The single biopsy approach is reliable for the measurement of muscle protein synthesis rates in vivo in older men

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Burd NA, Pennings B, Groen BB, Gijsen AP, Senden JM, van Loon LJ. The single biopsy approach is reliable for the measurement of muscle protein synthesis rates in vivo in older men. J Appl Physiol 113: 896–902, 2012. First published July 19, 2012; doi:10.1152/japplphysiol.00513.2012.—We aimed to assess the reliability of the single biopsy approach for calculating muscle protein synthesis rates compared with the well described sequential muscle biopsy approach following a primed continuous infusion of L-[ring-2H5]phenylalanine and GC-MS analysis in older men. Two separate experimental infusion protocols, with differing stable isotope amino acid incorporation times, were employed consisting of n = 27 (experiment 1) or n = 9 (experiment 2). Specifically, mixed muscle protein FSR were calculated from baseline plasma protein enrichments and muscle protein enrichments obtained at 90 min or 50 min (1BX SHORT), 210 min or 170 min (1BX LONG), and between the muscle protein enrichments obtained at 90 and 210 min or 50 and 170 min (2BX) of the infusion for experiments 1 and 2, respectively. In experiment 2, we also assessed the error that is introduced to the single muscle biopsy approach when nontracer naïve subjects are recruited for participation in a primed continuous infusion of isotope-labeled amino acids. In experiment 1, applying the individual plasma protein enrichment values to the single muscle biopsy approach resulted in no differences in muscle protein FSR between the 1BX SHORT (0.031 ± 0.003%·h⁻¹), 1BX LONG (0.032 ± 0.002%·h⁻¹), or the 2BX approach (0.034 ± 0.002%·h⁻¹). A significant correlation in muscle protein FSR was observed only between the 1BX LONG and 2BX approach (r = 0.8; P < 0.001). Similar results were observed in experiment 2. In addition, using the single biopsy approach in nontracer naïve state results in a muscle protein FSR that is negative for both the 1BX SHORT (−0.67 ± 0.051%·h⁻¹) and 1BX LONG (−0.19 ± 0.051%·h⁻¹) approaches. This is the first study to demonstrate that the single biopsy approach, coupled with the background enrichment of L-[ring-2H5]phenylalanine of mixed plasma proteins, generates data that are similar to using the sequential muscle biopsy approach in the elderly population.

stable isotope; amino acid; phenylalanine

THE GRADUAL LOSS OF SKELETAL muscle mass often accompanies healthy aging, a process commonly termed sarcopenia (8). The underlying basis of the sarcopenia is not completely clear at this time. It may be related, at least in part, to a reduced capacity to stimulate muscle protein synthesis rates in response to the main anabolic stimuli (exercise and dietary amino acids) to human skeletal muscle tissue (3). Thus an interest to us, and many others, is determining changes in muscle protein synthesis rates after different feeding and exercise paradigms. The overall expectations of these acute experiments are to develop nutritional and exercise strategies to offset the age-related loss of muscle mass. Before the determination of postprandial muscle protein synthesis rates, it is common to measure postabsorptive muscle protein synthesis rates. The accurate determination of postabsorptive muscle protein synthesis rates is important since it serves as a control measurement for the subsequent feeding- or exercise-induced stimulation of muscle protein synthesis rates. Thus experiments designed to include both the postabsorptive and postprandial/exercise recovery states would result in an accumulation in the number of collected muscle biopsies required during an infusion trial. It seems reasonable to suggest that a method that minimizes the number of muscle biopsies collected during acute experiments may be of more relevance in an aging model, since the number of collected muscle biopsies is generally more restricted in compromised and frail populations. Such a method may also be viewed more favorably by the medical ethical review committees since it further minimizes participant discomfort.

