

Basal protein synthesis rates differ between *vastus lateralis* and *rectus abdominis* muscle

Joey S.J. Smeets¹ , Astrid M.H. Horstman¹ , David P.J. van Dijk², Astrid G.M. van Boxtel³, Joost F. ter Woort³, Steven W.M. Olde Damink^{2,4} , Olaf E.M.G. Schijns^{5,6,7} & Luc J.C. van Loon^{1*} 

¹Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre+, Maastricht, The Netherlands, ²Department of Surgery, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre+, Maastricht, The Netherlands, ³Department of Cardiothoracic Surgery, Catharina Hospital, Eindhoven, The Netherlands, ⁴Department of General, Visceral and Transplantation Surgery, RWTH University Hospital Aachen, Aachen, Germany, ⁵Department of Neurosurgery, Maastricht University Medical Centre+, Maastricht, The Netherlands, ⁶School of Mental Health and Neuroscience (MHeNS), Maastricht University, Maastricht, The Netherlands, ⁷Academic Center for Epileptology, location Maastricht, Maastricht University Medical Centre+, Maastricht, The Netherlands

Abstract

Background *In vivo* muscle protein synthesis rates are typically assessed by measuring the incorporation rate of stable isotope labelled amino acids in skeletal muscle tissue collected from *vastus lateralis* muscle. It remains to be established whether muscle protein synthesis rates in the *vastus lateralis* are representative of muscle protein synthesis rates of other muscle groups. We hypothesized that post-absorptive muscle protein synthesis rates differ between *vastus lateralis* and *rectus abdominis*, *pectoralis major*, or *temporalis* muscle *in vivo* in humans.

Methods Twenty-four patients (62 ± 3 years, 42% female), scheduled to undergo surgery, participated in this study and underwent primed continuous intravenous infusions with L-[ring-¹³C₆]-phenylalanine. During the surgical procedures, serum samples were collected, and muscle tissue was obtained from the *vastus lateralis* as well as from the *rectus abdominis*, *pectoralis major*, or *temporalis* muscle. Fractional mixed muscle protein synthesis rates (%/h) were assessed by measuring the incorporation of L-[ring-¹³C₆]-phenylalanine into muscle tissue protein.

Results Serum L-[ring-¹³C₆]-phenylalanine enrichments did not change throughout the infusion period. Post-absorptive muscle protein synthesis rates calculated based upon serum L-[ring-¹³C₆]-phenylalanine enrichments did not differ between *vastus lateralis* and *rectus abdominis* (0.032 ± 0.004 vs. 0.038 ± 0.003%/h), *vastus lateralis* and *pectoralis major*, (0.025 ± 0.003 vs. 0.022 ± 0.005%/h) or *vastus lateralis* and *temporalis* (0.047 ± 0.005 vs. 0.043 ± 0.005%/h) muscle, respectively ($P > 0.05$). When fractional muscle protein synthesis rates were calculated based upon tissue-free L-[ring-¹³C₆]-phenylalanine enrichments as the preferred precursor pool, muscle protein synthesis rates were significantly higher in *rectus abdominis* (0.089 ± 0.008%/h) compared with *vastus lateralis* (0.054 ± 0.005%/h) muscle ($P < 0.01$). No differences were observed between fractional muscle protein synthesis rates in *vastus lateralis* and *pectoralis major* (0.046 ± 0.003 vs. 0.041 ± 0.008%/h) or *vastus lateralis* and *temporalis* (0.073 ± 0.008 vs. 0.083 ± 0.011%/h) muscle, respectively.

Conclusions Post-absorptive muscle protein synthesis rates are higher in *rectus abdominis* when compared with *vastus lateralis* muscle. Post-absorptive muscle protein synthesis rates do not differ between *vastus lateralis* and *pectoralis major* or *temporalis* muscle. Protein synthesis rates in muscle tissue samples obtained during surgery do not necessarily represent a good proxy for appendicular skeletal muscle protein synthesis rates.

Keywords Protein turnover; Stable isotope methodology; Cancer surgery; Rectus abdominis; Pectoralis major; Temporalis

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*Correspondence to: Prof. Luc J. C. van Loon, Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre+, PO Box 616 6200 MD, Maastricht, The Netherlands. Phone: +31 43 388 1397, Email: l.vanloon@maastrichtuniversity.nl
Joey S. J. Smeets and Astrid M. H. Horstman contributed equally to this work.

