Timing Protein Intake Increases Energy Expenditure 24 h after Resistance Training

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ABSTRACT

HACKNEY, K. J., A. J. BRUENGER, and J. T. LEMMER. Timing Protein Intake Increases Energy Expenditure 24 h after Resistance Training. Med. Sci. Sports Exerc., Vol. 42, No. 5, pp. 998-1003, 2010. Purpose: To determine whether protein supplementation (PRO) before an acute bout of heavy resistance training (HRT) would influence postexercise resting energy expenditure (REE) and the nonprotein respiratory exchange ratio (RER). Hypothesis: REE would be increased and RER would be decreased up to 48 h after timed PRO and HRT compared with CHO supplementation and HRT. Methods: Eight resistance-trained subjects (five men and three women) participated in a double-blind two-trial crossover design, where REE and RER were measured (7:00 a.m.) on four consecutive days. On the second day of trial 1, subjects consumed 376 kJ of either PRO (18 g of whey protein, 2 g of carbohydrate, 1.5 g of fat) or CHO (1 g of whey protein, 19 g of carbohydrate, 1 g of fat) 20 min before a single bout of HRT (nine exercises, 4 sets, 70%-75% 1-repetition maximum). REE and RER were measured 24 and 48 h after HRT. During trial 2, the same protocol was followed except subjects consumed the second supplement before HRT. Results: Compared with baseline, REE was elevated significantly in both CHO and PRO at 24 and 48 h after HRT (P < 0.05). At 24 h after HRT, REE in response to PRO was significantly greater compared with CHO (P < 0.05). RER decreased significantly in both CHO and PRO at 24 h after HRT compared with baseline (P < 0.05). No differences were observed in total energy intake, macronutrient intake, or HRT volume (P > 0.05). Conclusions: Timing PRO before HRT may be a simple and effective strategy to increase energy expenditure by elevating REE the day after HRT. Increasing REE could facilitate reductions in body fat mass and improve body composition if nutritional intake is stable. Key Words: RESTING METABOLIC RATE, METABOLISM, EPOC, SUBSTRATE UTILIZATION, BODY FAT COMPOSITION

t is well established that heavy resistance training (HRT) is an effective mode of exercise to increase L muscle mass and strength (12). However, the influence of HRT on energy expenditure and substrate utilization has been less characterized. Several investigations have shown that a single bout of HRT can significantly elevate resting energy expenditure (REE) anywhere from 14.5 to 72 h after HRT (11,14,16,21,26,30). In addition, studies report that the nonprotein repiratory exchange ratio (RER), an indirect assessment of substrate utilization, is decreased 24 h after an acute bout of HRT (21,30). Taken together, these acute alterations could be critical for managing body composition during a longitudinal period (30). For instance, REE has been estimated to account for 60%-75% of total daily energy expenditure (26); therefore, elevating the amount of energy needed to maintain homeostasis at rest could influ-

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ence whether there is a net positive or negative energy balance. Decreasing the RER has also been interpreted as an increase in fat oxidation (21,30). Thus, it seems that HRT has an influence on energy and substrate utilization for an extended period after the exercise session has been completed.

During the first 24 h after an acute bout of HRT, the elevation in REE is theorized to be associated with components of excess postexercise oxygen consumption (EPOC) (5). It is suggested that EPOC results from factors such as elevated body temperature, resynthesis of glycogen from lactate, ion redistribution, replenishment of oxygen stores in blood and muscle, resynthesis of adenosine triphosphate and creatine phosphate, increased circulation and ventilation, and residual hormone effects (1,5,11,27). However, it seems that the majority of these factors have acute affects and do not explain the more prolonged elevations in REE that have been reported 24 h after HRT (11,16,21,30).

The primary mechanism hypothesized to account for the delayed (i.e., \geq 24 h) elevations in REE is increased muscle protein synthesis (11,20). Muscle protein synthesis is an energy-consuming process that requires four adenosine triphosphate (ATP) equivalent molecules for every amino acid that is incorporated into the peptide chain during translation (6,9). Thus, it is estimated that, in a well-trained male, the amount of energy expended from this process can account for up 20% of REE (38) and be as high as 2030 kJ·d⁻¹ (40).

