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Influence of Tracer Selection on Protein Synthesis Rates at Rest and Post-Exercise in Multiple Human Muscles

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Abstract

The goal of this investigation was to assess the influence of tracer selection on mixed muscle fractional synthesis rate (FSR) at rest and post-exercise during amino acid infusion in multiple human skeletal muscles. FSR was measured before and 24h after 45-min of running using simultaneous infusion of [$^2\text{H}_5$]-phenylalanine (Phe) and [$^2\text{H}_3$]-leucine (Leu) coupled with muscle biopsies from the vastus lateralis and soleus in aerobically-trained men ($n=8$; age 26 ± 2 yr). Mixed muscle protein FSR was analyzed by gas chromatography-mass spectrometry combined with a standard curve using the enriched muscle tissue fluid (MTF) 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 ($P>0.05$). FSR measured 24h post-exercise was higher ($P<0.05$) compared to rest and was similar between tracers for the vastus lateralis (Phe: 0.110 ± 0.010 ; Leu: $0.109\pm 0.005\ \%\cdot\text{h}^{-1}$) and soleus (Phe: 0.123 ± 0.008 ; Leu: $0.122\pm 0.005\ \%\cdot\text{h}^{-1}$). These data demonstrate that tracer selection does not influence the assessment of resting or post-exercise FSR, thereby supporting the use of both [$^2\text{H}_5$]-phenylalanine and [$^2\text{H}_3$]-leucine for the measurement of FSR in exercise based studies of human skeletal muscle.

Keywords

fractional synthesis rate; [$^2\text{H}_5$]-phenylalanine; [$^2\text{H}_3$]-leucine

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 utilizing gas chromatography-mass spectrometry (GC-MS) to detect very low isotopic enrichments. Measuring the incorporation of a multi-labeled stable

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Conflicts of Interest: None

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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amino acid tracer with GC-MS combined with a standard curve was originally reported using [$^2\text{H}_5$]-phenylalanine (1) and later refined to be used with [$^2\text{H}_3$]-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 [$^2\text{H}_5$]-phenylalanine as the tracer of choice. Carbon-based leucine tracers (i.e., ^{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 due to the instrumentation required. Use of a multi-labeled leucine tracer, such as [$^2\text{H}_3$]-leucine, negates the need for IRMS analysis, however, to our knowledge, only three studies have used [$^2\text{H}_3$]-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 two primary objectives: 1) to compare muscle protein FSR values obtained with stable isotope tracers of two different commonly used amino acids (phenylalanine and leucine); and 2) to examine the influence of aerobic exercise on protein metabolism. The first objective was examined at rest and post-exercise during amino acid infusion using simultaneous infusion of both [$^2\text{H}_5$]-phenylalanine and [$^2\text{H}_3$]-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 post-exercise protein synthesis during amino acid infusion at 24h post-exercise in order to maximally stimulate protein synthesis (6). Findings from this investigation provide important insight into the feasibility of multiple approaches for measuring skeletal muscle protein synthesis in exercise-based human studies.

MATERIALS AND METHODS

Subjects and Study Overview

Eight male subjects (age 26 ± 2 yr) volunteered to participate in this investigation. All subjects were aerobically trained ($\text{VO}_{2\text{max}}$ $63 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and able to complete a continuous run of 45 minutes at 75% of their $\text{VO}_{2\text{max}}$. 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 underwent a treadmill test for the determination of $\text{VO}_{2\text{max}}$ as we have described previously (7-9) and to determine the speed necessary to elicit ~75% of $\text{VO}_{2\text{max}}$. Subjects then completed three experimental trials in a fixed order. The first trial consisted of the measurement of resting muscle protein synthesis rate. Approximately one week later, subjects performed an exercise trial followed the next morning (~24h post-exercise) by the measurement of post-exercise protein synthesis. The experimental trials are described in more detail below. Prior to the measure of resting protein synthesis and the exercise trial (described in detail below), subjects were asked to refrain from any exercise for 72h. 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.