Background

Preservation of appendicular skeletal muscle mass is important to remain ambulatory throughout our lifespan. As we age, the progressive loss of skeletal muscle mass and strength, termed sarcopenia, is accompanied by impairments in functional capacity and an increased risk of developing chronic metabolic diseases.^{1,2} Especially in clinical populations, skeletal muscle loss is associated with negative health outcomes such as increased complication rates, lengthened hospitalization, and increased mortality.^{3–5} Hence, many research groups investigate the regulation of skeletal muscle metabolism in both health and disease.^{6–17}

Skeletal muscle mass is maintained by a dynamic balance between muscle protein synthesis and breakdown rates, with protein turnover rates varying between 0.02% and 0.09% per hour (i.e. 0.5–2.2% per day).¹⁸ Both post-absorptive and post-prandial muscle protein synthesis rates are influenced by anabolic stimuli such as nutrition and physical activity, and catabolic stimuli such as fasting and disuse.¹⁹ In order to assess muscle protein synthesis rates, contemporary stable isotope methodology has been applied in combination with sampling of *vastus lateralis* muscle tissue. The latter has always been an obvious choice because of the convenience of sampling *vastus lateralis* muscle using the Bergström percutaneous needle biopsy technique²⁰ in contrast to the difficulty of accessing tissue from other skeletal muscles. Although some studies have previously assessed muscle protein synthesis rates in muscle groups other than the *vastus lateralis*,^{21–25} only few have compared protein synthesis rates of *vastus lateralis* muscle with other muscle groups within the same individual.^{22,23} Differences have been found between *triceps brachii* and *vastus lateralis* muscle protein synthesis rates,²⁶ with differences based upon protein synthesis rates of the myofibrillar fraction as opposed to mixed muscle protein. Similar muscle protein synthesis rates were reported for *soleus* and *rectus abdominis* muscle when compared with *vastus lateralis* muscle protein synthesis rates.^{22,23} Currently, there is no consensus on the presence or absence of differences in tissue protein synthesis rates between different muscle groups *in vivo* in humans.

Investigating impairments in muscle protein metabolism in clinical populations is often complicated by the difficulty of obtaining skeletal muscle biopsies from tissue outside of the surgical area. Therefore, studies in clinically compromised populations frequently use muscle tissue that becomes accessible during a surgical procedure. As such, *rectus abdominis* muscle is frequently sampled during abdominal surgery and is often used to represent (appendicular) skeletal muscle tissue.^{27–32} However, it remains to be established whether anatomically distinct muscle groups such as *vastus lateralis*, *rectus abdominis*, *pectoralis major*, or *temporalis* muscle express different protein synthesis rates. Based upon

distinct anatomical location, locomotor function, and involvement in weight bearing, we hypothesize that post-absorptive muscle protein synthesis rates differ between these different muscle groups.

To test our hypothesis, we recruited 24 patients, scheduled to undergo surgery, to participate in a study in which we applied contemporary stable isotope methodology to assess post-absorptive muscle protein synthesis rates of *rectus abdominis*, *pectoralis major*, or *temporalis* muscle tissue. In addition, muscle biopsy samples of the *vastus lateralis* muscle were obtained using the Bergström percutaneous needle biopsy technique during these surgical procedures to allow a direct comparison of post-absorptive muscle protein synthesis rates between *vastus lateralis* and *rectus abdominis*, *pectoralis major*, or *temporalis* muscle tissue *in vivo* in humans.