Because increases in muscle protein synthesis have been implicated as one of the primary mechanisms associated with elevating REE after HRT (11,20), interventions that stimulate muscle protein synthesis may influence energy expenditure. Furthermore, because the preferred fuel to provide ATP in skeletal muscle at rest is derived through the oxidation of fat (21,22,30,40), substrate utilization may be altered if muscle protein synthesis is enhanced. In this regard, recent publications have reported that timing the intake of protein (i.e., amino acids) before or immediately after HRT can be an effective strategy to increase muscle protein synthesis (3,18,19,29,36). Specifically, Tipton et al. (36) demonstrated that the fractional rate of muscle protein synthesis was enhanced when an amino acid-carbohydrate supplement was ingested before HRT compared with when the supplement was ingested immediately after HRT. Although energy expenditure was not directly measured during this study, Giordano and Castellino (15) have shown that the rate of amino acid infusion is significantly correlated with energy expenditure (r = 0.79). Likewise, Hulmi et al. (20) demonstrated that energy expenditure, measured by EPOC, was significantly elevated (~23%) 1.5-2.0 h after exercise in a group who ingested a protein supplement before HRT compared with placebo controls. Therefore, timing the intake of protein before HRT could increase the rate of muscle protein synthesis and correspond to the changes in energy expenditure in the following HRT period (20).

The aforementioned investigation exploring the relationship between muscle protein synthesis and energy expenditure focused primarily on changes that occur immediately (i.e., <2 h) after HRT (20). However, MacDougall et al. (25) demonstrated that muscle protein synthesis was increased by 50% at 4 h and 109% at 24 h after HRT. Additional studies also support a delayed elevation in muscle protein synthesis, due to posttranscriptional events, that can persist up to 48 h after HRT (7,28). Thus, it has been suggested that when protein is consumed before HRT, energy expenditure and substrate utilization may be altered beyond the initial period explored. Therefore, the purpose of this investigation was to determine the effect of protein (PRO) supplementation before an acute bout of HRT on postexercise REE and RER. It was hypothesized that REE would be increased and RER would be decreased up to 48 h after HRT in those receiving timed PRO compared with isoenergetic CHO supplementation (CHO).

METHODS

Research design. A double-blind two-trial crossover design was implemented in this investigation. All participants completed both the PRO and CHO trials, which were separated by at least 30 d (mean \pm SD; men = 39 \pm 18 d, women = 43 \pm 23 d). When participants were not involved in a trial, they continued their normal exercise routine. Preliminary testing, which included body composition measurement, one-repetition maximum (1RM)

assessment, nutritional log training, and familiarization with metabolic testing was performed before trial 1. During the first trial, REE and RER were measured in the morning (\sim 7:00 a.m.) on four consecutive days. After measurements on the second day, either PRO or CHO was provided 20 min before a full-body HRT protocol. REE and RER were then measured at 24 and 48 h after HRT. In addition, total energy intake was recorded during the first 4-d trial, and participants were instructed to replicate their diet during the second 4-d trial. If it was not possible to replicate the diet exactly, participants recorded their actual dietary intake in the dietary journal. During trial 2, participants performed the same 4 d of REE and RER testing; however, the second supplement was consumed before HRT (Fig. 1).

Participants. Nine resistance-trained men (n = 6) and women (n = 3) were recruited for the study. Resistancetrained was defined as having participated in strength training or weight lifting for a minimum of 3 d wk⁻¹ for at least 6 months before testing and was self-reported by the subjects. One male subject was removed from data analysis because of an inability to adhere to the physical activity guidelines required during the REE testing period. The remaining five men (age = 23.0 ± 3.8 yr, height = 178 ± 6.4 cm, mass = 85.6 ± 11.4 kg, body fat = $12.6\% \pm 7.5\%$) and three women (age = 24.0 ± 1.5 yr, height = 162 ± 6.4 cm, mass = 65.1 ± 7.3 kg, body fat = $26.5\% \pm 6.7\%$) reported 100% compliance with REE testing guidelines (described in detail below). Before participating in this investigation, subjects received information on all risks and signed a written informed consent form approved by the Biomedical Institutional Review Board at Michigan State University.

