage.

Less is known regarding the chronic effects of protein supplementation in conjunction with resistance training (2,13,26,45,57). These studies have reported improvements in muscular strength (14,19,62), muscle cross-sectional area (CSA) (2,14,62), and stimulation of MPS (2,62) as adaptations to resistance training with and without protein supplementation. For example, Coburn et al. (14) reported that 8 weeks of unilateral resistance training with or without protein supplementation resulted in improvements in strength and muscle CSA in the trained limb for both groups, with the untrained limb also improving strength in the protein-supplemented group. Without supplementation, Ogasawara et al. (41) reported marked improvements in muscle CSA (mCSA) as confirmed by magnetic resonance imaging (MRI) after 6 and 15 weeks of periodic and continuous bench press (BP) training programs in previously untrained men. In contrast, Chromiak et al. (13) showed that there were no differences in body composition, strength, muscular endurance, or power after 10 weeks of resistance training with the consumption of a creatine, whey protein, and amino acid supplement, or a carbohydrate-only supplement after exercise in recreationally active young men. Additionally, differences among these studies in resistance exercise programming variables, such as load, volume, and progression, likely influenced the magnitude of strength changes (24). These differences emphasize the need to continually re-assess the chronic adaptations of resistance training that may be augmented by the addition of concomitant protein supplementation. Therefore, the purpose of this randomized, double-blinded, placebo-controlled clinical trial was to examine the effects of 2 different types of protein supplementation on thigh mCSA, blood markers, muscular strength, endurance and body composition after 8 weeks of low- or moderate-volume resistance training in healthy, recreationally trained, college-aged men.

METHODS

Experimental Approach to the Problem

This was an 8-week, randomized, placebo-controlled clinical trial during which the pretraining assessments were followed by 8 weeks of upper- and lower-body resistance training 3 d·wk⁻¹. The pretraining and posttraining assessments included a fasted blood draw, fasted body composition, upper- and lower-body strength and endurance performance, mCSA, and dietary analysis. After the pretesting assessments, the subjects were randomly assigned to 1 of 5 treatment groups: (a) bioenhanced whey protein with low-volume training (BWP_LV, n = 22), (b) BWP with moderate-volume training (BWP_MV, n = 20), (c) standard whey protein with moderate-volume training (SWP_MV, n = 22), (d) placebo with moderate-volume training (PLA, n = 21), or (e) control with moderate-volume training (CON, n = 21). During the testing and training phases, all the subjects (except the CON group) consumed 1 chocolate-flavored shake per day on the nontraining days and 2 chocolate shakes per day on training days (1 shake was taken 30 minutes before training and 1 shake was taken immediately after training).

Subjects

One hundred and six healthy, active men volunteered for this investigation. Subject demographics for each group are shown in Table 1. All the procedures were approved by the Institutional Review Board for the protection of human subjects, and a written informed consent was obtained from each participant before any testing. Supplement and exercise history was also recorded, and none of the participants had taken any nutritional supplements within the 9 weeks before their initial testing date. Sixty seven (63.2%) of the 106 subjects reported participating in 3.7 ± 2.2 hours of aerobic exercise, 49 (46.2%) of the 106 subjects participated in 3.3 ± 1.3 hours of resistance

| Table 1. Subject demographics split by treatment group.†‡ |
|-----------------|-----------------|-----------------|-----------------|
| Age             | Height (cm)     | Body mass (kg)  | Percent fat (%) |
| BWP_LV n = 22   | 21.1 ± 2.5      | 176.9 ± 6.8     | 78.3 ± 13.7     | 18.3 ± 7.4     |
| BWP_MV n = 20   | 21.2 ± 2.7      | 178.1 ± 7.9     | 79.2 ± 12.4     | 18.5 ± 6.5     |
| SWP_MV n = 22   | 21.1 ± 1.6      | 180.2 ± 7.2     | 76.5 ± 12.5     | 15.8 ± 4.3     |
| PLA n = 21      | 20.9 ± 1.7      | 178.1 ± 5.1     | 78.1 ± 13.8     | 16.3 ± 7.2     |
| CON n = 21      | 21.1 ± 2.4      | 178.8 ± 6.2     | 79.9 ± 14.6     | 18.4 ± 6.2     |

*BWP = bioenhanced whey protein; BWP_LV = low-volume resistance training with BWP; BWP_MV = moderate-volume resistance training with BWP; SWP_MV = moderate-volume resistance training with standard whey protein; PLA = moderate-volume resistance training with a placebo; CON = moderate-volume resistance training with no supplementation.

