Timed-daily ingestion of whey protein and exercise training reduces visceral adipose tissue mass and improves insulin resistance: the PRISE study

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The present study examined the effects of timed ingestion of supplemental protein (20-g servings of whey protein, 3×/day), added to the habitual diet of free-living overweight/obese adults and subsequently randomized to either whey protein only (P, n = 24), whey protein and resistance exercise (P + RT; n = 27), or a whey protein and multimode exercise training program [protein and resistance exercise, intervals, stretching/yoga/Pilates, endurance exercise (PRISE); n = 28]. Total and regional body composition and visceral adipose tissue (VAT) mass (dual-energy X-ray absorptiometry), insulin sensitivity [homeostasis model assessment-estimated insulin resistance (HOMA-IR)], plasma lipids and adipokines, and feelings of hunger and satiety (visual analog scales) were measured before and after the 16-wk intervention. All groups lost body weight, fat mass (FM), and abdominal fat; however, PRISE lost significantly (P < 0.01) more body weight (3.3 ± 0.7 vs. 1.1 ± 0.7 kg, P + RT) and FM (2.8 ± 0.7 vs. 0.9 ± 0.5 kg, P + RT) and gained (P < 0.05) a greater percentage of lean body mass (2.0 ± 0.5 vs. 0.9 ± 0.3 and 0.6 ± 0.4%, P + RT and P, respectively). Only P + RT (0.1 ± 0.04 kg) and PRISE (0.21 ± 0.07 kg) lost VAT mass (P < 0.05). Fasting glucose decreased only in P + RT (5.1 ± 2.5 mg/dl) and PRISE (15.3 ± 2.1 mg/dl), with the greatest decline occurring in PRISE (P < 0.05). Similarly, HOMA-IR improved (0.6 ± 0.3, 0.6 ± 0.4 units), and leptin decreased (4.7 ± 2.2, 4.7 ± 3.1 ng/dl), and adiponectin increased (3.8 ± 1.1, 2.4 ± 1.1 μg/ml) only in P + RT and PRISE, respectively, with no change in P. In conclusion, we find evidence to support exercise training and timed ingestion of whey protein added to the habitual diet of free-living overweight/obese adults, independent of caloric restriction on total and regional body fat distribution, insulin resistance, and adipokines.

THE USE OF DIETARY MANIPULATION to manage energy intake effectively, as well as body weight and composition, is proving to be an effective lifestyle strategy to treat overweight and obesity. One such example is the combination of caloric restriction and increased dietary protein intake that is often associated with enhanced satiety, body weight, and composition during both weight loss and weight-loss maintenance (7, 19, 32, 53). Indeed, our lab showed recently that consuming increased protein (up to 35% of total energy intake), evenly spaced throughout the day (~20-g servings up to six meals/day), decreased total and abdominal fat, increased lean body mass (LBM) and the thermic response to a meal, and favorably altered adipokines, more than current recommendations for protein in overweight adults during both energy balance and energy deficit (7). Additionally, protein source is an important factor in the success of these weight-loss interventions. For example, ingestion of whey protein throughout the day, along with an ad libitum diet independent of caloric restriction, may mediate the increased satiety and enhanced body weight loss and composition changes compared with isoenergetic soy or carbohydrate (CHO) (9). Proposed mechanisms for the enhanced satiety, weight loss, and body fat (BF) distribution with increased whey protein consumption, in the absence of caloric restriction, may be due to reductions in fasting ghrelin (9) and feeding-induced stimulation of muscle protein synthesis brought on by whey protein and amino acid intake (12, 13, 27, 35), respectively. Indeed, a growing body of evidence supports an enhanced rate of protein synthesis (muscle and whole body) from protein ingestion at rest and during exercise (16, 20, 29, 46), and this effect appears to be augmented by distributing the protein in ~20-g servings throughout the day (8, 34). To what extent this feeding pattern of protein impacts body weight and abdominal BF distribution in free-living overweight adults, in the absence of caloric restriction, is of paramount importance.

Resistance training (RT) is another potent stimulus for enhanced skeletal muscle protein synthesis (10, 40) and body composition changes (17, 23, 26, 46, 51). Interestingly, the timing (approximately every 3 h) and amount (~20 g of whey protein/serving) of protein consumed at rest and in relation to resistance exercise further enhance this response (8, 34, 35). Additionally, recent findings from our laboratory provide compelling, new data in support of beginning and ending the day with a 20- to 30-g protein feeding to reduce abdominal fat and favorably alter adipokines (7).

Given the above findings, it is of interest to examine the coupling of quantity and timing of protein ingestion over an extended period of time (16 wk), with and without an exercise training stimulus on body weight, total and regional body composition, adipokines, and hunger in free-living overweight and obese adults. Recently, healthy lifestyle routines that combine multiple fitness components into one training program [resistance exercise, interval sprint exercise, stretching/yoga/Pilates, and endurance exercise (RISE)] have become increasingly popular but lack scientific data to confirm their effectiveness in favorably altering body weight and BF distribution in free-living overweight and obese adults. As such, it is important to compare the relative effectiveness of these combined lifestyle exercise regimens [protein and RISE (PRISE)] with well-established exercise routines involving RT exercise, both

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of which include timed ingestion of whey protein (P) evenly spaced throughout the day on body weight and fat distribution, adipokines, and hunger in ad libitum diet, free-living adults.