Experimental Trials (Figure 1)

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 6h. Shortly after arrival to the laboratory, a catheter was inserted into an antecubital vein for the simultaneous infusion of [$^2\text{H}_5$]-phenylalanine (prime: $2\ \mu\text{mol}\cdot\text{kg}^{-1}$; rate: $0.05\ \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and [$^2\text{H}_3$]-leucine (prime: $4.8\ \mu\text{mol}\cdot\text{kg}^{-1}$; rate: $0.12\ \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) (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 6h of the infusion for the measurement of plasma isotope enrichment. Muscle biopsies were obtained from the vastus lateralis and soleus muscles at 2 and 6h during the isotope infusion for determination of the incorporation of [$^2\text{H}_5$]-phenylalanine and [$^2\text{H}_3$]-leucine into mixed muscle protein.

The exercise trial consisted of a 45 minute treadmill run at $\sim 75\%$ VO_2max . Expired gas samples were obtained at two periods during the exercise bout to determine exercise intensity (5).

Post-exercise protein synthesis was measured the following morning, $\sim 24\text{h}$ after the running bout. This timepoint corresponds to the peak increase in protein synthesis after aerobic exercise (6). The post-exercise procedures were similar to the resting measure, except for the administration of unlabeled amino acids (10% Travasol; Clintec Nutrition, Deerfield, IL) which were initiated immediately following the 2h biopsies at a rate of $1.35\ \text{ml}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ following a prime of $0.45\ \text{ml}\cdot\text{kg}^{-1}$ (5,11). Additionally, the infusion rate of [$^2\text{H}_5$]-phenylalanine was increased to $0.10\ \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ with a second priming dose of $0.6\ \mu\text{mol}\cdot\text{kg}^{-1}$ and the infusion rate of [$^2\text{H}_3$]-leucine was increased to $0.24\ \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ with a second priming dose of $1.6\ \mu\text{mol}\cdot\text{kg}^{-1}$ at the onset of the unlabeled amino acid infusion to maintain steady plasma tracer enrichment.

Muscle Biopsies

A total of eight muscle biopsies (four each from the vastus lateralis and soleus muscles) were obtained during the study protocol. At each timepoint, percutaneous needle biopsies were obtained under local anesthetic (15). Muscle samples were dissected free of any visible connective and adipose tissue and divided into $\sim 20\ \text{mg}$ sections and immediately frozen and stored in liquid nitrogen (-190°C) until analysis.

Sample Preparation and Derivatization

The rate of mixed muscle protein synthesis was determined by quantifying the muscle tissue fluid (MTF) and protein bound [$^2\text{H}_5$]-phenylalanine and [$^2\text{H}_3$]-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 ($\sim 20\text{mg}$) was weighed at -35°C and then homogenized in $500\ \mu\text{l}$ of ice cold 14% perchloric acid. Muscle was homogenized on ice with a Teflon-coated pestle for one minute and then centrifuged at $21,000\ \text{g}$ for 10 minutes at 4°C . The supernatant was then collected and this process was repeated two more times, with all three supernatants combined as the muscle tissue fluid for that sample. The remaining pellet was washed once in deionized MilliQ (Millipore, Billerica, MA) water and three times in ethanol. Each wash was followed by centrifugation at $21,000\ \text{g}$ for 10 minutes at 4°C . The pellet, representative of mixed protein, was dried overnight at 50°C and hydrolyzed in $5\ \text{ml}$ of 6N hydrochloric acid (HCl) for 24 hours at 100°C . Each plasma sample was deproteinized with a 1:1 ratio of 15% sulfosalicylic acid (SSA) and then centrifuged at $21,000\ \text{g}$ for 10 minutes at 4°C .

Prior to derivatization, the muscle tissue fluid, 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 Labs, Hercules, CA). Amino acids were eluted from the column with 4N ammonium hydroxide, and this elute was collected and dried under vacuum (SC210A SpeedVac Plus, ThermoSavant, Holbrook, NJ). Once dried, samples were derivatized with 100 μ l of acetonitrile and N-methyl-N-(t-butyldimethylsilyl) trifluoroacetamide (MTBSTFA; Pierce Chemical, Rockford, IL) at a 1:1 ratio. All samples were derivatized at 100 °C: muscle tissue fluid and plasma for 10 minutes, and mixed muscle protein for 30 minutes.

