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ScienceDirect
Metabolism Clinical and Experimental 60 (2011) 689 – 697
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Influence of tracer selection on protein synthesis rates at rest and postexercise in multiple human muscles

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Received 15 February 2010; accepted 1 July 2010

Abstract

The goal of this investigation was to assess the influence of tracer selection on mixed muscle fractional synthesis rate (FSR) at rest and postexercise during amino acid infusion in multiple human skeletal muscles. Fractional synthesis rate was measured before and 24 hours after 45 minutes of running using simultaneous infusion of [$^2H_5$]-phenylalanine (Phe) and [$^2H_3$]-leucine (Leu) coupled with muscle biopsies from the vastus lateralis and soleus in aerobically trained men (n = 8; age, 26 ± 2 years). Mixed muscle protein FSR was analyzed by gas chromatography–mass spectrometry combined with a standard curve using the enriched muscle tissue fluid as the precursor pool. To control for potential analytical differences between tracers, all samples and standards for both tracers were matched for $m + 0$ abundance. Tracer selection did not influence resting FSR for the vastus lateralis or soleus ($P > .05$). Fractional synthesis rate measured 24 hours postexercise was higher ($P < .05$) compared with rate at rest and was similar between tracers for the vastus lateralis (Phe, 0.110% ± 0.010%·h^{-1}; Leu, 0.109% ± 0.005%·h^{-1}) and soleus (Phe, 0.123% ± 0.008%·h^{-1}; Leu, 0.122% ± 0.005%·h^{-1}). These data demonstrate that tracer selection does not influence the assessment of resting or postexercise FSR, thereby supporting the use of both [$^2H_5$]-phenylalanine and [$^2H_3$]-leucine for the measurement of FSR in exercise-based studies of human skeletal muscle.
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1. Introduction

Assessing the rate of muscle protein synthesis in response to exercise interventions is important for continued discovery into the plasticity of skeletal muscle. The feasibility of measuring the fractional synthesis rate (FSR) of mixed muscle protein was enhanced with the introduction of a technique using gas chromatography–mass spectrometry (GC-MS) to detect very low isotopic enrichments. Measuring the incorporation of a multilabeled stable amino acid tracer with GC-MS combined with a standard curve was originally reported using [$^2H_5$]-phenylalanine [1] and later refined to be used with [$^2H_3$]-leucine [2]. A review of the literature reveals that the vast majority of studies using the GC-MS combined with standard curve approach have used [$^2H_5$]-phenylalanine as the tracer of choice. Carbon-based leucine tracers (ie, ^{13}C) are also commonly used for the assessment of muscle protein synthesis; however, analysis of [^{13}C]-leucine requires the use of isotope ratio mass spectrometry (IRMS), which limits the practicality of the method because of the instrumentation required. Use of a multilabeled leucine tracer, such as [$^2H_3$]-leucine, negates the need for IRMS analysis; however, to our knowledge, only 3 studies have used [$^2H_3$]-leucine to measure human muscle protein synthesis, none incorporating an exercise stimulus.

Exercise presents a dynamic stimulus to protein metabolism by altering protein synthesis, breakdown, and oxidation [3]. Furthermore, specific amino acids, namely, phenylalanine and leucine, are differentially metabolized in skeletal muscle, particularly in response to exercise [4]. Interestingly, despite inherent differences in phenylalanine and leucine metabolism within skeletal muscle, it is unknown if leucine- and phenylalanine-based tracers yield similar quantitative and qualitative responses to exercise in investigations of human skeletal muscle protein metabolism.

Therefore, we designed this investigation with 2 primary objectives: (1) to compare muscle protein FSR values obtained with stable isotope tracers of 2 different commonly used amino acids (phenylalanine and leucine) and (2) to examine the influence of aerobic exercise on protein

Institutional approval: 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.

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0026-0495/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2010.07.003

metabolism. The first objective was examined at rest and postexercise during amino acid infusion using simultaneous infusion of both [$^2H_5$]-phenylalanine and [$^2H_3$]-leucine, and the results of this investigation are presented herein. The findings pertaining to the influence of aerobic exercise on protein metabolism have been previously published [5]. We chose to measure postexercise protein synthesis during amino acid infusion at 24 hours postexercise to maximally stimulate protein synthesis [6]. Additionally, we made these comparisons in the vastus lateralis and soleus muscles, which display divergent fiber-types composition [24], metabolic capacity [27], contractile properties [28], response to unloading [5,11,13,24,25] and response to exercise [13]. Findings from this investigation provide important insight into the feasibility of multiple approaches for measuring skeletal muscle protein synthesis in exercise-based human studies.

2. Materials and methods

2.1. Subjects and study overview

Eight male subjects (age, 26 ± 2 years) volunteered to participate in this investigation. All subjects were aerobically trained (maximum oxygen consumption [V_{O_2}max], 63 mL·kg^{-1}·min^{-1}) and able to complete a continuous run of 45 minutes at 75% of their V_{O_2}max. In addition, 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 written informed consent was obtained from all subjects before their participation.