Therefore, the primary objective of the present study was to examine the effects of timed ingestion (3×/day) of supplemental whey protein (P) added to the habitual diet of free-living overweight and obese adults, independent of caloric restriction, with and without exercise training (P + RT; PRISE) on body weight and fat distribution, insulin resistance (IR), and hunger. A secondary objective was to assess differences between the exercise training groups (P + RT vs. PRISE) on the above outcome measures. We hypothesized that three, 20-g servings of P ingested within 1 h of waking in the morning, mid-day (or within 30 min following an exercise bout; P + RT, PRISE), and within 2 h of going to bed, in addition to an ad libitum diet, would elicit significant improvements in total and regional (abdominal) body composition, adipokines, and hunger ratings over a 16-wk period in overweight and obese, healthy men and women.

METHODS

Participants

A total of 221 individuals were recruited from the Saratoga Springs, New York, area through newspaper advertisements and flyers and initially screened for participation, of which 97 were eligible for participation. Before randomization, 18 individuals declined to continue with the study, resulting in 79 participants who started the study. Participants were nonsmoking, healthy men and women with no overt cardiovascular or metabolic diseases (type 2 diabetes mellitus, thyroid disease, etc.), as assessed by a medical history and medical examination by their physicians. All participants were inactive (<30 min, 2 days/wk of structured physical activity, with no RT experience within the last 10 years), overweight or obese [body mass index (BMI) = 28.6 ± 5.4 kg/m²; percent BF (%BF) = 36.6 ± 7.4%], middle aged (46 ± 9.4 yr), and weight stable (±2 kg) for at least 6 mo before beginning the study. Each participant provided informed, written consent in adherence with the Skidmore College Human Subjects Review Board before participation, and the study was approved by the Human Subjects Institutional Review Board of Skidmore College. All experimental procedures were performed in accordance with the Federal Wide Assurence and related New York State regulations, which are consistent with the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research and in agreement with the Helsinki Declaration as revised in 1983. This trial was registered at clinicaltrials.gov as NCT01960335.

Experimental Design

Participants were assessed for BMI and body weight and randomly assigned to one of three free-living groups providing whey protein supplementation (20-g servings, 3×/day; 60 g total) to the habitual diet (without energy restriction), with and without an exercise training intervention: 1) whey protein only (P; n = 24); 2) whey protein combined with high-intensity RT (4×/wk; P + RT; n = 27); and 3) whey protein plus a combined exercise training program (4×/wk; P + PRISE; n = 28). Of note, exclusion of a CHO control group was purposefully designed, due to recent literature reporting negative changes to body composition in overweight and obese adults (9). All testing procedures were measured at baseline and following the 16-wk intervention period.

Whey protein supplementation. The whey protein supplement (classic whey, optimum nutrition) was distributed to each participant in 20-g servings (serving size: 21 g protein, 120 kcal, 360 kcal/day total). The timing of the whey protein supplement was an important component of the study. Specifically, the 20-g servings of whey protein supplement needed to be consumed in the following manner for all participants: serving one within 1 h of waking in the morning; serving two mid-afternoon or within 30 min following an exercise session on exercise days (P + RT, PRISE); and serving three within 2 h of going to bed at night. Otherwise, all participants were instructed to consume their habitual diet ad libitum throughout the 16-wk intervention.

Compliance. All participants were provided detailed verbal and written instructions regarding whey protein supplement intake, as well as exercise training guidelines and techniques (see below; P + RT and PRISE). Whey protein supplement compliance was reinforced and monitored through daily subject-researcher contact, including telephone conversations, weekly inspection of whey protein intake journals, distribution of weekly whey protein supplement containers and return of empty whey containers, monthly group meetings, and 3-day food diary analysis (see below). The principal investigator and/or an investigator met weekly with study participants to answer questions, clarify exercise and dietary guidelines, and verify compliance with the protein supplements. Verification of whey protein supplement intake and timing revealed a high compliance rate (>90%), defined as consuming ≥18 (or >85%) of the 21 weekly whey protein-supplemented feedings. Participants were considered noncompliant if they missed consuming greater than or equal to three servings of whey protein/wk for ≥2 consecutive wk at a time.

Self-reported energy intake, 3-day food diaries, and physical activity. Throughout the 16-wk intervention, participants maintained a daily food log that included all food and beverages consumed each day, including meal timing. To verify compliance further, a representative 3-day period, before beginning the intervention, and end of the study (wk 16) were analyzed using the Food Processor SQL Edition (version 10.12.0; 2012; ESHA Research, Salem, OR), as described previously (23). All dietary analyses were performed by the same laboratory technician and reported in Table 1.

Participants completed an activity questionnaire before the beginning and end of the 16-wk intervention detailing the intensity and types of physical activity during the previous 16 wk. This also served as a form of compliance.