Body composition. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body mass was assessed to the nearest 0.01 kg using an electric scale that was included in the BOD POD system (Version 1.69 Life Measurement, Inc., Concord, CA). Body density was estimated from the measurement of body volume using air displacement plethysmography via the BOD POD (10). Body fat percentage was calculated accordingly using the equation of Siri (31).

1RM strength testing. 1RM strength was measured on six free-weight exercises (squat, bench press, bent-over





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row, biceps curl, lateral raise, and shoulder press) and three machine exercises (leg extension, leg curl, and triceps extension). Before testing, participants warmed up for 5 min on a stationary cycle ergometer. For each HRT exercise, the participants selected a mass they felt they could easily lift for 10 repetitions (reps). A second set was then completed using a heavier mass for five reps. After these sets, mass was added, and the participants attempted to complete one rep using a full range of motion. If the participants were successful, additional mass was added and another attempt was performed after approximately 1 min of rest. This routine was repeated until the participants were unable to successfully complete the movement using a full range of motion. A research assistant determined the success or failure of each attempt and recorded the final mass that was successfully lifted as the 1RM. To limit the effect of fatigue, only five attempts were allowed to determine 1RM after the initial warm-up sets. In addition, the maximum number of abdominal sit-ups that could be performed in one set was determined to assess abdominal muscular endurance.

Dietary journal. Participants were educated on serving sizes, nutritional label reading, and food recording before the study by a member of the research team. Participants then kept a 4-d dietary journal during each trial in which they recorded meal time, food description, and amount of each food consumed. They were also encouraged to cut out food labels and turn them in with their dietary journals at the end of each trial. Dietary journals were analyzed for total energy intake and macronutrient intake using NutritionCalc Plus software (Version 2.0 ESHA Research, Salem, OR).

Nutritional supplementation. On day 2 of both trials, subjects consumed 376 kJ (either PRO or CHO) 20 min before HRT. Each supplement was dissolved in 300 mL of water. The PRO supplement consisted of 18 g of whey protein, 2 g of carbohydrate, and 1.5 g of fat (Designer Whey; NEXT Proteins, Inc., Manhattan Beach, CA). The PRO trial was matched with a common nutritional product that contained opposite protein and carbohydrate composition to increase ecological and external validity. Thus, the CHO trial consisted of 1 g of protein, 19 g of carbohydrate, and 1 g of fat (Nesquik; Nestlé, Glendale, CA).

Resistance training bout. The HRT protocol was adopted from Jamurtas et al. (21) because their protocol has been previously shown to elicit increases in REE at 24 h after HRT (21). Twenty minutes after ingesting the PRO or CHO supplement, participants warmed up by pedaling for 5 min on a stationary cycle ergometer. They also completed one set of 10 repetitions on each HRT exercise using 30% of their predetermined 1RM. After these activities, participants performed four sets on nine HRT exercises using 70%–75% of their predetermined 1RM. One hundred twenty seconds of rest was allowed between sets of bench press and squat, whereas all other exercises had 105 s of rest between sets. In addition, four sets of abdominal sit-ups were performed where participants attempted to reach a target goal of 70% of their predetermined abdominal endurance max-

imum. The same two members of the research team trained all subjects, and water was provided throughout the workout *ad libitum*.

REE and RER. REE was measured on four consecutive mornings (\sim 7:00 a.m.) using recommendations described previously (8). Briefly, CO₂ and O₂ were measured using a mask technique connected to a SensorMedics (Vmax Series 2900, Homestead, FL) metabolic system. Before beginning each trial, participants refrained from aerobic or HRT exercise for 72 h. In addition, before each REE, participants 1) refrained from alcohol and caffeine use for 24 and 12 h, respectfully, and 2) avoided eating or drinking anything but water for 10 h. In addition, female participants scheduled both trials during days 5–10 of the follicular phase of their menstrual cycle (35). Compliance was verified by a signed questionnaire each morning and examination of dietary logs.

On the morning of each test, the metabolic system was calibrated using gases of known concentration and a 3-L syringe. Each participant rested in the supine position for at least 10 min in a quiet, dark, thermoneutral environment (20°–25°C) on entering the laboratory (8). During this time, participants breathed normally, minimized movement, and remained as quiet as possible. After the rest period, the mask was placed on the subject, and the system measured REE for ~ 30 min of the testing period. The first 5 min of data collection was discarded because participants had to shift their position when putting on the mask, which could have elevated their energy expenditure for a brief amount of time (8). REE was then determined by taking the mean of 10 min during which the subject was in steady state (defined as a coefficient of variation (CV) in VO₂ and $\dot{V}CO_2 \le 10\%$ (8). The within-subject (day 1 vs day 2) and between-subject (PRO day 1 vs CHO day 1) CV for REE $(kJ \cdot d^{-1})$ were 1.5% and 3.9%, respectively. Fat and carbohydrate oxidation were determined indirectly by monitoring the RER ($\dot{V}CO_2 \cdot \dot{V}O_2^{-1}$). RER was determined from the same 10-min period as REE, and it was assumed that protein oxidation was similar between trials because fat is the primary fuel during rest (22). The within-subject (day 1 vs day 2) and between-subject (PRO day 1 vs CHO day 1) CV for RER were 1.5% and 3.9%, respectively.

Statistical analysis. During each trial, the mass (kg) lifted and the number of sets completed during all HRT exercises were held constant. Subsequently, only the number of reps performed was allowed to vary to calculate total HRT volume (sets reps kilogram lifted). Total HRT volume (an indicator of performance) was compared between CHO and PRO trials using paired Student's *t*-tests. Paired *t*-tests were also used to compare day 1 and day 2 values of REE (kJ·kg^{-1·d⁻¹}) and RER ($\dot{V}CO_2 \cdot \dot{V}O_2^{-1}$), respectively. There were no significant differences for these measures (mean ± SEM: REE, CHO day 1 = 90.7 ± 2.4 vs day 2 = 91.0 ± 2.4; PRO day 1 = 92.6 ± 2.6 vs day 2 = 93.0 ± 2.7; RER, CHO day 1 = 0.78 ± 0.03 vs day 2 = 0.77 ± 0.01, PRO day 1 = 0.79 ± 0.02 vs day 2 = 0.79 ± 0.01, P > 0.05 for all comparisons). There was a nonsignificant trend for



FIGURE 2—HRT volume during PRO and CHO trials. HRT volume = sets reps kilogram lifted; mean \pm SEM, P > 0.05.

day 2 REE values to be higher than day 1, which may have been due to the anticipation of performing the HRT session because it is known to elevate HR (32). Therefore, day 1 values were used to represent baseline for REE and RER analyses. Separate two (trial: CHO and PRO) \times three (time: days 1, 3, and 4) ANOVA with repeated measures were performed for REE and RER. Because the investigation was not counterbalanced, we also investigated the effect of trial order to ensure that any potential significance was not explained by trial order (trial 1 vs trial 2). Total energy intake $(kJ\cdot kg^{-1}\cdot d^{-1})$ and macronutrient intake $(g\cdot kg^{-1}\cdot d^{-1})$ were also analyzed using separate two (trial: CHO and PRO) \times four (time: days 1, 2, 3, and 4) ANOVA with repeated measures. All statistical tests were performed using SPSS (version 16.0; SPSS, Inc., Chicago, IL). Post hoc analyses were performed using Bonferroni tests for multiple comparisons. Significance was set at P < 0.05, and all reported values are mean ± SEM.