Stable Isotope Tracer Analysis

All samples were analyzed using gas chromatography-mass spectrometry (GC-MS, GC-6890N GC coupled with 5973 inert MSD, Agilent Technologies, Wilmington, DE) in duplicate (plasma samples) or triplicate (MTF and protein bound samples) using electron impact ionization and selected ion monitoring. For [²H₅]-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 [²H₃]-leucine, m/z 200 (m+0), 202 (m+2), and 203 (m+3) were monitored. Plasma and MTF [²H₅]-phenylalanine enrichments were measured using the m+5/m+0 ratio. Plasma and MTF [²H₃]-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 [²H₅]-phenylalanine and the m+3/m+2 ratio and a single linear curve from mixtures of known m+3/m+0 ratios for [²H₃]-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 (i.e., similar m+0 abundances) were injected for all samples and standards. Initial measurements were made on various amounts of phenylalanine and leucine to assure the amount of phenylalanine and leucine injected for all samples would be below saturation levels of the detector and would produce Gaussian-shaped peaks. The average m+0 abundance for the [²H₅]-phenylalanine and [²H₃]-leucine standard curves was 37,849,926 $\pm$ 232,413 and 37,803,619 $\pm$ 292,912, respectively. The average m+0 abundance for the muscle protein bound fractions was 37,595,787 $\pm$ 486,535 and 36,934,838 $\pm$ 437,253 for [²H₅]-phenylalanine and [²H₃]-leucine, respectively. With regards to instrument sensitivity, with the m+0 at ~37 million, the m+5 for [²H₅]-phenylalanine was 16,297 $\pm$ 1658 and m+3 abundance for [²H₃]-leucine was 295,993 $\pm$ 3541 on average in muscle protein bound samples when considering all four biopsy timepoints.

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

Mixed muscle protein FSR was calculated as the rate of [²H₅]-phenylalanine or [²H₃]-leucine tracer incorporated into muscle protein using the MTF phenylalanine enrichment or MTF leucine enrichment as the precursor and the following equation:

$$FSR \left(\% \bullet h^{-1} \right) = \left\{ \left[\left(Et_1 - Et_0 \right) / \left[E_p \bullet \left(t_1 - t_0 \right) \right] \right] \right\} \bullet 100$$

Where Et₁ and Et₀ are the phenylalanine or leucine tracer enrichments in the protein-bound fraction, (t₁ – t₀) is the phenylalanine or leucine tracer incorporation time, and E_p is the precursor.

Statistical Analysis

A two-way (tracer X time) analysis of variance (ANOVA) with repeated measures was used to determine the influence of tracer selection on protein synthesis rates at rest and post-exercise for each muscle independently. A one-way ANOVA was used to compare the absolute change from rest to post-exercise between tracers. Bonferonni's pos 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 $P < 0.05$. Data are presented as mean $\pm$ SE.

RESULTS

Plasma and muscle tissue fluid tracer enrichments

Plasma [$^2\text{H}_5$]-phenylalanine and [$^2\text{H}_3$]-leucine enrichments were stable during the infusion periods for the resting and post-exercise trials. The average plasma enrichments for [$^2\text{H}_5$]-phenylalanine and [$^2\text{H}_3$]-leucine during the incorporation period of the infusion for both the resting and post-exercise trials are presented in Table 2. During the resting protein synthesis measure, muscle tissue fluid enrichment of the soleus was lower ($P < 0.05$) than the vastus lateralis, independent of tracer (Table 3). No differences existed between muscles, for either tracer, during the post-exercise trial.

Influence of tracer selection on mixed muscle protein synthesis

Resting mixed muscle protein synthesis was similar for both tracers for both muscles (Figure 2). At rest, protein synthesis rates for the vastus lateralis were 0.080 ± 0.007 and 0.085 ± 0.004 $\% \cdot \text{h}^{-1}$ for [$^2\text{H}_5$]-phenylalanine and [$^2\text{H}_3$]-leucine, respectively. Protein synthesis rates for the soleus were 0.086 ± 0.008 and 0.094 ± 0.008 for [$^2\text{H}_5$]-phenylalanine and [$^2\text{H}_3$]-leucine, respectively. Mixed muscle protein synthesis was higher ($P < 0.05$) post-exercise, regardless of tracer, for both muscles (Figure 2). Further, choice of tracer did not influence post-exercise protein synthesis rates. Post-exercise protein synthesis rates for the vastus lateralis were 0.110 ± 0.010 and 0.109 ± 0.005 $\% \cdot \text{h}^{-1}$ for [$^2\text{H}_5$]-phenylalanine and [$^2\text{H}_3$]-leucine, respectively. Post-exercise protein synthesis rates for the soleus were 0.123 ± 0.008 and 0.122 ± 0.005 $\% \cdot \text{h}^{-1}$ for [$^2\text{H}_5$]-phenylalanine and [$^2\text{H}_3$]-leucine, respectively. The absolute change in FSR from rest to post-exercise was not different ($P > 0.05$) between tracers for the vastus lateralis (Phe: 0.030 ± 0.007 ; Leu: 0.023 ± 0.006 $\% \cdot \text{h}^{-1}$) and soleus (Phe: 0.037 ± 0.012 ; Leu: 0.028 ± 0.008 $\% \cdot \text{h}^{-1}$) (Figure 3).