Methods

Subjects

Twenty-four patients (age: 62 ± 3 years; body weight: 78 ± 4 kg), scheduled to undergo surgery, were selected to participate in the present study. Subjects had no history of participating in any stable isotope infusion studies prior to this experiment. Exclusion criteria included comorbidities and neuromuscular disorders of the lower limbs severely interacting with mobility with limited or no opportunity for improvement (e.g. cerebral palsy), peripheral arterial disease Fontaine III or IV, chronic obstructive pulmonary disease GOLD III or IV, use of systemic steroids other than indicated for the specific type of surgery or use of anti-inflammatory biologicals (e.g. TNF- α blockers) in the past 4 weeks, phenylketonuria, insulin dependent diabetes mellitus, total parenteral nutrition on the day of surgery, pregnancy or surgical intervention, neoadjuvant chemotherapy or radiotherapy in the past 4 weeks, medical history of ischemic heart disease, heart failure, or kidney transplantation. Patients were recruited at Maastricht University Medical Centre+ and Catharina Hospital Eindhoven, and were scheduled to undergo a nephrectomy due to renal cell carcinoma ($n = 4$), a pancreaticoduodenectomy due to pancreatic cancer ($n = 8$), an aortic valve replacement due to aortic valve stenosis ($n = 6$), or a temporal lobectomy due to drug-resistant temporomesial epilepsy ($n = 6$). For a complete overview of which muscle samples were obtained during which surgical procedures, please refer to *Table 1*. All subjects were informed about the nature and possible risks of the experimental procedures, before their written informed consent was obtained. The study was approved by the Medical Ethical Committee of Zuyderland Medical Centre, Heerlen, and

Table 1 Surgical populations and sample collection

Population (n)	Muscle samples (n)	Sex (M:F)
Renal cell carcinoma (n = 4)	<i>Vastus lateralis</i> (n = 4) <i>Rectus abdominis</i> (n = 4)	3:1
Pancreatic cancer (n = 8)	<i>Vastus lateralis</i> (n = 8) <i>Rectus abdominis</i> (n = 8)	5:3
Aortic valve stenosis (n = 6)	<i>Vastus lateralis</i> (n = 5) <i>Pectoralis major</i> (n = 6)	4:2
Drug-resistant temporomesial epilepsy (n = 6)	<i>Vastus lateralis</i> (n = 6) <i>Temporalis</i> (n = 6)	2:4

Maastricht University Medical Centre+, Maastricht, The Netherlands. The study conformed to the principles outlined in the declaration of Helsinki for use of human subjects and tissue.

Study design

The experimental protocol is outlined in Figure 1. Before and during surgery patients were subjected to primed continuous intravenous infusions with L-[ring-¹³C₆]-phenylalanine. Blood and muscle samples were collected throughout the surgical procedures to assess fractional mixed muscle protein synthesis rates [fractional synthesis rate (FSR); %/h]. Muscle biopsies from the *vastus lateralis* were obtained using the Bergström percutaneous needle biopsy technique.²⁰ This study is part of a greater project studying organ tissue protein synthesis rates *in vivo* in humans. Some of the data, such as *temporalis*³³ and *vastus lateralis* muscle,^{33,34} have been presented in previous publications.

Infusion protocol

All patients were fasted for at least 8 h prior to surgery. About 2.5 h before surgery, a Teflon catheter was inserted into an antecubital vein for stable isotope infusion. A second Teflon catheter was inserted into a dorsal hand vein of the contralateral arm for perioperative blood sampling. After taking a baseline blood sample at $t = -150$ min, the serum phenylalanine pool was primed with a single dose of L-

[ring-¹³C₆]-Phenylalanine (2 μmol/kg), after which continuous intravenous L-[ring-¹³C₆]-phenylalanine (0.05 μmol/kg/min) infusion was initiated. Subsequently, blood samples were collected before and during the surgical procedures (Figure 1).

To determine post-absorptive mixed muscle protein synthesis rates, tissue samples of the *vastus lateralis* plus *rectus abdominis*, *pectoralis major*, or *temporalis* muscle were obtained throughout the surgical procedures nephrectomy and pancreaticoduodenectomy, aortic valve replacement, or temporal lobectomy, respectively. All samples were collected through surgical excision, except for the *vastus lateralis* muscle that was collected from the middle region of the *vastus lateralis*, approximately 15 cm above the patella and 3 cm below entry through the fascia, using the standard percutaneous needle biopsy technique.²⁰ Samples were freed from any visible blood, immediately frozen in liquid nitrogen, and stored at -80°C until subsequent analysis. In addition, blood samples were collected at frequent intervals to determine L-[ring-¹³C₆]-phenylalanine enrichments in serum. Blood samples were collected in serum tubes and centrifuged at 3500g for 15 min at 20°C. Aliquots of serum were frozen in liquid nitrogen and stored at -80°C . For a schematic representation of the infusion protocol, please refer to Figure 1.

