Muscle protein synthesis and gene expression during recovery from aerobic exercise in the fasted and fed states

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Harber MP, Konopka AR, Jemiolo B, Trappe SW, Trappe TA, Reidy PT. Muscle protein synthesis and gene expression during recovery from aerobic exercise in the fasted and fed states. Am J Physiol Regul Integr Comp Physiol 299: R1254–R1262, 2010. First published August 18, 2010; doi:10.1152/ajpregu.00348.2010.—The purpose of this investigation was to assess mixed-muscle fractional synthesis rate (FSR) and the expression of genes involved in skeletal muscle remodeling after aerobic exercise in the fasted and fed states. Eight recreationally active males (25 ± 1 yr; VO₂ max: 52 ± 2 ml·kg⁻¹·min⁻¹) performed 60-min of cycle ergometry at 72 ± 1% VO₂ max on two occasions in a counter-balanced design. Subjects ingested a noncaloric placebo (EX-FAST) or a beverage containing (per kg body wt): 5 kcal, 0.83 g carbohydrate, 0.37 g protein, and 0.03 g fat (EX-FED) immediately and 1 h after exercise. FSR was assessed at rest and following exercise with the use of a L-[ring 2H5]-phenylalanine infusion combined with muscle biopsies at 2 and 6 h postexercise. mRNA expression was assessed at 2 and 6 h postexercise via real-time RT-PCR. FSR was higher (P < 0.05) after exercise in both EX-FAST (0.112 ± 0.010%/h⁻¹) and EX-FED (0.129 ± 0.014%/h⁻¹) compared with rest (0.071 ± 0.005%/h⁻¹). Feeding attenuated the mRNA expression (P < 0.05) of proteolytic factors MuRF-1 (6 h) and calpain-2 (2 and 6 h) postexercise but did not alter FOXO3A, calpain-1, caspase3, or myostatin mRNA expression compared with EX-FAST. Myogenic regulatory factor (MRF4) mRNA was also attenuated (P < 0.05) at 2 and 6 h postexercise in EX-FED compared with EX-FAST. These data demonstrate that nonevocative bout of aerobic exercise stimulates skeletal muscle FSR in the fasted state and that feeding does not measurably enhance FSR between 2 and 6 h after aerobic exercise. Additionally, postexercise nutrient intake attenuates the expression of factors involved in the ubiquitin-proteosome and Ca2⁺-dependent protein degradation pathways. These data provide insight into the role of feeding on muscle protein metabolism during recovery from aerobic exercise.

protein turnover; endurance; proteolysis; muscle-specific RING finger protein-1; calpain

AEROBIC EXERCISE ACUTEly (8, 44) and chronically (41, 45) alters skeletal muscle protein metabolism. The influence of nutrient supply (i.e., feeding) on skeletal muscle protein kinetics following exercise that is considered to be aerobic or endurance-natured remains relatively unexplored. Levenhagen et al. (29) initially showed that ingestion of minimal amounts of carbohydrate (8 g) combined with 10 g of protein creates a positive protein balance across the leg following aerobic exercise, apparently through promoting muscle protein synthesis with little influence on protein breakdown. Recently, it was reported that coinestion of protein and carbohydrate following aerobic exercise stimulates muscle protein synthesis greater than carbohydrate intake alone and yields a net positive whole-body protein balance (24). While the study by Howarth et al. (24) described the important role of protein on muscle protein synthesis following aerobic exercise, the lack of a resting or noncaloric (i.e., fasting) trial prevents a complete understanding of the stimulatory effects of feeding on muscle protein synthesis after exercise. Despite continued interest in the role of nutrition on muscle recovery from aerobic exercise, the direct effect of feeding on muscle protein synthesis after exercise has yet to be fully elucidated.