† Data represent mean ± SD.
exercise, and 62 (58.5%) of the 106 subjects participated in 3.3 ± 2.3 hours of recreational sports per week. The remaining participants reported participating in no additional physical activity, and none of the participants were competitive athletes. All the participants were instructed to maintain their current exercise regimen and to not increase nor decrease their total amount of physical activity. There was no regulation of extramural physical activity. The subjects were asked to maintain aerobic activities throughout the duration of the study and refrain from additional resistance exercise outside of their supervised study-related training sessions.

Procedures

Body Composition Assessment. After an 8- to 10-hour fast, residual volume was determined with the subject in a seated position using the oxygen dilution method via a metabolic cart with residual volume software (True One 2,400, Parvo-Medics, Inc., Sandy, UT, USA). The subjects completed a minimum of 2 trials, and the average of the closest 2 trials within 5% was used to estimate residual lung volume (liters).

Body volume (BV) was assessed from hydrostatic weighing (HW). Underwater weight was measured to the nearest 0.025 kg in a submersion tank in which a seat was suspended from an analog scale. Previous test-retest reliability for percent body fat (%BF) determined from HW in our laboratory indicated that for 11 young adults (24.0 ± 2.4 years) measured 24 hours apart, the intraclass correlation coefficient (ICC) for %BF was 0.99 (SEM = 0.8%BF), whereas the ICC for BV was 0.99 (SEM = 0.34 L). In addition, there were no significant differences (p > 0.05) from day 1 to day 2 for either %BF or BV.

Blood Marker Analysis. To assess the safety of consuming the different shakes (BWP, SWP, and PLA) over 8 weeks and ensure that all the subjects were healthy before commencing the study, all the subjects had fasted blood draws conducted at the university’s student health center. The subject’s sample was tested by LabCorp (Dallas, TX, USA) for a full chemistry panel (CHEM: glucose [Glu], uric acid, blood urea nitrogen [BUN], creatinine [creat], BUN/creat ratio, sodium [Na], potassium [K], chloride [Cl], carbon dioxide [CO₂], calcium [Ca], phosphorus [phos], total protein [TP], albumin [A], globulin [G], A/G ratio, bilirubin [bili], alkaline phosphatase, lactate dehydrogenase, aspartate aminotransferase, alanine transaminase, and gamma-glutamyl transpeptidase, iron) and lipid profile (total cholesterol, triglycerides, high-density lipoproteins [HDL], very low-density lipoprotein, low-density lipoprotein [LDL], LDL/HDL ratio). Estimated glomerular filtration rate (eGFR) was also calculated using age and creatinine, as a marker of kidney health. Blood was sampled before and after the intervention.

Muscle CSA. Two-dimensional images of the right thigh were obtained using a peripheral quantitative computed tomography (pQCT) scanner (XCT 3000, Orthometrix, White Plains, NY, USA). Before the scan, the femur length was measured manually using a Gulick anthropometric measuring tape from the greater trochanter to the lateral epicondyle of the femur. The subjects were then seated upright in the chair of the pQCT with the right thigh flexed at 90° and leg extended. The right leg was supported by a custom-built plastic support device (Bone Diagnostics, Fort Atkinson, WI, USA) between the chair and gantry of the pQCT. The foot was secured on the opposite side of the gantry with a Velcro strap placed over the metatarsals (Figure 1).

Using a longitudinal scout view scan, the pQCT software positioned the gantry at 50% of the measured length of the femur from the distal surface in the medial knee joint cleft (OrthoMetrix, Inc., Naples, FL, USA). The cross-sectional image obtained from the pQCT was calculated by subtracting the bone, skin, and subcutaneous fat CSA from the total CSA, which only left the muscle CSA (centimeters; Figure 2). This calculation was performed by the pQCT software (Stratec XCT 3000 software v. 6.00, Pforzheim, Germany). Previously published ICCs for test-retest reliability for muscle CSA measured with the pQCT ranged from 0.996 to 0.998 with an SEM of 1.660–1.101 cm² and no significant differences among the day-to-day mean CSA values (p ≤ 0.05) (16).

