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Comparison of bolus injection and constant infusion methods for measuring muscle protein fractional synthesis rate in humans

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ABSTRACT

Background. The use of stable isotope tracer techniques to measure muscle protein fractional synthesis rate (FSR) has been well established and widely used. The most common method that has been utilized so far is a primed constant infusion (CI) method, which requires 3–4 h of tracer infusion. However, recently our group has developed a bolus injection (BI) method, which requires an injection of bolus of tracer and can be completed within 1 h. In this study, we compared calf (gastrocnemius) muscle protein FSR measured using these two different methods — CI and BI.

Method. FSRs were measured in eight people (5 men and 3 women; age: 62.3 ± 6.9 years (mean ± SD); body weight: 75.4 ± 21.5 kg) at basal, postabsorptive state using L-[ring-$^2H_5$]-phenylalanine. In the CI protocol, a primed continuous infusion was given for 4 h, and muscle biopsies were taken at 120 and 240 min; in the BI, a bolus injection of the tracer was given at 0 min and biopsies were taken at 5 and 60 min. Tracer enrichments in blood and muscle tissue were determined by gas chromatography–mass spectrometry. Data are expressed as mean ± SE; *t*-test, linear regression and Levene Median equal variance test analyses were performed.

Results. CI FSR was 0.066 ± 0.006%/h, whereas BI FSR was 0.058 ± 0.008%/h, p = NS. The linear regression analysis showed a significant relationship between BI and CI, $p = 0.038$. The intra-class correlation coefficient was 0.83. The standard deviation of the differences in the measurements was 0.015%/h. The Levene Median equal variance test demonstrated no difference in variance between the CI and BI measurements ($p = 0.722$).

Conclusion. No difference could be detected in calf muscle protein FSR measured by CI and BI methods; the BI method can be used for the measurement of muscle protein FSR in humans.

Abbreviations: BI, bolus method; CI, constant infusion method; FD, flooding dose; FBR, fractional breakdown rate; FSR, fractional synthesis rate; GC-MS, gas chromatography–mass spectrometry; PAD, peripheral arterial disease.

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1. Introduction

In vivo measurement of muscle protein synthesis is an essential element in metabolic studies evaluating lean body mass metabolism in health and pathology. Primed constant infusion (CI) and flooding dose (FD) methods have been introduced to measure muscle protein fractional synthesis rate (FSR) [1]. The CI method is well established and a proven approach in this area. However, it requires isotopic steady-state conditions, which take at least an hour to achieve depending on the tracer, and then at least another two or three hours to achieve adequate change in protein-bound enrichment over time for accurate measurements. Therefore, if the goal of the experiment is to determine the basal postabsorptive muscle protein FSR, the length of the infusion protocol may take up to four hours [1]. The FD method eliminates this issue; however, the total amount of amino acid given with the flooding dose exceeds the endogenous free amino acid level several-fold [2]. This flooding dose can stimulate protein synthesis by itself when an essential amino acid is used [3–5]. The usage of a non-essential amino acid has not shown any effect on FSR; however, intracellular physiological production of non-essential amino acid (from breakdown or *de novo* synthesis) will dilute the pool [4,5].

Recently, a new bolus injection (BI) method was introduced that uses a much smaller dose than the flooding dose method [6]. FSR calculation by the BI method is based on the precursor–product principle similar to both CI and FD approaches, using muscle intracellular free and bound tracer enrichments as precursor and product, respectively. The design of the BI method includes a bolus injection of a tracer with subsequent blood draws and muscle biopsies at five and sixty minutes. Zhang et al. [6] showed that this method does not affect muscle protein kinetics in rabbits, is suitable to be used with essential amino acid tracer (e.g., $^2H_5$-phenylalanine), gives reliable results and can be completed within one hour. In humans, to our best knowledge only one study using the BI method has been reported [7], and it has not been validated in comparison with the CI method in humans. Therefore, the purpose of this study was to use the BI method to measure *in vivo* muscle protein FSR in humans and compare the results with FSR measured by the CI method.

2. Materials and methods

2.1. Subjects

Eight elderly adults (5 men and 3 women; age: 62.3 ± 6.9 years old (mean ± SD); body weight: 75.4 ± 21.5 kg) participated in the study. Two subjects were healthy elderly; the other six had been diagnosed with peripheral arterial disease (PAD). All subjects underwent measurements of their calf muscle protein FSR by the CI and BI methods at two different occasions. This study is a part of a larger clinical study on muscle protein metabolism in patients with PAD and a part of the study has been already reported [8]. However, none of the subjects reported here underwent any interventions between the study trials. Written informed consent was obtained from all subjects before the participation in the study. The protocol was approved by the Institutional Review Board (IRB) and the General Advisory Committee of the General Clinical Research Center (GCRC) at the University of Texas Medical Branch (UTMB).

2.2. Tracer infusion/injection protocol

Subjects were admitted to the GCRC at UTMB the day before the study. After an overnight fast, a polyethylene catheter was inserted into a forearm vein for infusion or injection of the tracer L-[ring-$^2H_5$]-phenylalanine (Cambridge Isotope Laboratories, Andover, MA, USA). Another catheter was inserted in retrograde fashion into the hand vein of the other arm for arterialized blood sampling, as described previously [8,9]. Blood samples were obtained for measurement of blood tracer enrichment and pO_2.

2.3. Constant infusion protocol

A background blood sample was obtained before the start of the tracer infusion, after which a primed (2 μmol/kg) constant infusion (0.05 μmol/kg/min) of labeled phenylalanine was started and maintained throughout the study [8]. The study design is illustrated in Fig. 1A. Arterialized blood samples from the retrograde dorsal vein catheter were collected hourly. Muscle biopsies were obtained from gastrocnemius muscle of one leg of each participant under sterile conditions and local anesthesia using a 5-mm Bergstrom biopsy needle, as previously described [8]. Biopsies were performed at 120 and 240 min after start of the tracer infusion (Fig. 1A).

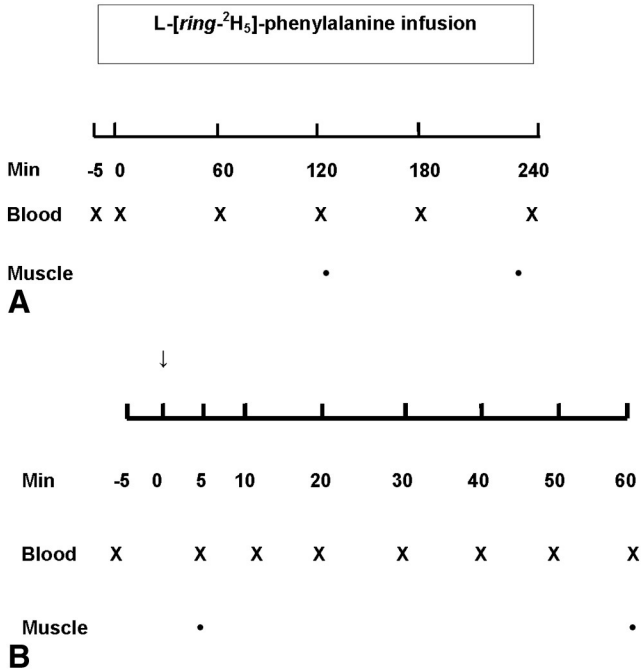

Fig. 1 – Experimental protocol. (A) Constant infusion protocol; (B) Bolus injection protocol. *Min*, minutes of study; *Blood*, blood sampling from arterialized dorsal hand vein; *Muscle*, gastrocnemius muscle biopsy. ↓, Bolus injection of stable isotope.

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