Qualified subjects underwent a treadmill test for the determination of V_{O_2}max as we have described previously [7-9] and to determine the speed necessary to elicit approximately 75% of V_{O_2}max. Subjects then completed 3 experimental trials in a fixed order. The first trial consisted of the measurement of resting muscle protein synthesis rate. Approximately 1 week later, subjects performed an exercise trial followed the next morning (~24 hours postexercise) by the measurement of postexercise protein synthesis. The experimental trials are described in more detail below. Before the measure of resting protein synthesis and the exercise trial (described in detail below), subjects were asked to refrain from any exercise for 72 hours. 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 weight.

2.2. 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 hours. Shortly after arrival to the laboratory, a catheter was inserted into an antecubital vein for the simultaneous infusion of [$^2H_5$]-phenylalanine (prime, 2 μmol kg^{-1}; rate, 0.05 μmol kg^{-1} min^{-1}) and [$^2H_3$]-leucine (prime, 4.8 μmol kg^{-1}; rate, 0.12 μmol kg^{-1} min^{-1}) (Cambridge Isotopes, Andover, MA) as we have previously performed [5,10-14]. A second catheter was placed in an antecubital vein of the contralateral arm for blood sampling at 0, 2, 3, 4, 5, and 6 hours 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 hours during the isotope infusion for determination of the incorporation of [$^2H_5$]-phenylalanine and [$^2H_3$]-leucine into mixed muscle protein (Fig. 1).

The exercise trial consisted of a 45-minute treadmill run at approximately 75% V_{O_2}max. Expired gas samples were obtained at 2 periods during the exercise bout to determine exercise intensity [5].

Postexercise protein synthesis was measured the following morning, approximately 24 hours after the running bout. This time point corresponds to the peak increase in protein synthesis after aerobic exercise [6]. The postexercise procedures were similar to the resting measure, except for the administration of unlabeled amino acids (10% Travasol; Clintec Nutrition, Deerfield, IL) that was initiated immediately following the 2-hour biopsies at a rate of 1.35 mL kg^{-1} h^{-1} following a prime of 0.45 mL kg^{-1} [5,11]. In addition, the infusion rate of [$^2H_5$]-phenylalanine was increased to 0.10 μmol kg^{-1} min^{-1} with a second priming dose of 0.6 μmol kg^{-1} and the infusion rate of [$^2H_3$]-leucine was increased to 0.24 μmol kg^{-1} min^{-1} with a second priming dose of 1.6 μmol kg^{-1} at the onset of the unlabeled amino acid infusion to maintain steady plasma tracer enrichment.

2.3. Muscle biopsies

A total of 8 muscle biopsies (4 each from the vastus lateralis and soleus muscles) were obtained during the study protocol. At each time point, percutaneous needle biopsies were obtained under local anesthetic [15]. Muscle samples were dissected free of any visible connective and adipose tissue and divided into approximately 20-mg sections and immediately frozen and stored in liquid nitrogen (−190°C) until analysis.

2.4. Sample preparation and derivatization

The rate of mixed muscle protein synthesis was determined by quantifying the muscle tissue fluid (MTF) and protein-bound [$^2H_5$]-phenylalanine and [$^2H_3$]-leucine enrichment (tracer to tracee ratio) in muscle samples from the vastus lateralis and soleus muscles as we have previously described [5,10-14]. Each muscle sample (~20 mg) was weighed at −35°C and then homogenized in 500 μL of ice-cold 14% perchloric acid. Muscle was homogenized on ice