Muscular Strength and Endurance. The subjects performed tests to determine 1-repetition maximums (1RMs) for the incline leg press (LP) and BP exercises based on the National Strength and Conditioning Association’s 1RM testing guidelines (15). The BP exercise was performed on a standard free-weight bench (TuffStuff, Pomona, CA, USA) with an Olympic bar. The LP exercise was performed using...
a plate-loaded hip sled with a 45° incline (Paramount Fitness Corp., Los Angeles, CA, USA). The subjects sat in the seat with their back flat against the backrest and were instructed to grasp the handles of the device tightly to avoid the buttocks losing contact with the seat during the exercise. The subjects placed their feet in the middle of the platform about shoulder-width apart, and this foot position remained constant for all the subsequent LP tests. The subjects were instructed to lower the platform until the legs reached 90° of flexion at which point they were instructed to fully extend the legs (i.e., 0° of leg flexion). The subjects were given 2–3 warm-up sets at progressively heavier workloads in preparation for the 1RM attempts. If a repetition for either the LP or BP exercises did not meet the aforementioned criteria, it was not counted, and another attempt was allowed. Two minutes of rest was allowed between all 1RM trials. Previously published ICCs for test-retest reliability for LP and BP 1RM testing was 0.997 and 0.997 for LP and 0.997 and 1.000 for BP in men and women, respectively, with a coefficient of variation of 0.235 and 0.315 for LP and 0.290 and 0.535 for BP in men and women, respectively (49).

Ten minutes after the 1RM procedure, the subjects were instructed to complete as many repetitions as possible (repetitions to failure; REP_MAX) while maintaining the aforementioned exercise technique with a load of 80% of the predetermined 1RM for both the BP and LP exercises (BP_REPS and LP_REPS, respectively).

**Supplementation Protocol.** Once pretesting was completed, the subjects were randomly assigned to 1 of 5 groups (BWP_LV, BWP_MV, SWP_MV, PLA, or CON). The subjects assigned to a supplement group (BWP_LV, BWP_MV, SWP_MV, PLA) were instructed to consume 1 shake in the laboratory 30 minutes before their training session and a second shake immediately after their training session. The contents of each supplement are described in Table 2. Each serving of all supplements were chocolate-flavored powder packaged individually in opaque white disposable packets with no writing other than labels with removable stickers showing the subject number, clinical trial number, and mixing instructions. The stickers were removed and placed in each subject’s case report form to document that the supplement had been consumed (compliance mean ± SD: 99.4 ± 2.1%; range: 83–100%). The subjects were instructed to tear open the packet, empty its contents (powder) into an opaque black shaker cup, mix with 8 oz. of water, and drink the shake under the supervision of the study personnel.

Each subject in a supplement group was also given 32 supplement packets and another opaque shaker cup to take home. They were instructed to consume 1 packet in the morning of each nontraining day on an empty stomach by mixing and drinking the shake in the same manner as done in the laboratory. They were also instructed to return all empty packets to the laboratory to account for consumption compliance. Acceptable supplementation compliance was set at ≥70% of packet consumption. If a subject returned <70% of their originally distributed packets, they were to be

<table>
<thead>
<tr>
<th>Table 2. Supplement ingredient breakdown.*</th>
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<tbody>
<tr>
<td>BWP 20 g of Polyethylene glycosylated whey</td>
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<tr>
<td>protein concentrate and 7 g of leucine</td>
</tr>
<tr>
<td>SWP 20 g of Whey protein concentrate</td>
</tr>
<tr>
<td>PLA 27 g of Maltodextrin</td>
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*BWP = bioenhanced whey protein; SWP = standard whey protein; PLA = placebo.
Three to 5 days after completing the pretesting assessments, the subjects returned to the laboratory to begin the resistance training protocol. All the subjects were supervised in the laboratory by certified personal trainers using the BP and LP exercises at 80% 1RM 3 times per week for 8 weeks. The first 3 weeks progressively increased the number of sets performed, whereas the repetitions remained constant (6 repetitions). The BWP<sub>LV</sub> group completed 1 set during week 1, 2 sets during week 2, and 3 sets during weeks 3–8, whereas all other groups completed 3 sets during week 1, 4 sets during week 2, and 5 sets during weeks 3–8. If a subject could not complete 6 repetitions, the number of repetitions completed was recorded on their training log from which total training volume (weight lifted × number of sets completed × number of repetitions completed) could be calculated. The subjects were given 2 minutes of rest between sets and 2 warm-up sets for both the BP and LP exercises at 20–40% 1RM. Physical activity outside of the study-supervised training sessions were not documented although the subjects were instructed to maintain their regular daily activity as reported at the start of the study.