Exercise training protocols. Participants assigned to the exercise groups (P + RT and PRISE) consumed whey protein as outlined above. In addition, on exercise days, one of the whey protein servings was consumed within 30 min following the exercise bout. Participants were familiarized with each individual exercise movement (see below; P + RT or PRISE) by participating in eight individually supervised exercise sessions (four times/wk), the first 2 wk of the intervention (weeks 1 and 2), and then were monitored by a member of the research team in a group setting for the remainder of the intervention (weeks 3–16) at the Skidmore College Fitness Center. Throughout the entire 16-wk intervention, participants assigned to the P + RT and PRISE groups performed four exercise sessions/wk of the following exercises: 1) RT and two sessions of high-intensity intervals on aerobic exercise equipment (P + RT) or 2) exercise training consisting of functional RT (R); interval sprint training (I); stretching/yoga/Pilates training (S); and endurance exercise (E) (“RISE”), respectively. Exercise compliance was reinforced through weekly inspection of exercise journals, monthly group meetings, and daily exercise monitoring and subject-researcher contact. In the event that a subject missed a scheduled exercise session, it was made up on an alternate day that week so that all exercise sessions were fully completed for each week. Investigators were in contact with each participant by phone, email, or in person on a daily basis. Participants were considered noncompliant if they missed greater than or equal to one exercise session for 2 consecutive wk. Compliance with the exercise training sessions remained high (~85%).

All exercise training sessions followed the American College of Sports Medicine Training Principles and Guidelines, whereby exercise intensity was based on individual fitness level and health status.
(47a). In addition, we used an intensity-level scale for all exercises that consisted of: 1 = no effort at all; 5 = moderate effort; and 10 = all-out maximal effort. The total exercise time/wk for the P + RT and PRISE was identical (~4 h/wk). All participants in P + RT and PRISE were provided with training journals that included pictures and descriptions [and DVDs for stretching (S) routines] of all of the exercises to assist them in completing the routines with proper form and technique. Each participant was provided with a heart-rate monitor and instructed to wear the monitor for every exercise session to assist with the proper intensity level. The combination of the heart-rate monitor and use of the intensity-level scale (1–10) provided a consistent level of effort for each exercise session that was tailored to their level of fitness. The specific routines for each exercise training group included P + RT and PRISE.

**Protein and RT.** The P + RT sessions were designed to target larger muscle groups first, followed by smaller muscle groups, and were a similar RT program used previously in our laboratory (6). Specific details regarding the intensity, duration, and specific exercise for P + RT training are included in Table 1. Two exercises were performed for each muscle group, consisting of two sets of the first exercise, increasing the resistance (kg) and decreasing the repetitions (12–10), followed by two sets of the second exercise following the same repetition scheme (12–10) for a total of four sets/muscle group. Each P + RT session was designed to take 60 min, including warm-up, RT and sprint intervals, and a cool-down, and was monitored by a member of the research team.

**PRISE.** PRISE sessions alternated the four types of exercise on a weekly basis, such that participants performed each of the four exercises, 1 day/wk for a total of four exercise sessions/wk (RISE).

1) **RESISTANCE EXERCISE (R).** For details regarding intensity, duration, and specific exercise for R, please refer to Table 1. Each specific exercise was performed at a resistance to induce muscular fatigue in 10–15 repetitions or for a set time, 30–60 s. A 60-s recovery was allowed between exercises. Each exercise session was preceded and followed by a 5- to 7-min dynamic warm-up and a stretching cool-down, respectively. The R routine was completed within 60 min. As participants progressed, they were instructed to increase the resistance and intensity in which they performed the exercises so the level of intensity or resistance ranged from 7 to 9 on the intensity-level scale.

2) **INTERVAL SPRINT EXERCISE (I).** For details regarding intensity, duration, and specific exercises for I, please see Table 1. Each I session was completed within 45 min.

3) **STRETCHING/YOGA/PILATES EXERCISES (S).** This was based primarily on traditional yoga “asanas,” or poses, with the modern elements of Pilates training for a total body stretching, flexibility, and strengthening workout. All S routines were led by a certified yoga instructor (P. J. Arciero) and included balance, stamina, strengthening, and toning poses, including basic sun salutations, the use of 1- to 3-lb. hand-held weights, a core-strengthening portion, light stretching, and a final resting-relaxation phase. In addition to the training journal that contained pictures and descriptions, participants were provided with DVDs containing all of the poses to assist them in completing the routines with proper form and technique. As participants progressed, they were instructed to increase the amount of weights and intensity in which they performed the poses so the level of intensity ranged from 7 to 9 on the intensity scale. Please refer to Table 1 for additional details regarding intensity and duration of the S routine.

4) **ENDURANCE EXERCISE (E).** Please refer to Table 1 for specific details regarding intensity, duration, and modes of exercise. Our laboratory has previously shown that a similar E exercise routine in obese, middle-age men and women is efficacious (5).

**Laboratory Testing Procedures**

All testing procedures (see below) were administered preintervention (week 0) and postintervention (week 17) and were conducted between 0600 and 0900, following a 12-h overnight fast and a 48-h abstinence of caffeine and alcohol intake, and 48–72 h after the last exercise session to eliminate the effect of the last acute bout of exercise on laboratory measures.