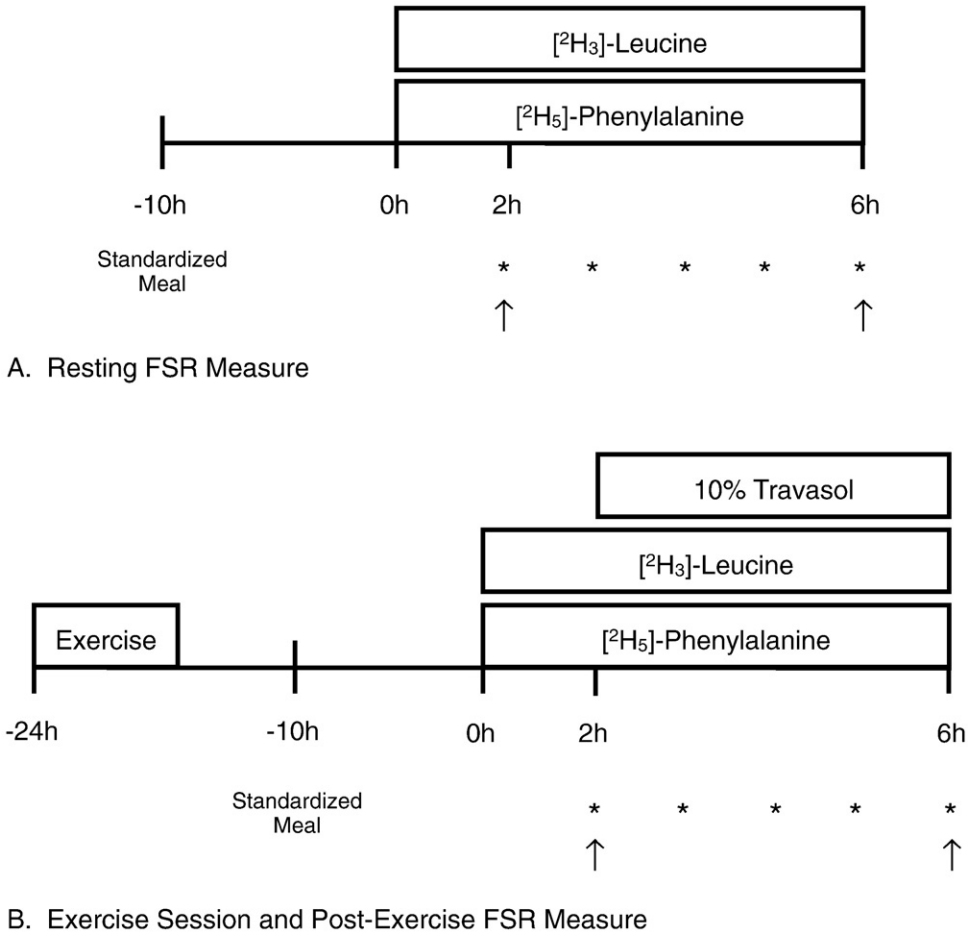

Fig. 1. Schematic of experimental design outlining (A) the resting protein synthesis (FSR) measure and (B) the exercise session and postexercise FSR measure. The exercise session consisted of a 45-minute treadmill run at 75% Vo_2max and occurred approximately 1 week following the resting FSR measure. *, blood sampling. ↑, vastus lateralis and soleus muscle biopsy.

with a Teflon-coated pestle for 1 minute and then centrifuged at 21 000*g* for 10 minutes at 4°C. The supernatant was then collected; and this process was repeated 2 more times, with all 3 supernatants combined as the MTF for that sample. The remaining pellet was washed once in deionized MilliQ (Millipore, Billerica, MA) water and 3 times in ethanol. Each wash was followed by centrifugation at 21 000*g* for 10 minutes at 4°C. The pellet, representative of mixed protein, was dried overnight at 50°C and hydrolyzed in 5 mL of 6 N hydrochloric acid for 24 hours at 100°C. Each plasma sample was deproteinized with a 1:1 ratio of 15% sulfosalicylic acid and then centrifuged at 21 000*g* for 10 minutes at 4°C.

Before derivatization, the MTF, mixed muscle protein hydrolysates, and plasma samples were washed over a cation exchange column (Dowex AG 50W-8X, 100-200 mesh, H+ form; Bio-Rad Laboratories, Hercules, CA). Amino acids were eluted from the column with 4 N ammonium hydroxide, and this elute was collected and dried under vacuum (SC210A SpeedVac Plus; ThermoSavant, Holbrook, NJ). Once dried, samples were derivatized with 100 μL of acetonitrile and *N*-methyl-*N*-(*t*-butyldimethylsilyl)trifluoroacetamide (Pierce Chemical, Rockford, IL) at a 1:1 ratio. All samples were derivatized at 100°C: MTF and plasma for 10 minutes, and mixed muscle protein for 30 minutes.

2.5. Stable isotope tracer analysis

All samples were analyzed using GC-MS (GC-6890N GC coupled with 5973 inert MSD; Agilent Technologies, Wilmington, DE) in duplicate (plasma samples) or triplicate

Table 1
Mean coefficient of variation (percentage) for multiple injections of each sample for plasma, muscle tissue fluid, and muscle protein-bound tracer enrichment

	Leucine	Phenylalanine
Plasma	0.28 ± 0.03	0.76 ± 0.34
MTF	0.49 ± 0.05	2.14 ± 0.19
Protein bound	0.20 ± 0.01	1.35 ± 0.19

Data are mean ± SE.

Table 2
Plasma [$^2H_5$]-phenylalanine and [$^2H_3$]-leucine enrichments

	3 h	4 h	5 h	6 h
[$^2H_5$]-phenylalanine				
Rest	0.053 ± 0.002	0.053 ± 0.002	0.055 ± 0.002	0.051 ± 0.002
Postexercise	0.050 ± 0.001	0.053 ± 0.001	0.054 ± 0.002	0.054 ± 0.002
[$^2H_3$]-leucine				
Rest	0.065 ± 0.003	0.063 ± 0.002	0.065 ± 0.002	0.061 ± 0.003
Postexercise	0.067 ± 0.001	0.071 ± 0.001	0.074 ± 0.001	0.073 ± 0.001

Data are mean ± SE. Data reflect the tracer to tracee ratio ($m + 5/m + 0$ for [$^2H_5$]-phenylalanine and $m + 3/m + 0$ for [$^2H_3$]-leucine). Data are from an n = 8.