Our previous work demonstrated a reliable method to reduce the number of muscle biopsies collected during a stable isotope infusion. Specifically, we reported that the single biopsy approach, together with a primed continuous infusion of L-[U-13C6]phenylalanine and GC-C-IRMS analysis, results in comparable group averages of muscle protein synthesis rates to those produced by the sequential biopsy approach (2). The single biopsy approach assumes that the pre-infusion mixed plasma protein 13C enrichments are similar to that of muscle protein (7) and that muscle protein labeling is linear after the onset of the tracer infusion (14). A caveat of the single biopsy approach is that its reliability (or so it is presumed) is dependent on whether the subject has not participated in previous amino acid tracer infusions (i.e., tracer naïve); otherwise the synonymous relationship between muscle protein and mixed plasma protein enrichment is no longer present. To date, it remains to be investigated whether the single biopsy approach can be successfully applied to other amino acid tracers (e.g., L-[ring-2H5]phenylalanine) and using gas chromatography mass spectrometry (GC-MS) analysis to measure muscle protein synthesis rates in vivo in humans. Due to the widespread availability of GC-MS analysis, compared with gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) analysis, the information with regard to how reliable deuterated amino acid tracers are for the determination of postabsorptive muscle protein synthesis rates should yield valuable information (e.g., minimize tracer cost and an ethical perspective) for biomedical laboratories in the field of muscle protein metabolism.

In the present investigation, our first aim (experiment 1) was to use a primed continuous infusion of L-[ring-2H5]phenylalanine
and a different form of analysis (GC-MS) from our previous work (2) to assess the reliability of the single biopsy approach for calculating muscle protein synthesis rates compared with the well described sequential muscle biopsy procedure in a more compromised elderly population. The second aim (experiment 2) of our investigation was to determine the error that is introduced in the single muscle biopsy approach when non-tracer naive subjects are recruited for participation in a primed continuous infusion of isotope-labeled amino acids. Therefore, we studied a group of individuals that visited the laboratory twice and received primed, continuous infusions of L-[ring-\(^2\)H\(_5\)]phenylalanine (with 2 wk between each visit). In addition, we assessed the impact of the timing of the initial single biopsy for the determination of muscle protein synthesis rates (for both experiments 1 and 2).

**METHODS**

**Subjects**

Thirty-six, healthy older men were examined in the present investigation. The subjects were part of a larger ongoing investigation being conducted in our laboratory. We chose to study older subjects since skeletal muscle mass declines with age (15). Thus the clinical/healthy aging population may represent a subject cohort in which the reduction of the number of muscle biopsies collected during a stable isotopic infusion trial is exceptionally relevant. Subjects were not involved in a regular exercise program and had no history of participating in any stable isotopic infusion studies before these experiments. Subjects were informed of the nature and possible risks of the experimental procedures before written, informed consent was obtained. The study was approved by the Medical Ethics Committee of the Maastricht University Medical Centre, Maastricht, the Netherlands, and conformed to standards for the use of human subjects in research as outlined in the fifth Declaration of Helsinki.

**Experimental protocol.** In experiment 1, 27 healthy older men (age: 73 ± 1 yr; body weight: 77 ± 1 kg; body mass index: 25 ± 0.3 kg/m\(^2\)) were studied to determine the relationship between using the baseline mixed plasma protein enrichment and the muscle protein L-[ring-\(^2\)H\(_5\)]phenylalanine enrichment at 90 min (i.e., short tracer incorporation time; 1BX SHORT) or 210 min (i.e., long tracer incorporation time; 1BX LONG) against the well described sequential muscle biopsy approach (2BX) (11). In experiment 2, a separate group of nine healthy older men (age: 74 ± 2 yr; body weight: 79 ± 1 kg; body mass index: 26 ± 0.2 kg/m\(^2\)) were studied with the same objective of experiment 1 with the exception of shorter tracer incorporation times for 1BX SHORT (50 min) and 1BX LONG (170 min). In addition, the subjects in experiment 2 returned to the laboratory 2 wk after the initial tracer experiment and repeated the identical experiment as performed in visit 1 to determine the impact of tracer exposure from previous experiments on protein-bound enrichments and the subsequent determination of muscle protein synthesis rates. The subjects maintained their normal habitual physical activities (no exercise or nutritional intervention) during the 2 wk before visit 2. The experimental protocols are presented in Fig. 1.