RESULTS

There were no significant differences in HRT volume (P > 0.05; Fig. 2) or total energy and macronutrient intake between PRO and CHO trials (P > 0.05; Table 1). Furthermore, there was no significant interaction for trial order, indicating that differences in REE and RER were deter-

TABLE 1. Total energy and macronutrient intake during CHO and PRO trials

Time	Trial	Energy (kJ·kg ^{-1.} d ⁻¹)	Protein (g·kg ⁻¹ ·d ⁻¹)	Carbohydrate (g·kg ⁻¹ ·d ⁻¹)	Fat (g·kg ⁻¹ ·d ⁻¹)
Day 1	СНО	115 ± 29	1.6 ± 0.6	3.4 ± 0.9	0.8 ± 0.4
	PRO	117 ± 25	1.1 ± 0.4	3.8 ± 0.3	1.0 ± 0.5
Day 2	CHO	111 ± 29	1.3 ± 0.4	3.6 ± 0.9	0.8 ± 0.4
	PR0	$106~\pm~25$	1.3 ± 0.3	$3.7\ \pm\ 0.9$	$0.7\ \pm\ 0.3$
Day 3	CHO	121 ± 33	1.5 ± 0.7	$3.7\ \pm\ 0.9$	0.9 ± 0.5
	PR0	$104~\pm~20$	1.1 ± 0.3	3.3 ± 0.9	0.9 ± 0.3
Day 4	CHO	$112~\pm~20$	1.2 ± 0.4	3.8 ± 0.8	$0.7\ \pm\ 0.4$
	PRO	116 ± 29	1.3 ± 0.5	$4.0\ \pm\ 0.1$	0.8 ± 0.4

Values are presented as mean \pm SD. P > 0.05

Carbohydrate, carbohydrate intake; Energy, energy intake; Fat, fat intake; Protein, protein intake.



FIGURE 3—Postexercise REE in PRO and CHO trials. Mean \pm SEM. *Statistically greater than baseline, P < 0.05. #Statistically greater than CHO, P < 0.05.

mined; it was facilitated by the interaction between the supplement and the acute HRT session.

A significant trial × time interaction was determined for REE (P < 0.05, $\eta_p^2 = 0.20$). Post hoc analysis showed that REE (kJ·kg⁻¹·d⁻¹) was significantly greater at 24 h after HRT after PRO (99.7 ± 2.2) compared with CHO (94.6 ± 2.9; Fig. 3). There was also a significant main effect of time (P < 0.05, $\eta_p^2 = 0.45$). Post hoc analysis determined that REE (kJ·kg⁻¹·d⁻¹) was significantly elevated at 24 and 48 h after HRT (CHO = 97.7 ± 3.4, PRO = 95.0 ± 2.9) compared with baseline (CHO = 90.7 ± 2.4, PRO = 92.6 ± 2.7).

In addition, a significant main effect for time was observed for RER (P < 0.05, $\eta_p^2 = 0.25$). *Post hoc* analysis demonstrated that RER was significantly decreased 24 h after HRT (CHO = 0.74 ± 0.05, PRO = 0.74 ± 0.02) compared with baseline (CHO = 0.78 ± 0.03, PRO = 0.79 ± 0.02).

DISCUSSION

In the present investigation, both PRO and CHO showed significant elevations in REE at 24 and 48 h after a single bout of HRT compared with baseline. The main finding was that at 24 h after HRT, REE was greater after PRO compared with CHO. RER also decreased in both PRO and CHO at 24 h after the acute HRT bout compared with baseline. The observed alterations in REE and RER were shown to be independent of energy intake, macronutrient intake, or HRT volume.

RER has traditionally been used as an indirect assessment of substrate utilization after HRT (20,21,30). Previous studies have shown that RER can decline 24 h after an acute bout of HRT (21,30). Our results are in agreement with these investigations because RER in both PRO and CHO declined the morning after an HRT session. In this capacity, the decrease in RER at rest has been interpreted as an increased reliance on lipid as a fuel source to generate ATP.

REE represents the minimum amount of energy required to maintain cellular processes at rest, and it has been

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regarded as a gauge for metabolic homeostasis (39). Previous research has demonstrated that laboratory measures of REE on consecutive days are reliable and stable, with reported CV between 1.5% and 4.5% (17,33,34). In the present study, the aforementioned observations were supported because the CV values were within the defined range. Thus, we believe that the increase in REE observed was facilitated because of the combined nutrient timing and HRT intervention.