(MTF and protein-bound samples) using electron impact ionization and selected ion monitoring. For [$^2H_5$]-phenylalanine, m/z 234 ($m + 0$), 235 ($m + 1$), 237 ($m + 3$), and 239 ($m + 5$) were monitored, with $m + 0$ representing the lowest molecular weight of the ion. For [$^2H_3$]-leucine, m/z 200 ($m + 0$), 202 ($m + 2$), and 203 ($m + 3$) were monitored. Plasma and MTF [$^2H_5$]-phenylalanine enrichments were measured using the $m + 5/m + 0$ ratio. Plasma and MTF [$^2H_3$]-leucine enrichments were measured using the $m + 3/m + 0$ ratio. Enrichments of the protein-bound samples were determined using the $m + 5/m + 3$ ratio and a single linear standard curve from mixtures of known $m + 5/m + 0$ ratios for [$^2H_5$]-phenylalanine and the $m + 3/m + 2$ ratio and a single linear curve from mixtures of known $m + 3/m + 0$ ratios for [$^2H_3$]-leucine, as previously described [1,2].

To accurately compare protein synthesis rates between tracers and for elimination of bias due to any potential concentration dependency, nearly equal amounts of phenylalanine and leucine (ie, similar $m + 0$ abundances) were injected for all samples and standards. Initial measurements were made on various amounts of phenylalanine and leucine to ensure that the amount of phenylalanine and leucine injected for all samples would be less than the saturation levels of the detector and would produce Gaussian-shaped peaks. The average $m + 0$ abundance for the [$^2H_5$]-phenylalanine and [$^2H_3$]-leucine standard curves was 37 849 926 ± 232 413 and 37 803 619 ± 292 912, respectively. The average $m + 0$ abundance for the muscle protein-bound fractions was 37 595 787 ± 486 535 and 36 934 838 ± 437 253 for [$^2H_5$]-phenylalanine and [$^2H_3$]-leucine, respectively. With regard to instrument sensitivity, with the $m + 0$ at approximately 37 million, the $m + 5$ for [$^2H_5$]-phenylalanine was 16 297 ± 1658 and $m + 3$ abundance for [$^2H_3$]-leucine was 295 993 ± 3541 on average in muscle protein-bound samples when considering all 4 biopsy time points.

Coefficient of variation for enrichment analysis of multiple injections on the GC-MS of the same plasma, MTF, and muscle protein-bound samples is presented in Table 1.

Mixed muscle protein FSR was calculated as the rate of [$^2H_5$]-phenylalanine or [$^2H_3$]-leucine tracer incorporated into muscle protein using the MTF phenylalanine enrichment or MTF leucine enrichment as the precursor and the following equation:

$$\mathrm{FSR}\left(\%\bullet \mathrm{h}^{-1}\right) = \left\{\left[\left(\mathrm{Et}_1 - \mathrm{Et}_0\right) / \left[\mathrm{E_p}\bullet\left(t_1 - t_0\right)\right]\right\}\bullet 100,$$

where Et_1 and Et_0 are the phenylalanine or leucine tracer enrichments in the protein-bound fraction, ($t_1 - t_0$) is the phenylalanine or leucine tracer incorporation time, and E_p is the precursor.

2.6. Statistical analysis

A 2-way (tracer × time) analysis of variance with repeated measures was used to make within-subjects comparisons to determine the influence of tracer selection on protein

Table 3
[$^2H_5$]-phenylalanine and [$^2H_3$]-leucine enrichments of the MTF during the rest and postexercise protein synthesis measures

	Rest		Postexercise[a]	
	2 h	6 h	2 h	6 h
Vastus lateralis				
[$^2H_5$]-PHE	0.036 ± 0.004	0.041 ± 0.003	0.038 ± 0.001	0.055 ± 0.001
[$^2H_3$]-LEU	0.045 ± 0.003	0.050 ± 0.002	0.044 ± 0.001	0.071 ± 0.002
Soleus*				
[$^2H_5$]-PHE	0.030 ± 0.002	0.033 ± 0.001	0.032 ± 0.002	0.054 ± 0.001
[$^2H_3$]-LEU	0.039 ± 0.001	0.042 ± 0.001	0.038 ± 0.001	0.069 ± 0.002

Data are mean ± SE. Data reflect the tracer to tracee ratio ($m + 5/m + 0$ for [$^2H_5$]-phenylalanine and $m + 3/m + 0$ for [$^2H_3$]-leucine). Data are from an n = 8. PHE, phenylalanine; LEU, leucine.

* MTF enrichments of the soleus were lower ($P < .05$) compared to vastus lateralis at 2 and 6 h during Rest.

[a] Because of the initiation of the intravenous amino acids infusion immediately following the 2-hour biopsy during the postexercise measure, the MTF at 6 hours was used for the calculation of the FSR during the postexercise measure, as we have previously performed [5,11].

synthesis rates at rest and postexercise for each muscle independently. A 1-way analysis of variance was used to compare the absolute change from rest to postexercise between tracers. Bonferroni post hoc test was used when necessary to determine pairwise differences. A paired *t* test was used to compare muscle-specific tissue fluid enrichment for each tracer. Significance for all analyses was set at $P < .05$. Data are presented as mean ± SE.

3. Results

3.1. Plasma and MTF tracer enrichments

Plasma [$^2H_5$]-phenylalanine and [$^2H_3$]-leucine enrichments were stable during the infusion periods for the resting and postexercise trials. The average plasma enrichments for [$^2H_5$]-phenylalanine and [$^2H_3$]-leucine during the incorporation period of the infusion for both the resting and postexercise trials are presented in Table 2. During the resting protein synthesis measure, MTF enrichment of the soleus was lower ($P < .05$) than the vastus lateralis, independent of tracer (Table 3). No differences existed between muscles, for either tracer, during the postexercise trial.

3.2. Influence of tracer selection on mixed muscle protein synthesis

Protein bound [$^2H_5$]-phenylalanine (Phe) and [$^2H_3$]-leucine enrichments in the vastus lateralis and soleus muscles at rest and postexercise are presented in Table 4. Resting mixed muscle protein synthesis was similar for both tracers for both muscles (Fig. 2). At rest, protein synthesis rates for the vastus lateralis were 0.080% ± 0.007%·h^{-1} and 0.085% ± 0.004%·h^{-1} for [$^2H_5$]-phenylalanine and [$^2H_3$]-leucine, respectively. Protein synthesis rates for the soleus were 0.086% ± 0.008%·h^{-1} and 0.094% ± 0.008%·h^{-1} for [$^2H_5$]-phenylalanine and [$^2H_3$]- leucine, respectively. Mixed muscle protein synthesis was higher ($P < .05$) postexercise, regardless of tracer, for both muscles (Fig. 2). Furthermore, choice of tracer did not influence postexercise protein synthesis rates. Postexercise protein synthesis rates for the vastus lateralis were 0.110% ± 0.010%·h^{-1} and 0.109% ± 0.005%·h^{-1} for [$^2H_5$]-phenylalanine and [$^2H_3$]-leucine, respectively. Postexercise protein synthesis rates for the soleus were 0.123% ± 0.008%·h^{-1} and 0.122% ± 0.005%·h^{-1} for [$^2H_5$]-phenylalanine and [$^2H_3$]-leucine, respectively. The absolute change in FSR from rest to postexercise was not different ($P > .05$) between tracers for the vastus lateralis (Phe, 0.030% ± 0.007%·h^{-1}; Leu, 0.023% ± 0.006%·h^{-1}) and soleus (Phe, 0.037% ± 0.012%·h^{-1}; Leu, 0.028% ± 0.008%·h^{-1}) (Fig. 3).

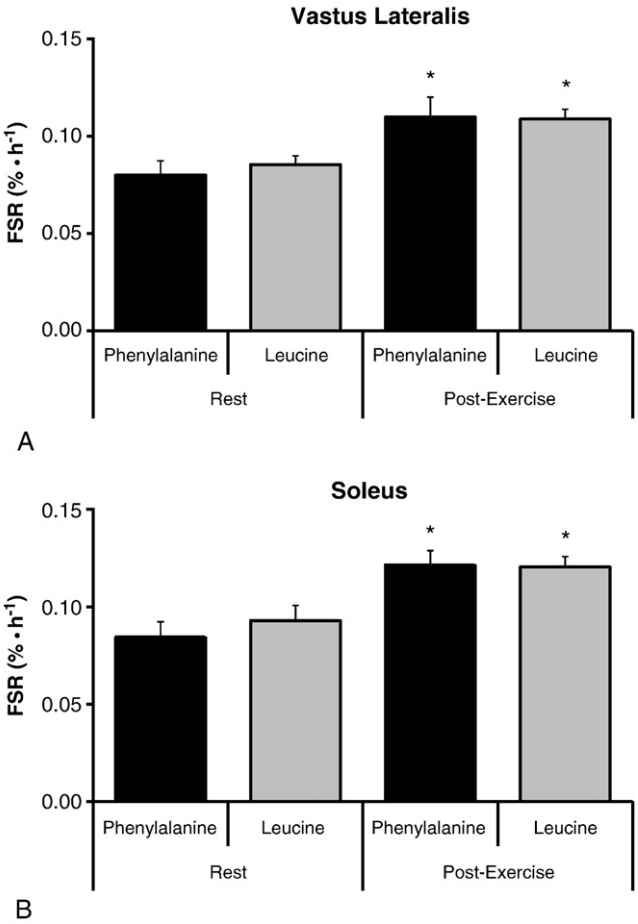

Fig. 2. Mixed muscle protein synthesis rates at rest and 24 hours postexercise for (A) vastus lateralis and (B) soleus. Fractional synthesis rates (FSR) were determined using [$^2H_5$]-phenylalanine and [$^2H_3$]-leucine with the MTF amino acid enrichment as the precursor pool. Data are mean ± SE. Data are from an n = 8. *$P < .05$ compared with rest.

Table 4
[$^2H_5$]-phenylalanine and [$^2H_3$]-leucine enrichments of the protein-bound fractions during the rest and postexercise protein synthesis measures

	Rest		Postexercise	
	2 h	6 h	2 h	6 h
Vastus lateralis				
[$^2H_5$]-PHE	0.000131 ± 0.000011	0.000252 ± 0.000017	0.000473 ± 0.000033	0.000706 ± 0.000038
[$^2H_3$]-LEU	0.000299 ± 0.000047	0.000461 ± 0.000054	0.000691 ± 0.000067	0.000992 ± 0.000077
Soleus				
[$^2H_5$]-PHE	0.000145 ± 0.000012	0.000256 ± 0.000011	0.000444 ± 0.000018	0.000770 ± 0.000034
[$^2H_3$]-LEU	0.000370 ± 0.000040	0.000517 ± 0.000037	0.000659 ± 0.000047	0.001009 ± 0.000050

Data are mean ± SE. Data reflect the tracer to tracee ratio ($m + 5/m + 0$ for [$^2H_5$]-phenylalanine and $m + 3/m + 0$ for [$^2H_3$]-leucine).

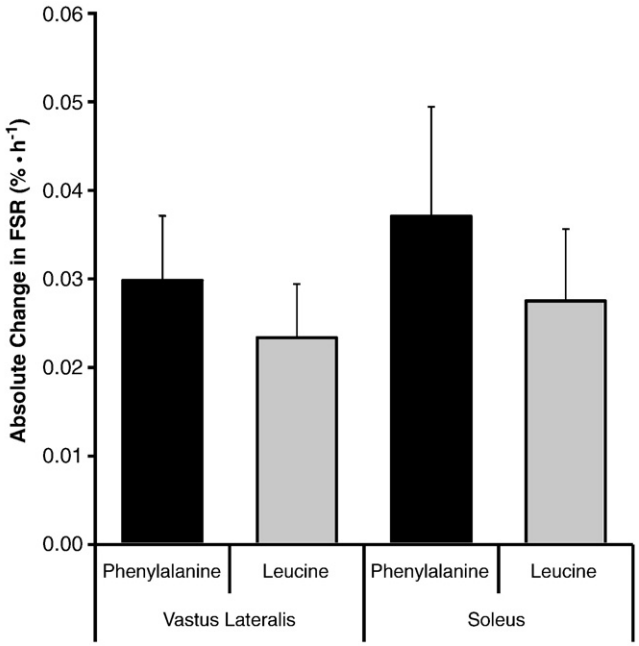

Fig. 3. Absolute change in mixed muscle protein synthesis rate from rest to 24 hours postexercise in the vastus lateralis and soleus muscles. Fractional synthesis rates were determined using [$^2H_5$]-phenylalanine and [$^2H_3$]-leucine. Data are from an n = 8. Data are mean ± SE.

4. Discussion

The primary finding from this study is that 2 different multilabeled amino acid isotope tracers ([$^2H_5$]-phenylalanine or [$^2H_3$]-leucine) yield similar absolute values of mixed muscle protein synthesis rates at rest and after aerobic exercise during amino acid stimulation measured via GC-MS combined with the standard curve approach when the MTF enrichment is used as the precursor. In addition, these 2 tracers yield similar qualitative changes in response to exercise plus amino acid stimulation. Furthermore, these trends are consistent between 2 leg muscles with distinct differences in morphology and metabolic characteristics. These data suggest that the use of [$^2H_5$]-phenylalanine and [$^2H_3$]-leucine yields similar rates of mixed muscle protein synthesis and should both be considered appropriate and feasible methods for measuring muscle protein synthesis in exercise-based human studies.

To our knowledge, this is the first study to report human skeletal muscle protein synthesis rates in response to an exercise intervention by measuring the direct incorporation of [$^2H_3$]-leucine into muscle proteins using GC-MS combined with a standard curve. Since the introduction of the GC-MS analytical technique [1], [$^2H_5$]-phenylalanine has been the most commonly used tracer, likely because it was the initial tracer selected and it cannot be oxidized in skeletal muscle [4]. In addition, phenylalanine has a low abundance (4%) in skeletal muscle relative to other amino acids, which minimizes the amount of tracer (and therefore costs) necessary to reach detectable levels [16]. Carbon-based leucine tracers have also been widely used for the measurement of muscle protein synthesis, but are typically analyzed using IRMS. Patterson et al [2] validated the use of [$^2H_3$]-leucine analyzed with GC-MS combined with a standard curve approach against protein synthesis rates measured using IRMS. Our findings extend this work to demonstrate that [$^2H_3$]-leucine yields similar results to [$^2H_5$]-phenylalanine when used for the quantitative assessment of protein synthesis in exercise-based human studies.

Although phenylalanine- and leucine-based tracers are commonly used for measuring human muscle protein synthesis, there is relatively little information comparing the two. During completion of the current study, Smith et al [17] reported that the use of [$^2H_3$]-leucine yields approximately 20% higher protein synthesis rates compared with phenylalanine-based tracers ($^2H_5$ and $^{13}C_6$) using GC-MS analysis with the standard curve approach. These findings are not completely in agreement with our results, which suggest that tracer selection does not influence the quantification of protein synthesis rates at rest or postexercise in the presence of exogenous amino acids. Although an explanation for this discrepancy is not readily at hand, the a priori tracer comparison intention and analytical control appear to be different between the 2 studies. In support of this notion, the same authors have recently reported data that appear to contrast with their original work, suggesting that leucine-based tracers yield higher FSR values [18].

The comparison of protein synthesis rates determined with multiple tracers may potentially be influenced by the analytical approach. Isotopic enrichments determined with GC-MS are known to be influenced by concentration dependency [1,2,19,20], which can be accounted for by matching sample abundance to a standard curve of known isotopic enrichments [20]. However, our pilot work and the work of Calder et al [1] reveal that the amount of amino acid loaded alters the slope and *y*-intercept of the standard curves for both phenylalanine and leucine, which influences the calculated synthesis rate. To control for potential influences of concentration dependency on measured enrichment [20], we loaded equal amounts of phenylalanine and leucine into the GC-MS for both standard curves and all samples by matching the $m + 0$ abundance. We felt that this approach was necessary to adequately compare protein synthesis rates between tracers by accounting for the potential effects of concentration dependency on isotopic enrichment and by standardizing the influence of sample abundance on the slope of the standard curves. Using this approach, we report that the calculated rate of mixed muscle protein synthesis is not influenced by tracer selection.

Another unique aspect of this investigation is the assessment of mixed muscle protein FSR after aerobic exercise. Although the role of aerobic exercise on muscle protein metabolism is not as clearly defined as resistance exercise, several investigations have reported higher muscle

protein FSR values following aerobic exercise under fed [6,21] and fasted [22,23] conditions. Therefore, the results of the current investigation reporting higher rates of mixed muscle protein following aerobic exercise are consistent with previous studies.

We assessed the vastus lateralis and soleus muscles because of our interest in examining the muscle-specific response to changes in activity patterns such as exercise and unloading [5,11,13,24,25]. Interestingly, the MTF enrichment of the soleus at rest was lower than that of the vastus lateralis, independent of tracer (Table 3). Resting protein synthesis rates were similar between muscles; therefore, the lower MTF enrichment in the soleus may be a reflection of a higher protein breakdown that resulted in a dilution of the labeled amino acids in the MTF. The difference in MTF enrichment between these 2 muscles may be specific to endurance-trained athletes, as we have recently reported no muscle-specific differences in sedentary, untrained subjects [26]. Although the mixed muscle protein FSR was not different between the vastus lateralis and soleus muscles in the current study, these muscles display divergent fiber-type composition [24], metabolic capacity [27], contractile properties [28], and response to exercise [13]. Collectively, these factors highlight the heterogeneity between human muscles and warrant the need to use caution when interpreting and extrapolating muscle-based studies in humans. Furthermore, the difference in MTF enrichment between muscles argues against the use of a plasma-derived precursor pool when making comparisons across muscles.

There is wide variability in resting mixed muscle protein synthesis rates reported in the literature. This variability can be potentially attributed to tracer selection (although the current data suggest otherwise), analytical approach (GC-MS vs GC-C-IRMS), subject characteristics, or precursor pool (tRNA, MTF, or plasma enrichment). The resting rates of protein synthesis in the current study are slightly higher than we have previously reported [11,12]. However, this may be explained by subject training status, as aerobic training significantly increases resting muscle protein synthesis [29,30]. In addition, an exhaustive review of the literature reveals that several studies using a multitude of tracers including [^{13}C]-leucine [31-34], [$^{2}H_5$]-phenylalanine [14,29,35-37], [^{13}C]-phenylalanine [38,39], or α-KIC [40,41] have reported fasted-state resting mixed muscle protein synthesis rates comparable to or greater than our resting values. Furthermore, using the plasma-derived precursor pool (ie, plasma phenylalanine or α-KIC for leucine), our protein synthesis rates are 0.059 ± 0.006 and 0.056 ± 0.004 (vastus lateralis; phenylalanine and leucine, respectively) and 0.054 ± 0.006 and 0.053 ± 0.004 (soleus; phenylalanine and leucine, respectively). These results further highlight the variability in resting muscle protein synthesis values that is likely due to nuances in analytical approach.