Serum analyses

Serum amino acid concentrations and enrichments were determined by gas chromatography–mass spectrometry (GC–MS; Agilent 7890A GC/5975C; MSD, Little Falls, DE, USA), as described in detail previously.^{33,34} The serum was

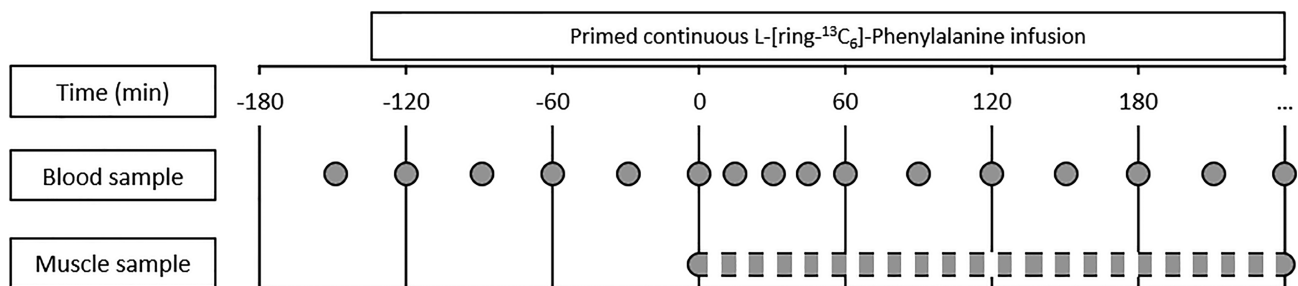


Figure 1 Schematic representation of the infusion protocol. $t = 0$ min represents the start of surgery. ● represents the time of blood sampling. ■■■ represents the time period during which the muscle samples were collected (dependent on the progress of the various surgical procedures). Mean incorporation times (hh:mm:ss) for *vastus lateralis*, *rectus abdominis*, *pectoralis major*, and *temporalis* muscle were 06:10:57 ± 00:16:01, 06:15:20 ± 00:18:57, 05:02:50 ± 00:26:52, and 06:24:20 ± 00:45:42, respectively.

deproteinized on ice with dry 5-sulfosalicylic acid, and internal standards were added for amino acid concentration measurements. Specifically, $^*C_6H_5^*CH_2^*CH(^*NH_2)^*COOH$ internal standard ($m + 10$) was added to the samples (Cambridge Isotope Laboratories, Tewksbury, Massachusetts, USA). Free amino acids were purified using cation exchange AG 50W-X8 resin [mesh size: 100–200, ionic form: hydrogen (Bio-Rad Laboratories, Hercules, CA, USA)] columns. The free amino acids were eluted with 2 M NH_4OH and dried under a continuous N_2 -stream for 48 h. The dried amino acids were converted to their *tert*-butyl dimethylsilyl derivative before analysis by GC–MS. The amino acid concentrations were determined using electron impact ionization by monitoring ions at mass/charge (m/z) 336, 342, and 346 for unlabelled phenylalanine and internal standards, respectively. Phenylalanine enrichments were determined using selective ion monitoring at m/z 336 and 342 for unlabelled and L-[ring- $^{13}C_6$]-labelled phenylalanine, respectively. Standard regression curves were applied from a series of known standard enrichment values against the measured values to assess the linearity of the mass spectrometer and to account for any isotope fractionation that may have occurred during the analysis.

Muscle tissue analyses

As described in detail previously,^{33,34} for this study, the single biopsy approach was used to determine mixed muscle protein synthesis rates. Therefore, serum proteins were extracted from serum samples by adding 20% perchloric acid (PCA). Samples were centrifuged at 3500g for 20 min at 4°C, and the supernatants were removed. The serum protein pellet was washed with 2% PCA and centrifuged at 3500g for 10 min at 4°C. Amino acids were liberated by adding 6 M HCl and were heated at 120°C for 15–18 h. Thereafter, the hydrolysed serum protein samples were processed via the same procedures (purification and derivatization) as the muscle protein-bound samples (described below). All muscle samples were freeze-dried, weighed, and crushed. Subsequently, samples were homogenized in ice-cold 2% PCA using ultrasonic disintegration (Soniprep; MSE, London, UK). Samples were incubated on ice for 10 min. Following centrifugation, the supernatant was collected for determination of L-[ring- $^{13}C_6$]-phenylalanine enrichments in the muscle-free amino acid pool using GC–MS analysis. Therefore, the supernatant was processed in the same manner as the serum samples. The muscle protein pellet was washed three times with 1.5 mL of ice-cold 2% PCA and hydrolysed in 3 mL of 6 M HCl overnight at 120°C. The free amino acids were then dissolved in 50% acetic acid solution and passed over cation exchange AG 50W-X8 resin [mesh size: 100–200, ionic form: hydrogen (Bio-Rad Laboratories, Hercules, CA, USA)] columns. The amino acids were eluted with 2 M NH_4OH and dried under a continuous N_2 -stream for 48 h for measurement of