All subjects consumed the same standardized meal [providing 55 energy% (En%) carbohydrate, 15% En% fat, and 30 En% of protein] the evening before the tracer infusion. Subjects reported to the laboratory at 0800 after an overnight fast. A Teflon catheter was inserted into a vein in the hand, which was placed in a hot-box, to allow arterialized blood sampling. In all subjects, a baseline blood sample was collected to determine L-[ring-\(^2\)H\(_5\)]phenylalanine enrichment in plasma protein. A second Teflon catheter was inserted into an antecubital vein in the contralateral arm, and a primed, continuous infusion of L-[ring-\(^2\)H\(_5\)]phenylalanine (prime: 2 μmol/kg; 0.05 μmol·kg\(^{-1}\)·min\(^{-1}\); Cambridge Isotope Laboratories, Andover, MA) was initiated while subjects rested comfortably in the supine position for 210 min or 170 min for experiments 1 and 2, respectively. Muscle biopsies were obtained during the continuous intravenous infusion in the middle region of the vastus lateralis (15 cm above the patella) with a Bergström needle after 90 or 50 min (first muscle biopsy) and 210 or 170 min (second muscle biopsy) for experiments 1 and 2, respectively (Fig. 1). All samples were freed from any visible adipose tissue and blood, immediately frozen in liquid nitrogen, and stored at −80°C until subsequent analysis. The described experimental design allows us to compare mixed muscle protein synthesis rates (within the same subjects) using both the single muscle biopsy and the sequential muscle biopsy approach.

**Plasma analyses.** Blood samples (8 ml) were collected in EDTA-containing tubes and centrifuged at 1,000 × g for 10 min. Aliquots of plasma were frozen in liquid nitrogen and stored at −80°C until analysis. Mixed plasma proteins were extracted from blood samples by adding 2% perchloric acid (PCA). Samples were centrifuged at 1,000 g at 4°C for 10 min, and the supernatants were removed. The mixed plasma protein pellet was washed and lyophilized to dryness. Amino acids were liberated by adding 6 M HCl and were heated at 120°C for 15–18 h. Thereafter, the hydrolyzed mixed plasma protein samples were processed via the same procedures as the muscle protein-bound samples (described below). Plasma enrichments of the \(t\)-butyldimethyl-silyl (\(t\)-BDMS) derivative (12) of L-[ring-\(^2\)H\(_5\)]phenylalanine was measured by GC-MS analysis (described below).

**Muscle analyses.** Mixed muscle protein L-[ring-\(^2\)H\(_5\)]phenylalanine enrichments were extracted from 50 mg of wet muscle tissue. After the muscle was freeze-dried, collagen, blood, and other nonmuscle fibers were removed from the muscle fibers under a light microscope. The isolated muscle fiber mass (~10 mg) was weighed, and 35 volumes (7 × dry wt of isolated muscle fibers × wet/dry ratio 5:1) of ice-cold 2% PCA were added, and the sample was homogenized and centrifuged. The mixed muscle protein pellet was washed with 1.5 ml of 2% PCA, and the pellet was lyophilized. Amino acids were liberated by adding 6 M HCl after which the hydrolyzed protein fraction was dried under a nitrogen stream while being heated to 120°C. The protein fraction was then dissolved in a 25% acetic acid solution and passed over a Dowex exchange resin (AG 50W-X8, 100–200 mesh hydrogen form; Biorad, Hercules, CA). The amino acids were eluted with 2 M NH₄OH, dried, and the purified amino acids were analyzed by GC-MS.
acids were derivatized into MTBSTFA-phenylethylamines (5, 12) to determine the L-[ring-2H5]phenylalanine enrichment of mixed muscle protein as described below.

Gas chromatography-mass spectrometry. Plasma-free and mixed protein samples were analyzed by GC-MS (6890N GC coupled with a 5973 inert MSD; Agilent Technologies, Little Falls, DE) in duplicate (plasma-free samples) or triplicate (mixed protein samples) using electron impact ionization and selected ion monitoring for measurement of isotope ratios. Specifically, plasma free L-[ring-2H5]phenylalanine enrichments were measured from the mass-to-charge ratios at 336 (m + 1) and 341 (m + 5), with m + 0 representing the lowest molecular weight of the ion. Mixed protein enrichments were determined by selected ion monitoring for phenylalanine mass to charge ratio at 183 (m + 0) and a single linear standard curve from mixtures of known m + 5-to-m + 0 ratios (10).

