Protein synthesis and the expression of growth-related genes are altered by running in human vastus lateralis and soleus muscles

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Harber MP, Crane JD, Dickinson JM, Jemiolo B, Raue U, Trappe TA, Trappe SW. Protein synthesis and the expression of growth-related genes are altered by running in human vastus lateralis and soleus muscles. Am J Physiol Regul Integr Comp Physiol 296: R708–R714, 2009. First published December 31, 2008; doi:10.1152/ajpregu.90906.2008.— Recent evidence suggests that aerobic exercise may help preserve soleus muscle mass during unloading. The purpose of this investigation was to examine the muscle-specific metabolic response to running as it relates to muscle growth. Mixed-muscle protein synthesis [fractional synthetic rate (FSR)] and gene expression (GE) were examined in the vastus lateralis (VL) and soleus (SOL) muscles from eight men (26 ± 2 yr; V̇O₂max 63 ± 2 ml·kg⁻¹·min⁻¹) before and after a 45-min level-grade treadmill run at 77 ± 1% intensity. Muscle glycogen utilization was similar between muscles. Resting FSR was similar between the VL (0.080 ± 0.007%/h) and SOL (0.086 ± 0.008%/h) and was higher (P < 0.05) at 24 h postexercise compared with rest for both muscles. The absolute change in FSR was not different between muscles (0.030 ± 0.007%/h vs. 0.037 ± 0.012%/h for VL and SOL). At baseline, myostatin GE was approximately twofold higher (P < 0.05) in SOL compared with VL, while no other muscle-specific differences in GE were present. After running, myostatin GE was suppressed (P < 0.05) in both muscles at 4 h and was higher (P < 0.05) than baseline at 24 h for VL only. Muscle regulatory factor 4 mRNA was elevated (P < 0.05) at 4 h in both SOL and VL; MyoD and peroxisome-proliferator-activated receptor-gamma coactivator-1α (PGC-1α) were higher (P < 0.05) at 4 h, and forkhead box [FOXO]3A was higher at 24 h in SOL only, while muscle-RING-finger protein-1 (MuRF-1) was higher (P < 0.05) at 4 h in VL only. Myogenin and atrogin-1 GE were unaltered. The similarity increases between muscles in FSR support running as part of the exercise countermeasure to preserve soleus mass during unloading. The subtle differences in GE suggest a potential mechanism for muscle-specific adaptations to chronic run training.

MATERIALS AND METHODS

Subjects and study overview. Eight male subjects volunteered to participate in this investigation (Table 1). All subjects were aerobically trained and able to complete a continuous run of 45 min at 75% of their V̇O₂max. Seven subjects had a competitive run history of > 5 yr. Additionally, all subjects were nonsmokers, not overweight or obese, and apparently healthy as determined from a detailed medical history questionnaire. This study was approved by the Institutional Review Board of Ball State University, and informed consent was obtained from all subjects prior to their participation. Qualified subjects completed three experimental trials in a fixed order. The first trial consisted of the measurement of resting protein synthesis. Approximately 1 wk later, subjects performed an exercise trial for the measurement of muscle glycogen and mRNA expression.

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The morning after the exercise trial (~24 h postexercise) subjects returned to the laboratory for the measurement of postexercise protein synthesis and gene expression. The experimental trials are described in more detail below. Prior to the experimental trials, qualified subjects underwent a treadmill test for the determination of VO_{2\text{max}} as we have described previously (12, 13, 38) and to determine the speed necessary to elicit ~75% of VO_{2\text{max}}. Prior to the measurement of resting protein synthesis and the exercise trial (described in detail below), subjects were asked to refrain from any exercise for 72 h. The evening before each experimental trial, subjects consumed a standardized meal (Ensure Plus; Abbott Laboratories, Abbot Park, IL) with a macronutrient composition of 57% carbohydrate, 15% protein, and 28% fat with a caloric content of 17 kcal/kg body wt.