Training Protocol. Three to 5 days after completing the pretesting assessments, the subjects returned to the laboratory to begin the resistance training protocol. All the subjects were supervised in the laboratory by certified personal trainers using the BP and LP exercises at 80% 1RM 3 times per week for 8 weeks. The first 3 weeks progressively increased the number of sets performed, whereas the repetitions remained constant (6 repetitions). The BWP<sub>LV</sub> group completed 1 set during week 1, 2 sets during week 2, and 3 sets during weeks 3–8, whereas all other groups completed 3 sets during week 1, 4 sets during week 2, and 5 sets during weeks 3–8. If a subject could not complete 6 repetitions, the number of repetitions completed was recorded on their training log from which total training volume (weight lifted × number of sets completed × number of repetitions completed) could be calculated. The subjects were given 2 minutes of rest between sets and 2 warm-up sets for both the BP and LP exercises at 20–40% 1RM. Physical activity outside of the study-supervised training sessions were not documented although the subjects were instructed to maintain their regular daily activity as reported at the start of the study.

### Table 3. Preintervention and postintervention values represented as mean ± SE for body composition and performance variables.*

<table>
<thead>
<tr>
<th></th>
<th>BWP&lt;sub&gt;LV&lt;/sub&gt;</th>
<th></th>
<th>BWP&lt;sub&gt;MV&lt;/sub&gt;</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>LM</td>
<td>63.4 ± 1.8</td>
<td>64.6 ± 1.7†</td>
<td>64.2 ± 1.9</td>
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<tr>
<td>BM</td>
<td>78.3 ± 2.9</td>
<td>77.5 ± 3.2</td>
<td>79.2 ± 2.8</td>
</tr>
<tr>
<td>%BF</td>
<td>18.3 ± 1.6</td>
<td>17.8 ± 1.6†</td>
<td>18.5 ± 1.5</td>
</tr>
<tr>
<td>mCSA</td>
<td>149.0 ± 4.8</td>
<td>153.4 ± 4.6†</td>
<td>149.4 ± 5.2</td>
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<tr>
<td>BP&lt;sub&gt;MAX&lt;/sub&gt;</td>
<td>93.2 ± 5.2</td>
<td>95.0 ± 4.9†</td>
<td>86.2 ± 4.4</td>
</tr>
<tr>
<td>BP&lt;sub&gt;REPS&lt;/sub&gt;</td>
<td>7.1 ± 0.4</td>
<td>14.5 ± 0.6†</td>
<td>7.6 ± 0.3</td>
</tr>
<tr>
<td>LP&lt;sub&gt;MAX&lt;/sub&gt;</td>
<td>256.0 ± 13.7</td>
<td>323.1 ± 14.2†</td>
<td>254.9 ± 9.4</td>
</tr>
<tr>
<td>LP&lt;sub&gt;REPS&lt;/sub&gt;</td>
<td>12.3 ± 1.3</td>
<td>37.1 ± 3.8†</td>
<td>9.1 ± 1.1</td>
</tr>
<tr>
<td>BP&lt;sub&gt;VOL&lt;/sub&gt;</td>
<td>55,750.9 ± 3,492.2†</td>
<td>101,002 ± 5,025.5</td>
<td>300,873 ± 11,005.0</td>
</tr>
<tr>
<td>LP&lt;sub&gt;VOL&lt;/sub&gt;</td>
<td>171,226 ± 9,205.0</td>
<td>328,401 ± 16,560.4</td>
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<table>
<thead>
<tr>
<th></th>
<th>PLA</th>
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<th>CON</th>
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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>LM</td>
<td>64.2 ± 2.0</td>
<td>65.2 ± 2.0†</td>
<td>64.7 ± 1.7</td>
<td>65.8 ± 1.6†</td>
</tr>
<tr>
<td>BM</td>
<td>78.5 ± 2.7</td>
<td>77.4 ± 2.5</td>
<td>78.1 ± 3.0</td>
<td>79.4 ± 3.0</td>
</tr>
<tr>
<td>%BF</td>
<td>15.8 ± 0.9</td>
<td>15.6 ± 1.0†</td>
<td>16.3 ± 1.6</td>
<td>16.3 ± 1.5†</td>
</tr>
<tr>
<td>mCSA</td>
<td>151.3 ± 5.1</td>
<td>158.9 ± 4.9†</td>
<td>155.7 ± 5.5</td>
<td>162.8 ± 5.6†</td>
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<tr>
<td>BP&lt;sub&gt;MAX&lt;/sub&gt;</td>
<td>79.5 ± 5.3</td>
<td>94.4 ± 5.1†</td>
<td>80.0 ± 3.1</td>
<td>95.0 ± 3.5†</td>
</tr>
<tr>
<td>BP&lt;sub&gt;REPS&lt;/sub&gt;</td>
<td>8.0 ± 0.5</td>
<td>15.6 ± 0.9†</td>
<td>7.8 ± 0.4</td>
<td>15.4 ± 0.8†</td>
</tr>
<tr>
<td>LP&lt;sub&gt;MAX&lt;/sub&gt;</td>
<td>246.4 ± 12.5</td>
<td>316.9 ± 12.7†</td>
<td>257.3 ± 12.8</td>
<td>331.6 ± 12.1†</td>
</tr>
<tr>
<td>LP&lt;sub&gt;REPS&lt;/sub&gt;</td>
<td>12.1 ± 1.1</td>
<td>32.6 ± 2.4†</td>
<td>12.5 ± 1.0</td>
<td>32.3 ± 2.5†</td>
</tr>
<tr>
<td>BP&lt;sub&gt;VOL&lt;/sub&gt;</td>
<td>59,204 ± 6,265.3</td>
<td>94,625 ± 4,138.8</td>
<td>87,431 ± 5,633.3</td>
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<tr>
<td>LP&lt;sub&gt;VOL&lt;/sub&gt;</td>
<td>289,998 ± 14,721.5</td>
<td>302,547 ± 14,848.0</td>
<td>328,401 ± 16,560.4</td>
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</tr>
</tbody>
</table>

