A new method to study in vivo protein synthesis in slow- and fast-twitch muscle fibers and initial measurements in humans

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Dickinson JM, Lee JD, Sullivan BE, Harber MP, Trappe SW, Trappe TA. A new method to study in vivo protein synthesis in slow- and fast-twitch muscle fibers and initial measurements in humans. J Appl Physiol 108: 1410–1416, 2010. First published March 4, 2010; doi:10.1152/japplphysiol.00905.2009.—The aim of this study was to develop an approach to directly assess protein fractional synthesis rate (FSR) in isolated human muscle fibers in a fiber type-specific fashion. Individual muscle fibers were isolated from biopsies of the vastus lateralis (VL) and soleus (SOL) obtained from eight young men during a primed, continuous infusion of [5,5,5-2H3]leucine performed under basal conditions. To determine mixed protein FSR, a portion of each fiber was used to identify fiber type, fibers of the same type were pooled, and the [5,5,5-2H3]leucine enrichment was determined via GC-MS. Processing isolated slow-twitch [myosin heavy chain (MHC) I] and fast-twitch (MHC IIa) fibers for mixed protein bound [5,5,5-2H3]leucine enrichment yielded mass ion chromatographic peaks that were similar in shape, abundance, and measurement reliability as tissue homogenates. In the VL, MHC I fibers exhibited a 33% faster (P < 0.05) mixed protein FSR compared with MHC IIa fibers (0.068 ± 0.006 vs. 0.051 ± 0.003%/h), MHC I fibers from the SOL (0.060 ± 0.005%/h) and MHC II fibers from the VL displayed similar (P > 0.05) mixed protein FSR. Feasibility of processing isolated human muscle fibers for analysis of myofibrillar protein [5,5,5-2H3]leucine enrichment was also confirmed in non-fiber-typed pooled fibers from the VL. These methods can be applied to the study of fiber type-specific responses in human skeletal muscle. The need for this level of investigation is underscored by the different contributions of each fiber type to whole muscle function and the numerous distinct adaptive functional and metabolic changes in MHC I and MHC II fibers originating from the same muscle.

Fractional synthesis rate; [5,5,5-2H3]leucine; myosin heavy chain

Human skeletal muscle is comprised of a mixture of different fiber types that have unique metabolic and contractile characteristics. For instance, slow-twitch fibers possess a high oxidative capacity and are less prone to fatigue, whereas fast-twitch fibers are more anaerobic in nature and are capable of generating substantially more power (up to 20 times) than slow-twitch fibers (16, 46, 54, 59). Due to these contrasting properties, each fiber type contributes very differently to whole muscle function (8, 57), and thus adaptations occurring at the whole muscle level are influenced by the specific alterations of each fiber type. As a result, fiber type-specific responses to acute and chronic interventions have been of scientific interest, and over the past 35 years it has been well documented that in response to a given stimulus each fiber type often adapts differently with respect to metabolic, structural, and contractile properties (18, 23, 27, 51, 56). Further, these differential fiber type adaptations are commonly associated with conditions of muscle wasting and decreased muscle function, such as aging and long-term unloading (15, 32, 53, 57–59). While the importance of investigating fiber type-specific adaptation is evident in human skeletal muscle, our current understanding of the underlying mechanisms responsible for differential fiber type adaptation is limited due to the lack of existing methodologies to examine in vivo metabolic processes in a fiber type-specific fashion.

Monitoring changes in the rate of muscle protein synthesis in response to a given stimulus can provide important insight into the adaptive state of skeletal muscle (5, 21). This technique has proven useful for the examination of tissue homogenates obtained from human skeletal muscle biopsies (2, 6, 41); however, a limitation to the current method is that it does not differentiate between the protein synthesis rates occurring in different fiber types. Consequently, the current methodologies cannot be utilized in humans to provide insight into the adaptive processes taking place specifically in slow- and fast-twitch fibers originating from the same muscle.