Elimination of bias due to any potential concentration dependency (5, 10) was accomplished by injecting nearly identical amounts of phenylalanine for all samples and standards. Initial measurements were made on various amounts of phenylalanine to ensure that the amount of phenylalanine injected for all samples would be below saturation levels of the detector and would produce Gaussian-like peaks.

Calculations. Mixed muscle protein FSRs were calculated using the standard precursor-product equation: FSR (%h\(^{-1}\)) = Ep2 - Ep1/[Ep1]precursor × t / 100. In experiment 1, for the 1BX SHORT condition, Ep2 and Ep1 are the protein-bound enrichments measured in the muscle biopsy collected at 90 min and mixed plasma proteins (at t = 0 min), respectively. For 1BX LONG, Ep2 and Ep1 represent the protein-bound enrichments measured in the muscle biopsy collected at 210 min and mixed plasma protein (at t = 0 min), respectively. For 2BX, Ep2 and Ep1 are the protein-bound enrichments measured in muscle biopsies collected at 210 and 90 min, respectively. The “study population” average was determined from the background (pre-infusion) mixed plasma protein enrichments in experiment 1 subjects. In addition, zero background enrichment was used to assess the variability that is introduced in the single muscle biopsy approach when no background value (i.e., Ep2 - 0) is applied in the calculation of FSR.

In experiment 2 (both visits), for the 1BX SHORT condition, Ep2 and Ep1 are the protein-bound enrichments measured in the muscle biopsy collected at 50 min and mixed plasma proteins (at t = 0 min), respectively. For 1BX LONG, Ep2 and Ep1 represent the protein-bound enrichments measured in the muscle biopsy collected at 170 min and mixed plasma protein (at t = 0 min), respectively. For 2BX, Ep2 and Ep1 are the protein-bound enrichments measured in muscle biopsies collected at 170 and 50 min, respectively. In all cases, [Ep1]precursor is the average plasma free L-[ring-2H5]phenylalanine, and t indicates the tracer incorporation time.

Statistics. A one-factor (condition) ANOVA with repeated measures was used to compare differences in mixed muscle protein FSR between conditions. Linear regression analyses were performed on the plasma enrichments to assess the existence of any deviation in enrichment. Pearson’s r product moment correlation was used to compare differences in mixed muscle protein FSR and incorporation times for experiment 1 (n = 27) and experiment 2 (n = 9). Values represent means ± SE.

RESULTS

Tracer enrichments and incorporation times. As shown in Fig. 2, plasma free L-[ring-2H5]phenylalanine enrichments did not deviate from tracer steady-state during experiment 1 (P = 0.13) or in experiment 2 on visit 1 (P = 0.71) and visit 2 (P = 0.60). Background L-[ring-2H5]phenylalanine enrichment of mixed plasma protein in experiment 1 averaged −0.000023 ± 0.0000018 tracer-to-tracee ratio (TTR). The mixed plasma protein L-[ring-2H5]phenylalanine enrichments ranged from −0.000033 to 0.000021 TTR in the tracer naive subjects. In experiment 2, the background L-[ring-2H5]phenylalanine enrichment of mixed plasma proteins averaged −0.0000178 ± 0.0000005 TTR (range −0.000034 to 0.000010 TTR) in visit 1, and the background (pre-infusion) plasma protein enrichments had increased ~76-fold to 0.0000726 ± 0.0000059 TTR on the subsequent visit to the laboratory (P < 0.001). Presented in Table 1 are the average changes in protein enrichments (TTR) and incorporation times for visits 1 and 2.