*LM = lean mass; BM = body mass; %BF = percent body fat; mCSA = muscle cross-sectional area; BP<sub>MAX</sub> = bench press maximum; BP<sub>REPS</sub> = bench press repetitions; LP<sub>MAX</sub> = leg press maximum; LP<sub>REPS</sub> = leg press repetitions; BP<sub>VOL</sub> = bench press volume; LP<sub>VOL</sub> = leg press volume.
†Indicates a significant difference from prevalue.
‡Indicates a significant difference from the values indicated in bold.
transposed the recorded food records into Diet Analysis Plus (DA+, Version 7.0, Thompson Learning 2008) to determine total kilocalorie intake and total grams of protein consumed (gPRO), percentage of kilocalories from protein consumed (%PRO), and protein intake relative to body weight (PROREL).

Statistical Analyses. Analysis of variance (ANOVA) and analysis of covariance (ANCOVA) statistical models were used to analyze the data. Two-way (time: pre vs. post × group: BWP_LV vs. BWP_MV vs. SWP_MV vs. PLA vs. CON) repeated measures ANOVAs were used to determine group differences in body composition, performance, and blood marker variables across groups. Tests for homogeneity of slopes were conducted a priori between the pretraining and posttraining and between self-reported training status (SRTS) and posttraining values for each treatment group (BWP_LV, BWP_MV, SWP_MV, PLA, and CON). If there were no differences among the slopes, ANCOVA was used with the pretraining (PRE) and SRTS as the 2 covariates. Two 1-way ANOVAs were used to compare BP and LP volume among the groups. Two-way (time: pre vs. post × group: BWP_LV vs. BWP_MV vs. SWP_MV vs. PLA vs. CON) repeated measures ANOVAs were used to compare group differences in kilocalories, gPRO, %PRO, and PROREL across groups. Post hoc analyses were conducted using planned comparisons. Before all statistical analyses, the alpha level was set to $p \leq 0.05$ to determine statistical significance. Data were analyzed using SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL, USA).

**RESULTS**

Two-way mixed factorial ANOVA results including preintervention and postintervention values for performance and body composition variables are represented in Table 3. There were no significant interactions ($p > 0.05$) and no main effect for group ($p > 0.05$); however, there was a significant main effect for all body composition variables (%BF, lean mass, BM, and mCSA), or muscular performance variables (BP_MAX, BP_REPS, LP_MAX, and LP_REPS).

The results of the ANCOVAs indicated there were no differences ($p > 0.05$) among the group adjusted mean values for the body composition variables, or muscular performance variables. There were no main effects for 28 of the 29 measured blood markers ($p > 0.05$). However, the A:G ratio for the SWP_MV group was lower than the BWP_LV or BWP_MV groups (adjusted mean values: 1.81, 1.83, 1.68, 1.76, and 1.76 for BWP_LV, BWP_MV, SWP_MV, PLA, and CON, respectively; $p = 0.037$), with no differences among the other groups. The 1-way ANOVA indicated the BWP_LV treatment group lifted half as much as the other 4 groups ($p < 0.001$ and $p < 0.001$ for BP and LP volume, respectively) as indicated in Table 3.