To expand on our laboratory’s long interest in characterizing fiber type-specific functional (33), structural (12), and molecular (29) responses to aging (54), spaceflight (50), bed rest (52, 53, 57), and aerobic (51, 56) and resistance (48, 55, 58) exercise, the purpose of this investigation was to develop a method to directly assess protein synthesis rates in a fiber type-specific manner. Further, as an initial use of the method we compared resting protein synthesis rates between slow-twitch [myosin heavy chain (MHC) I] and fast-twitch (MHC IIa) fibers from the vastus lateralis and between MHC I fibers from the vastus lateralis and soleus.

Materials and Methods

Subjects and Study Design

A total of eight men (23 ± 1 yr; 178 ± 3 cm; 81 ± 4 kg) were recruited to take part in this investigation. All subjects were considered recreationally active (i.e., were not involved in any regular aerobic or resistance exercise program), nonobese, nonsmokers, and apparently healthy as determined from a detailed medical history questionnaire. This investigation was approved by the Institutional Review Board at Ball State University, and each subject provided written consent before participation.

Each subject underwent one experimental trial (detailed below), with the primary aim of this investigation being to develop a method to directly assess mixed protein fractional synthesis rates (FSR) in MHC I and MHC IIa fibers isolated from human muscle biopsies. During analysis of the first five subjects, we were able to demonstrate the feasibility of both isolating individual muscle fibers and processing isolated MHC I and MHC IIa fibers for stable isotope enrichment analysis. Additionally, in both fiber types we demonstrated the ability to achieve m + 0 ion abundance values on the gas chromatography-mass spectrometry (GC-MS) instrument similar to that in muscle tissue homogenate samples. With the achievement of our primary
objectives, we chose to expand the method development to determine whether myofibrillar protein isotopic enrichment could be assessed in isolated human muscle fibers in a separate subset of five subjects. As a result of the different analyses, the two subsets of five subjects were categorized into subject groups A and B. The analyses performed on the muscle from subject group A included determination of mixed protein FSR in MHC I and MHC IIA fibers from the vastus lateralis, MHC I fibers from the soleus, and tissue homogenates from the vastus lateralis and soleus. The analysis performed on the muscle from subject group B included determination of myofibrillar protein isotopic enrichment in isolated fibers (non-fiber typed) from the vastus lateralis.

Dietary and Activity Control

Subjects were asked to maintain their normal dietary habits and refrain from any exercise for 3 days before the experimental trial. The evening before the experimental trial, subjects consumed a standardized liquid meal (Ensure Plus: Abbott Laboratories, Abbott Park, IL) with a macronutrient content of 57% carbohydrate, 15% protein, and 28% fat. The liquid meal provided 50% of the subjects estimated caloric need (1.5 times the subjects predicted resting metabolic rate) to standardize the composition, amount, and timing (i.e., duration of fast) of the final meal consumed before the experimental trial (13, 22). Subjects remained in the fasted state throughout the experimental trial.

Experimental Trial

The precursor-product model was used for the determination of protein FSR via incorporation of [5,5,5-\textsuperscript{2}H\textsubscript{3}]leucine into proteins in skeletal muscle fibers and skeletal muscle tissue homogenates at rest during a 6-h primed, continuous tracer infusion. On the morning of the experimental trial each subject had an 18-gauge catheter placed in an antecubital vein for infusion of the stable, isotopically labeled amino acid [5,5,5-\textsuperscript{2}H\textsubscript{3}]leucine (Cambridge Isotopes, Andover, MA). [5,5,5-\textsuperscript{2}H\textsubscript{3}]leucine was dissolved in 0.9% saline, passed through a 0.2-µm filter before infusion, and infused with a calibrated infusion pump (PHD 2000, Harvard Apparatus, Natick, MA) at 0.125 µmol·kg\textsuperscript{-1}·min\textsuperscript{-1} preceded by a priming dose of 4.8 µmol·kg\textsuperscript{-1} (13, 22). The tracer was confirmed sterile and pyrogen free before use. A total of three muscle biopsies were obtained following local anesthetic (Lidocaine HCI 1%) using a 5-mm Bergstrom needle with suction (4). A background muscle biopsy was taken from the vastus lateralis before initiating the infusion for the determination of natural [5,5,5-\textsuperscript{2}H\textsubscript{3}]leucine tracer enrichment in muscle protein. All subjects were tracer naïve (i.e., had not previously received a tracer infusion), and therefore the protein enrichment from this background biopsy was used to represent the beginning of the isolated fiber and muscle tissue homogenate protein FSR measurements for both fiber types (MHC I and MHC IIA) and muscles (vastus lateralis and soleus) examined (34, 35, 43). The remaining biopsies, one from the vastus lateralis (opposite leg) and one from the soleus, were taken at 6 h into the tracer infusion and were used to represent the conclusion of the isolated fiber and muscle tissue homogenate protein FSR measurement for each respective muscle. All muscle samples were cleaned of visible fat and blood, separated into muscle bundles of parallel running fibers for fiber isolation (6 h only), and immediately frozen and stored in liquid nitrogen (−190°C) until fiber isolation or determination of protein FSR (homogenate).