Experimental trials. Subjects reported to the laboratory after an overnight fast on the morning of each experimental trial. Mixed-muscle protein synthesis was assessed using a primed constant infusion for 6 h. Shortly after arrival at the laboratory, a catheter was inserted into an antecubital vein for the infusion of [2H_5]phenylalanine (Cambridge Isotopes, Andover, MA) at a rate of 0.05 μmol·kg⁻¹·h⁻¹ following a priming dose of 2 μmol/kg as we have previously performed (7). A second catheter was placed in an antecubital vein of the contralateral arm for blood sampling at 0, 2, 3, 4, 5, and 6 h of the infusion for the measurement of plasma isotope enrichment. Muscle biopsies were obtained from the vastus lateralis and soleus muscles at 2 and 6 h during the isotope infusion for determination of the incorporation of [1H_2]phenylalanine into mixed-muscle protein.

The exercise trial consisted of a 45-min treadmill run at ~75% VO_{2\text{max}}. Muscle biopsies from the vastus lateralis and soleus muscles were obtained prior to and 4 h after the exercise bout for the determination of muscle glycogen concentration and mRNA expression. Expired gas samples were obtained at two periods during the exercise bout to determine exercise intensity. Subjects remained fasted through the 4-h postexercise biopsy.

Postexercise protein synthesis was measured the following morning, ~24 h after the running bout. The experimental procedures were similar to the resting measure, except for the administration of unlabeled amino acids (10% Trasoval; Clintec Nutrition, Deerfield, IL), which were initiated immediately following the 2-h biopsies at a rate of 1.35 ml·kg⁻¹·h⁻¹ following a prime of 0.45 ml/kg (7). Additionally, the infusion rate of [1H_2]phenylalanine was increased to 0.10 μmol·kg⁻¹·h⁻¹ with a second priming dose of 0.6 μmol/kg at the onset of the unlabeled amino acid infusion to maintain steady plasma tracer enrichment. Muscle biopsies. A total of 12 muscle biopsies (6 each from the vastus lateralis and soleus muscles) were obtained for the entire study protocol. At each time point, percutaneous needle biopsies were obtained under 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 RNALater (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.

Stable isotope tracer analysis. The rate of mixed-muscle protein synthesis was determined by quantifying the tissue fluid and protein-bound [1H_2]phenylalanine enrichment (tracer to tracee) in muscle samples (~20 mg) from the vastus lateralis and soleus muscles as we have previously described (7, 30, 41). All samples were analyzed using gas chromatography-mass spectrometry (GC-MS; GC-6890N GC coupled with 5973 inert MSD; Agilent Technologies, Wilmington, DE) in triplicate using electron impact ionization and selected ion monitoring of m/z 234 (m+0), 235 (m+1), 237 (m+3), and 239 (m+5), with m+0 representing the lowest molecular weight of the ion. Plasma and muscle intracellular [1H_2]phenylalanine enrichments were measured using the m+5-to-m+0 ratio. Enrichments of the protein-bound samples were determined using the m+5-to-m+3 ratio and a single linear standard curve from mixtures of known m+5-to-m+0 ratios, as previously described (5, 28). Elimination of bias due to any potential concentration dependency was accomplished by injecting nearly equal amounts of phenylalanine (i.e., similar m+0 abundances) for all samples and standards.

Mixed-muscle fractional synthetic rate (FSR) was calculated as the rate of [1H_2]phenylalanine tracer incorporated into muscle protein using the muscle tissue fluid phenylalanine enrichment as the precursor and the following equation: FSR (%/h) = [(E_t - E_0)/E_0](t_1 – t_0) where E_t and E_0 are the phenylalanine tracer enrichments in the protein-bound fraction, (t_1 – t_0) is the phenylalanine trace incorporation time, and E_p is the muscle intracellular-free phenylalanine enrichment.

Muscle glycogen concentration. Muscle glycogen content was determined from muscle biopsy samples from the vastus lateralis and soleus muscles before and 4 h following the run exercise. Glycogen content was determined from the measurement of glucose after acid hydrolysis of muscle samples with hydrochloric acid (27).

Myosin heavy chain composition. Myosin heavy chain (MHC) composition was determined from muscle homogenates by SDS-PAGE as described previously (11). Briefly, 100 ng protein were run overnight at 4°C on a Hoeffer SE 600 gel electrophoresis unit utilizing a 3.5% acrylamide stacking gel with a 5% separating gel. After electrophoresis, gels were silver stained as we have described previously. MHC isoforms (I, IIa, IIx) were identified according to migration rate, and the relative proportion of each MHC isoform was determined by densitometry.