L-[ring- $^{13}C_6$]-phenylalanine enrichment in mixed muscle protein. To determine the L-[ring- $^{13}C_6$]-phenylalanine enrichment of mixed muscle protein, the purified amino acids were derivatized into their *N*(O,S)-ethoxycarbonyl ethyl ester derivatives with ethyl chloroformate. The derivatives were then measured by gas chromatography isotope ratio mass spectrometry (MAT 253; Finnigan, Bremen, Germany) using an Agilent J&W DB-5 ms (30 m) or DB-17 ms (60 m) GC-column (Agilent Technologies, Santa Clara, CA, USA), and monitoring of ion masses 44 and 45. A series of known standards was used to assess the linearity of the mass spectrometer and to control for the loss of tracer.

Amino acid concentrations

Quantification of amino acids in the different muscle samples was performed using ultra-performance liquid chromatograph mass spectrometry (ACQUITY UPLC H-Class with QDa; Waters, Saint-Quentin, France), as described in detail previously.^{33,34} At least 5 mg of freeze-dried muscle tissue was hydrolysed in 3 mL of 6 M HCl for 12 h at 120°C and dried under a continuous N_2 -stream. Five microlitres of 0.1 M HCl was used to reconstitute the hydrolysates after which 50 μ L of each protein hydrolysate was deproteinized using 100 μ L of 10% SSA with 50 μ M of MSK-A2 internal standard (Cambridge Isotope Laboratories, Tewksbury, Massachusetts, USA). Subsequently, 50 μ L of ultra-pure demineralized water was added, and samples were centrifuged. After centrifugation, 10 μ L of supernatant was added to 70 μ L of Borate reaction buffer (Waters, Saint-Quentin, France). In addition, 20 μ L of AccQ-Tag derivatizing reagent solution (Waters, Saint-Quentin, France) was added after which the solution was heated to 55°C for 10 min. Of this, 100 μ L derivative 1 μ L was injected and measured using ultra-performance liquid chromatograph mass spectrometry.

Calculations

Mixed muscle protein FSRs were calculated using the standard precursor-product equation and the single biopsy approach⁶:

$$FSR(\%/h) = \frac{E_{p2} - E_{p1}}{E_{precursor} \times t} \times 100\%$$

E_{p2} is the protein-bound enrichments measured in the muscle samples collected during surgery and E_{p1} is the enrichment at $t = -150$ min in serum protein (before the start of the tracer infusion; Figure 1).⁶ $E_{precursor}$ is the weighted average serum or tissue-free L-[ring- $^{13}C_6$]-phenylalanine enrichment, and t indicates the tracer incorporation time (measured from the start of the tracer infusion until sampling of each individual muscle tissue sample).

Statistics

All data are expressed as means \pm SEM. Paired *t*-tests were used to compare both the intracellular free and protein-bound L-[ring- $^{13}\text{C}_6$]-phenylalanine enrichments and protein synthesis rates of the different above-mentioned muscle samples with *vastus lateralis* muscle intracellular free and protein-bound L-[ring- $^{13}\text{C}_6$]-phenylalanine enrichments and protein synthesis rates, respectively. Pairwise comparisons were made between *vastus lateralis* muscle and *rectus abdominis* ($n = 12$), *pectoralis major* ($n = 5$), and *temporalis* ($n = 6$) muscle. For all analyses, significance was set at $P < 0.05$. All calculations were performed using SPSS (Version 25.0, IBM Corp., Armonk, NY, USA). Because of the exploratory nature of the experiment, post-hoc power calculations were performed in order to provide an objective means to interpret the reported data. The results of these calculations are presented in the captions of the corresponding figures and table.

Results

Serum enrichments

Serum L-[ring- $^{13}\text{C}_6$]-phenylalanine enrichments, as shown in Figure 2, did not change throughout the infusion period despite the surgical setting of the experiments. Throughout the surgical procedures, serum L-[ring- $^{13}\text{C}_6$]-phenylalanine enrichments averaged 7.47 ± 0.09 , 9.05 ± 0.24 , and 8.55 ± 0.21 mole percent excess (MPE) during the

nephrectomy/pancreaticoduodenectomy (sampling *rectus abdominis* muscle), aortic valve replacement (sampling *pectoralis major* muscle), and temporal lobectomy (sampling *temporalis* muscle).

Muscle-free and protein-bound enrichments

Muscle tissue free L-[ring- $^{13}\text{C}_6$]-phenylalanine enrichment levels did not differ between *vastus lateralis* and *rectus abdominis*, *pectoralis major*, or *temporalis* muscle (4.41 ± 0.28 vs. 3.71 ± 0.35 , 5.04 ± 0.31 vs. 4.64 ± 0.33 , and 5.14 ± 0.35 vs. 4.28 ± 0.54 MPE, respectively; $P > 0.05$; Table 2). Protein-bound L-[ring- $^{13}\text{C}_6$]-phenylalanine enrichment levels in *vastus lateralis* muscle did not differ from *rectus abdominis*, *pectoralis major*, or *temporalis* muscle (0.01 ± 0.00 vs. 0.02 ± 0.00 , 0.01 ± 0.00 vs. 0.01 ± 0.00 , and 0.03 ± 0.01 vs. 0.02 ± 0.00 MPE, respectively; $P > 0.05$; Table 2).

Muscle protein synthesis rates

Muscle-specific protein synthesis rates are shown in Figure 3, using serum L-[ring- $^{13}\text{C}_6$]-phenylalanine enrichments as the precursor pool. Post-absorptive *vastus lateralis* muscle protein synthesis rates averaged $0.034 \pm 0.003\%/h$. Protein synthesis rates of *vastus lateralis* did not differ from *rectus abdominis*, *pectoralis major*, and *temporalis* muscle (0.032 ± 0.004 vs. $0.038 \pm 0.003\%/h$ ($n = 12$), 0.025 ± 0.003 vs. $0.022 \pm 0.005\%/h$ ($n = 5$), and 0.047 ± 0.005 vs. $0.043 \pm 0.005\%/h$ ($n = 6$), respectively; $P > 0.05$). Protein

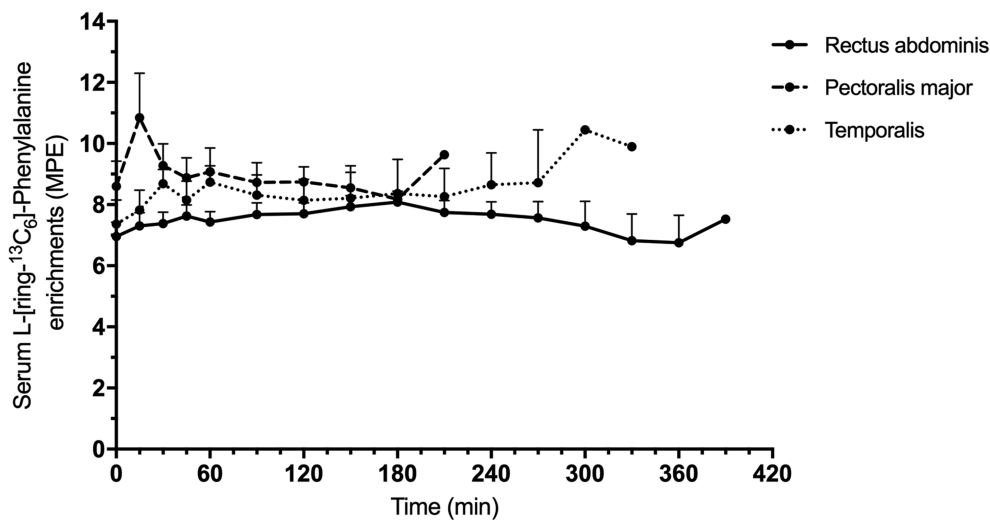


Figure 2 Serum L-[ring- $^{13}\text{C}_6$]-phenylalanine enrichments. Serum L-[ring- $^{13}\text{C}_6$]-phenylalanine enrichments are expressed as mole percent excess (MPE). $t = 0$ min represents the start of the surgical procedures: nephrectomy/pancreaticoduodenectomy (sampling *rectus abdominis* muscle), aortic valve replacement (sampling *pectoralis major* muscle), and temporal lobectomy (sampling *temporalis* muscle). Values represent means \pm SEM. Serum L-[ring- $^{13}\text{C}_6$]-phenylalanine enrichments did not change significantly throughout the experiments.