Furthermore, there were no differences ($p > 0.05$) among the group adjusted mean values for any of the dietary analysis variables (kilocalories, gPRO, or %PRO; Table 4).

### Table 4. Dietary analysis as mean ± SE at baseline, postintervention without supplementation, and postintervention with supplementation.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>BWP_Lv</th>
<th>BWP_Mv</th>
<th>SWP_Mv</th>
<th>PLA</th>
<th>CON</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre (no supp) kcal</td>
<td>2,786.8±321.5</td>
<td>2,346.0±200.5</td>
<td>2,766.9±213.1</td>
<td>2,320.4±129.2</td>
<td>2,402.1±164.2</td>
</tr>
<tr>
<td>gPRO</td>
<td>125.1±23.8</td>
<td>105.0±9.02</td>
<td>104.7±9.2</td>
<td>100.5±8.6</td>
<td>105.1±9.2</td>
</tr>
<tr>
<td>%PRO</td>
<td>18.4±1.4</td>
<td>18.2±0.8</td>
<td>15.9±0.6</td>
<td>17.5±1.3</td>
<td>17.9±1.2</td>
</tr>
<tr>
<td>PROREL</td>
<td>1.6±0.3</td>
<td>1.3±1.1</td>
<td>1.4±0.1</td>
<td>1.3±0.1</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>Post (w/o supp) kcal</td>
<td>2,455.2±194.3</td>
<td>2,624.2±19.8</td>
<td>2,869.4±181.0</td>
<td>2,469.5±168.7</td>
<td>2,431.7±188.7</td>
</tr>
<tr>
<td>gPRO</td>
<td>98.9±10.0</td>
<td>102.2±7.2</td>
<td>112.5±6.3</td>
<td>97.9±4.7</td>
<td>100.1±9.9</td>
</tr>
<tr>
<td>%PRO</td>
<td>16.4±0.8</td>
<td>15.7±0.6</td>
<td>16.1±0.6</td>
<td>16.8±1.0</td>
<td>16.7±0.9</td>
</tr>
<tr>
<td>PROREL</td>
<td>1.3±0.1</td>
<td>1.3±0.1</td>
<td>1.5±0.1</td>
<td>1.3±0.1</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>Post (inc. supp) kcal</td>
<td>2,615.2±194.3</td>
<td>2,784.2±219.8</td>
<td>3,029.4±181.0</td>
<td>2,685.5±168.7</td>
<td>2,431.7±188.7</td>
</tr>
<tr>
<td>gPRO</td>
<td>138.9±10.0</td>
<td>140.2±7.2</td>
<td>152.5±6.3</td>
<td>97.9±4.7</td>
<td>100.1±9.9</td>
</tr>
<tr>
<td>%PRO</td>
<td>21.9±1.0</td>
<td>21.2±0.9</td>
<td>20.9±0.8</td>
<td>15.2±0.8</td>
<td>16.7±0.9</td>
</tr>
<tr>
<td>PROREL</td>
<td>1.8±0.1</td>
<td>1.8±0.1</td>
<td>2.0±0.1</td>
<td>1.3±0.1</td>
<td>1.3±0.1</td>
</tr>
</tbody>
</table>

*BWP = Bioenhanced whey protein; BWP_Lv = low-volume resistance training with BWP; BWP_Mv = moderate-volume resistance training with BWP; SWP_Mv = moderate-volume resistance training with standard whey protein; PLA = moderate-volume resistance training with a placebo; CON = moderate-volume resistance training with no supplementation; gPRO = total grams of protein consumed; %PRO = percentage of kilocalories from protein consumed; PROREL = protein intake relative to body weight.

* Indicates a significant difference from prevalue.
† Indicates a significant difference from post w/o supplement.
§ Indicates a significant difference from supplemented groups.
However, the 2-way ANOVA indicated a significant interaction ($p < 0.000$) where BWP$_{1V}$, BWP$_{MV}$, and SWP$_{MV}$ consumed significantly more than PLA and CON at post. The range of PRO$_{REL}$ per group was 1.12–2.84, 1.04–2.43, 1.26–2.86, 0.76–1.91, and 0.69–1.96 g·kg$^{-1}$ for BWP$_{1V}$, BWP$_{MV}$, SWP$_{MV}$, PLA, and CON, respectively, and not excessively greater than the current recommendation for active men (1.4–2.0 g·kg$^{-1}$) (11), although some individuals reported consuming greater PRO$_{REL}$ than what may be considered “safe” per previous literature (44).