A catheter was also placed in the antecubital vein of the opposite arm for blood sampling. Blood samples were obtained before initiating the infusion (background) and at 0.5, 1.5, 2.5, 3.5, 4.5, and 5.5 h into the infusion for the measurement of plasma fluid α-ketosocaprate (α-KIC) and [5,5,5-\textsuperscript{2}H\textsubscript{3}]leucine enrichment. Additionally, the natural [5,5,5-\textsuperscript{2}H\textsubscript{3}]leucine enrichment in plasma protein was measured in background blood samples. This measurement was performed to determine whether [5,5,5-\textsuperscript{2}H\textsubscript{3}]leucine enrichment in plasma protein could be used in tracer naïve individuals to accurately represent baseline muscle protein [5,5,5-\textsuperscript{2}H\textsubscript{3}]leucine enrichment for the determination of muscle protein FSR (see Calculation of protein FSR).

Isolation of Muscle Fibers

A bundle of muscle fibers (~7 mm in length) previously frozen in liquid nitrogen was placed into 400 µl of RNAlater-ICE (Ambion, Austin, TX) and stored at −20°C overnight. The bundle was then washed in 500 µl of RNAlater (Ambion, Austin, TX) by lightly shaking for 2 min, placed into a second aliquot of 500 µl of RNAlater, and stored at −20°C until isolation of muscle fibers. The bundle of fibers was placed into a Petri dish containing RNAlater. Approximately 190 individual muscle fibers (total of vastus lateralis and soleus fibers) per subject were isolated at room temperature under a light microscope (29, 63) using fine tweezers, and approximately one-quarter of each isolated fiber was clipped and placed into 40 µl of SDS sample buffer for fiber type identification via SDS-PAGE. The remaining isolated muscle fiber (approximately three-quarters of original fiber length) was placed into 75 µl of RNAlater and stored at −20°C until fiber pooling and determination of protein [5,5,5-\textsuperscript{2}H\textsubscript{3}]leucine enrichment.

RNAlater was chosen as the storage and isolation medium for three reasons. First, RNAlater preserves the quantity of proteins equivalent to, if not better than, liquid nitrogen (14). Second, RNAlater preserves molecular components of the cell at room temperature for an extended period of time (1, 30). Third, RNAlater denatures cellular proteins (1), which would eliminate any additional protein turnover during the fiber isolation process.

Isolated Muscle Fiber MHC Identification

The myosin isoform of each individual isolated muscle fiber was determined via SDS-PAGE, which has been described in detail previously by our laboratory (62). Briefly, the clipped portion of each isolated fiber was solubilized in 40 µl of 1% SDS sample buffer (1% SDS, 6 mg/ml EDTA, 0.06 M Tris, pH 6.8, 2 mg/ml bromophenol blue, 15% glycerol, and 5% β-mercaptoethanol), heated, and subjected to SDS-PAGE and silver staining. The MHC isoforms were identified according to migration distance. A representative MHC gel is shown in Fig. 1.