Several studies have used the plasma amino acid enrichment as the precursor pool when calculating muscle protein FSR in human subjects [30,36,42-44]. This approach may be appealing when accurate assessments of the enrichment of the aminoacyl-tRNA and MTF are not feasible. Because the enrichments of plasma amino acids are higher than amino acid enrichments in MTF, using the plasma enrichment as the precursor pool typically reduces the calculated FSR. Furthermore, muscle protein FSR during amino acid infusion appears to be overestimated when the plasma enrichment is used as a precursor [45]. In the current study, calculating mixed muscle protein FSR using plasma amino acid enrichment as the precursor method resulted in lower resting FSR values for both tracers, as stated previously. Despite these quantitative differences, these 2 tracers yield similar FSR values at rest and postexercise when plasma enrichment is used as the precursor pool (Fig. 4). These findings extend the tracer comparison to indicate that these 2 tracers yield similar FSR values and qualitative response to exercise plus amino acid stimulation when the plasma enrichment is used as the precursor pool.

In conclusion, the measurement of human skeletal muscle protein synthesis in response to exercise will

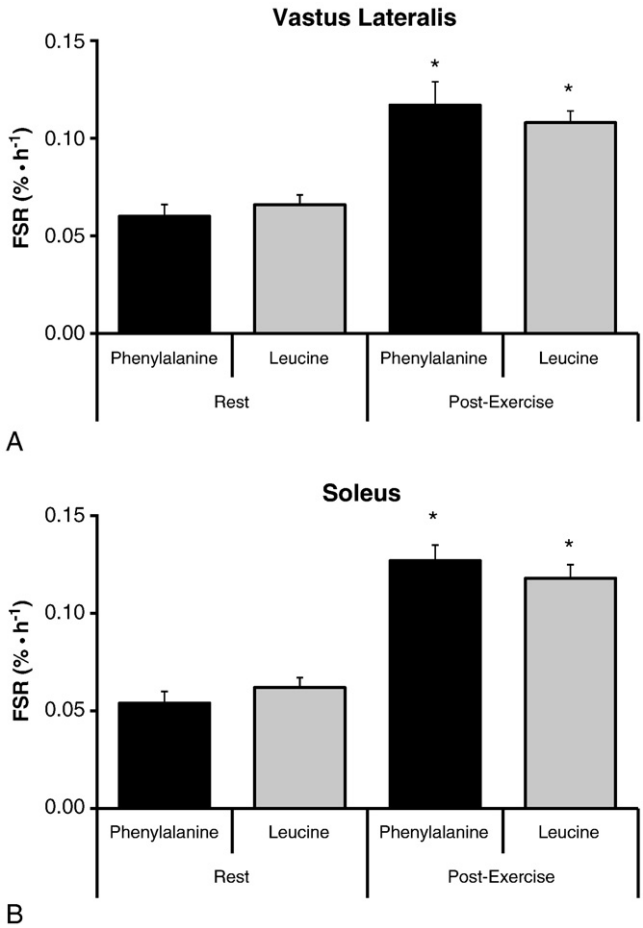

Fig. 4. Mixed muscle protein synthesis rate at rest and 24 hours postexercise for (A) vastus lateralis and (B) soleus. Fractional synthesis rates were determined using [$^{2}H_5$]-phenylalanine and [$^{2}H_3$]-leucine with plasma amino acid enrichment as the precursor pool. Data are mean ± SE. Data are from an n = 8. *$P < .05$ compared with rest.

continue to be of great scientific importance. A limitation to these types of studies is that various analytical approaches to measuring protein synthesis constrain the comparison between investigations and among laboratories. Our results suggest that, when matched for loading abundance on the GC-MS, the use of [$^2H_5$]-phenylalanine and [$^2H_3$]-leucine yields similar quantitative results at rest and postexercise during amino acid stimulation in multiple human skeletal muscles. A novel aspect of this study was the use of a constant infusion of [$^2H_3$]-leucine to assess muscle protein synthesis in response to an exercise intervention, which to our knowledge has not been previously reported. Importantly, there is strong agreement between absolute changes in protein synthesis determined with the tracers, supporting the use of [$^2H_3$]-leucine in human-based exercise studies. In addition, multiple GC-MS injections of [$^2H_3$]-leucine demonstrated less variability (Table 1) compared with [$^2H_5$]-phenylalanine for plasma, MTF, and protein-bound samples, which may lead to less measurement variability and improve experimental ability to detect small perturbations in protein synthesis.

Acknowledgment

This investigation was supported by National Aeronautics and Space Administration grant NNJ06HF59G and National Institutes of Health grant R01AG020532 (TT).

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