Mixed muscle protein synthesis. In experiment 1, using the background L-[ring-2H5]phenylalanine enrichment of mixed plasma protein coupled with a single muscle biopsy enrichment resulted in similar calculated mixed muscle protein synthesis rates compared with the sequential muscle biopsy approach (P = 0.17; Fig. 3A). However, there was no significant correlation between the 1BX SHORT using 90 min of tracer incorporation time and the 2BX condition in mixed muscle protein synthesis rates (r = 0.34; P = 0.08; Fig. 3B). The intercept for the linear regression of best fit between the 1BX SHORT and 2BX was 0.012 ± 0.010 and the slope of the regression was 0.5 ±
In contrast, a significant correlation in mixed muscle protein synthesis rates was observed between the 1BX LONG and 2BX conditions ($r = 0.80; P < 0.001$) (Fig. 3C). The intercept for the linear regression of best fit between the 1BX LONG and 2BX was 0.0055 ± 0.0004, and the slope of the regression was 0.8 ± 0.1. The Bland-Altman analysis indicates that the 95% limits of agreement between the 1BX LONG and 2BX methods ranged from −0.009 to 0.012%/h (plot not shown). The level of disagreement (the bias) between the 1BX LONG/2BX approaches was 0.002 ± 0.001%/h. The relative or absolute differences in mixed muscle protein synthesis rates between 1BX SHORT and 2BX were 38 ± 10 or 0.010 ± 0.002%/h and between 1BX LONG and 2BX were 17 ± 7 or 0.004 ± 0.001%/h. Also, using the study population background plasma enrichment average (i.e., −0.000023 ± 0.00000018 TTR) resulted in a significant difference in muscle protein synthesis rates between the 1BX SHORT and 2BX ($P = 0.03$). However, no significant differences in muscle protein synthesis rates were observed between the 1BX LONG and 2BX approaches (Fig. 4A). A significant correlation in mixed muscle protein synthesis rates was only observed between the 1BX LONG and 2BX conditions using the population reference value ($r = 0.76; P < 0.001$) and not between the 1BX SHORT and 2BX conditions ($r = 0.29; P = 0.14$). The use of zero as the background enrichment for the calculation of muscle protein synthesis rates resulted in significant differences between the 2BX and the 1BX SHORT/LONG approaches (Fig. 4B).

In experiment 2, using the background L-[ring-$^2$H$_5$]phenylalanine enrichment of mixed plasma proteins coupled with the shorter incorporation times for 1BX SHORT/LONG approaches in visit 1 resulted in similar calculated mixed muscle protein synthesis rates compared with sequential muscle biopsy approach ($P = 0.9$). However, there was no significant correlation between the 1BX SHORT using 50 min of incorporation time and 2BX conditions in mixed muscle protein synthesis rates ($r = −0.08; P = 0.86$). Conversely, a significant correlation in mixed muscle protein synthesis rates were observed between the 1BX LONG using 170 min of tracer incorporation time and the 2BX conditions ($r = 0.80; P < 0.001$). The relative or absolute differences in mixed muscle protein synthesis rates between 1BX SHORT or 2BX approach were 231 ± 149 or 0.028 ± 0.007%/h and between 1BX LONG or 2BX approach were 31 ± 6 and 0.008 ± 0.002%/h. In visit 2 of experiment 2, there were significant differences ($P < 0.001$) in muscle protein synthesis rates between the 1BX SHORT (−0.669 ± 0.051%/h) and the 1BX LONG (−0.189 ± 0.051%/h) conditions when compared against the 2BX condition (0.024 ± 0.010%/h). The relative difference in mixed muscle protein synthesis rates between the 1BX LONG (visit 1) and 2BX approach (visit 2) was 63 ± 13%.

The muscle protein-bound enrichments were significantly greater ($P < 0.05$) in visit 2 compared with visit 1 (Fig. 5A). There was an ~25% difference in postabsorptive muscle protein synthesis rates between the sequential biopsy approaches of visit 1 and visit 2 (Fig. 5B). However, this difference did not reach significance ($P = 0.47$).