Stable Isotope Enrichment Measurements

Fiber type-specific mixed protein bound [\textsuperscript{2}H\textsubscript{3}]leucine labeling. Isolated MHC I and MHC IIA fibers from the vastus lateralis and isolated MHC I fibers from the soleus of subject group A were analyzed for mixed protein bound [5,5,5-\textsuperscript{2}H\textsubscript{3}]leucine enrichment. Following fiber type identification, ~20 corresponding MHC I fibers or ~20 MHC IIA fibers were homogenized in 60 µl of ice cold 14% perchloric acid (PCA) for 1 min with a Teflon-coated pestle and centrifuged at 21,000 g for 10 min at 4°C, and the supernatant was
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discarded. The remaining pellet was washed once in 100 μl of purified and deionized water and twice in 100 μl of ethanol, with each wash followed by centrifugation at 21,000 g for 10 min at 4°C. Following the last ethanol wash, the remaining pellet, taken as the isolated fiber mixed protein, was hydrolyzed in 1 ml of 6 N HCl for 24 h at 100°C.

Isolated fiber myofibrillar protein bound [5,5,5–2H3]leucine labeling. Isolated fibers from the vastus lateralis of subject group B were analyzed for myofibrillar protein bound [5,5,5–2H3]leucine enrichment using well-established techniques to isolate the myofibrillar protein fraction from muscle tissue homogenates (6, 11, 36, 61). Further, given that myofibrillar protein constitutes approximately half of the mixed protein fraction (54), we doubled the number of isolated fibers to achieve the necessary m + 0 abundance on the GC-MS. However, our goal was not to compare myofibrillar protein FSR between fiber types, but rather to evaluate the necessary steps to successfully measure myofibrillar protein enrichment in isolated human muscle fibers that will allow future investigations to examine fiber type-specific myofibrillar protein synthesis. Therefore, we did not determine the fiber type of isolated fibers used for this aspect of the project.

Forty non-fiber-typed isolated fibers were homogenized in 100 μl of homogenizing buffer (250 mM sucrose, 100 mM potassium chloride, 20 mM imidazole, 5 mM EDTA, pH = 6.8) for 2 min with a Teflon-coated pestle and then centrifuged at 1,600 g for 30 min at 4°C. The pellet contained myofibrillar proteins and was washed once with 100 μl of homogenizing buffer and once with 100 μl of purified and deionized water, with each wash followed by centrifugation at 1,600 g for 20 min at 4°C. Myofibrillar proteins were dissolved by adding 150 μl of 0.3 N NaOH to the remaining pellet, and insoluble proteins (i.e., collagen) were removed by centrifugation at 4,000 g for 20 min at 4°C. The supernatant was transferred to a new tube and added to 100 μl of 1 N PCA, centrifuged at 4,000 g for 20 min at 4°C, and the remaining pellet, taken as the myofibrillar protein, was hydrolyzed in 1 ml of 6 N HCl for 24 h at 100°C.

Plasma [5,5,5–2H3]leucine and [5,5,5–2H3]α-KIC labeling. Each plasma sample was deproteinated with a 1:1 ratio of 15% sulfosalicylic acid (SSA) and centrifuged at 21,000 g for 10 min at 4°C, and plasma fluid [5,5,5–2H3]leucine enrichment was determined from the supernatant. The pellet from the background blood sample was subsequently washed twice more in 300 μl of SSA and once with 300 μl of ethanol, with each wash followed by centrifugation at 21,000 g for 10 min at 4°C. Following the ethanol wash, the pellet, taken as the plasma protein, was dried overnight at 37°C and then hydrolyzed in 5 ml of 6 N HCl for 24 h at 100°C. Plasma fluid α-KIC enrichment was determined as previously described (24).

Muscle tissue homogenate and muscle tissue fluid [5,5,5–2H3]leucine labeling. Muscle tissue homogenate samples (24.56 ± 0.80 mg) were analyzed for mixed protein bound and muscle tissue fluid [5,5,5–2H3]leucine enrichment as previously described (41, 60).

Sample washing and derivatization. All samples were washed over a cation-exchange column (Dowex AG 50W-8X, 100 –200 mesh, H+ form, Bio-Rad Laboratories, Hercules, CA) and dried under vacuum (SC210A SpeedVac Plus, ThermoSavant, Holbrook, NY) (60). Once dried, samples were derivatized with a 1:1 mixture of acetoneitrile (ACN) and N-methyl-N-(t-butyldimethylsilyl)triﬂuoroacetamide (MTBSTFA; Pierce Chemical, Rockford, IL): isolated fiber mixed and myofibrillar samples with 50 μl, and muscle tissue fluid, plasma fluid, muscle protein, and muscle tissue homogenate samples with 100 μl. All samples were derivatized at 100°C; isolated fiber mixed and myofibrillar samples for 45 min, muscle tissue fluid and plasma fluid samples for 10 min, and plasma protein and muscle tissue homogenate samples for 30 min.

GC-MS. All samples were analyzed by GC-MS (6890N GC coupled with 5973 inert MSD. Agilent Technologies, Wilmington, DE) in triplicate (muscle tissue fluid and protein bound samples) or duplicate (plasma fluid samples) using electron impact ionization and selected ion monitoring of m/z 200 (m + 0), 201 (m + 1), 202 (m + 2), and 203 (m + 3) for leucine, and m/z of 301 (m + 0), 302 (m + 1), 303 (m + 2), and 304 (m + 3) for α-KIC, with m + 0 representing the lowest molecular weight of the ion. Plasma fluid [5,5,5–2H3]leucine and α-KIC enrichment and muscle tissue fluid [5,5,5–2H3]leucine enrichment were measured from the m + 3 to m + 0 ratio. [5,5,5–2H3]leucine enrichment of the protein bound samples was determined using the m + 3 to m + 2 ratio and a linear standard curve from mixtures of known m + 3 to m + 0 ratios, as previously described (10, 39). Elimination of bias due to any potential concentration dependency (39, 40) was accomplished by injecting nearly identical amounts of leucine (i.e., similar m + 0 and m + 2 abundances) for all samples (Fig. 2) and standards. Initial measurements were made on various amounts of leucine to ensure the amount of leucine injected for all samples would be below saturation levels of the detector and would produce Gaussian-shaped peaks.

Calculation of protein FSR. Protein FSR was calculated as the rate of [5,5,5–2H3]leucine incorporated into protein using the plasma fluid α-KIC or muscle tissue fluid [5,5,5–2H3]leucine enrichment as the precursor pool and the following equation:

\[
\text{FSR}\% / h = \frac{(E_t - E_0)}{E_p (t_1 - t_0) - 1} \times 100
\]

where \(E_0\) and \(E_t\) is the [5,5,5–2H3]leucine enrichment in the protein bound fraction from background and 6-h muscle biopsies, \((t_1 - t_0)\) is the [5,5,5–2H3]leucine tracer incorporation time, and \(E_p\) is the precursor enrichment. Mixed protein FSR in vastus lateralis MHC I and MHC IIa fibers were also calculated, where \(E_0\) was the [5,5,5–2H3]leucine enrichment in background plasma protein samples (20, 28, 49).

Statistical Analysis

A paired t-test was used for all protein FSR comparisons between vastus lateralis MHC I and MHC IIa fibers, and to compare the vastus lateralis and soleus for MHC I fiber protein FSR. [5,5,5–2H3]leucine muscle tissue fluid enrichment, and muscle tissue homogenate protein FSR. A paired t-test was also used to compare the protein FSR values determined using plasma and muscle protein to represent baseline [5,5,5–2H3]leucine enrichment within a given fiber type. Additionally, we calculated the statistical power (\(\beta\)) for the comparison of protein synthesis rates between MHC I and MHC IIa fibers from the vastus lateralis. For all variables, significance was accepted at \(P < 0.05\). Data are presented as means ± SE.