**DISCUSSION**

This study results indicated that although the BWP$_{1V}$, BWP$_{MV}$, and SWP$_{MV}$ groups consumed an overall higher protein diet because of supplementation, the effects of training were the most prominent among all groups. Furthermore, similar results were seen when individuals completed a lower-volume training program in conjunction with protein supplementation, indicating the importance of including additional protein to a diet when a moderate or high-volume program cannot be completed. Earlier studies have shown that resistance training alone increases MPS on a molecular level (35,47), reflecting the anabolic changes that appeared in the CON and PLA groups.

Despite some differences in study design compared to the previous 10- to 21-week resistance training interventions (2,13,19,26,45,57,62), the results of this study reflect the disparity among results on the effects of whey protein plus 8 weeks of resistance training in healthy young men. Because the sample in this study consisted of untrained or recreationally trained healthy young men, it is possible that the profound effects of the resistance training alone—as indicated by the improvements in the CON or PLA groups—may overshadow any additional, smaller benefits of whey protein supplementation. Additionally, there was an increase of 4.6% across all the groups, suggesting a hypertrophic response in the present population.

Chromiak et al. (13) investigated the effects of a whey protein, amino acid, and creatine-containing drink vs. carbohydrate placebo during 10 weeks of resistance training in men. Similar to this study, there were no differences between the 2 groups; however, the authors reported a trend toward greater increases in fat-free mass for the active supplement group. Additionally, Andersen et al. (2) reported that a 25 g of protein-containing drink before and after resistance training sessions over 14 weeks increased type I and II muscle fiber hypertrophy and improved performance compared with a carbohydrate placebo. Willoughby et al. (62) studied the effects of a 20-g protein or placebo drink before and after training sessions and reported improvements in markers of MPS, body composition, and performance after 10 weeks of resistance training with the protein drink. Similar to this study, Ogasawara et al. (41) reported hypertrophic responses after similar training volume was implemented in previously trained individuals using BP, as indicated by changes in mCSA measured by MRI. Conversely, studies by Verdijk et al. (57) and DeNysschen et al. (19) reported no differences in muscle hypertrophy, performance, or body composition among protein and placebo or whey protein and soy protein groups, respectively, after 12 weeks of resistance training.

Furthermore, young healthy men may have such a high basal level of MPS (60,63) that 8 weeks of whey protein and leucine supplementation may do little to further enhance MPS amidst the anabolic stimulus of resistance training. This may partially explain why this study showed no significant effects of protein supplementation beyond the resistance training during the 8-week study period, regardless of treatment group. Of note, one group in this study (BWP$_{1V}$) completed 55–59% of the resistance training volume that the other groups completed (226,976 in BWP$_{1V}$ vs.401,875 in BWP$_{MV}$, 383,202 in SWP$_{MV}$,415,832 in CON, and 397,172 in PLA). Because there were no interactions for body composition, mCSA, muscular strength, or muscular endurance, these findings suggest that when a moderate- or high-volume of resistance training is not possible, consuming protein and amino acids in conjunction with a low-volume resistance training program may be sufficient for achieving equivalent results. Regardless of the training program or supplementation status, the average change in mCSA was 4.89% in this study, similar gains as reported previously with different types of training regimens. Recent studies have indicated hypertrophy responses to occur as early as 3–4 weeks (1) as opposed to the characteristic 6- to 8-week (4,32) onset of hypertrophy using linear periodization strategies. Additionally, DeFreitas et al. (18) recently reported improvements in strength and thigh mCSA 6 weeks into an 8-week training program including leg extension and LP in untrained men. These findings are also consistent with recent studies showing that a reduced-volume resistance training program combined with supplementation achieved similar increases in strength and hypertrophy compared with that of traditional-volume resistance exercise without supplementation (45,54,62). This may be particularly useful when resistance training volume must remain low, such as for athletes during their preseason and in-season periodization schedules.

The BWP in this study included polyethylene glycosylated (PEG) amino acids. Polyethylene glycosylation is often used to increase the absorption and uptake efficiency of various compounds into the muscle cell (5,23,42,56,58). Specifically, PEG is believed to enhance the gastrointestinal absorption by increasing permeability and transport across the sarcolemma (23). Polyethylene glycosylation has been reported to make the peptide more water soluble, because PEG is hydrophilic (52,59), increases molecular stability (58), and increases the size of the combined glycosylated structure preventing the degradation of the actual peptide from endogenous enzymes (59). Overall, these mechanisms may extend the newly glycosylated compound’s half-life and bioavailability (59).

A recent study by Fry et al. (23) reported an increase in skeletal muscle uptake efficiency when subjects consumed
PEG creatine vs. creatine monohydrate. Creatine monohydrate is often criticized for having poor uptake efficiency into skeletal muscle, particularly after the muscle has reached its theoretical creatine capacity (43). Additionally, Herda et al. (25) reported an increase in upper- and lower-body strength for all creatine groups, but without weight gain in the 2 PEG creatine groups (25). Camic et al. (10) also reported an increase in upper-body strength with 28 days of 5 g·d$^{-1}$ PEG creatine compared with a dose-matched placebo; however, lower-body strength and Wingate performance remained unchanged. Thus, for creatine supplementation, the efficacy of polyethylene glycosylation has been observed in both basic (23) and applied (10,25) research. However, the results of this study did not show additional benefits for the PEG protein supplements (BWP$_{LV}$ or BWP$_{MV}$) in conjunction with resistance training for 8 weeks. Although we did not measure uptake efficiency, absorption, or bioavailability, the glycosylation may not have been capable of improving amino acid bioavailability in this study, because amino acids can readily be absorbed without delivery aids (22,30,31); however, future studies are necessary to confirm this hypothesis.

This study included no adverse health effects due to increased protein consumption as confirmed by blood markers of liver and kidney health. Previous literature has suggested that protein supplementation may have negative effects on kidney health (27,33). Serum markers that are used to assess renal function include creatinine, BUN, and eGFR (29,37). In this study, there were no changes in serum creatinine or BUN, and there was no change in the eGFR based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (34). These findings are largely consistent with those of several previous studies that have reported no negative impact of high-protein diets on these markers in healthy individuals with normal renal function (7,36,48,61). Other serum markers that may be affected by protein consumption include albumin, globulin, and TP (9). Albumin is synthesized by the liver after dietary protein consumption, and low albumin concentrations could be a result of protein insufficiency (9). Serum globulins are also synthesized by the liver and may also be decreased by malnutrition or dehydration (9). Based on reference ranges for healthy young adults, the optimal ratio of albumin to globulin is around 1.1–2:1 (28). In this study, the albumin to globulin ratio was higher in both BWP groups than in the SWP group after 8 weeks, but no group was outside the reference range for young men. Although it is unclear how the difference in albumin to globulin ratio may be related to polyethylene glycosylation, there seemed to be no blood marker-related health concerns after 8 weeks of supplementation in this study. Although the dietary intakes of protein were within normal ranges at baseline, individuals in the groups that consumed a whey protein shake had significantly increased PRO$_{REL}$ compared with the nonsupplemented groups. However, the increased protein intake did not negatively alter any blood markers of liver or kidney function.

**Practical Applications**

As previously discussed, several studies have reported that acute protein supplementation can reduce the magnitude of muscle protein breakdown at rest and after resistance exercise, and of the amino acids in whey protein, leucine may be the most effective. This study indicated that although 8 weeks of low- or moderate-volume resistance exercise increased strength across all groups, these changes may partially be of neural nature. This does not take away from the fact there was also hypertrophy across all groups, regardless of supplementation status. There were also no adverse health-related effects of any protein supplementation (BWP$_{LV}$, BWP$_{MV}$, or SWP$_{MV}$) suggesting that the amounts and duration of protein supplements consumed were relatively safe for the healthy young male population. Furthermore, athletes could benefit from a low-volume regimen in conjunction with protein supplementation while recovering from injury and completing their prescribed rehabilitation program. This may potentially speed up the recovery process and decrease the event of postinjury complications. As active adults age, they are encouraged to maintain or increase activity. However, less is known about how older adults may respond to whey protein and leucine supplementation in conjunction with chronic resistance exercise. A lower-volume of resistance exercise plus supplementation can potentially benefit untrained or detrained individuals, similar to moderate-volume without protein supplementation. It is also possible that older adults and elderly patients may have a higher aptitude to respond to the anabolic effects of protein supplementation and resistance exercise. Additionally, it is possible that PEG may be more beneficial for the absorption of the amino acids in those with difficulties digesting nutrients, rather than healthy young men that already have a high basal MPS rate.

**Acknowledgments**

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**References**


Resistance Training and Protein Supplementation