**Body weight, body composition, and visceral adipose tissue mass.** Body weight was measured under identical conditions (shorts, t-shirt, same time of day) for each subject using a digital scale (Beaumont, TX). Waist circumferences were obtained using a standard tape measure placed around the waist at the level of the umbilicus and top of the iliac crest, ensuring the tape measure is parallel with the floor, as described previously (7). Total [fat mass (FM), LBM] and regional (abdominal) FM body composition was determined by dual-energy X-ray absorptiometry (iDXA; Lunar iDXA; GE Healthcare, Madison, WI; analyzed using encore software version 13.6) with subjects in the supine position as described previously (27). iDXA scans were performed at baseline (week 0) and at the end of the 16-wk intervention (week 17). Total body adiposity (FM) was expressed as %BF, and LBM was expressed as kilograms. Appendicular composition (arm and leg) was determined using the iDXA software analysis. Regional abdominal adiposity was determined by creating a region of interest (ROI) for the abdomen using the ROI option (with ruler option) within the manual analysis menu of the Lunar software. Abdominal BF (AbFat) adiposity is expressed as percent AbFat (%AbFat) and total (kg) AbFat, as described previously (27). Test-retest intraclass correlation (£) and coefficient of variation (CV) for body composition analysis using iDXA in our laboratory, with n = 12, are: LBM and FM, r = 0.99 and CV = 0.64%; r = 0.98 and %CV = 2.2%, respectively, and for regional abdominal body composition analysis: percent fat (%Fat), r = 0.99 and CV = 2.4%.

Estimation of visceral adipose tissue (VAT) mass using the iDXA has been validated previously (30). VAT is computed by subtracting subcutaneous AT (SAT) from the total AT mass in the android region. The geometric assumptions of the model are based on comparing

<table>
<thead>
<tr>
<th>Group</th>
<th>Exercise Type</th>
<th>Exercise modality</th>
<th>Work</th>
<th>RPE</th>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
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</thead>
<tbody>
<tr>
<td>P + RT</td>
<td>Resistance training (RT)</td>
<td>UB, LB</td>
<td>2 sets/exercise 10–12 reps</td>
<td>7–9 UB</td>
<td>LB</td>
<td>Rest</td>
<td>UB</td>
<td>LB</td>
<td></td>
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<tr>
<td>Sprint</td>
<td>RT</td>
<td>C</td>
<td>3 sets 30 s/4 min rest</td>
<td>10/3 X</td>
<td>X</td>
<td></td>
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<tr>
<td>PRISE</td>
<td>Resistance (R)</td>
<td>WB</td>
<td>2 sets/exercise 10–15 reps</td>
<td>7–9 WB</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Intervals (I)</td>
<td>WB</td>
<td>C</td>
<td>5–7 sets 30 s/4 min rest</td>
<td>10/3 WB</td>
<td>X</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Stretching (S)</td>
<td>UB</td>
<td>S</td>
<td>≤ 60 min</td>
<td>7–9</td>
<td></td>
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<tr>
<td>Endurance (E)</td>
<td>UB</td>
<td>C</td>
<td>≥ 60 min</td>
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RPE, rating of perceived effort; P + RT, whey protein and RT; UB, upper-body resistance exercise; LB, lower-body resistance exercise; Sprint, sprint interval training; C, choice of exercise modality; X, exercise day; PRISE, protein and resistance exercise, intervals, stretching/yoga/Pilates, endurance exercise; WB, whole-body exercise; S, stretching exercise. P + RT exercises incorporated the following upper-body muscle groups: chest, shoulders, biceps, triceps, and back. P + RT lower-body muscle groups included: quadriceps, hamstrings, calves, and abdomen. PRISE (R) exercises used medicine balls, physioballs, rubber tubes, and bands, which were incorporated into a dynamic warm-up, footwork and agility drills, resistance and power movements, and core and body weight exercises (e.g., lunges, squats, and jumping rope). Exercise modalities available for C include: walking, jogging, running, cycling, swimming, elliptical, rowing, rollerblading, cross-country skiing, etc.
iDXA and computed tomography image volumes from a training set of images obtained from two independent clinical trials (30). Cardiometabolic and plasma biomarkers. A 12-h fasted venous blood sample (~20 ml) was obtained at baseline (week 0) and postintervention (week 17). Blood was collected into EDTA-coated Vacutainer tubes and centrifuged (Rotina 46R5; Hettich, Beverly, MA) for 15 min at 2,500 rpm at 4°C. Plasma was then separated and subsequently stored at ~70°C in small aliquots until analyzed. Leptin (LEP), adiponectin (ADI), and insulin were determined using commercially available ELISA kits (Millipore, Billerica, MA, and Diagnostic Systems Laboratories, Webster, TX). Total cholesterol (TC), HDL cholesterol (HDL-C), triglycerides (TRG), and glucose (GLU) concentrations (mg/dl) were assessed using the Cholestech LDX blood analysis system (Cholestech, Hayward, CA). Test-retest r and CV in our laboratory with n = 15 are: TC, GLU, and HDL-C (mg/dl), r = 0.95 and CV = 3.2%; r = 0.94 and CV = 2.5%; and r = 0.97 and CV = 5.3%, respectively.

Insulin sensitivity was estimated with the homeostasis model assessment-estimated (HOMA)-IR (52), which has a strong correlation (r = 0.69; P < 0.05) with the hyperinsuliminc-euglycemic clamp technique. HOMA-IR values were calculated from fasting GLU and insulin levels using a computer program available online at https://www.dtu.ox.ac.uk/homacalculator/. This method of calculating HOMA-IR is more accurate than other formulas, as it takes into account variations in hepatic and peripheral GLU resistance, increases in the insulin-secretion curve for plasma GLU concentrations >10 mmol/l (180 mg/dl), and the contribution of circulating proinsulin (52).

Heart rate and blood pressure. Resting heart rate and blood pressure were obtained in the supine position, as described previously (4, 6, 7). Heart rate and blood pressure were obtained by the same investigator (P. J. Arciero) following 10 min of quiet resting.