RESULTS

The plasma fluid α-KIC enrichment data reflect steady intracellular tracer enrichment during the protein synthesis measurement (0.5 h: 0.0704 ± 0.0021; 1.5 h: 0.0726 ± 0.0016; 2.5 h: 0.0738 ± 0.0014; 3.5 h: 0.0779 ± 0.0018; 4.5 h: 0.0791 ± 0.0016; 5.5 h: 0.0776 ± 0.0017).

Processing isolated MHC I and MHC IIa fibers for mixed protein bound [5,5,5–2H3]leucine enrichment yielded quantifiable m + 3 peaks and similar m + 0 and m + 2 abundances to that produced when examining muscle tissue homogenates (Fig. 2). The resulting mixed protein FSR of vastus lateralis MHC I fibers was 33% faster (\(P < 0.05\); \(\beta = 0.89\)) compared with vastus lateralis MHC IIa fibers (Fig. 3). Further, within a given fiber type a similar (\(P > 0.05\)) mixed protein FSR was obtained when baseline [5,5,5–2H3]leucine enrichment was determined from background muscle protein (MHC I: 0.068 ± 0.006; MHC IIa: 0.051 ± 0.003%/h) or background plasma protein (MHC I: 0.070 ± 0.009; MHC IIa: 0.053 ± 0.005%/h). The mixed protein FSR in MHC I fibers from the vastus lateralis and MHC I fibers from the soleus was similar (\(P > 0.05\)) (Fig. 4). Mixed protein FSR was similar (\(P > 0.05\)) between vastus lateralis (0.045 ± 0.003%/h) and soleus (0.051 ± 0.006%/h) tissue homogenates.
The feasibility of processing isolated (non-fiber typed) fibers for myofibrillar protein \([5,5,5-\text{H}_3]\)leucine enrichment was also confirmed as we observed quantifiable \(m/z\) 1001 peaks and similar \(m/z\) 0 abundances to that yielded by analysis of muscle tissue homogenates (Fig. 2), suggesting that various protein subfractions found within muscle fibers can be analyzed if the appropriate number of muscle fibers are isolated.

Plasma fluid \([5,5,5-\text{H}_3]\)leucine enrichment was also stable over the 6 h (data not shown), and the muscle tissue fluid \([5,5,5-\text{H}_3]\)leucine enrichment was similar (\(P > 0.05\)) in the vastus lateralis \((0.0572 \pm 0.0029)\) and soleus \((0.0559 \pm 0.0011)\). All the same mixed protein FSR results were obtained for the fiber type-specific (vastus lateralis: MHC I 33% higher than MHC IIa, \(P < 0.05\), \(\beta = 0.95\); similar MHC I in the vastus lateralis and soleus, \(P > 0.05\)) and tissue homogenate (similar vastus lateralis and soleus, \(P > 0.05\)) comparisons when the muscle tissue fluid \([5,5,5-\text{H}_3]\)leucine enrichment was used as the precursor, but with a somewhat higher absolute FSR (~30%) as has been previously shown (2, 3, 25, 26, 44).

Similar coefficient of variation values were observed for MHC I mixed protein bound, MHC IIa mixed protein bound, isolated fiber myofibrillar protein bound, and all tissue homogenate mixed protein bound samples (Table 1), which is attributed to equal amounts of leucine (i.e., similar \(m/z\) 0 abundances) injected into the GC-MS for all protein bound samples.

Based upon SDS-PAGE analysis performed on the individual fibers that were isolated for mixed protein FSR analysis, the Fig. 2. Representative mass ion chromatographs for \(m/z\) of 200 (\(m + 0\)), 202 (\(m + 2\)), and 203 (\(m + 3\)) for analysis of \([5,5,5-\text{H}_3]\)leucine protein bound enrichment in mixed protein from isolated vastus lateralis MHC I fibers (\(m + 0\) abundance, 38,033,184; \(m + 2\) abundance, 2,346,335) (A), mixed protein from isolated vastus lateralis MHC IIa fibers (\(m + 0\), 37,684,253; \(m + 2\) abundance, 2,286,659) (B), myofibrillar protein from isolated vastus lateralis non-fiber-typed fibers (\(m + 0\) abundance, 39,529,898; \(m + 2\) abundance, 2,447,519) (C), and mixed protein from a vastus lateralis tissue homogenate (\(m + 0\) abundance, 38,455,981; \(m + 2\) abundance, 2,355,590) (D). The peak corresponding to ~12.30 min (x-axis) represents the time of entry for leucine into the mass spectrometer, which was verified with pure leucine standard injections. The processing of isolated MHC I and MHC IIa fibers for mixed protein bound \([5,5,5-\text{H}_3]\)leucine enrichment and isolated non-fiber-typed fibers for myofibrillar protein bound \([5,5,5-\text{H}_3]\)leucine enrichment yielded quantifiable \(m + 3\) peaks, similar \(m + 0\) and \(m + 2\) abundances, and similar peak shapes to that produced when examining muscle tissue homogenates.

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**Fig. 3.** Mixed protein fractional synthesis rates (FSR) in MHC I and MHC IIa fibers from the vastus lateralis. A faster mixed protein FSR (*\(P < 0.05\)) was observed in MHC I vs. MHC IIa fibers from the vastus lateralis. Data are means ± SE from subject group A. Plasma fluid α-KIC enrichment was used as the precursor for the calculation of FSR.

**Fig. 4.** Mixed protein FSR in MHC I fibers from the vastus lateralis and soleus. No differences in mixed protein FSR (\(P > 0.05\)) were observed between MHC I fibers from the vastus lateralis and MHC I fibers from the soleus. Data are means ± SE from subject group A. Plasma fluid α-KIC enrichment was used as the precursor for the calculation of FSR.
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The primary findings from the present investigation were 1) human muscle fibers can be isolated and the enrichment of \([5,5,5-\text{H}_3]\)leucine determined in the mixed and myofibrillar 1 fiber type distribution in the vastus lateralis was as follows: MHC I, 36 ± 6%; MHC I/IIa, 14 ± 2%; MHC IIa, 32 ± 5%; MHC IIax, 16 ± 4%; and MHC I/IIax, 2 ± 1%. The fiber type distribution in the soleus was as follows: MHC I, 65 ± 4%; MHC I/IIa, 7 ± 2%; MHC IIa, 17 ± 2%; MHC IIax, 9 ± 4%; and MHC I/IIax, 1 ± 0.4%.

DISCUSSION

The primary findings from the present investigation were 1) human muscle fibers can be isolated and the enrichment of \([5,5,5-\text{H}_3]\)leucine determined in the mixed and myofibrillar 1 protein of pooled muscle fibers, 2) in muscle fibers isolated from the vastus lateralis, MHC I fibers exhibited a significantly faster (33%) rate of mixed protein synthesis compared with MHC IIa fibers, and 3) MHC I fibers from the vastus lateralis and soleus had similar mixed protein synthesis rates. These findings indicate that mixed and myofibrillar protein synthesis rates can be successfully assessed in muscle fibers isolated from human skeletal muscle biopsies, and these methods can be applied to the study of fiber type-specific responses in human skeletal muscle.

We used a 6-h infusion protocol to ensure adequate tracer incorporation into the muscle fibers, considering the relatively small amount of protein within each muscle fiber and that the measurements were conducted during basal conditions. However, in all isolated fiber samples (mixed and myofibrillar) we observed m + 0 abundances that were similar and m + 3 peaks that were equivalent in shape (Fig. 2) and measurement reliability (Table 1) to that observed during analysis of much larger muscle tissue homogenate samples. These findings suggest the methodology presented in the present investigation could certainly be utilized in a variety of study designs that incorporate much shorter infusion periods, that study fewer muscle fibers, or that examine specific protein components of the muscle fibers.

Individual muscle fiber isolation and determination of protein synthesis rate in the pure fiber types (i.e., fibers containing only one type of MHC) examines the muscle in a fundamentally different way than previous tissue homogenate approaches (5, 41), even when subfractions of these muscle tissue homogenates are examined (2, 6, 25, 26). The present approach allows the protein components contained within each muscle fiber of a given fiber type to be examined without the influence of protein components from other pure or hybrid fiber types, as well as proteins found between the muscle fibers. To this end, 32 ± 5% and 17 ± 3% of the vastus lateralis and soleus muscle fibers, respectively, were hybrid fibers in the untrained subjects in the present study, which is consistent with previous findings (19). Therefore, caution must be used when comparing the protein synthesis findings from the present isolated fiber measurements to homogenates of muscle protein (total or subfractions) reported in the present and previous studies (13, 22, 35). Similar comparisons across two (13, 22) or three (35) muscles becomes even more unwieldy. It is known that muscles of the lower leg, upper leg, and upper body have very different chronic activity patterns (i.e., training status), and chronic muscle activity influences fiber type (19, 56) and basal protein synthesis rate (17, 37, 38, 42, 47). However, it is unknown how these two adaptations are linked to each other. Regardless of the aforementioned issues, each approach provides useful but fundamentally different information and underscores the need for both types of investigations.

The present findings of a higher rate of protein synthesis in isolated MHC I fibers compared with MHC Ia fibers from the vastus lateralis are supported by recent human investigations utilizing immunohistochemistry to examine the phosphorylation status of key proteins involved in the regulation of protein synthesis. Specifically, the phosphorylation of eukaryotic elongation factor 2 (eEF2) and AMP-activated protein kinase (AMPK) is lower in MHC I fibers compared with MHC II fibers from the vastus lateralis at rest (31, 45). The phosphorylation of these proteins has been shown to attenuate protein synthesis (7, 9), and therefore the lower basal phosphorylation of both eEF2 and AMPK in MHC I fibers is in agreement with the faster basal protein synthesis rate in MHC I compared with MHC Ia fibers found in the present study.

Due to our interest in investigating the muscle-specific responses that occur with changes in loading pattern (19, 22, 52, 53, 60), we assessed protein synthesis rates in isolated fibers from both the vastus laterals and soleus. However, given the relatively low percentage of pure MHC Ia fibers in the soleus, only MHC I fiber protein synthesis rate was assessed in this muscle. In the untrained individuals studied in the present investigation we observed similar basal protein synthesis rates in MHC I fibers from the vastus lateralis and soleus, which is likely a function of the orderly recruitment and the role of MHC I fibers from both muscles during daily ambulatory movement. However, MHC I fibers from the vastus lateralis and soleus differentially adapt to changes in activity level (52, 53), and therefore further examination of the protein synthesis response in fibers from both muscles is warranted.

We realize the methods outlined here are somewhat time intensive, and we provide initial measurements for only pure fiber types from two human leg muscles of untrained individuals. However, the ability to make metabolic measurements at the level that defines the characteristics of a given human skeletal muscle (i.e., the isolated fiber type-specific muscle fiber) is extremely valuable if we are to better understand the adaptive processes that ultimately alter human muscle function. Further, the present methods can also be utilized to examine the various hybrid fiber populations, which we know can change in abundance in humans with a variety of circumstances (19, 56, 62). Future work is necessary to further our understanding of not only the basal protein synthesis rates, but the adaptive responses of the various fiber types in other human muscles, other subject populations (e.g., elderly, women), and in response to various perturbations (e.g., exercise, inactivity, microgravity exposure, pharmaceutical interventions).
In conclusion, we present new methodology to assess the rates of mixed and myofibrillar protein synthesis in fibers isolated from human skeletal muscle biopsies. These methods can be applied to the study of fiber type-specific responses in human skeletal muscle and therefore can be used to gain important new insight into the adaptive processes occurring in each fiber type. This level of investigation is critical given the different contributions of MHC I and MHC II fibers to whole muscle function, and the distinct metabolic and functional alterations of each fiber type in response to stimuli such as exercise training, aging, and long-term unloading. Finally, this new information has the potential to be utilized in a manner that could prove to enhance training programs and therapies for human skeletal muscle.

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GRANTS

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DISCLOSURES

No conflicts of interest are declared by the authors.

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