Total RNA extraction and RNA quality check. All the methods for RNA extraction and real-time RT-PCR have been described in detail previously by our laboratory (32, 46). Total RNA was extracted in TRI reagent (Molecular Research Center, Cincinnati, OH). The quantity and integrity of extracted total RNA was evaluated using an RNA 6000 Nano LabChip kit on an Agilent 2100 Bioanalyzer.

RT and real-time PCR. Oligo(dt) primed first-strand complementary DNA (cDNA) was synthesized using SuperScript II RT (Invitrogen, Carlsbad, CA) optimized for sensitive RT-PCR on low amounts of RNA. Quantification of messenger RNA (mRNA) transcription (in duplicate) was performed in a 72-well Rotor-Gene 3,000 Centrifugal Real-Time Cycler (Corbett Research, Mortlake, NSW, Australia). GAPDH was used as a housekeeping gene (HKG) for internal control after validation (32). All primers used in this study were mRNA specific (on different exons and/or crossing over an intron) and designed for gene expression real-time PCR analysis using Vector NTI Advance 9 software (Invitrogen). Primers for MyoD, myogenin, muscle regulatory factor 4 (MRF4), atrogin-1, muscle-RING-finger protein-1 (MuRF-1), and forkhead box [FOXO]3A have been reported previously by our laboratory (21, 46). The primer sequences for peroxisome proliferator-activated receptor-gamma coactivator 1-α (PGC-1α): forward primer, 5′-GGCCCAGGTATGACGCTACGGAGGA-3′ and reverse primer, 5′-TCAATTGGCITCTGCTCCTGGCCTCTC-3′.

A melting curve analysis was generated at the end of each real-time PCR assay. A single-melt peak was observed for each sample, validating that only one product was present. The presence of PCR inhibitors within our biological samples was assessed using SPUD assay [an artificial amplicon-based Solanum tuberosum phyB gene of potato root (26)]. The assay generated CT values (22.5–22.9) characterizing...
teristic of an uninhibited SPUD assay showing that no inhibitors were present in the cDNA generated from the muscle tissue samples in the current study.

Relative quantification of real-time PCR assay. The gene expression in relation to muscle and exercise was evaluated by a relative quantification method as described by us previously (32, 46). To avoid interassay variability, each real-time PCR run contained samples from both muscles (vastus lateralis and soleus) and both experimental conditions (pre- and postexercise).

The data were analyzed using $2^{-\Delta\Delta Ct}$ and $2^{-\Delta Ct}$ methods (20). As described by us previously (32), to compare the relative gene expression between the vastus lateralis and soleus muscles at baseline, the $2^{-\Delta\Delta Ct}$ method was used. This method generates a value in arbitrary units (AU) of the gene of interest (GOI) expression normalized to the HKG expression ($\Delta\Delta Ct = C_{T\ GOI} - C_{T\ HKG}$). The $2^{-\Delta Ct}$ method was used to calculate the fold change in gene expression as a result of the run exercise. In this method, GOI expression was normalized to HKG expression and calibrated to control preexercise value ($C_{T\ GOI\ time\ X}$) within each muscle. Using this analysis, the fold changes at time 0 (control = pre), if not influenced by external stimuli, should be very close to 1.

Statistical analysis. A two-way analysis of variance (ANOVA) with repeated measures on muscle (vastus lateralis vs. soleus) and condition (rest vs. postexercise) was used to compare muscle protein synthesis and muscle glycogen content. A paired $t$-test was used to compare MHC composition, absolute and relative changes in protein synthesis, baseline muscle glycogen content, and baseline gene expression differences between the muscles. The changes in mRNA levels with respect to preexercise levels at 4 and 24 h postexercise for each gene were analyzed using the nonparametric Friedman test. Significant differences were determined using the pairwise comparison post hoc test. Significance for all analysis was set at $P < 0.05$. Data are presented as means ± SE.

RESULTS

Exercise trial. All subjects successfully completed the exercise trial at an average intensity of 77 ± 1% $\bar{V}_{O_2\max}$. Energy expenditure during the 45-min run was 771 ± 24 kcal at an respiratory exchange ratio of 0.94 ± 0.11, representing substrate utilization of 81% of the total energy expenditure from carbohydrate and 19% from fat. Additionally, average heart rate during the exercise trial was 170 ± 5 beats/min. Assuming an average running cadence of 180 steps/min, we estimate that the muscles examined performed over 4,000 muscle contractions during the exercise session.

Plasma $[^2\text{H}_5]\text{phenylalanine enrichment and mixed-muscle protein synthesis.}$ Plasma $[^2\text{H}_5]\text{phenylalanine enrichments were stable during the infusion periods for resting and postexercise trials (Table 2). Resting mixed-muscle FSR was similar between the vastus lateralis (0.080 ± 0.007 %/h) and soleus (0.086 ± 0.008 %/h) muscles (Fig. 1). FSR was higher ($P < 0.05$) postexercise compared with rest for both muscles (Fig. 1). The absolute change in FSR from rest to postexercise was not different between muscles

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<th>Time</th>
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Values are means ± SE. Data reflect the tracer-to-tracee ratio ($m_{5}/m_{0}$).
These data are the first to examine the muscle-specific protein synthetic and gene responses to acute aerobic exercise. Resting mixed-muscle protein synthesis rates were not different between muscles in our subjects, which is in agreement with previous data from our laboratory (7) and others (25) in untrained subjects. However, the rates of protein synthesis observed in our subjects are slightly higher than we have reported previously (7), which is likely a function of the training status of our subjects. Endurance-trained individuals have elevated nonoxidized leucine disposal (19) and short-term endurance training has been shown to increase resting mixed-muscle protein synthesis in the vastus lateralis (31). Interestingly, myostatin transcript level was approximately twofold higher in the soleus muscle compared with the vastus lateralis muscle at baseline. Myostatin is a negative regulator of muscle growth, and its expression has been inversely associated with protein synthesis in humans (29) and rodents (43). Furthermore, it has been postulated that myostatin expression is positively associated with muscle mass (17). Soleus muscle fibers are typically larger than fibers from the vastus lateralis, which may account for the greater myostatin expression in the soleus (9, 22). Despite previous reports linking myostatin gene expression to muscle protein synthesis, our data do not support this relationship as we report similar resting synthetic rates despite drastically different myostatin transcript levels. Interestingly, no baseline muscle-specific differences in mRNA expression were evident for the other myogenic and proteolytic genes examined, suggesting the differences in baseline morphology between muscles do not alter the expression of these genes involved in muscle remodeling.

There are limited data describing how various muscles respond to the same exercise bout. Trappe et al. (41) showed that the resistance exercise-induced increase in mixed-muscle protein synthesis is partially blunted in the soleus compared with the vastus lateralis. The authors suggest this differential response to an acute exercise session may underlie the impaired adaptive response of the soleus muscle to chronic resistance exercise (42, 47). In response to prolonged unloading, the soleus muscle displays a greater degree of atrophy relative to the vastus lateralis and is less responsive to resistance exercise countermeasures (1). The addition of aerobic exercise to the countermeasure is more effective than resistance exercise alone, suggesting that aerobic exercise may prevent the atrophic effect of unloading on the soleus (40). The similar increase in mixed-muscle protein synthesis between muscles suggests that the soleus is acutely more responsive to aerobic exercise, as the soleus has previously shown a diminished response to resistance exercise.

We examined the mRNA levels of select genes involved in muscle growth and breakdown processes in both muscles in response to run exercise. Overall, the soleus muscle appears...
more responsive to run exercise, as five of the eight genes examined were altered after the exercise session compared with three of eight in the vastus lateralis. Muscle glycogen utilization patterns were similar between muscles, suggesting that both muscles were subjected to comparable levels of metabolic stress during the run exercise. Therefore, differences in gene expression after the run exercise are likely a reflection of a muscle-specific response to run exercise rather than different levels of muscle use.

Combining the current data with prior work from our laboratory (21, 46) permits the comparison of the mRNA response to run exercise in three different leg muscles. We have previously reported that an acute bout of running does not alter myogenic gene expression while inducing the expression of proteolytic markers atrogin-1 and MuRF-1 in the gastrocnemius (21, 46). These findings are consistent with a reduction in gastrocnemius fiber size that has been reported following run training (38). In contrast, the current data reveal an increase in the myogenic genes MRF4 (both soleus and vastus lateralis) and MyoD (soleus only) 4 h after run exercise with limited induction of proteolytic genes. A reduction in myostatin post-exercise is consistent between the three muscles; however, elevated expression at 24 h is specific to the vastus lateralis. It is possible that the greater eccentric load on the vastus lateralis compared with the calf muscles during running (4) led to the differential response in myostatin gene expression between muscles, as eccentric muscle actions induce a greater myostatin response than concentric muscle actions (14). Another possible explanation is the morphological difference between muscles as fiber-type-specific responses after exercise have been reported in rodent models (23). Overall, these data indicate a muscle-specific response in gene expression in the immediate hours following run exercise, which suggest that musclespecific differences in adaptations in response to training are possible.

Alterations in protein metabolism following aerobic exercise are not well characterized and are understudied relative to resistance exercise. We chose to assess protein synthesis 24 h postexercise based on data from Miller et al. (24) who reported that fed-state protein synthesis peaks at 24 h after aerobic exercise and remains elevated for 48–72 h. Unlike resistance exercise (41), it appears aerobic exercise induces similar changes in translational processes at the whole muscle level. It is possible that the various protein fractions (i.e., mitochondrial, myofibrillar, sarcoplasmic) that comprise mixed-muscle protein synthesis were stimulated differently in the two muscles. In support of this, PGC-1α expression was significantly upregulated after exercise in the soleus only. PGC-1α is a regulator of mitochondrial biogenesis; therefore, it is feasible that the increased protein turnover after exercise was geared more toward oxidative processes (i.e., mitochondrial fraction) in the soleus compared with the vastus lateralis (45). Such a disparity could lead to muscle-specific adaptations over time, independent of similar changes in mixed-muscle protein synthesis.
We chose to examine postexercise protein synthesis in the fed state to maximize any potential differences between muscles and because previous data from our laboratory have shown that mixed-muscle protein synthesis is elevated similarly in both muscles during amino acid infusion (7). Interestingly, the absolute increase in protein synthesis (0.037 ± 0.012) and 0.030 ± 0.007 %/h, for soleus and vastus lateralis, respectively) we report after run exercise in the current study is similar to what has been previously shown in response to the identical amino acid infusion independent of an exercise stimulus (7). We cannot fully discount the possibility that the increase in postexercise protein synthesis was not appreciably influenced by the exercise session. However, several previous studies have reported increases in rates of mixed-muscle (6, 34), sarcoplasmic and myofibrillar (24), mitochondrial (44), or whole-body (8, 33) fractional synthesis rates after a single session of aerobic exercise, suggesting that our observed change in synthesis rates was influenced by the run exercise. It is possible that the training status of our subjects and their familiarity with the exercise mode contributed to a diminished exercise-stimulated mixed-muscle protein synthesis response as trained subjects display a refined exercise-stimulated response in protein synthesis (18, 35, 44). Interestingly, the resting protein synthesis rates in our subjects are similar to values reported by Bolster et al. (3) in trained runners in the hours immediately following an acute running session. The lack of a resting measure of protein synthesis in their study makes it difficult to interpret the influence of the exercise bout. Although our study design does not permit us to delineate the individual effects of run exercise and amino acid infusion on mixed-muscle protein synthesis, pursuant to our original purpose, our results indicate that the soleus and vastus lateralis muscles are equally responsive to run exercise at the level of protein synthesis.

Perspectives and Significance

This is the first investigation to report the protein synthesis and gene response to an acute exercise session in two distinctly different leg muscles within the same individual. Our data reveal that despite differences in anatomical location, loading patterns during running (4), MHC composition, and oxidative capacity (15) postexercise protein synthesis was not different between muscles. Furthermore, unlike the muscle-specific response to resistance exercise (41) and unloading (40), the vastus lateralis and soleus muscles appear to be similarly responsive, at the mixed-muscle protein synthesis level, to run exercise. In addition to basal differences in myostatin gene expression, subtle differences in gene expression existed in response to the exercise session, which if accumulated over repeated exercise sessions, may lead to muscle-specific adaptations in response to chronic run training. These findings support the role of aerobic exercise as a key component of the exercise countermeasure for preserving soleus muscle mass during unloading.

REFERENCES