Muscle protein breakdown is also elevated acutely (44) and chronically (41) in response to aerobic exercise. Skeletal muscle protein breakdown is primarily mediated through the ATP-dependent ubiquitin-proteosome pathway (UPP), which degrades myofibrillar and sarcoplasmic proteins in concert with the calpain system (1, 16). Atrogin-1 and muscle-specific RING finger protein-1 (MuRF-1) are the primary atrogenes of the UPP and are induced through Forkhead box (FOXO) signaling (16). These factors are acutely responsive to aerobic exercise, presumably as part of the muscle remodeling process (20, 32). Furthermore, the mRNA expression of these atrogenes is elevated in sarcopenic muscle (43) and reduced following muscle hypertrophy (51), suggesting a role in the regulation of muscle mass. The calpains are components of the Ca2⁺-dependent protein degradation pathway and have been implicated in muscle proteolysis following muscle-damaging exercise (14, 28). The role of these factors in regulating exercise-mediated protein breakdown to nondamaging exercise is not well defined. Collectively, these markers of muscle proteolysis are a potential mechanism by which feeding may alter muscle protein metabolism following exercise; however, to our knowledge, this interaction has not been investigated.

Therefore, the purpose of this study was to examine the influence of postexercise feeding (0.83 g carbohydrate, 0.37 g protein, 0.03 g fat/kg body wt) on muscle protein synthesis and the mRNA expression of proteolytic markers following a session of aerobic exercise. Our primary hypothesis was that feeding would result in higher muscle protein synthesis rates following exercise compared with exercise–fast (EX-FAST), similar to what was observed with resistance exercise (34). We further hypothesized that postexercise feeding would attenuate the mRNA expression of proteolytic markers in skeletal muscle, which would be mediated primarily through a feeding-induced elevation in circulating insulin levels.

METHODS

Subjects and Experimental Overview

Eight recreationally active males (means ± SE; 25 ± 1 yr, 177 ± 3 cm, 74 ± 3 kg) volunteered to participate in this investigation. Prior to enrollment, subjects participated in an introductory visit to the...
laboratory, where they were given an overview of the study protocol and a medical history questionnaire, including exercise and dietary screening. Subjects were excluded if they had a body mass index ≥ 28 kg/m²; any acute or chronic illness; cardiac, pulmonary, liver, kidney, or metabolic disorder(s); uncontrolled hypertension; or were smokers. None of the subjects were taking medications or supplements known to affect protein metabolism. This investigation was approved by the Institutional Review Board at Ball State University, and written informed consent was obtained from each subject prior to participation.

Each subject completed the protocol over the period of ~4 wk. Subjects performed a cycle ergometer test for the assessment of maximum aerobic capacity (VO₂ max), which was used to determine the exercise intensity during the exercise trials. Each subject then performed the resting trial followed by the two exercise trials in a counter-balanced study design. The two exercise trials differed only in the type of beverage consumed after exercise. Subjects refrained from exercise for 3 days prior to all experimental trials. Additionally, subjects consumed a standardized meal the evening prior to all experimental trials and reported to the laboratory in the fasted state on the morning of each trial. Approximately 1 wk was allocated between each of the three experimental trials.

Aerobic Capacity

Prior to the experimental trials, subjects performed a test for the assessment of aerobic capacity (VO₂ max) using an electronically braked cycle ergometer (Velotron; RacerMate, Seattle, WA). During the test, subjects performed a warm-up consisting of 2 min at 100 W and 4 min at 150 W. Thereafter, the workload was increased in 25-W increments every 2 min until exhaustion. During the test, subjects’ perceived exertion and heart rate were recorded and respiratory gases were measured with gas analyzers (Applied Electrochemistry, Amteck S3A and CD3A).

Resting Assessment of Muscle Protein Synthesis

The resting trial served as a baseline assessment for protein synthesis, gene expression, and muscle glycogen content. Subjects reported to the laboratory in the fasted state following a standardized meal the evening before. A catheter was placed in an antecubital vein for infusion of the stable, isotopically labeled amino acid (Cambridge Isotopes, Andover, MA). The amino acid tracer (³H₅-phenylalanine) was dissolved in 0.9% saline, passed through a 0.2-µm filter, and infused at a constant rate after an initial priming dose, as we have previously described (6, 9, 20). The stable isotope infusion was administered for five continuous hours to ensure that steady state tracer enrichment in the plasma and muscle was maintained. During the infusion, two muscle biopsies were obtained from the vastus lateralis muscle. Both the 2-h and 5-h biopsies were used for the measurement of resting protein synthesis. The 2-h biopsy was obtained for the assessment of basal gene expression and preexercise muscle glycogen.

Additionally, a venous catheter was placed in the contralateral arm for blood sampling. Blood samples (5 ml) were taken at 0, 2, 3, 4, and 5 h for the measurement of plasma amino acid tracer enrichment.

Exercise Trials

Each subject performed two exercise trials in a counterbalanced design (Fig. 1). The exercise trials differed only in the composition of the postexercise beverage. During each trial, subjects performed 60 min of cycle ergometry at ~70% of their predetermined VO₂ max. Indirect calorimetry was used to assess energy expenditure and exercise intensity every 15 min during the exercise period. Immediately following exercise, a catheter was placed in an antecubital vein for postexercise blood draw and infusion of the stable, isotopically labeled amino acid tracer (³H₅-phenylalanine) (Cambridge Isotopes). The stable isotope infusion was administered for six continuous hours. Following the initiation of the infusion, subjects consumed a beverage containing 5 kcal/kg body wt (exercise-fed, EX-FED: 0.83 g carbohydrate/kg body wt, 0.37 g protein/kg body wt, 0.028 g fat/kg body wt), or an isovolumetric, noncaloric placebo beverage (EX-FAST). On average, subjects consumed 62 ± 3 g of carbohydrate, 27 ± 3 g of protein, and 2.1 ± 0.1 g of fat at each feeding. [³H₅]-phenylalanine was added to the EX-FED beverage to maintain steady plasma enrichment. The protein component of the supplement was a milk protein isolate containing the proteins of milk (both whey and casein) in their original proportions. The amino acid profile of the protein was (in g/100 g of amino acids): 3.09 alanine, 3.28 arginine, 7.13 aspartic acid, 0.63 cystine, 19.72 glutamic acid, 1.80 glycine, 2.57 histidine, 5.19 isoleucine, 8.93 leucine, 7.77 lysine, 2.49 methionine, 4.55 phenylalanine, 9.95 proline, 5.47 serine, 4.71 threonine, 1.56 tryptophan, 4.98 tyrosine, and 6.18 valine. Following the exercise session, and beverage consumption, subjects rested in the supine position within the laboratory for the duration of infusion. One hour after the exercise session, subjects consumed another beverage identical to the one provided immediately after exercise. At each time point, subjects consumed the beverage within a 15-min period.

A second catheter was placed in an antecubital vein of the contralateral arm for blood sampling at 2, 3, 4, 5, and 6 h during the isotope enrichment for muscle glycogen content. Muscle biopsies were obtained from the vastus lateralis at 2 and 6 h during the isotope infusion for determination of the incorporation of [³H₅]-phenylalanine into mixed-muscle protein, gene expression, and muscle glycogen content.

Muscle Biopsy

For each subject, a total of six muscle biopsies were obtained for the entire study protocol. At each time point, percutaneous needle biopsies were obtained under a local anesthetic (2). Muscle samples were dissected free of any visible connective and adipose tissue and divided into ~20-mg sections. Sections to be used for mRNA analysis were placed in 0.5 ml of RNLater (Ambion, Austin, TX) and stored at −20°C until RNA extraction. The other muscle sections were immediately frozen and stored in liquid nitrogen (−190°C) until analysis.
Plasma Substrates and Insulin

Plasma glucose (ThermoDMA, Arlington, TX) and fatty acid (Wako Chemicals, Neuss, Germany) concentrations were measured using commercially available colorimetric assay kits. Plasma insulin was determined using an ELISA immunoassay kit (Alpco Diagnostics, Salem, NH).

Muscle Glycogen

Muscle glycogen content was determined from muscle biopsy samples from the vastus lateralis muscle during the resting trial and 2 and 6 h following the cycle exercise. Glycogen content was determined from the measurement of glucose after acid hydrolysis of muscle samples with hydrochloric acid (37).

Analysis of Mixed-Muscle Protein Synthesis

The rate of mixed-muscle protein synthesis was determined by quantifying the tissue fluid and protein-bound $[^{2}H]_5$-phenylalanine enrichment (tracer to tracee) in muscle samples (~20 mg) from the vastus lateralis, as we have previously described (6, 9, 20). All samples were analyzed using GC-MS (6890N GC coupled with 5973 inert MSD; Agilent Technologies, Wilmington, DE) in triplicate using samples were analyzed using GC-MS (6890N GC coupled with 5973 inert MSD; Agilent Technologies, Wilmington, DE) in triplicate using selected ion monitoring of m/z 234 in triplicate using samples).

Plasma Phenylalanine Rate of Appearance

Whole body protein synthesis was measured as phenylalanine rate of appearance (Ra) into plasma under steady-state conditions using the following formula: $Ra = F/E_{Pr}$, where $Ra$ is the rate of appearance ($\mumol\cdot kg^{-1}\cdot min^{-1}$), $F$ is the infusion rate, and $E_{Pr}$ is the plasma enrichment.

mRNA Expression

Total RNA extraction and RNA quality check. Total RNA was extracted in TRI reagent (Molecular Research Center, Cincinnati, OH). The quality and integrity [RNA Integrity Number of 8.35 ± 0.07 (SE)] of extracted RNA (165.15 ng/µl) was evaluated using an RNA 6000 Nano LabChip kit on an Agilent 2100 Bioanalyzer.

RT and real-time PCR. Oligo (dT) primed first-strand cDNA was synthesized (150 ng total RNA) using SuperScript II RT (Invitrogen, Carlsbad, CA). Quantification of mRNA transcription (in duplicate) was performed in a 72-well Rotor-Gene 3000 Centrifugal Real-Time Cycler (Corbett Research, Mortlake, NSW, Australia). HKG GAPDH was used as a reference gene. The validation of GAPDH was performed to ensure that its expression was unaffected by the experimental treatments, as we have previously described (25).

All primers used in this study were mRNA specific (on different exons and crossing over an intron) and designed for real-time PCR analysis using Vector NTI Advance 9 software (Invitrogen). Primers for muscle regulatory factor 4 (MRF4), muscle-RING-finger protein-1 (MuRF-1), forhead box [FOXO3A], peroxisome proliferator-activated receptor-gamma coactivator 1-α (PGC-1α), mitochondrial transcription factor A (TFAM), calpain-1, calpain-2, caspase-3, and myostatin have been reported previously by our laboratory (20, 32, 55).

A melting curve analysis was generated to validate that only one product was present. The details about RT and PCR reaction parameters have been reported previously (32, 42).

The data were analyzed using $2^{-\Delta\Delta Ct}$ method (30). The $2^{-\Delta\Delta Ct}$ method was used to calculate the fold change (compared to rest) in gene expression at 2 and 6 h in each of the experimental trials. In this method, gene of interest (GOI) expression was normalized to housekeeping gene (HKG) expression and calibrated to control resting value ($\Delta\Delta Ct = Ct_GOI\ text{x}_{\text{traces}} - Ct_{\text{x}_{\text{HKG}}\ text{x}_{\text{traces}}}$). In using this analysis, the fold changes at time 0 (control = rest), if not influenced by external stimulus, should be very close to 1.

Statistical Analysis

Data from the exercise trials (i.e., exercise intensity, energy expenditure) were compared using a paired Student’s t-test. A one-way ANOVA with repeated measures was used to compare muscle protein synthesis rates and plasma Ra. A two-way ANOVA with repeated measures on trial (EX-FAST vs. EX-FED) and time (postexercise, 2 h, 3 h, 4 h, 5 h, 6 h) was used to compare plasma substrates and insulin. A two-way ANOVA with repeated measures on trial (rest vs. EX-FAST vs. EX-FED) and time (2 h vs. 6 h) was used to compare muscle glycogen concentration. Changes in mRNA expression (expressed as fold-change from rest) between the exercise trials were analyzed with a two-way ANOVA with repeated measures on trial (EX-FAST vs. EX-FED) and time (2 h vs. 6 h). In the presence of a main effect, a Bonferroni post hoc analysis was performed to make pairwise comparisons. Significance for all analysis was set at $P < 0.05$. Data are presented as means ± SE.

RESULTS

Exercise Trials

Aerobic capacity, assessed as maximum oxygen consumption ($\text{VO}_{2\ max}$) during cycling, was 3.9 ± 0.2 l/min and 52 ± 2 ml·kg$^{-1}$·min$^{-1}$. All subjects successfully completed all three experimental trials. There were no differences in external power output (W), average $\text{VO}_{2}$, exercise intensity, average heart rate, or energy expenditure between the two exercise trials (Table 1).

Plasma Glucose and Fatty Acids

No differences were present in plasma glucose concentration immediately postexercise or during the 6-h postexercise recov-

Table 1. Performance and physiological measures from each exercise trial

<table>
<thead>
<tr>
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<th>EX-FAST</th>
<th>EX-FED</th>
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<tr>
<td>Average power output, W</td>
<td>187 ± 11</td>
<td>187 ± 11</td>
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<tr>
<td>Average $\text{VO}_{2}$, l/min</td>
<td>2.8 ± 0.1</td>
<td>2.8 ± 0.1</td>
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<tr>
<td>Intensity, % $\text{VO}_{2\ max}$</td>
<td>71.9 ± 1.2</td>
<td>72.6 ± 1.2</td>
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<tr>
<td>Average HR, bpm</td>
<td>160 ± 4</td>
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<td>Energy expenditure, kcal</td>
<td>832 ± 36</td>
<td>840 ± 40</td>
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<tr>
<td>RER</td>
<td>0.95 ± 0.01</td>
<td>0.94 ± 0.01</td>
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Data are expressed as means ± SE. Subjects performed 60-min of cycle ergometry during each trial. EX-FAST, exercise–fast (noncaloric placebo ingestion); EX-FED, exercise–fed (5 kcal, 0.83 g carbohydrate, 0.37 g protein, and 0.03 g fat); HR, heart rate; RER, respiratory exchange ratio.
ery period between trials (Table 2). Plasma fatty acids were lower at 2 h, 3 h, and 4 h postexercise in EX-FED compared with EX-FAST (Table 2).

**Plasma Insulin**

Plasma insulin concentrations were similar between trials immediately postexercise. Plasma insulin was higher at 2 h, 3 h, and 4 h postexercise in EX-FED compared with EX-FAST (Table 2).

**Muscle Glycogen**

Resting muscle glycogen concentration (468 ± 30 mmol/kg dry wt) was higher (*P < 0.05) compared with postexercise during EX-FAST and EX-FED, suggesting that the exercise session significantly lowered muscle glycogen. Muscle glycogen was higher (*P < 0.05) at 2 h and 6 h during EX-FED (223 ± 36 and 231 ± 38 mmol/kg dry wt, respectively) compared with EX-FAST (162 ± 29 and 165 ± 28 mmol/kg dry wt for 2 h and 6 h, respectively).

**Mixed-Muscle Protein Synthesis**

Plasma [2H5]-phenylalanine enrichments were stable during infusion periods for REST, EX-FAST, and EX-FED trials, demonstrating isotope steady-state (Fig. 2). The average plasma [2H5]-phenylalanine enrichment was 0.051 ± 0.001, 0.052 ± 0.001, and 0.065 ± 0.002 for REST, EX-FAST, and EX-FED, respectively. Muscle intracellular free [3H]-phenylalanine enrichments were 0.040 ± 0.003, 0.044 ± 0.002, and 0.054 ± 0.001 for REST, EX-FAST, and EX-FED, respectively.

Mixed muscle FSR was higher (*P < 0.05) during both EX-FED (0.112 ± 0.010%·h⁻¹) and EX-FED (0.129 ± 0.014%·h⁻¹) compared with REST (0.071 ± 0.005%·h⁻¹) (Fig. 3). No significant differences were apparent between EX-FAST and EX-FED.

**Plasma Ra**

Whole-body proteolysis, reflected by phenylalanine Ra into plasma, was lower (*P < 0.05) in EX-FED (0.83 ± 0.03 µmol·kg⁻¹·min⁻¹) compared with REST (1.06 ± 0.02 µmol·kg⁻¹·min⁻¹) and EX-FAST (1.05 ± 0.02 µmol·kg⁻¹·min⁻¹).

**mRNA Expression**

All gene expression data are presented as fold change from REST. MuRF-1 was higher (*P < 0.05) at 2 h and 6 h after exercise during EX-FAST and only at 2 h during EX-FED (Fig. 3). There was an interaction between trials at 6 h, indicating that MuRF-1 mRNA expression was attenuated at 6 h during EX-FED. FOXO3A was lower (*P < 0.05) at 6 h postexercise for both EX-FAST and EX-FED (Fig. 4). Calpain-1 was unchanged (*P > 0.05) after exercise in both EX-FAST and EX-FED (Fig. 4). There was an interaction (*P < 0.05) between EX-FAST and EX-FED for calpain-2 at 2 h and

<table>
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<th>Glucose, mM</th>
<th>Postexercise</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
<th>6 h</th>
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<tr>
<td>EX-FAST</td>
<td>5.9 ± 0.3</td>
<td>5.2 ± 0.2</td>
<td>5.2 ± 0.1</td>
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<tr>
<td>EX-FED</td>
<td>6.3 ± 0.3</td>
<td>5.2 ± 0.4</td>
<td>5.3 ± 0.3</td>
<td>5.1 ± 0.1</td>
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<th>Fatty Acids, mM</th>
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<tr>
<td>0.68 ± 0.08</td>
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<td>0.77 ± 0.11</td>
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<td>0.64 ± 0.12</td>
<td>0.19 ± 0.01*</td>
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<td>0.58 ± 0.05</td>
<td>0.42 ± 0.06*</td>
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<tr>
<td>0.53 ± 0.06</td>
<td>0.58 ± 0.06</td>
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<td>0.61 ± 0.07</td>
<td>0.62 ± 0.05</td>
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<th>Insulin, µU/ml</th>
<th>EX-FAST</th>
<th>EX-FED</th>
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<td>9.3 ± 2.5</td>
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<td>5.5 ± 2.0</td>
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<tr>
<td>5.7 ± 2.6*</td>
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<td>5.3 ± 1.7</td>
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Data are expressed as means ± SE. *P < 0.05 compared to EX-FAST.

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Fig. 2. Plasma [2H5]-phenylalanine enrichments during the resting and exercise trials. TTR, tracer-to-tracee ratio (m + 5/m + 0). Data are expressed as means ± SE.

Fig. 3. Mixed muscle protein fractional synthesis rate (FSR) at rest and following exercise during exercise-fasting (EX-FAST) and exercise-fed (EX-FED) conditions. *P < 0.05 compared with rest. Data are expressed as means ± SE.
6 h postexercise, suggesting that calpain-2 mRNA expression was attenuated during EX-FED (Fig. 4). Caspase-3 remained unchanged ($P > 0.05$) after exercise in both EX-FAST and EX-FED. Myostatin was lower ($P < 0.05$) at 2 h and 6 h postexercise during both EX-FAST and EX-FED. However, no differences were apparent between EX-FAST and EX-FED (data not shown).

MRF4 was elevated ($P < 0.05$) at 2 h and 6 h during EX-FAST only (Fig. 5). Additionally, an interaction ($P < 0.05$) between EX-FAST and EX-FED was apparent at both 2 h and 6 h, indicating that MRF4 was blunted during EX-FED (Fig. 5).

PGC-1α was elevated ($P < 0.05$) at 2 h and 6 h postexercise during both EX-FAST and EX-FED (Fig. 6). Conversely, TFAM was unchanged after exercise in both trials.

**DISCUSSION**

The primary findings from this investigation were that 1) mixed muscle protein synthesis is elevated in the immediate hours following one session of aerobic exercise performed with moderate duration (60 min) and intensity (72% $V_{O_2}$ max) in the fasted state; 2) ingestion of carbohydrate plus protein (i.e., feeding) after exercise did not measurably stimulate mixed muscle FSR above fasted state values between 2 and 6 h postexercise; 3) feeding after exercise attenuated skeletal muscle mRNA expression of proteolytic markers MuRF-1 and calpain-2; and 4) feeding after exercise blunted skeletal muscle mRNA expression of the myogenic marker MRF4. These findings indicate that aerobic exercise stimulates muscle FSR, even in the absence of exogenous nutrient provision. While feeding in the postexercise period did not significantly further augment muscle FSR, the suppression of whole-body proteolysis and proteolytic markers suggests that feeding may reduce muscle protein breakdown. Collectively, these data indicate that nutritional intake following aerobic exercise may create a net anabolic environment that would enhance muscle recovery and potentially optimize adaptations to exercise training.
To our knowledge, this is the first study to report muscle FSR after aerobic exercise in both the fasted and fed states. Several studies have reported muscle FSR following aerobic exercise (5, 8, 20, 24, 33, 44, 48, 50); however, most have induced hyperaminoacidemia through feeding or infusion of amino acids (20, 24, 33, 50) or have not included a resting measure (5). There is precedent in human skeletal muscle that aerobic exercise stimulates muscle FSR in the absence of feeding (8, 44); however, both of these studies used relatively low exercise intensities (i.e., 45% \( V_{\text{O}_2}\) max) and walking exercise, which incorporates both concentric and eccentric loads on the muscle examined (i.e., vastus lateralis). Our results extend these findings to show that cycling exercise performed at moderate intensity (72% \( V_{\text{O}_2}\) max) also significantly stimulates muscle FSR in the hours immediately postexercise. Interestingly, providing a meal containing adequate carbohydrate for glycogen resynthesis (0.83 g·kg\(^{-1}\)·h\(^{-1}\)) and sufficient protein (0.37 g·kg body wt\(^{-1}\)·h\(^{-1}\) or \(\sim 27\) g/h) to stimulate muscle FSR did not result in a further increase in mixed muscle FSR above values obtained in the fasted state. In comparison, postexercise feeding has been shown to exert a stimulatory influence on mixed-muscle protein FSR following resistance exercise (34). However, it is unclear whether the divergent response between studies is due to the mode of exercise (aerobic vs. resistance) or the timeframe of feeding and FSR assessment after exercise.

Our aim was to examine a practical feeding regimen that provides physiological relevance to real-world applications. To achieve this, we used a two-bolus-feeding regimen of a commercially available product instead of inducing hyperaminoacidemia via intravenous infusion of nutrients or with consistent pulse feedings throughout the entire assessment timeframe, as we and others have previously performed (20, 24, 33, 50). A similar single-bolus-feeding approach has been used previously to assess the influence of varying protein amounts on muscle FSR following resistance exercise (34). The stimulatory effect of amino acids on muscle FSR is transient (4), although this relationship may be prolonged in the postexercise state (35). Therefore, it is possible that the feeding response may not have been robust enough to be detected during the 2–6 h timeframe after exercise, which was initiated 1 h after feeding. Despite this possibility, these data provide novel insights into the interactions between feeding and muscle metabolism during recovery from aerobic exercise.

In addition to the influence of exercise on muscle protein synthesis, muscle protein breakdown is elevated after both aerobic (44) and resistance (3, 40) exercise. We have previously shown that the mRNA transcripts of proteolytic factors (e.g., FOXO3A, MuRF-1) are elevated following exercise (20, 32), which may serve as a mechanism through which exercise elevates muscle proteolysis. MuRF-1 and FOXO3A are components of the ubiquitin-proteosome pathway that is responsible for the majority of skeletal muscle protein degradation (16). Specifically, these factors target myofibrillar proteins for degradation and have been implicated in the degradation of select mitochondrial proteins (52, 53). To our knowledge, these are the first data to report that feeding after exercise attenuates MuRF-1 mRNA expression after aerobic exercise. Glynn et al. (17) recently reported that feeding after resistance exercise did...
not alter MuRF-1 mRNA or protein levels 2 h postexercise, which is consistent with the current data reporting a blunting of MuRF-1 mRNA at 6 h but not 2 h after exercise. Because of the role of MuRF-1 in mediating protein breakdown, these data suggest that the feeding regimen may inhibit or reduce protein breakdown after aerobic exercise. Future studies are warranted to further explore this relationship, incorporating direct measures of protein breakdown.

Calpain-1 and -2 (also known as -a and -m) are components of the Ca\(^{2+}\)-activated protein degradation that appear to be initiators of myofibrillar protein degradation (26). Little is known regarding the role of calpains in response to exercise; however, they are generally thought to play a pivotal role in the muscle remodeling process after muscle-damaging exercise (1). In support of this, calpain-2 mRNA abundance is elevated 2 days after exhaustive jumping exercise (28), 1 day after eccentric exercise (14), and in response to muscle reloading following hindlimb suspension (46). Our data reveal that calpain-2 mRNA is elevated in the immediate hours following cycling exercise, which is not typically associated with muscle damage. Furthermore, feeding after exercise blunted the increase in calpain-2 mRNA, providing another potential mechanism suggesting that feeding may influence myofibrillar breakdown following exercise. The influence of exercise on myofibrillar breakdown has not been clearly elucidated; however, our laboratory has previously reported that myofibrillar breakdown is not altered after aerobic exercise in the fed state (22). The blunting of calpain-2 mRNA induction after exercise by feeding is consistent with a lack of elevation of myofibrillar breakdown.