Feelings of hunger, satiation, and desire to eat. Visual analog scales (VAS) were used to evaluate hunger, satiation, and desire to-eat scores. Each VAS was 100 mm in length and anchored at each end. Participants were instructed to place a mark on the 100-mm line to indicate their levels of hunger, satiety, and desire to eat. A mark at 0 indicated a complete lack of hunger, satiety, or desire to eat, and a mark at 100 indicated extreme hunger, satiety, or desire to eat for each VAS, respectively. For each of the three measures (hunger, satiety, and desire to eat), the degree to which each sensation was felt was quantified by measuring how far the mark was from the 0-mm mark. For this measurement, a standard millimeter ruler was used, and all scores were computed by the same investigator. VAS were completed at baseline (week 0) and postintervention (week 17) in a fasted state.

Statistical analysis. Statistical analyses were performed using SPSS software (Version 21; IBM-SPSS, Armonk, NY). Significance was set at P < 0.05. All values are reported as means ± SE unless noted otherwise. Before the start of the study, subject number was determined from a power analysis based on the major outcome variables (total and regional body composition, visceral FM, adipokines), as reported by our previous study (4), with an alpha level set to 0.05 and a power of 0.8. This analysis determined that we would require n = 45 participants (15/treatment group) to detect significant differences. Absolute changes in total and regional body mass and composition were calculated as the baseline value (week 0) subtracted from the postintervention (week 17) values (see Figs. 1 and 2, respectively). A 3 × 2 factor repeated-measures ANOVA (diet: P, P + RT, PRISE; time: week 0, week 17) was run to determine differences among groups and time points (time and group × time interactions). Where significant main effects were identified, post hoc comparisons [paired sample t-test (time effects) and Tukey’s test (group differences)] were performed to locate differences. Based on our hypothesis of a reduction in all physiological variables (with the exception of LBM), we used one-tailed t-tests for investigation of time effects for each group. Given the differences between the number of men and women within each group, we performed an analysis of covariance (ANCOVA) using gender as the covariate to test for differences among the groups for the major outcome variables.

RESULTS

Participants and Compliance

Of the 79 subjects who started the study, 22 were not included in the data analysis, due to noncompliance with the whey protein supplementation and/or the exercise training sessions for more than 1 consecutive wk or drop-out, resulting in 75% adherence (Fig. 1). There were no differences among the groups in the rate of noncompliance or drop-outs (P, n = 6; P + RT, n = 5; PRISE, n = 11). Thus baseline physical characteristics of the n = 57 subjects who completed testing (n = 36 women, n = 21 men) are presented in Table 2. As noted, no differences existed among the three groups for any variable.

Self-Reported Energy Intake, 3-Day Food Diaries, and Physical Activity

Dietary intakes and physical activity levels are reported in Table 3. At baseline, PRISE %fat intake was higher vs. P + RT (P = 0.032). We observed a significant main effect of time for protein intake (P = 0.000). Moreover, post hoc testing revealed significant increases in protein intake for all three groups. No group × time interactions were present among any

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Fig. 1. Participant flow chart. PRISE, protein and resistance exercise, intervals, stretching/yoga/Pilates, endurance exercise.
dietary variables. Self-reported physical activity increased significantly ($P < 0.05$) in each group by $65.5 \pm 26.8\%$, $137.6 \pm 62.6\%$, and $114.9 \pm 27.2\%$ for P, P + RT, and PRISE, respectively. However, we observed no group × time interactions. This is obviously an underestimation for the exercise groups, as we have shown previously that physical activity energy expenditure increases substantially more than this for similar exercise training protocols (4, 6).

**Body Weight, Body Composition, and VAT Mass**

Mean changes in body weight, FM, AbFat, VAT, SAT, LBM, percent LBM (%LBM), arm/leg LBM, and waist circumference are shown in Table 4. We observed that main time and group × time effects for weight loss (Fig. 2) with post hoc analysis demonstrating PRISE resulted in greater weight loss ($2.6 \pm 0.9\%$) relative to P + RT ($P = 0.009$), whereas P was not different from P + RT or PRISE. Main time and group × time effects were also present for FM (see Table 4 and Fig. 3). Specifically, PRISE resulted in a greater loss of FM vs. P + RT (6.6%; $P = 0.009$) and P (5.2%; $P = 0.059$).

There was no main time or group × time effect for LBM, although post hoc analysis of LBM time effects revealed a small (0.5 kg) loss of LBM in P but no change with P + RT or PRISE. In an attempt to elucidate further the changes in LBM, we examined arm and leg LBM and showed a significant main time effect for both arm and leg LBM. Specifically, arm LBM increased with P + RT and PRISE and remained unchanged in P, whereas leg LBM decreased with P but did not change in P + RT and PRISE. There were no group × time effects for either arm or leg LBM. We observed main time and group × time effects for %LBM. Post hoc analysis revealed significant increases in %LBM in P + RT and PRISE. Moreover, the increase in %LBM with PRISE was significantly greater than both P ($P = 0.027$) and P + RT ($P = 0.035$).

We also observed a main time effect for AbFat without any differences among groups over time (Fig. 3 and Table 4). Interestingly, we observed main time effects for SAT and VAT mass. Specifically, P + RT and PRISE resulted in significant reductions in VAT, whereas SAT decreased only with P and PRISE. There were no group × time interactions for VAT or SAT. However, when the data were analyzed using gender as the covariate, there was a significant group × time interaction, whereby PRISE resulted in a greater loss of VAT compared with P and P + RT ($P = 0.01$), and P + RT reduced VAT to a greater extent than P ($P = 0.01$). There were no differences among the groups for any other variable using the ANCOVA analysis. Similarly, there was a main time effect for waist circumference without any group × time interactions. Specifically, waist circumference dropped by 8.7 ± 1.0 cm, 7.2 ± 0.8 cm, and 10.3 ± 1.1 cm for P, P + RT, and PRISE, respectively (Table 4).

**Cardiometabolic and Plasma Biomarkers**

Results of plasma biomarkers are presented in Table 5. A significant main time and group × time effect was observed for fasting GLU. Specifically, fasting GLU was reduced in P + RT and PRISE with the greatest reduction in fasting GLU levels with PRISE vs. P ($P = 0.0001$) and P + RT ($P = 0.002$). Although baseline insulin was slightly higher in P + RT, insulin decreased significantly over time with P + RT and PRISE, without any group × time interactions. Main time effects were also present for HOMA-IR, a surrogate measure of insulin sensitivity. Specifically, HOMA-IR improved dra-
interactions. However, because we found group reported for heart rate or blood pressure. Additionally, there were no time effects or group increased in P, *effect for plasma TRG, with significant reductions in P and group time interactions between PRISE and P did not change. Additionally, we observed a main group strong trends toward group P did not change over time in P (Table 5). Additionally, there were no group × time interactions for HOMA-IR.

We observed a main time effect for LEP. Similar to insulin, LEP was slightly elevated at baseline in P + RT and decreased significantly over time with P + RT and PRISE, with no change in P. There were no group × time interactions present for LEP. There was also a main time effect for ADI, with significant increases in ADI with P + RT and PRISE, whereas P did not change. Additionally, we observed a main group × time interaction for ADI, with post hoc analysis revealing strong trends toward group × time interactions between P + RT (P = 0.072) and PRISE (P = 0.073) vs. P. We observed a significant time effect in the LEP:ADI ratio (see Table 5), although there were no group × time interactions. Additionally, there were no time effects or group × time interactions reported for heart rate or blood pressure.

We observed no main time effects for plasma total and LDL-C or HDL-C levels. However, because we found group time interactions, we performed post hoc testing for both time and group × time to understand these effects. We found significant reductions in total and LDL-C levels with PRISE and an increase with P + RT, resulting in group × time interactions between PRISE and P + RT (P = 0.005) for both variables. HDL-C remained unchanged in PRISE but decreased in P + RT and P. We observed a significant main time effect for plasma TRG, with significant reductions in P and PRISE, but not with P + RT, and there were no group × time interactions.

**DISCUSSION**

This study examined the effects of whey protein consumption added to the normal diet of free-living overweight and obese adults, with or without an exercise intervention. Novel findings of this study were that: 1) whey protein consumption, alone or combined with exercise training, was associated with a reduced body mass, FM, %BF, AbFat, and waist circumference; 2) why protein combined with an exercise intervention (P + RT and PRISE) resulted in significant reductions of visceral FM; 3) PRISE resulted in the greatest reductions of body mass, %BF, and FM; 4) why protein combined with exercise training (P + RT and PRISE) improved HOMA-IR and reduced the LEP:ADI ratio; and 5) P and PRISE significantly reduced plasma TRG. P + RT and PRISE participants followed a program of progressive exercise training, whereas P participants were required to maintain their habitual level of physical activity (sedentary) throughout the 16-wk study.

Interestingly, P alone was associated with reduced total and regional body FM and waist circumference. These findings are consistent with recent findings from our own laboratory (7) as well as those from others (9). Baer and colleagues (9) reported reduced body mass, FM, and waist circumference, with twice-daily whey protein (P; ~56 g total/day) added to the normal diet of free-living adults over a 23-wk intervention period. Additionally, we recently reported that consuming increased

![Fig. 2. Body mass and lean body mass (LBM) changes. A: percent change (%Change) in body mass. B: %Change in percent LBM (%LBM). Data are presented as means ± SE. P (20 g/day × 16 wk) added to normal diet in free-living adults; P + RT, with sprint interval training; PRISE, P combined with an exercise and healthy eating lifestyle intervention. *Significantly reduced from baseline (P < 0.05); †significantly different from P + RT (P < 0.01); ‡significantly different from P and P + RT (P < 0.05).](https://doi.org/10.1152/japplphysiol.00152.2014)
protein (up to 35% of total energy intake) evenly spaced throughout the day (~20-g servings up to six meals/day) decreased total and abdominal fat (7). A novel finding of this study is that P was associated with reduced SAT but only decreased VAT when combined with a comprehensive exercise program (PRISE). Although reductions in SAT are associated with many health benefits, VAT losses may be of greater importance for disease prevention (39) (discussed below). Regardless, the observed benefits of P on body mass, FM, %BF, AbFat, and waist circumference could be partially attributed to an increase in physical activity. However, the physical activity data should be interpreted with caution, as we did not measure it directly but rather, estimated it based on questionnaires only.

Of particular interest in the current study was the finding of reduced VAT with P + RT and PRISE. VAT, or visceral adipose tissue (VAT), is highly associated with metabolic syndrome and IR (11). Reductions in VAT seem to be more important than reductions in other adipose species (e.g., SAT) for attenuating risk factors associated with metabolic syndrome (39). The current finding of reduced VAT corroborates others (28, 54), suggesting an essential role for exercise in preventing the onset of metabolic syndrome. To address whether gender differences were responsible for VAT reductions, the data were analyzed, controlling for differences in gender among the groups (using gender as a covariate). The analysis revealed that PRISE resulted in substantial reductions in VAT compared with P and P + RT, and P + RT reduced VAT to a greater extent than P. These findings lend further support to a multidimensional exercise training program combined with protein supplementation to enhance BF distribution compared with traditional exercise training programs. We observed changes in LBM that also appear to be influenced by the inclusion of exercise training. Specifically, P + RT and PRISE maintained total LBM, with an increase in arm LBM, whereas P resulted in a very modest (~0.5 kg) loss in total LBM, likely due to a reduction in leg LBM. These body composition changes are reflected further in %LBM increases in both exercise groups. Indeed, changes in %LBM were greatest in PRISE compared with P and P + RT. The LBM findings were expected, as the effects of RT on LBM are well documented (17, 23, 26, 46). The mechanism for these effects on LBM with RT and PRISE is likely enhanced protein synthesis (10, 40), which may be increased further with consumption of P in close proximity to RT and RISE, as was done in the current study (8, 34, 35). We observed differential responses of arm and leg LBM to both exercise protocols, whereby arm increased, and leg LBM (kg) remained unchanged. It is convenient to postulate that these differences may be due to more conditioned leg muscles of our participants, due to increased muscle activation to support excess body weight for ambulation, and more conditioned muscles

![Figure 3](image_url)

**Fig. 3.** Changes in total body fat (BF), abdominal fat mass (AbFat), and visceral adipose tissue (VAT). **A:** %Change in total BF. **B:** %Change in AbFat. **C:** %Change in VAT. Data are presented as means ± SE. *Significantly different from baseline (P < 0.05); †significantly different from P + RT (P < 0.01); ‡trending different from P (P < 0.06).
study was the beneficial effects of \( P + RT \) and PRISE on insulin sensitivity. The insulin-sensitizing effects of exercise are well known (21), and \( P + RT \) and PRISE improved insulin sensitivity (via reductions in fasting GLU and insulin), whereas \( P \) showed no effect. Interestingly, fasting GLU concentrations were reduced to a greater degree with PRISE vs. \( P + RT \). Previous work from our laboratory has shown that endurance exercise, similar to that used in the current PRISE program, induces significant improvement in insulin sensitivity using an oral GLU tolerance test (15) and lends further support to the potential health benefits of this lifestyle intervention.

Improvements in insulin sensitivity and body composition may also be related to changes observed in LEP and ADI concentrations. LEP is typically positively correlated with FM and is thought to regulate hunger negatively. ADI may be an important regulator of fatty acid metabolism (56), as well as IR and inflammation (49). Changes in these variables, like VAT and HOME-IR, seem to be exercise dependent, as LEP and ADI did not change with \( P \). Indeed, a recent study indicates that exercise may affect LEP and ADI to a greater extent than dietary changes alone (1). As such, in the current study, we observed decreases and increases with \( P + RT \) and PRISE for LEP and ADI, respectively. As a consequence, both exercise conditions reduced the LEP/ADI ratio, which has a high correlation with type 2 diabetes risk (48). Thus these findings confirm the importance of exercise for type 2 diabetes prevention or treatment.

The majority of cardiometabolic biomarkers (i.e., TC, HDL, LDL, or blood pressure) did not change over the course of the intervention. It should be noted that exercise training effects on blood lipids are equivocal with some studies showing improvement (22, 42, 55) and others not (43, 45). Furthermore, in the current population of overweight/obese adult men and women, means for all groups at baseline were within healthy physiological ranges and likely explain the lack of change in most of these variables. Moreover, HDL-C and LDL-C seem resistant to change in response to exercise (18). We did observe significant reductions in plasma TRG following \( P \) and PRISE but not \( P + RT \). Although some studies have reported no changes in blood lipid profiles of healthy subjects following RT (50), our finding of no change with \( P + RT \) was unexpected, because the

exhibit attenuated protein synthesis in response to exercise (31). Perhaps a greater stimulus was required to induce lean mass increases in the legs. Whereas this is a logical explanation, it is purely speculative. Nevertheless, our data indicate that PRISE is the most effective way to enhance LBMI compared with the other two groups and suggest that future exercise training protocols should consider a combination of fitness components.