DISCUSSION

The primary finding from this study is that two different multi-labeled amino acid isotope tracers ([$^2\text{H}_5$]-phenylalanine or [$^2\text{H}_3$]-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 muscle tissue fluid enrichment is used as the precursor. Additionally, these two tracers yield similar qualitative changes in response to exercise plus amino acid stimulation. Further, these trends are consistent between two leg muscles with distinct differences in morphology and metabolic characteristics. These data suggest that use of [$^2\text{H}_5$]-phenylalanine and [$^2\text{H}_3$]-leucine yield 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 [$^2\text{H}_3$]-leucine into muscle proteins using GC-MS combined with a standard curve. Since the

introduction of the GC-MS analytical technique (1), [$^2\text{H}_5$]-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). Additionally, 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 [$^2\text{H}_3$]-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 [$^2\text{H}_3$]-leucine yields similar results to [$^2\text{H}_5$]-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 [$^2\text{H}_3$]-leucine yields ~20% higher protein synthesis rates compared to phenylalanine based tracers ($^2\text{H}_5$ and $^{13}\text{C}_6$) 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 post-exercise 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 appears to be different between the two studies. In support of this notion, the same authors have recently reported data that appears to contrast 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 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. While the role 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 due to 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 muscle tissue fluid enrichment of the soleus at rest was lower than the vastus lateralis, independent of tracer (Table 3). Resting protein synthesis rates were similar between muscles, therefore the lower muscle tissue fluid enrichment in the soleus may be a reflection of a higher protein breakdown which resulted in a dilution of

the labeled amino acids in the muscle tissue fluid. The difference in muscle tissue fluid enrichment between these two 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 muscle tissue fluid 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, muscle tissue fluid, 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). Additionally, an exhaustive review of the literature reveals several studies utilizing a multitude of tracers including [^{13}C]-leucine (31-34), [$^2\text{H}_5$]-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 (i.e., plasma phenylalanine or α -KIC for leucine); our protein synthesis rates are 0.059 ± 0.006 and 0.056 ± 0.004 (VL; 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 are 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 muscle tissue fluid are not feasible. Because the enrichments of plasma amino acids are higher than amino acid enrichments in muscle tissue fluid, 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 two tracers yield similar FSR values at rest and post-exercise when plasma enrichment is used as the precursor pool (Figure 4). These findings extend the tracer comparison to indicate that these two 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 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 amongst laboratories. Our results suggest that, when matched for loading abundance on the GC-MS, the use of [$^2\text{H}_5$]-phenylalanine and [$^2\text{H}_3$]-leucine yield similar quantitative results at rest and post-exercise during amino acid stimulation, in multiple human skeletal muscles. A novel aspect of this study was the use of a constant infusion of [$^2\text{H}_3$]-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 [$^2\text{H}_3$]-leucine in human based exercise studies. Additionally, multiple GC-MS injections of [$^2\text{H}_3$]-leucine demonstrated less variability (Table 1) compared to [$^2\text{H}_5$]-phenylalanine for plasma, muscle tissue fluid, and protein bound samples, which may lead to less measurement variability and improve experimental ability to detect small perturbations in protein synthesis.

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Figure 1.

Schematic of experimental design outlining A. The resting protein synthesis (FSR) measure and B. The exercise session and post-exercise FSR measure. The exercise session consisted of a 45 minute treadmill run at 75% VO_2max and occurred ~1 week following the resting FSR measure. *, blood sampling. ↑, vastus lateralis and soleus muscle biopsy

Figure 2. Mixed muscle protein synthesis at rest and 24h post-exercise for A. vastus lateralis and B. soleus. Fractional synthesis rates (FSR) were determined using [$^2\text{H}_5$]-phenylalanine and [$^2\text{H}_3$]-leucine with the muscle tissue fluid amino acid enrichment as the precursor pool. Data are mean $\pm$ SE. Data are from an n=8. *P<0.05 compared to Rest

Figure 3.