**DISCUSSION**

In this investigation, we report that using the background L-[ring-$^2$H$_5$]phenylalanine enrichment of mixed plasma proteins for the initial enrichment in the calculation of basal mixed muscle protein synthesis rates generates reliable data for determining group averages for basal mixed muscle FSR in older adults. The use of the single biopsy approach in subjects that have received L-[ring-$^2$H$_5$]phenylalanine in previous stable isotope amino acid tracer infusion experiments resulted in erroneous muscle protein synthesis rates (i.e., negative values).

An aim of the present investigation was to determine whether a detectable background enrichment of L-[ring-$^2$H$_5$]phenylalanine would be present in mixed plasma protein samples obtained from tracer naive subjects, and whether these plasma protein enrichment values could be used in the single biopsy approach for the determination of muscle protein synthesis rates. Interestingly, the average background enrichment of L-[ring-$^2$H$_5$]phenylalanine was essentially nonexisting in experiment 1 (−0.000023 ± 0.0000018 TTR) and in experiment 2 of visit 1 (−0.0000178 ± 0.0000005 TTR). Such a finding indicates that L-[ring-$^2$H$_5$]phenylalanine in the examined (pre-infusion) mixed plasma protein samples were not within the detection limits of the GC-MS analysis (i.e., the m + 5 abundance is low in a non-enriched sample). We applied these background enrichment values in the calculation of FSR and found that the single biopsy approach can still generate reliable reference postabsorptive muscle protein synthesis rates (Fig. 3A). These findings do not seem different from using a carbon-labeled

### Table 1. Average changes in protein enrichments and incorporation times

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<tr>
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<th>1BX SHORT</th>
<th>1BX LONG</th>
<th>2BX</th>
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<tr>
<td><strong>Incorporation time, min</strong></td>
<td>85 ± 1</td>
<td>205 ± 1</td>
<td>120 ± 1</td>
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<tr>
<td>ΔProtein, TTR</td>
<td>0.000035 ± 0.000003</td>
<td>0.000096 ± 0.000006</td>
<td>0.000061 ± 0.000004</td>
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**Visit 1**

|                  | 50 ± 3    | 170 ± 3  | 119 ± 1        |
| ΔProtein, TTR     | 0.000022 ± 0.000007 | 0.000068 ± 0.000009 | 0.000052 ± 0.000010 |

**Visit 2**

|                  | 44 ± 2    | 166 ± 2  | 123 ± 2        |
| ΔProtein, TTR     | −0.000359 ± 0.000050 | −0.000429 ± 0.000071 | 0.000048 ± 0.000019 |

Values are means ± SE. TTR, tracer-to-tracee ratio; 1BX SHORT, single biopsy approach coupled with a short incorporation time; 1BX LONG, single biopsy approach coupled with a longer incorporation time; 2BX, sequential biopsy approach.
amino acid tracer (e.g., L-[ring-\(^{13}\)C\(_6\)]phenylalanine). In accordance, we report an \(\sim 17\%\) variability within our measurement of mixed muscle protein synthesis rates between the 1BX LONG and 2BX conditions using L-[ring-\(^2\)H\(_5\)]phenylalanine. This variability is similar to previous observations using L-[ring-\(^{13}\)C\(_6\)]phenylalanine and GC-C-IRMS analysis to assess the reliability of the single muscle biopsy approach in young men (4).

In the present investigation, calculating the muscle protein FSR using the “study” population reference background enrichment (Fig. 4A) also resulted in a significant correlation between the single biopsy and the sequential muscle biopsies approaches, provided the amino acid tracer incorporation time was longer in duration (i.e., 1BX LONG condition). This finding is not surprising given that our population reference value originated from the same cohort of subjects that it was later applied to for the determination of muscle protein synthesis rates. Of course, simply using zero as the background enrichment for the calculation of muscle protein synthesis, irrespective of whether it is used in the 1BX SHORT or 1BX LONG approaches, results in erroneous muscle protein FSRs (Fig. 4B). These results, and those of others (14), underpin the notion that some reference background value is required to

