Dietary protein intake impacts human skeletal muscle protein fractional synthetic rates after endurance exercise

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Bolster, Douglas R., Matthew A. Pikosky, P. Courtney Gaine, William Martin, Robert R. Wolfe, Kevin D. Tipton, David Maclean, Carl M. Maresh, and Nancy R. Rodriguez. Dietary protein intake impacts human skeletal muscle protein fractional synthetic rates after endurance exercise. Am J Physiol Endocrinol Metab 289: E678–E683, 2005. First published May 24, 2005; doi:10.1152/ajpendo.00060.2005.—This investigation evaluated the physiological impact of different dietary protein intakes on skeletal muscle protein synthesis postexercise in endurance runners. Five endurance-trained, male runners participated in a randomized, crossover design diet intervention, where they consumed either a low (0.8 g/kg; LP)-, moderate (1.8 g/kg; MP)-, or high (3.6 g/kg; HP)-protein diet for 4 wk. Diets were designed to be eucaloric with carbohydrate, fat, and protein approximating 60, 30, and 10%; 55, 30, and 15%; and 40, 30, and 30% for LP, MP, and HP, respectively. Substrate oxidation was evaluated via indirect calorimetry at 3 wk of the dietary interventions. Mixed-muscle protein fractional synthetic rate (FSR) was measured after an endurance run (75 min at 70% V˙O2peak) and using a primed, continuous infusion of [2H5]phenylalanine. Protein oxidation increased with increasing protein intake, with each trial being significantly different from the other (P < 0.01). FSR after exercise was significantly greater for LP (0.083%/h) and MP (0.078%/h) than for HP (0.052%/h; P < 0.05). There was no difference in FSR between LP and MP. This is the first investigation to establish that habitual dietary protein intake in humans modulates skeletal muscle protein synthesis after an endurance exercise bout. Future studies directed at mechanisms by which level of protein intake influences skeletal muscle turnover are needed.

THE EFFECT OF ACUTE EXERCISE on protein metabolism is well documented. The majority of investigations regarding protein metabolism and endurance exercise to date have examined whole body protein turnover. These findings suggest the protein turnover response during this mode of exercise is catabolic in nature (9, 25, 27, 35), with the postexercise environment being more anabolic (or at least less catabolic; see Refs. 12, 25, and 29). The majority of studies examining skeletal muscle protein turnover have focused on the effect of resistance exercise. Few published studies have directly assessed the effect of acute aerobic exercise on skeletal muscle protein synthesis in humans (10, 26, 29). Skeletal muscle protein synthesis [fractional synthetic rate (FSR)] is increased after resistance exercise; however, the simultaneous increase in breakdown [fractional breakdown rate (FBR)] results in a NET balance (FSR − FBR) that remains negative (3, 23). Leg protein kinetic data after a 60-min cycling bout indicates that a negative NET is noted after aerobic exercise in the fasted state (20). It is generally accepted that, in the fasted state, NET muscle balance will remain negative. However, the acute provision of nutrients (primarily amino acids) can influence skeletal muscle protein turnover, such that NET becomes positive. These studies provide substantial evidence that, when amino acids (alone or with carbohydrates) are administered immediately postexercise, a shift to a positive NET balance results (4, 5, 24, 28, 30, 33). A similar positive NET balance has been reported when a protein-containing supplement was consumed after endurance exercise (20). Therefore, provision of exogenous amino acids creates a more anabolic environment by increasing muscle protein synthesis and decreasing the reliance on amino acids from muscle protein breakdown.

No studies to date have determined whether or not postexercise skeletal muscle protein synthesis can be modulated in response to chronic consumption of increased dietary protein. Theoretically, increased or high protein intakes could expand the free amino acid pool, decreasing reliance on breakdown to supply the amino acids required for skeletal muscle protein synthesis. At this time, crucial questions remain largely unexplored. First, can the chronic consumption of different amounts of dietary protein augment the free amino acid pool after exercise? And if so, will consuming increased amounts of dietary protein impact skeletal muscle protein synthesis during recovery from an endurance exercise bout? The purpose of the present investigation was to establish whether the habitual consumption of varying levels of dietary protein would alter skeletal muscle protein synthesis after an acute aerobic exercise session in endurance athletes. We hypothesized that increased dietary protein intake would expand the free amino pool, thereby enhancing FSR postexercise.

METHODS

Participants. After project approval by the Institutional Review Board at the University of Connecticut, five male endurance athletes aged 22–29 yr were recruited from the University community and local health/running clubs to participate in the study. Each participant provided a complete medical history, training schedule, and a record of dietary intake. Subjects were required to be running a minimum of 56 km/wk for inclusion in the study. Individuals reporting metabolic or cardiovascular abnormalities, gastrointestinal disorders (i.e., lactose intolerance), use of nutritional/sports supplements or anabolic steroids, or who considered themselves...
vegan were excluded from the study. Informed, written consent was obtained from all subjects.

Experimental design. This study was a crossover design, and volunteers served as their own controls. After an initial baseline testing period, volunteers were randomly assigned to a diet containing either 0.8 g protein (low protein; LP), 1.8 g protein (moderate protein; MP), or 3.6 g protein (high protein; HP) per kilogram of body weight (g·kg⁻¹·day⁻¹) for 4 wk. After 3 wk of each diet intervention, 24-h nitrogen balance and substrate oxidation were assessed. After 4 wk, mixed-muscle protein FSR was determined postexercise. After an ~2 wk “washout” period, athletes crossed over and consumed the other diets, and all measurements were repeated.

Preliminary measurements. Baseline testing included assessment of aerobic capacity (V\text{O}_2\text{peak}), anthropometry (height and weight), body composition (hydrostatic weighing), resting energy expenditure (REE-indirect calorimetry), 3-day diet records, and training records. Height and weight were measured on a balance beam scale (Health-o-meter, Bridgeview, IL) to the nearest 0.5 kg and 0.5 cm, respectively. Percent body fat was estimated through hydrostatic weighing, and values were calculated from body density according to equations by Brozek et al. (8). V\text{O}_2\text{peak} testing was conducted before the start of the study and was determined via breath by breath analysis of expired gases during testing using an open circuit respiratory apparatus (MedGraphics CPX/D; Medical Graphics, St. Paul, MN) on a treadmill (MedTrack ST55; Quinton, Bothell, WA) containing a ventilation flowmeter, oxygen analyzer, and carbon dioxide analyzer (15). Later, participants were asked to return to the treadmill for determination of appropriate workloads for exercise testing during the study. The speed was manipulated to elicit ~70% of their calculated V\text{O}_2\text{peak}.

Baseline dietary records. Three-day dietary records were collected from all study participants during the baseline period to assess initial nutrient intake for calories, carbohydrates, protein, and fat. All dietary records were analyzed using Nutritionist Pro Software (First Data Bank, version 1.1).

Training journals. All athletes kept training journals that detailed their daily and weekly totals for running mileage. The majority of the participants were distance runners at the University and were included in the study during their competitive season, thus ensuring consistent training patterns between the feeding interventions. These journals were collected and analyzed at the end of each dietary intervention.

Resting metabolic rate. REE was assessed at baseline and then after 3 wk of consuming each respective diet. REE at baseline was used to more accurately predict caloric needs and provide supplemental information to ensure weight stability and energy balance in these athletes. REE assessed during the dietary intervention phases at week 3 was used to determine the effect of the diets on substrate oxidation. Twenty-four-hour excreted nitrogen values were used in conjunction with indirect calorimetry for estimates of substrate oxidation.

REE was determined by indirect calorimetry using a metabolic cart (MedGraphics CPX/D; Medical Graphics). On the morning of testing, each participant was given a car ride to the laboratory to minimize physical activity. All testing was done after an overnight fast, and participants were in at least a 10-h postabsorptive state.

Diet interventions. Protein intakes were set at either a “low,” “moderate,” or “high” protein intake level (0.8, 1.8, or 3.6 g·kg⁻¹·day⁻¹, respectively). Diet interventions were designed such that the percentage of total calories contributed by the macronutrients (carbohydrate, 60%; fat, 30%; and protein, 10%) were preserved for the LP, 55% carbohydrate, 30% fat, and 7% protein for the MP, and 40% carbohydrate, 30% fat, and 30% protein, for the HP diet. The diets were eucaloric with an isoeenergetic exchange between protein and carbohydrate. The predominant protein source at each meal was beef. Additionally, participants on the HP diet received two commercially available protein bars (Protein Plus; Met-Rx, Irvine, CA) per day that provided ~300 kcal and 32 g protein (15 g carbohydrate, 8 g fat) to increase their protein intake to the prescribed level. Menus incorpo-

Protein intake and muscle protein synthesis after exercise. Participants were fed at a designated dining room through the Department of Catering at the University of Connecticut. Research assistants were present at all meals to weigh and serve the appropriate foods for each participant. Participants were not food restricted, per se, with any food eaten in excess or less than prescribed at each meal. After each exercise, an additional catheter was placed in a contralateral hand vein for arterialized blood sampling obtained at 15-min intervals. An intravenous saline drip was maintained to keep the line patent. The participant’s hand was covered with a heating pad and was heated to ~70°C such that arterialized blood samples could be obtained. Previous research established that arterialized blood samples significantly correlated with arterial blood values (11). Subsequently, participants were prepped under sterile conditions for a muscle biopsy that was taken from the lateral portion of the vastus lateralis (~20 cm above the knee). The skin and subcutaneous layer surrounding the area to be biopsied was locally anesthetized with 1% lidocaine (Eiklns-Sinn, Cherry Hill, NJ). All biopsies were made with Bergström 5-mm cannulas (Depuy Orthopedics, Warsaw, IN) with suction. The tissue sample was immediately blotted dry, removed of any visible fat or connective tissue, and frozen in liquid nitrogen. Samples were then stored at ~80°C until further processing. Subsequent biopsies were taken from the same leg, and mild pressure was applied between procedures.

Blood. Blood samples obtained from the arterialized hand vein for determination of phenylalanine enrichment were immediately precipitated in tubes containing 15% sulfosalicyclic acid and thoroughly mixed. The whole blood samples were spun in a centrifuge, and the supernatant was frozen at ~80°C until further analysis. To determine the enrichment of labeled phenylalanine in whole blood, the t-butyldimethylsilis1 (BDMS) derivative of phenylalanine was made according to previous methods used by Phillips et al. (23). Analysis of t-BDMS phenylalanine by gas chromatography-mass spectrometry (Hewlett-Packard 5890, series II) was performed using electron-impact ionization and selected-ion [mass-to-charge ratio (m/z)] monitoring of m/z 234, 235, 239, and 240, for the m + 0, m + 1, m + 5, and m + 6 ions, respectively. Appropriate corrections were made for any spectra that overlapped and contributed to the tracer-to-tracee ratio (34).
**Muscle.** Data presented represent mixed-muscle protein synthesis, and specific muscle proteins were not measured in this study. Muscle tissue samples were analyzed for both protein-bound and free intracellular enrichment, as previously described (23).

**Calculations.** FSR was calculated from the rate of tracer incorporation in skeletal muscle protein and with the use of the muscle intracellular free phenylalanine enrichment as the precursor, according to the following equation (see Ref. 2):

\[
FSR(\%/h) = \left( \frac{E_{t} - E_{0}}{E_{p}} \times (t_{1} - t_{0}) \right) \times 100
\]

where \( E_{0} \) was the enrichment in the protein-bound phenylalanine tracer from the first biopsy at time \( t = 0 \) (preexercise), \( E_{t} \) was the enrichment of the protein-bound phenylalanine tracer from the second or third biopsy at 300 min, \( t_{1} - t_{0} \) was the incorporation time (~3 h); and \( E_{p} \) was the mean intracellular \( (t = 120, 280, \text{and} 300 \text{ min}) \) \( ^{2} \text{H}_{5} \text{-phenylalanine} \) enrichment for the time period of protein incorporation.

**Nutritional analyses-diet interventions.** Dietary records were analyzed for energy and macronutrient composition using Nutritionist Pro Software (First Data Bank, version 1.1). Nutrient intakes are reported as the daily average over the entire 4-wk dietary intervention.

**Plasma amino acids.** Plasma amino acids were analyzed at baseline \((t = 0)\) after 4 wk of the diets by derivatizing with phenylisothiocyanate and HPLC (14).

**Statistical analysis.** Volunteers were described by common descriptive statistics for measures of height, weight, age, body composition, and \( V_{O2\text{peak}} \). A repeated-measures ANOVA was used to determine if differences were present in the various criterion measures at baseline, postexercise, and in recovery between LP, MP, and HP. When significant differences were noted, a Tukey’s post hoc analysis was performed. The \( \alpha \)-level for significance was set at \( P < 0.05 \). Data are expressed as means \( \pm \) SE.

**RESULTS**

**Baseline subject characteristics.** A homogenous group of fit young males participated in this study \((n = 5)\). Subject characteristics are shown in Table 1. Baseline dietary intakes revealed that the runners consumed \(~2,831\) kcal, \(52\%\) carbohydrate \((5.4 \text{ g/kg})\), \(17\%\) protein \((1.7 \text{ g/kg})\), and \(31\%\) fat.

**Diet interventions.** Table 2 summarizes the mean nutrient intakes for the LP, MP, and HP dietary protein interventions. The macronutrient breakdown was \(48\%\) carbohydrate, \(26\%\) fat, and \(26\%\) protein for MP, \(60\%\) carbohydrate, \(26\%\) fat, and \(14\%\) protein for MP, and \(66\%\) carbohydrate, \(27\%\) fat, and \(7\%\) protein for LP. Mean protein intake in grams per kilogram body weight was \(0.87, 1.78, \text{and} 3.12\), and carbohydrate intake was \(8.3, 7.4, \text{and} 5.4\) for LP, MP, and HP, respectively.

**Substrate oxidation.** Substrate oxidation data reflect the subject’s dietary compliance. Protein oxidation increased as dietary protein increased, with dietary interventions being significantly different from each other \((54 \pm 7 \text{ vs.} 25 \pm 2 \text{ vs.} 14 \pm 2 \% \text{ for HP, MP, and LP respectively,} P < 0.001)\). There were no significant differences in carbohydrate \((28 \pm 7 \text{ vs.} 55 \pm 10 \text{ vs.} 52 \pm 10 \% \text{ for HP, MP, and LP respectively})\) or fat \((18 \pm 8 \text{ vs.} 19 \pm 10 \text{ vs.} 34 \pm 9 \% \text{ for HP, MP and LP, respectively})\) oxidation.

**Table 1. Baseline subject characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>Body fat, %</th>
<th>( V_{O2\text{peak}} ), ml·kg(^{-1})·min(^{-1})</th>
<th>Running distance, km/wk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, yr</strong></td>
<td>21.3±0.3</td>
<td>179.1±1.6</td>
<td>70.6±0.1</td>
<td>8.7±0.4</td>
<td>70.6±0.1</td>
<td>56±3</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE; \( n = 5 \) subjects.

**Table 2. Actual dietary intakes for LP, MP, and HP**

<table>
<thead>
<tr>
<th></th>
<th>kcal</th>
<th>Carbohydrate, g</th>
<th>Fat, g</th>
<th>Protein, g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LP</strong></td>
<td>3,498±33</td>
<td>584±4*†</td>
<td>107±3</td>
<td>64±1*†</td>
</tr>
<tr>
<td><strong>MP</strong></td>
<td>3,463±87</td>
<td>524±13†‡</td>
<td>98±3‡</td>
<td>125±3‡‡</td>
</tr>
<tr>
<td><strong>HP</strong></td>
<td>3,347±19</td>
<td>391±10‡‡</td>
<td>97±2‡</td>
<td>220±3‡‡</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE. LP, low-protein diet; MP, moderate-protein diet; HP, high-protein diet. \( P < 0.05 \), significantly different from ‡LP, *MP, and †HP.
The purpose of this investigation was to determine if habitual changes in dietary protein intake could modulate skeletal muscle protein synthesis after an acute aerobic exercise session in runners. Data were obtained across a wide range of protein intakes reflective of the current dietary reference intakes (DRIs) (14a). The highest level reflects the amount of protein intake recommended for these young men would enhance the skeletal muscle protein synthetic response after an endurance exercise bout. This investigation marks the first study to demonstrate that variations in habitual protein intake can directly impact skeletal muscle protein synthesis in the fasted state after endurance exercise. Postexercise FSR was significantly higher for both the LP and MP diets compared with the HP diet.

To date, no investigations have examined the impact of varying levels of dietary protein in humans on skeletal muscle protein synthesis after an endurance exercise bout. Furthermore, few published studies have assessed changes in skeletal muscle protein synthesis after acute aerobic exercise in humans (10, 26, 29). Direct comparisons between these studies and the present investigation are not warranted, given the differing objectives of the studies. The purpose of the previous studies was to determine the effect of a single aerobic exercise session on skeletal muscle protein synthesis by examining FSR at rest and immediately after exercise. In the present study, we did not determine FSR at rest, as the primary focus was instead to evaluate the impact of variations in habitual protein intake on skeletal muscle protein synthesis during the recovery from exercise. Therefore, comparisons of our findings with existing scientific literature are limited to investigations that have examined the effects of an acute exercise manipulation on whole body protein turnover (7, 13, 21) or those studies that have examined the effects of an acute aerobic exercise session on plasma and muscle BCAA (positive correlation between dietary protein intake and both baseline BCAA and EAA concentrations and FSR after exercise (r = −0.7, P < 0.01 for both BCAA and EAA). Therefore, higher plasma amino acid concentrations at rest after 4 wk of dietary intervention were correlated with decreased FSR values postexercise.

**FSR.** The postexercise skeletal muscle FSR data are presented in Fig. 2. Habitual consumption of HP resulted in a significantly lower FSR postexercise (P < 0.05) compared with MP and LP, with no difference noted between these latter two interventions (0.052 ± 0.009, 0.078 ± 0.006, and 0.082 ± 0.006%/h for HP, MP, and LP, respectively). Plasma and muscle enrichment data are shown in Fig. 3.

**DISCUSSION**

The purpose of this investigation was to determine if habitual changes in dietary protein intake could modulate skeletal muscle protein synthesis after an acute aerobic exercise session.
nutritional intervention on skeletal muscle protein utilization after exercise (all but one after resistance training; see Refs. 4, 5, 20, 22, 24, 28, 30, 31).

Several studies have examined the ability of habitual protein intake to modulate the whole body protein metabolism response to endurance exercise. Collectively, no significant differences have been found between rates of whole body synthesis and breakdown in the fasted state when protein intakes ranged from 0.9 to 2.5 g·kg\(^{-1}\)·day\(^{-1}\), before, during, or after exercise (7, 9, 13). The only consistent response is an increase in leucine oxidation with higher protein intakes (7, 13).

Based on the findings of these investigations, it does not appear that dietary protein intake can impact rates of whole body protein synthesis and breakdown in the fasted state, either before or after endurance exercise. However, it is important to note that these studies used whole body assessments of protein utilization, which are not likely to reflect skeletal muscle protein metabolism (1, 3, 10, 29). Indeed, the present study is unique in that it is the first investigation to document that variations in habitual protein intake can impact skeletal muscle protein synthesis during recovery from endurance exercise.

The major finding of the current study was that the highest protein intake resulted in decreased FSR in the postexercise period. This is in contrast to our original hypothesis that mixed-muscle FSR would be enhanced with higher intakes of protein. Feeding higher levels of dietary protein appeared to increase free amino acid availability, as suggested by the plasma amino acid data. Although significant differences were noted only in BCAA between LP and MP, the observed augmentation of EAA and BCAA concentrations may be of important physiological significance. Indeed, there was a significant negative correlation between increased plasma concentrations of EAA and BCAA and decreases in FSR postexercise. An increase in amino acid availability could potentially influence protein turnover in one of two ways: 1) increasing the protein synthetic response postexercise, similar to what has been documented with acute amino acid feedings after resistance training (4, 5, 28), or 2) limiting the body’s reliance on endogenous sources of these free amino acids, thereby limiting proteolysis during exercise (32). This scenario is analogous to previous research where infusion of amino acids stimulated protein synthesis postexercise without a simultaneous increase in muscle protein breakdown (4).

It is plausible that the increase in BCAA and EAA on the HP diet may have attenuated protein breakdown during exercise. Similarly, the increase in carbohydrate intakes on LP and MP may have enhanced glycogen availability and attenuated amino acid oxidation during the endurance bout. Nonetheless, we propose that skeletal muscle proteolysis was reduced during exercise and diminished the magnitude of the anabolic response postexercise, and this may offer insight regarding the observed decrease in FSR postexercise when subjects were habitually consuming the highest level of protein. Although we have no measurements during the exercise bout, we determined FBR in the postexercise period. Unfortunately, the low number of subjects (i.e., 2–3/diet) for whom these calculations were completed require that these observations be considered preliminary since statistical power was not sufficient. However, these preliminary findings provide supportive evidence that a stepwise decrease in FBR postexercise may exist with increasing levels of dietary protein (0.169, 0.134, and 0.068%/h for LP, MP and HP, respectively). Without question, further studies are needed to determine if habitual consumption of different levels (i.e., increased) of dietary protein intake can modulate endogenous proteolysis during exercise and recovery.

Overall, findings from this study support the proposal that rates of protein synthesis are, in part, dependent on the availability of free amino acids (20, 32). Amino acids are taken from the free amino acid pool to be used for promoting protein synthesis. In turn, amino acids are needed to replenish this pool. In the fasted state, these amino acids are solely available from endogenous protein breakdown. Therefore, the increase in protein breakdown that results from exercise can be viewed as necessary, to provide the free amino acids required to elicit the concurrent increase in synthesis. The habitual provision of exogenous amino acids may act to augment this free amino acid pool, thereby allowing synthesis to continue without a corresponding increase in breakdown.

The fact that FSR was significantly lower on the HP diet is in direct contrast to our original hypothesis. Based on previous findings that acute protein feedings immediately following resistance and aerobic training stimulated protein synthesis to a greater extent than that noted with exercise alone (4, 5, 20, 22, 24, 28, 30, 31), we assumed that the habitual consumption of higher protein would lead to a chronic expansion of the free amino acid pool, thereby allowing for a greater synthetic response postexercise, even in the fasted state. When the FSR data are examined alone, this was not the case. Further detailed investigations examining FBR with increased levels of dietary protein are necessary to fully elucidate skeletal muscle protein turnover under these conditions.

In summary, the present investigation provides the first comprehensive analysis of postexercise skeletal muscle protein synthesis in response to varying degrees of dietary protein intake. The fact that habitual variation in protein intake was associated with changes in skeletal muscle protein synthesis in the fasted state supports the notion that protein intake plays a direct role in regulating skeletal muscle protein metabolism. These findings reinforce the need to implement long-term, well-controlled diet interventions that reflect habitual intake to further characterize the relationships between protein intake, endurance exercise, and skeletal muscle protein turnover.

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