Absolute change in mixed muscle protein synthesis from rest to 24h post-exercise in the vastus lateralis and soleus muscles. Fractional synthesis rates (FSR) were determined using [²H₅]-phenylalanine and [²H₃]-leucine. Data are from an n=8. Data are mean±SE

Figure 4. Mixed muscle protein synthesis at rest and 24h post-exercise for A. vastus lateralis and B. soleus. Fractional synthesis rates (FSR) were determined using $[^2H_5]$ -phenylalanine and $[^2H_3]$ -leucine with plasma amino acid enrichment as the precursor pool. Data are mean $\pm$ SE. Data are from an n=8. * $P < 0.05$ compared to Rest

Table 1

Mean coefficient of variation (%) 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
Muscle Tissue Fluid	0.49±0.05	2.14±0.19
Protein Bound	0.20±0.01	1.35±0.19

Data are mean±SE

Table 2Plasma [$^2\text{H}_5$]-phenylalanine and [$^2\text{H}_3$]-leucine enrichments.

	3h	4h	5h	6h
[$^2\text{H}_5$]-phenylalanine				
Rest	0.053±0.002	0.053±0.002	0.055±0.002	0.051±0.002
Post-Exercise	0.050±0.001	0.053±0.001	0.054±0.002	0.054±0.002
[$^2\text{H}_3$]-leucine				
Rest	0.065±0.003	0.063±0.002	0.065±0.002	0.061±0.003
Post-Exercise	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 [$^2\text{H}_5$]-phenylalanine and m+3/m+0 for [$^2\text{H}_3$]-leucine). Data are from an n=8.

Table 3

[²H₅]-phenylalanine and [²H₃]-leucine enrichments of the muscle tissue fluid during the rest and post-exercise protein synthesis measures.

	Rest		Post-Exercise*	
	2h	6h	2h	6h
Vastus Lateralis				
[² H ₅]-PHE	0.036±0.004	0.041±0.003	0.038±0.001	0.055±0.001
[² H ₃]-LEU	0.045±0.003	0.050±0.002	0.044±0.001	0.071±0.002
Soleus				
[² H ₅]-PHE	0.030±0.002	0.033±0.001	0.032±0.002	0.054±0.001
[² H ₃]-LEU	0.039±0.001	0.042±0.001	0.038±0.001	0.069±0.002

Data are mean±SE. [²H₅]-PHE, [²H₅]-phenylalanine; [²H₃]-LEU, [²H₃]-leucine.

* Due to the initiation of the intravenous amino acids infusion immediately following the 2h biopsy during the post-exercise measure, the muscle tissue fluid at 6h was used the calculation of the fractional synthetic rate during the post-exercise measure, as we have previously performed (5,11).

Data reflect the tracer-to-tracee ratio (m+5/m+0 for [²H₅]-phenylalanine and m+3/m+0 for [²H₃]-leucine). Data are from an n=8.

Table 4

[²H₅]-phenylalanine and [²H₃]-leucine enrichments of the protein bound fractions during the rest and postexercise protein synthesis measures.

	Rest		Post-Exercise	
	2h	6h	2h	6h
Vastus Lateralis				
[² H ₅]-PHE	0.000131±0.00001 1	0.000252±0.00001 7	0.000473±0.00003 3	0.000706±0.00003 8
[² H ₃]-LEU	0.000299±0.00004 7	0.000461±0.00005 4	0.000691±0.00006 7	0.000992±0.00007 7
Soleus				
[² H ₅]-PHE	0.000145±0.00001 2	0.000256±0.00001 1	0.000444±0.00001 8	0.000770±0.00003 4
[² H ₃]-LEU	0.000370±0.00004 0	0.000517±0.00003 7	0.000659±0.00004 7	0.001009±0.00005 0

Data are mean±SE. [²H₅]-PHE, [²H₅]-phenylalanine; [²H₃]-LEU, [²H₃]-leucine. Data reflect the tracer-to-tracee ratio (m+5/m+0 for [²H₅]-phenylalanine and m+3/m+0 for [²H₃]-leucine).