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Fig. 3. Mixed muscle protein synthesis rates expressed as fractional synthesis rates (FSR) during fasting conditions calculated by using the plasma-free tracer enrichment as the precursor pool and using the mixed plasma protein enrichment and a muscle biopsy enrichment at 90 min (1BX SHORT), 210 min (1BX LONG), or sequential muscle biopsies (2BX) in experiment 1 (A). The relationship of mixed muscle protein FSR between the sequential muscle biopsy approach (2BX) and the single muscle biopsy approach coupled with 90 min (1BX SHORT) or 210 min (1BX LONG) of tracer incorporation time. No correlation was observed between the 1BX SHORT and 2BX approach (B; \(r = 0.34; P = 0.08\); \(y = 0.5 \pm 0.3x + 0.012 \pm 0.010\)). A significant correlation was observed between the 1BX LONG and 2BX approach (C; \(r = 0.8; P < 0.001\); \(y = 0.8 \pm 0.1x + 0.005 \pm 0.004\)). Dark line: line of linear best fit with a 95% confidence interval. Broken line: line of identity.

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Fig. 4. Mixed muscle protein FSR calculated assuming zero background labeling (A) or using the study population average background enrichment (B). Values are means ± SE. *Significantly different from the 1BX SHORT condition (\(P < 0.001\)). †Significantly different from the 1BX LONG condition muscle protein (\(P < 0.001\)).
incorporation time before the single muscle biopsy in both the
generated from the single muscle biopsy approach. What is
need to include a longer tracer incorporation time before the
those of others (2, 4), are that amino acid tracer experiments
practical recommendations from the present investigation, and
(visit 1) allowed us to assess the impact of allocating less tracer
approach (Fig. 3). The C
in muscle protein synthesis rates that were more closely related
extension time (210 min) before the single muscle biopsy resulted
A
approach (2, 4). We report that
fundamental impact on the reliability of the single muscle
experiment 1 and experiment 2
experiment 2
experiment 1
experiment 2
visit 1 or visit 2.
Values are means ± SEM. *Significantly different from the first muscle biopsy enrichment (P < 0.05). †Significantly different from visit 2 at that muscle biopsy time point (P < 0.05).

Fig. 5. A: muscle protein phenylalanine enrichment (TTR) measured from the first or second muscle biopsies collected during experiment 2 of visit 1 or visit 2. B: mixed muscle protein synthesis rates expressed as FSR calculated from the sequential muscle biopsy approach during experiment 2 of visit 1 or visit 2.

1BX SHORT (~44 min) and 1BX LONG (~177 min) conditions. We found that there were no differences in muscle protein synthesis rates between the 1BX SHORT/LONG conditions compared with 2BX condition. It is clear, however, there are substantial intra-individual variations between the 1BX SHORT and 2BX condition as noted by the 231% difference in muscle protein synthesis rates between the two approaches. We observed less variation in muscle protein synthesis rates (~31%) between the 1BX LONG and 2BX conditions. In fact, a significant correlation in muscle protein FSR was only observed between the 1BX LONG and 2BX approach on visit 1 (experiment 2). These data highlight that a prolonged tracer incorporation time before the single biopsy improves the reliability or the single muscle biopsy approach and that extending the tracer incorporation time beyond 180 min further reduces this intra-individual variation (noted by the 17% variation in the 1BX LONG condition in experiment 1).

As a proof-of-principle (experiment 2), we demonstrated that the recruitment of tracer naïve subjects is required for the single muscle biopsy approach to provide reliable muscle protein FSR results. Specifically, the pre-infusion mixed plasma protein enrichments were approximately twofold greater than the muscle protein-bound enrichments, and this finding ultimately resulted in the calculated muscle protein FSRs to be negative. Also in experiment 2, we assessed the measurement variability of the 2BX approach by measuring postabsorptive muscle protein synthesis rates in the same subjects (separated by 2 wk). We observed ~25% variation in mixed muscle protein synthesis rates between the 2BX conditions of visits 1 and 2, which is similar to the ~23% variation previously reported by Parise et al. (9). These findings are not trivial since they highlight that any detectable change in post-absorbptive muscle protein FSR induced after anabolic (exercise training) and/or catabolic (inactivity) interventions will need to be robust to overcome this variation. In contrast, it is likely that if only subtle changes in postabsorbptive muscle protein synthesis rates occur after a prolonged intervention, they may go undetected.