Total FM was reduced across all three groups, with a tendency for a greater reduction in PRISE relative to \( P + RT \) and \( P = 0.059 \). Thus significant reductions in body mass appear to be the result of combined losses in total FM and LBMI following \( P \) vs. larger reductions in total FM coupled with the maintenance of LBMI with PRISE. Alternatively, \( P + RT \) resulted in LBMI maintenance with relatively less total FM and total body mass loss (2.6 ± 0.9% less) vs. PRISE. These changes were also reflected in %BF loss, which was greater in PRISE compared with \( P + RT (P = 0.04) \) and \( P = 0.06 \). Losses in total and regional BF with \( P + RT \) and PRISE are in agreement with others reporting reductions in FM and %BF with various forms of exercise. Specifically, RT has been shown to increase fat oxidation in overweight and obese individuals (37), possibly as a result of increased subcutaneous and whole-body lipolysis (38) and/or elevations in resting metabolic rate (33). However, not all studies support the ability of \( RT \) to induce fat loss (24, 36) and may partly explain why reductions in total FM with \( P + RT \) were smaller vs. PRISE. In support of this, aerobic training has consistently been reported to reduce total FM (44, 54). Moreover, it may reduce total FM to a greater degree than RT (54). Willis and colleagues (54) recently reported that 10 wk aerobic training (calorically equivalent to ~12 miles/wk running at 65–80% peak maximal oxygen consumption) resulted in significantly more fat loss (4.7% vs. 0.8%) than the same duration of RT alone (3 days/wk, three sets/day, eight to 12 repetitions/set). Furthermore, the potential additive effects of high-intensity interval training, which has well-documented, beneficial effects on FM (14) combined with the possible benefits of weekly yoga (47) and Pilates (2), are likely explanations for the greater total fat loss with PRISE vs. \( P + RT \). Collectively, the findings of greater reductions in total body mass and enhanced body composition with PRISE lend further support to the notion that RT alone may be less effective than exercise interventions. Including multiple fitness components (54). As

**Table 5. Cardiometabolic biomarkers pre- and postintervention**

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>Δ</th>
<th>Group × Time, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGL, mg/dl</td>
<td>109.1 ± 18.5</td>
<td>95 ± 15.3a</td>
<td>−14.2 ± 9.0</td>
<td>0.059</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>173.5 ± 8.0</td>
<td>172.7 ± 7.6</td>
<td>−2.4 ± 5.1</td>
<td>0.011</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>49.4 ± 2.9</td>
<td>46.6 ± 3.2a</td>
<td>−3.6 ± 1.4</td>
<td>0.011</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>105.9 ± 7.9</td>
<td>111.1 ± 6.7</td>
<td>3.9 ± 4.4</td>
<td>0.018</td>
</tr>
<tr>
<td>GLU, mg/dl</td>
<td>92.2 ± 2.5</td>
<td>92.5 ± 2.6</td>
<td>0.6 ± 2.1</td>
<td>0.011</td>
</tr>
<tr>
<td>Insulin, μg/dl</td>
<td>19.2 ± 4.7</td>
<td>116.3 ± 3.4</td>
<td>−7.8 ± 6.5</td>
<td>0.011</td>
</tr>
<tr>
<td>HOMA-IR, units</td>
<td>2.3 ± 0.5</td>
<td>1.4 ± 0.4</td>
<td>−0.9 ± 0.8</td>
<td>0.011</td>
</tr>
<tr>
<td>LEP, ng/dl</td>
<td>21.4 ± 4.9</td>
<td>19.2 ± 5.8</td>
<td>−2.4 ± 4.0</td>
<td>0.011</td>
</tr>
<tr>
<td>ADI, mg/dl</td>
<td>22.7 ± 2.5</td>
<td>21.3 ± 2.0</td>
<td>−2.4 ± 1.5</td>
<td>0.011</td>
</tr>
<tr>
<td>LEP/ADI</td>
<td>1.19 ± 0.3</td>
<td>0.97 ± 0.2</td>
<td>−0.1 ± 0.1</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. TGL, plasma triglycerides; GLU, fasting plasma glucose; Insulin, fasting plasma insulin; HOME-IR, homeostasis model assessment-estimated insulin resistance; LEP, leptin; ADI, adiponectin; LEP/ADI, leptin-to-adiponectin ratio. Significantly different from baseline preintervention measurements (\( P < 0.05 \)); significantly different from \( P + RT (P < 0.05) \); significantly different from \( P (P < 0.05) \); significantly different from PRISE at baseline (\( P < 0.05 \)); significantly different from PRISE at baseline (\( P = 0.018 \)).
current subjects were untrained and participating in high-intensity training. Moreover, consuming a high-protein diet with or without exercise has been reported to have beneficial effects on the blood lipid profiles of rats (3). Thus collectively, the blood lipid results for P + RT are unusual and require further study.

One potential limitation to the current study was lack of a CHO-only control group. Recently, it was reported that 6 mo of whey protein supplementation (similar amount to the current study, 60 g/day total) resulted in significant reductions in body weight and FM in overweight and obese individuals compared with an isoenergetic CHO supplement (9). Therefore, we felt that inclusion of a CHO control group for this study would not be appropriate or ethical, given the lack of improvement in many of the same variables that we measured in the current study (body mass, composition, and hunger). Instead, the primary aims were to compare the independent effects of timed ingestion of 20-g servings of whey protein throughout the day (60 g total) and whether the addition of resistance exercise or a combination of exercise training modalities provides additional benefit.

In conclusion, P, with and without exercise, is associated with an enhanced total and regional body composition and cardiometabolic health. Specifically, P alone was associated with reduced total body mass, waist circumference, AbFat, SAT, and total FM. However, the combined effects of whey protein and 4 days/wk of exercise training (P + RT and PRISE) result in additional improvements in VAT mass, adipokines, and insulin sensitivity. We found that whey protein combined with a comprehensive exercise training program (PRISE) is associated with the greatest improvements in total and regional body composition, as well as cardiometabolic disease risk variables, and should be considered as an effective lifestyle intervention for overweight and obese adults.

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DISCLOSURES

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS


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