The mechanisms underlying the influence of feeding on the attenuation of proteolytic mRNA expression after exercise is likely mediated through insulin, as insulin exhibits an antiproteolytic influence on skeletal muscle (15, 18, 31). Greenhaff et al. (18) comprehensively investigated the influence of amino acids and insulin on muscle protein metabolism in the absence of exercise, incorporating measures of proteolytic mRNA expression. Interestingly, they reported an inhibitory influence of insulin on muscle protein breakdown without an appreciable change in the expression of proteolytic genes (i.e., MuRF-1, calpain-1, calpain-2) (18). In the current study, we report that feeding and the resultant increase in circulating insulin attenuates the transcriptional response of MuRF-1 and calpain-2 following exercise. MuRF-1 transcription was likely induced after exercise through NF-κB signaling (27), while MuRF-1 mRNA expression was likely suppressed by the presence of insulin through the Akt-FOXO signaling pathway (47) during the feeding trial. Another plausible mechanism for the attenuated MuRF-1 mRNA expression with feeding is through AMPK signaling as AMPK activation has recently been linked with myofibrillar degradation (36) and induction of MuRF-1 transcription (49). On the basis of previous research (10), it is reasonable to suspect that AMPK activity was elevated after the exercise protocol in the current study. AMPK is sensitive to muscle glycogen levels (11, 54), and therefore, the glycogen resynthesis during the feeding trial may have altered AMPK signaling and ultimately attenuated MuRF-1 expression. The suppression of calpain-2 mRNA with feeding was potentially mediated through a similar mechanism; however, this is only conjecture at this point, as relatively little is known regarding the regulation of calpain-2 expression by exercise and nutrition.

An unexpected finding from the current study was the attenuation of MRF4 mRNA with feeding. MRF is a member of the family of muscle regulatory factors and is involved in myogenesis and differentiation of myoblasts into mature myofibers (23). Consistent with our previous work (20, 55), we observed that MRF4 mRNA expression is upregulated during recovery from exercise. Contrary to our hypothesis, feeding attenuated MRF4 mRNA expression following exercise. Our laboratory has previously shown that both myogenic and proteolytic genes are upregulated in sarcopenic muscle (43), and it has been proposed that during muscle atrophy, MRF4 mRNA expression is upregulated in an attempt to offset the atrophic process. The exact role of MRF4 in adult human muscle remains unclear; however, it has been suggested that MRF4 is involved in the maintenance of terminally differentiated cells (39). Therefore, in the immediate postexercise period, MRF4 is upregulated in concert with proteolytic genes as part of the muscle remodeling process. Along these lines, it is possible that the same mechanism that attenuated MuRF-1 and calpain-2 mRNA expression with feeding may have also blunted MRF4 mRNA expression.

**Perspectives and Significance**

Aerobically trained skeletal muscle is characterized by a high oxidative capacity and in some cases muscle fiber hypertrophy (12, 13, 19, 21). While the mechanisms of exercise-induced mitochondrial biogenesis have been comprehensively investigated, the alterations in muscle protein metabolism during recovery from aerobic type exercise have been less characterized. Results from this study demonstrate that aerobic exercise performed with moderate duration and intensity significantly stimulates mixed-muscle protein FSR, even in the fasted state. Although we did not specifically assess the FSR of the various muscle protein fractions (i.e., myofibrillar, sarcoplasmic, mitochondrial), it is probable that the detectable change in mixed muscle protein FSR was primarily due to changes in mitochondrial protein FSR (50), although future work is needed to verify this point. This finding extends previous research reporting elevated protein FSR after low-intensity exercise (8, 44), to demonstrate that aerobic exercise performed at moderate to high intensity, which significantly reduces muscle glycogen levels, also stimulates mixed-muscle protein FSR. Interestingly, feeding after exercise did not further stimulate protein FSR, which may be due to the timing of postexercise FSR measurement or because the feeding effect was minimal and/or transient in nature and was, therefore, not detected. Postexercise feeding did attenuate the transcription of proteolytic markers MuRF-1 and calpain-2, suggesting a potential role of feeding after aerobic exercise on muscle protein breakdown. The association between mRNA expression of these proteolytic factors and their protein content or function was not directly assessed in the current study; therefore, the role of feeding on postexercise protein degradation remains speculative. A reduction of protein breakdown coupled with similar or slightly elevated levels of protein FSR in the presence of feeding would potentially enhance muscle recovery by creating a positive muscle-protein balance. Collectively, these data provide molecular insights into the regulation of muscle protein metabolism during feeding and aerobic exercise.
protein metabolism and recovery by feeding in the hours immediately following aerobic exercise.

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