What is the value of using the single biopsy approach for the determination of basal muscle protein synthesis rates? In our view, the single biopsy approach can provide a reliable reference value for postabsorbptive muscle protein synthesis rates compared with obtaining sequential muscle biopsies. Of course, an argument can be put forward that to calculate “true” muscle protein synthesis rates the protein-bound enrichments should likely originate from muscle proteins (e.g., 2BX approach) and not some other nonmuscle protein pool (e.g., 1BX SHORT/LONG approaches). However, certain experimental designs may require multiple muscle biopsies to be obtained to correctly address the research question. Reducing the number of muscle biopsies required in a stable isotope amino acid infusion experiment is often favorable for both the workers and, more importantly, the study participants. For example, the aim of many experimental infusion protocols is not to simply assess postabsorbptive muscle protein synthesis rates but to determine the subsequent muscle protein synthetic response after a particular feeding paradigm (an experimental approach that requires multiple muscle biopsies to be collected). Notable is that we do not expect the reliability of the data generated from the single muscle biopsy approach to differ with age. However, it is apparent that the single muscle biopsy approach

improve the reliability of the single muscle biopsy approach, and this value cannot simply be zero.

The present work (experiment 1) corroborates previous FSR data suggesting the timing of the initial muscle biopsy has a fundamental impact on the reliability of the single muscle biopsy approach (2, 4). We report that ~90 min of tracer incorporation time can generate muscle protein synthesis rates that are similar to the well described sequential muscle biopsy approach (Fig. 3A). However, extending the tracer incorporation time (~210 min) before the single muscle biopsy resulted in muscle protein synthesis rates that were more closely related to the sequential muscle biopsy approach (Fig. 3C). The practical recommendations from the present investigation, and those of others (2, 4), are that amino acid tracer experiments need to include a longer tracer incorporation time before the initial muscle biopsy to improve the reliability of the data generated from the single muscle biopsy approach. What is noteworthy is that the experimental approach in experiment 2 (visit 1) allowed us to assess the impact of allocating less tracer incorporation time before the single muscle biopsy in both the
may be a useful tool to apply in sarcopenic or the frail elderly where the number of muscle biopsies that can be collected may be limited.

As an advisory note, we suggest that the single muscle biopsy approach may not be an ideal method to use to compare postabsorptive muscle protein synthesis rates, for example, between the young and the old. In view of the notion that muscle protein synthesis rates may gradually decline (~3.5% per decade) over the ages of 20–80 yr (13), it would appear that any differences between the healthy young and old are subtle (especially considering muscle protein synthesis rates are often expressed as %/h). Thus the inherent variability (~17% in the present study) that is associated with the single muscle biopsy approach, compared with the 2BX approach, may only further contribute to the ongoing debate with regard to whether postabsorptive muscle protein synthetic rates differ between the young and the old (1, 6, 16). Finally, it is worth highlighting the work of Smith et al. (14) that demonstrated that the timing of the initial muscle biopsy does not matter with regard to the measured muscle protein synthesis rates. These data are important since they show that, to circumvent the tracer naive “problem,” a simple solution is to obtain a muscle biopsy before stable isotope amino acid infusion experiment, and this approach would also effectively function to minimize the duration of the stable isotope infusion experiment.

In conclusion, the single biopsy approach coupled with the background enrichment of L-[ring-2H5]phenylalanine of mixed plasma proteins (measured with GC-MS analysis) generates data that are similar to using the sequential muscle biopsy approach in older men. A prolonged tracer incorporation time should minimize the intra-individual subject variability and ultimately improves the reliability of the data generated from the single muscle biopsy approach. Our general recommendations are the single muscle biopsy approach can be used to generate a reliable reference value for postabsorptive muscle protein synthetic rates. Such muscle protein FSR data may be useful to provide a reference value for experimental protocols designed to assess the impact of exercise and nutritional and/or pharmaceutical interventions on the subsequent stimulation of muscle protein synthesis rates.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES