Effect of Protein Ingestion on Energy Expenditure and Substrate Utilization After Exercise in Middle-Aged Women

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Research suggests that ingesting protein after resistance exercise (RE) increases muscle protein synthesis and results in greater muscle gains. The effect on energy expenditure and substrate utilization, however, is unclear. This study evaluated the effect of RE and postexercise protein on recovery energy expenditure and substrate utilization in 17 women (age 46.5 ± 1.2 y). A whey-protein supplement (120 kcal, 30 g protein) was ingested immediately after 1 bout of RE (PRO) and a noncaloric placebo after another (PLA). VO$_2$ and respiratory-exchange ratio (RER) were measured before and for 120 min after each exercise session. RE resulted in a significant increase in VO$_2$ that persisted through 90 min of recovery ($P < 0.01$) and was not affected by protein supplementation. RE significantly lowered RER, resulting in an increase in fat oxidation for both PLA and PRO ($P < 0.01$). For PRO, however, RER returned to baseline values earlier than for PLA, resulting in a reduced fat-oxidation response ($P = 0.02$) and earlier return to preexercise baseline values than for PLA. Substrate utilization was significantly different between conditions ($P = 0.02$), with fat contributing 77.76% ± 2.19% for PLA and 72.12% ± 2.17% for PRO, while protein oxidation increased from 17.18% ± 1.33% for PLA to 20.82% ± 1.47% for PRO. Postexercise protein did not affect energy expenditure, but when protein was available as an alternate fuel fat oxidation was diminished. Based on these findings it might be beneficial for middle-aged women to delay protein intake after RE to maximize fat utilization.

Key Words: whey protein, respiratory-exchange ratio, nonprotein respiratory quotient

Resistance exercise (RE) has been shown to increase muscle mass even in individuals in their 90s (12). Muscle protein synthesis increases dramatically after an acute bout of high-intensity RE and can remain elevated as much as 100% 24 h after cessation of exercise (16). When amino acids are available immediately postexercise they provide an additional anabolic stimulus that further enhances muscle protein synthesis (20, 25). Recently it has been suggested that the timing of dietary-protein ingestion in relation to a resistance training (RT) program can
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influence its anabolic effect (19). Data from two 12-wk studies of elderly (10) and middle-aged men (7) appear to support this effect. A similar effect has not been found in women, however. Preliminary data from this lab have shown that women enrolled in a 12-wk high-intensity RT program gained similar amounts of lean mass whether a high-protein supplement was ingested immediately postexercise or delayed for 2 h (23). When protein supplementation was delayed for 2 h, however, significant decreases in fat mass were noted that did not occur when nutritional intake was provided within 5 min after each RE bout (23). Although no clear mechanism for this finding was identified, it was hypothesized that the availability of protein immediately after exercise might blunt the postexercise increases in energy expenditure and fat utilization that have been found to occur immediately after an acute bout of RE (2).

To date, the effect of postexercise protein supplementation on energy expenditure and substrate utilization during this critical postexercise recovery period has not been studied. Furthermore, although acute bouts of RE have been shown to result in transitory, yet significant, increases in energy expenditure and fat utilization in young men and women (2, 8), the response of middle-aged individuals has not been studied. Therefore, the purpose of this study was 2-fold: to examine the effect of an acute bout of RE on substrate utilization and energy expenditure during the 2-h recovery period in middle-aged women and to evaluate the effect of an oral protein supplement on that response.

Methods

Participants

Twenty-two women volunteered to participate in this study. All were healthy, nonsmokers, and between the ages of 40 and 55 y. Exclusion criteria included cardiovascular disease, diabetes, pregnancy (currently or planning to become pregnant), body-mass index $\geq 40$ kg/m$^2$, or any orthopedic condition that would impede safe participation in RE. Demographic and health questionnaires were completed before inclusion in the study. The study was approved by the institutional review board of Arizona State University.

Design

A single-sample, repeated-measures, double-blind design was used. After completion of the informed consent all participants were tested under 2 treatment conditions: RE followed immediately by ingestion of a placebo (PLA) and RE followed immediately by ingestion of a protein supplement (PRO). The order of assignment of the 2 treatments was counterbalanced and randomized. Each participant was tested at the same time of day for both conditions. To minimize hormonal variation, participants reporting regular menstrual activity within the preceding 6 months were tested in the early follicular phase of the menstrual cycle (days 3–5 after commencement of menstruation) during 2 consecutive menstrual cycles. Participants reporting natural or surgical menopause were tested with at least 72 h rest between exercise bouts. All participants were instructed to abstain from exercise, alcohol, and caffeine for 24 h before testing. To control for dietary influences, they were
asked to record all intake for the 24-h period before the first test and consume an identical diet during the 24-h period before the second test.

**Anthropometric Measurement**

Before testing, anthropometric measurements were obtained for all participants, including height, weight, body-mass index, waist and hip circumferences, and body composition using air-displacement plethysmography (Bod Pod). Participants were instructed not to eat or drink anything but water for 4 h and not to engage in exercise for 24 h before measurement.

**Strength Measurement**

Participants completed 3 sessions of maximal-strength testing with at least 24 h rest between sessions to identify the maximum weight they could lift 1 time (1RM) (1). To better gauge intensity, participants were also asked to rate each lift using a rating of perceived exertion as suggested by McGuigan and Foster (17). A maximal attempt was defined as actual failure or a rating of perceived exertion of 9 or 10 (on a 10-point scale) with a statement by the participant that another attempt could not be achieved. The 1RM was reported as the weight of the last successfully completed lift. To ensure that no carryover effect occurred as a result of the strength-testing sessions, the final session was scheduled at least 48 h before the first RE bout.

**Metabolic Testing**

Metabolic testing was done twice (before and after RE) for each treatment condition. Participants reported to the laboratory for baseline, preexercise metabolic testing in a well-hydrated state and having consumed no food for 4 h. Energy expenditure (VO$_2$) and fuel utilization (respiratory-exchange ratio [RER]) were measured by indirect calorimetry using a metabolic cart (MAX-2, AEI Technologies, Pittsburgh, PA) and mask. Before each test session the metabolic cart was calibrated using gases with known concentrations. During testing participants were positioned in a reclining chair with feet elevated and habituated to the open-circuit spirometry metabolic-analysis apparatus for 20 min in a semidarkened, quiet, temperature-controlled (22–24 °C) room. A 2-way nonrebreathing respiratory valve and mask (Hans Rudolph, Kansas City, MO) were placed snugly over the nose and mouth to ensure an adequate seal to prevent air leakage. Participants were instructed to remain awake and not move or talk once the mask was in place. After the 20-min habituation period VO$_2$ and RER were obtained from a mean of 10 min of continuous gas sampling via indirect calorimetry. Resting energy expenditure was calculated from VO$_2$ and the RER using the Weir formula, REE = [(3.9 × VO$_2$) + (1.1 × VCO$_2$)] × 1.44 (26). Substrate (fat and carbohydrate) oxidation was estimated using RER after correction for protein oxidation, as estimated by urinary excretion of urea (11, 13).

**Urinary Nitrogen**

Each participant provided a postexercise urine specimen for each metabolic test session. Participants were asked to void and discard urine immediately after ingesting
the postexercise protein or placebo supplement and then collect all urine through completion of the postexercise metabolic test. Immediately after the 120-min test the participants were asked to void again to complete the specimen. Urine specimens were assayed for urea nitrogen using photometric measurement of urease (Sonora Quest Laboratories, Tempe, AZ).

**Resistance Exercise**

After measurement of baseline resting energy expenditure, participants were escorted to the exercise area where they completed an acute bout of high-intensity RE. Each exercise bout consisted of 3 sets of 8–12 repetitions of 8 exercises designed to stimulate all major muscle groups. The first set of each exercise served as a warm-up set and consisted of 10 repetitions at 50% 1RM. Thereafter, participants completed 2 sets of each exercise at 80% 1RM with 1 min rest between sets. Exercises were counterbalanced to avoid fatigue (chest press, latissimus pull-down, leg press, shoulder press, seated row, leg extension, triceps pushdown, and biceps curl) and paired into supersets so that one muscle group could rest for 60 s while another was trained.

**Protein Supplementation**

Fourteen fluid ounces of a commercially available fruit-flavored liquid protein supplement (Isopure Zero Carb, Nature’s Best, Hauppauge, NY) consisting of 30 g whey protein (120 kcal) was ingested within 5 min of completion of 1 bout of RE. An equal amount of a fruit-flavored, calorie-free placebo drink (Aloe Splash, Scottsdale, AZ) was consumed immediately after the other bout. Water was provided ad libitum during and immediately after both bouts of RE.

**Postexercise Metabolic Testing**

Metabolic measurements were repeated immediately after completion of each bout of RE for an additional 120 min. Because the increase in energy expenditure that occurs immediately after an acute bout of RE has been found to peak within 30 min (4, 22), participants were connected to the metabolic apparatus within 10 min after completing each exercise bout. Energy expenditure and substrate utilization were obtained from mean 4-min intervals gathered at 4, 20, 40, 60, 90, and 120 min of the postexercise metabolic test. During testing participants were monitored continually to ensure that they remained awake. To help them stay awake, travel or historical videos were shown throughout the test period.

**Statistical Analysis**

Data were analyzed using SPSS version 14.0 software and reported as mean ± standard error. Energy expenditure (VO$_2$) and fuel utilization (RER) data were analyzed using a 2 × 7 repeated-measures ANOVA with conditions (placebo and protein) and time (pre- and postexercise) as factors. An alpha level of $P < 0.05$ was used to determine significance. Post hoc paired $t$-tests were run with a Bonferroni correction to evaluate differences between conditions for training volume, training time, delay between cessation of exercise and commencement of postexercise metabolic testing, and postexercise metabolic measurements at individual time points.
Results

Participants

Of the 22 women initially enrolled in the study, 3 were unable to continue because of time constraints and 2 because of complaints of knee pain that inhibited full participation in strength testing. Seventeen women (age 46.5 ± 1.2 y) completed the study. Their anthropometric characteristics are summarized in Table 1. Eleven participants had regular menses so were tested 2–5 d after onset of menstruation during 2 consecutive menstrual cycles. Six participants reported having had no menses for at least 6 months so were tested with a rest period of at least 72 h between exercise bouts. All participants refrained from exercise for at least 24 h before each metabolic test session. Because participants were instructed not to eat or drink anything but water for 4 h before reporting to the testing laboratory, all sessions were timed to allow them to eat a light snack at the beginning of that 4-h period (i.e., the tests were scheduled no earlier than 4 h after waking).

One participant self-reported a prior medical diagnosis of thalassemia minor, a clinically asymptomatic microcytic anemia, that was confirmed by blood analysis. A second participant reported a history of anemia treated with oral iron supplements. This was also confirmed by blood analysis. Because there were no contraindications for exercise in either condition, they were admitted to the study and monitored closely during test sessions. Both tolerated the RE protocol well, without undue fatigue. Study data were analyzed both with and without data from these 2 participants, and no differences were found, so they were included in the final data analysis.

Strength Measurement

All strength tests met the goal of attaining a 1RM measurement within 5 maximal attempts. A rest period of at least 24 h was provided with an average of 5 ± 0.6 d between strength tests. Because most participants were unfamiliar with the 8 resistance exercises, the 3 strength-testing sessions served the purpose of familiarization, as well as testing.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± standard error</th>
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</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>46.5 ± 1.2</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>67.3 ± 2.7</td>
</tr>
<tr>
<td>Body-mass index (kg/m²)</td>
<td>25.8 ± 1.1</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.80 ± 0.01</td>
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<tr>
<td>Lean mass—Bod Pod</td>
<td>42.0 ± 0.6</td>
</tr>
<tr>
<td>% Lean mass—Bod Pod</td>
<td>63.9 ± 2.4</td>
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<tr>
<td>% Body fat—Bod Pod</td>
<td>36.1 ± 2.4</td>
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Resistance Exercise

There were no significant differences between the 2 test conditions. Mean exercise time was 21.1 ± 0.5 min for PLA and 22.4 ± 0.8 min for PRO, and mean training volume was 951 ± 86 kg (2096 ± 189 lb) for PLA and 938 ± 84 kg (2067 ± 185 lb) for PRO. All participants consumed the 14-oz drink, returned immediately to the laboratory, and commenced postexercise metabolic testing within 6.9 ± 0.3 min after cessation of exercise. Not surprisingly, a typical and equivalent cardiovascular response to exercise was observed for PLA and PRO, with heart rate increasing significantly immediately postexercise and remaining elevated throughout the entire 120-min postexercise monitoring period (P < 0.01).

Energy Expenditure

Before exercise, baseline mean energy expenditure equaled 1372.2 ± 91.4 kcal/24 h for PLA and 1293.9 ± 64.6 kcal/24 h for PRO, and mean postexercise energy expenditure increased to the equivalent of 1595.9 ± 67.7 kcal/24 h for PLA and 1654.9 ± 75.2 kcal/24 hr for PRO (Table 2). There were no significant differences in VO₂ (mL·kg⁻¹·min⁻¹) between PLA and PRO at baseline or any time point during the 120-min recovery period (Figure 1). Compared with baseline, VO₂ was significantly increased immediately after cessation of exercise (P < 0.01) and remained elevated through 90 min of the postexercise period (P < 0.01) for both PLA and PRO. At 120 min postexercise VO₂ was no longer significantly different from preexercise baseline values for either PLA or PRO (P = 0.15). The combined mean VO₂ at rest for PLA and PRO was 2.76 mL·kg⁻¹·min⁻¹. Using this value, the predicted VO₂ for a rest period of 120 min would be 331.44 mL/kg, and the mean measured VO₂ for the recovery period was 402.59 mL/g. This represents an increase of 22% over predicted resting energy expenditure.

Substrate Utilization

RER was significantly different from baseline through 90 min of the recovery period (P < 0.01) for both PLA and PRO (Figure 2). Immediately after cessation of RE (time 0) for both PLA and PRO there was an immediate increase in RER (P < 0.01), followed within the first 20 min of recovery by a decrease (P < 0.01). For the PLA condition RER continued to decrease through 40 min postexercise and then began

Table 2  Postexercise Metabolic Measurements, Mean ± Standard Error, P < 0.05

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Placebo</th>
<th>Protein</th>
</tr>
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<tbody>
<tr>
<td>Postexercise energy expenditure (kcal/24 h)</td>
<td>1,595.9 ± 67.7*</td>
<td>1,654.9 ± 75.2*</td>
</tr>
<tr>
<td>Postexercise protein oxidation (kcal/24 h)</td>
<td>274.18 ± 0.9</td>
<td>344.55 ± 1.11†</td>
</tr>
<tr>
<td>Postexercise fat oxidation (kcal/24 h)</td>
<td>1240.97 ± 1.48</td>
<td>1193.51 ± 1.63†</td>
</tr>
<tr>
<td>Postexercise carbohydrate oxidation (kcal/24 h)</td>
<td>74.21 ± 1.11</td>
<td>116.84 ± 1.46</td>
</tr>
<tr>
<td>2-h postexercise urinary nitrogen (g)</td>
<td>0.832 ± 0.065</td>
<td>1.043 ± 0.08‡</td>
</tr>
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</table>

*Significantly different from baseline preexercise values (p < 0.01). †Protein significantly different from placebo (P = 0.02). ‡Protein significantly different from placebo (P < 0.01).
Figure 1 — Change in oxygen uptake (VO$_2$) over time. The preexercise measurement consisted of a mean of 10 min after a 20-min habituation period. Postexercise measurements consisted of 4-min means taken immediately after exercise (Post 0) and 20, 40, 60, 90, and 120 min postexercise. The mean time that elapsed between cessation of exercise and commencement of measurement (Post 0) was 6.9 ± 0.3 min for both placebo (PLA) and protein (PRO). *Significantly different from preexercise ($P < 0.01$).
a gradual increase that continued for the remainder of the 120 min, although at the end of the recovery period RER remained below preexercise values ($P < 0.05$). For PRO, however, there was a more immediate and profound increase in RER that began between the 20- and 40-min collection points and continued throughout the recovery period, so that RER remained consistently higher for PRO than for PLA. By the end of the 120-min postexercise period, RER for PRO had returned to baseline values and was significantly higher than for PLA ($P = 0.01$), perhaps indicating a greater reliance on fat as a fuel source for PLA during recovery.

Two-hour postexercise urinary nitrogen excretion was significantly different between conditions (PLA = 0.832 ± 0.065 g, PRO = 1.043 ± 0.08 g; $P < 0.01$; Table 2). After calculation of the nonprotein respiratory quotient there was a significant difference between conditions in the proportion of substrates oxidized during recovery ($P = 0.02$). For PRO the contribution of protein to energy expenditure was 20.82% ± 1.47%, and for PLA it was 17.18% ± 1.33%. This represented an increase in protein oxidation of 3.65% ± 1.42% ($P = 0.02$) when protein was available as a fuel source. Furthermore, for PRO there was a commensurate decrease in fat oxidation of 5.65% ± 2.18% ($P = 0.02$). When protein was not available during the PLA condition, fat contributed 77.76% ± 2.19% to total energy expenditure. When protein was available as an alternative fuel during the PRO condition, however, fat utilization was diminished to 72.12% ± 2.17% of total energy expenditure (Table 2).

**Discussion**

The purposes of this study were 2-fold: to evaluate the recovery energy expenditure and substrate utilization response of middle-aged women to an acute bout of RE and to determine the effect of postexercise protein ingestion on that metabolic response. Not surprisingly, middle-aged women responded to high-intensity RE in a manner similar to that of younger individuals. There was an immediate increase in energy expenditure ($\text{VO}_2$) after exercise that remained significantly elevated through 90 min of the 120-min postexercise recovery period. This finding is in agreement with earlier research that noted a similar response in young women (2, 24) and men (18, 22) after a comparable bout of RE. Apparently there are no gender or age differences in the metabolic response to brief, high-intensity RE.

Although the total postexercise increase in caloric expenditure was minimal when computed for a single exercise bout (approximately 18 kcal for a 50-kg woman), the accumulation over time might be significant, especially in view of research indicating a prolonged increase in metabolic rate for up to 48 h after exercise, which might well increase the total caloric deficit (8, 27). For a middle-aged woman weighing 50 kg, a similar RT program 4 times a week over a 1-y period could be expected to increase caloric expenditure approximately 3744 kcal or the equivalent of 0.5 kg (1 lb) of body fat, the average yearly weight gain observed in women 45–55 y old (5). RE might indeed be a viable solution for long-term weight management in middle-aged women.

The second aim of this study was to evaluate the effect of postexercise protein ingestion on the metabolic response to RE. The increase in postexercise caloric expenditure observed in this study occurred both in the fasting state and after protein supplementation. Dietary protein did not interfere with postexercise increases
Figure 2 — Change in respiratory-exchange ratio (RER) over time. There were no significant differences between groups. Postexercise values were significantly different from baseline for both placebo (PLA) and protein (PRO) conditions through 90 min of the recovery period ($P < 0.01$). By 120 min postexercise PRO had returned to baseline values and was significantly higher than PLA ($P = 0.01$). **Both PLA and PRO significantly different from preexercise ($P < 0.05$). *Only PLA significantly different from preexercise ($P < 0.05$). †PRO significantly different from PLA ($P = 0.01$).
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in VO₂. This is significant for individuals wishing to supplement with protein postexercise in anticipation of enhanced muscle protein synthesis. Although protein supplementation has been found to enhance muscle protein synthesis after RE (9), its application to a long-term RT program is not clear. Esmarck et al. (10) found that in elderly men, ingestion of a protein supplement immediately after RE resulted in greater accretion of lean mass than when supplementation was delayed by 2 h. This effect was not found in women, however (23). It is therefore possible that for women the choice of when to supplement with protein after RE might primarily depend on its desired metabolic effects (increase in energy expenditure and fat oxidation) rather than solely the anticipated increases in muscle protein synthesis.

During the 2-h recovery period, postexercise protein supplementation was observed to attenuate fat oxidation compared with PLA. Normally, recovery from RE is characterized by a shift toward fat as a fuel source. The current study found, however, that when protein was available this shift was blunted, resulting in a decrease of more than 5% in the total fat utilized for energy in the postexercise period. Because this decrease in fat oxidation was accompanied by an increase in protein oxidation, it is possible that dietary protein was selectively utilized as a fuel in favor of fat. This might have been a result of the choice of whey protein as a supplement. Whey has been found to have a more rapid rate of digestion and bioavailability than other proteins such as casein, resulting in an increase in protein oxidation when whey protein is available (6).

In absolute terms, the 5% difference in fat oxidation was calculated to represent an additional 6 kcal (0.67 g of fat) for a 50-kg woman during recovery. Although modest, this difference in fat oxidation can be physiologically significant when applied over time. At a training frequency of 3 times a week, an increase in fat oxidation of only 6 kcal/bout would result in a reduction of more than 0.1 kg (0.25 lb) of fat mass in 1 y with no additional intervention other than delaying intake for 2 h after RE. It should be recognized, as well, that at 120 min postexercise, when monitoring ceased, RER for PLA had not returned to baseline, so a greater actual amount of fat oxidation is likely to have occurred.

The current findings are similar to those of Hulmi et al. (15), who administered a protein supplement or noncaloric placebo before a bout of RE. RER was significantly higher for the protein condition than for the placebo, indicating decreased fat oxidation after ingestion of protein, although no significant differences in fat oxidation were found when absolute values were calculated (15). Because those authors did not report the absolute numbers, however, the possible long-term effects on fat mass cannot be estimated. It would appear, however, that whether protein is ingested before or immediately after RE there is a commensurate influence on substrate utilization that blunts the postexercise shift to fat as a primary fuel source.

In comparison, Bosher et al. (3) administered either a high-fat or a high-carbohydrate meal after RE and found no difference in fat utilization compared with a water-only condition. Postexercise supplementation was delayed, however, for 45 min after cessation of exercise. It is possible that this delay in intake was sufficient to allow the anticipated postexercise shift to fat oxidation to occur, so that when nutrients were finally consumed they had no effect on substrate utilization. If such were indeed the case, then a 2-h delay in supplementation after RE would facilitate fat oxidation while at the same time promoting the increased muscle protein synthesis observed by Rasmussen et al. (21) when oral amino acids were ingested up to 3 h postexercise.
A potential limitation of this study was that lactate concentration was not monitored. Although lactic acid produced in response to high-intensity exercise can decrease RER (14), in the current study both PLA and PRO conditions were identical in terms of exercise intensity so the effect of lactate on RER would have been identical between conditions. This is supported by the results of Hulmi et al. (15), who found that although blood lactate levels increased significantly during identical bouts of RE, this increase was not affected by ingestion of either a protein or placebo drink before exercise. It is therefore reasonable to conclude that any difference in RER between conditions in the current study was not influenced by lactate levels but was instead the result of true differences in fat oxidation.

In conclusion, a single bout of RE significantly increased VO\(_2\) and fat oxidation during the 120-min postexercise period in women 40–55 y old. Ingestion of a protein supplement immediately after RE did not affect total energy expenditure (VO\(_2\)) but did in fact inhibit fat oxidation, resulting in a decreased reliance on fat as a fuel source when protein was available. Based on these findings, women whose training goals include decreases in fat mass, as well as increases in lean mass, might benefit from delayed nutritional supplementation after RE. Administration of a protein supplement immediately after RE might not affect anticipated increases in energy expenditure but might in fact selectively inhibit fat oxidation and promote accretion of fat mass over time.

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**References**