Increased REE compared with baseline has been previously been reported to last 14-72 h after an acute bout of HRT (11,14,16,21,26,30). However, because different methods (e.g., sets, reps, exercises) were used in each investigation, it is difficult to make direct comparisons with the current findings. In the present study, we adopted the HRT protocol from Jamurtas et al. (21); thus, our results are comparable with their previous investigation. Similarly, both investigations observed a significant increase in REE and significant decrease in RER 24 h after HRT. Our investigation also showed a prolonged elevation in REE at 48 h after HRT compared with baseline in both PRO and CHO, although the aforementioned investigation showed a nonsignificant elevation. Overall, however, it seems that the within-group increases in REE are consistent with what others have reported using a similar HRT protocol.

The most notable finding of our investigation was that REE was increased in PRO at 24 h after HRT compared with CHO. We speculate that this elevation was mediated by preferentially increasing amino acid availability in skeletal muscles that were damaged during the acute HRT session. This hypothesis is supported by previous research, demonstrating that consuming protein or amino acids near the HRT session can increase muscle protein synthesis in the postexercise period (3,18,19,23,29,36,37). Although these studies have shown more short-term elevations (e.g., 2 h), it is important to remember that these investigations had predetermined end points. Studies examining a longer duration have shown that muscle protein synthesis can remain elevated up to 48 h after HRT (7,25,28), and this process may be the primary factor underlying delayed (i.e., >24 h) elevations in REE (11,20). In this regard, our investigation has shown that PRO before HRT increased REE 8.5% at 24 h after the exercise session compared with a 3.5% increase in CHO. Therefore, PRO before HRT resulted in an additional 5% increase in REE after a single session of HRT. For an individual who has an REE of 7536 kJ·d⁻¹, this would represent an additional 376 kJ·d⁻¹ of energy expended after HRT above the response demonstrated in CHO.

The mechanisms by which timing the intake of protein facilitates increases REE are unclear and require further re-

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search. However, it is postulated that this strategy increases amino acid delivery and uptake to the working muscles (36), leading to the activation of multiple cell signaling transduction pathways (e.g., mammalian target of rapamycin (mTOR) (4,9,13). For example, activation of the mTOR pathway can lead to acute (i.e., minutes to hours) and longterm (i.e., hours to days) up-regulation of muscle protein synthesis through alterations in mRNA translation and the biogenesis of ribosomes (18).

In addition, a combination of feeding and exercise may interact and influence hormone concentrations during exercise and in the postexercise period (37). For example, cortisol is released after HRT, and this hormone increases protein degradation and decreases protein synthesis in skeletal muscle cells (24). Recently, it was reported that cortisol concentrations were significantly reduced 24 h after an acute HRT session in a group using a protein timing strategy compared with a placebo (2). Thus, it is possible that a reduction in cortisol 24 h after HRT could increase muscle protein synthesis and elevate metabolic activity, leading to the elevation in REE that was observed. However, because we did not directly measure muscle protein synthesis or hormonal changes in combination with our REE measurements, we can only speculate which mechanisms were associated with the elevation in energy expenditure.

CONCLUSIONS

In summary, we have shown that RER was significantly reduced 24 h after HRT, which indicates that there is a greater reliance on fat oxidation at rest. Compared with baseline, both PRO and CHO trials also resulted in increased REE at 24 and 48 h after HRT. In addition, REE in PRO was greater than CHO 24 h after HRT. In this capacity, ingesting protein before HRT may be a simple yet effective strategy to increase energy expenditure. Over time, consistent increases in REE could facilitate reductions in body fat mass and improve composition if energy intake is controlled. Further research is needed to determine whether incorporating a protein timing strategy (i.e., whey, essential amino acids) during a long-term HRT program would lead to a chronic increase in REE and to improvements in body composition. In addition, it may be warranted to explore combining aerobic exercise with HRT and using this type of strategy to see if it could help promote reductions in body fat mass without decreasing muscle mass.

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