Table 2 Protein-bound and muscle-free L-[ring-¹³C₆]-phenylalanine enrichments

Muscle	L-[ring- ¹³ C ₆]-phenylalanine enrichment (MPE)	
	Protein-bound	Muscle tissue-free
<i>Vastus lateralis</i> (n = 23)	0.02 ± 0.00	4.74 ± 0.19
<i>Rectus abdominis</i> (n = 12)	0.02 ± 0.00 (P = 0.10)	3.71 ± 0.35 (P = 0.08)
<i>Pectoralis major</i> (n = 5)	0.01 ± 0.00 (P = 0.43)	4.64 ± 0.33 (P = 0.52)
<i>Temporalis</i> (n = 6)	0.02 ± 0.00 (P = 0.32)	4.28 ± 0.54 (P = 0.16)

Values represent means ± SEM. Post-hoc power calculations for pairwise comparisons between *vastus lateralis* and *rectus abdominis*, *vastus lateralis* and *pectoralis major*, and *vastus lateralis* and *temporalis* muscle resulted in a power of 0.37, 0.27, and 0.14, for protein-bound L-[ring-¹³C₆]-phenylalanine enrichments and a power of 0.43, 0.16, and 0.27 for muscle tissue free L-[ring-¹³C₆]-phenylalanine enrichments, respectively.

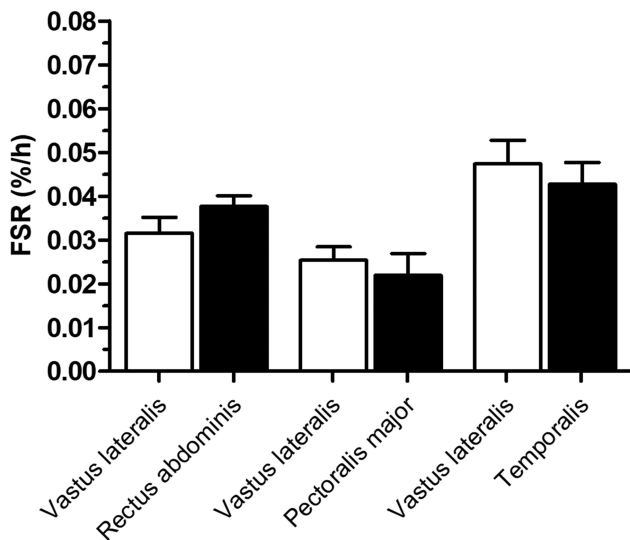


Figure 3 Muscle protein synthesis rates based upon serum L-[ring-¹³C₆]-phenylalanine enrichments as the precursor pool. Fractional mixed muscle protein synthesis rates (FSR) based on incorporation of L-[ring-¹³C₆]-phenylalanine in human skeletal muscle tissue protein with serum L-[ring-¹³C₆]-phenylalanine enrichments used as precursor pool. Pairwise comparisons were made between *vastus lateralis* muscle and *rectus abdominis* (n = 12), *pectoralis major* (n = 5), and *temporalis* (n = 6) muscle. Values represent means + SEM. Post-hoc power calculations for pairwise comparisons between *vastus lateralis* and *rectus abdominis*, *vastus lateralis* and *pectoralis major*, and *vastus lateralis* and *temporalis* muscle resulted in a power of 0.33, 0.09, and 0.11, respectively.

synthesis rates based upon muscle free enrichments as precursor pool, shown in Figure 4, averaged 0.054 ± 0.005 vs. 0.089 ± 0.008%/h (n = 12), 0.046 ± 0.003 vs. 0.041 ± 0.008%/h (n = 5), and 0.073 ± 0.008 vs. 0.083 ± 0.011%/h (n = 6), in the *vastus lateralis* vs. *rectus abdominis*, *pectoralis major*, or *temporalis* muscle, respectively. Post-absorptive mixed muscle protein synthesis rates were significantly higher in *rectus abdominis* compared with *vastus lateralis* muscle when fractional synthesis rates were calculated based upon muscle tissue free enrichments as the precursor pool (P < 0.01).

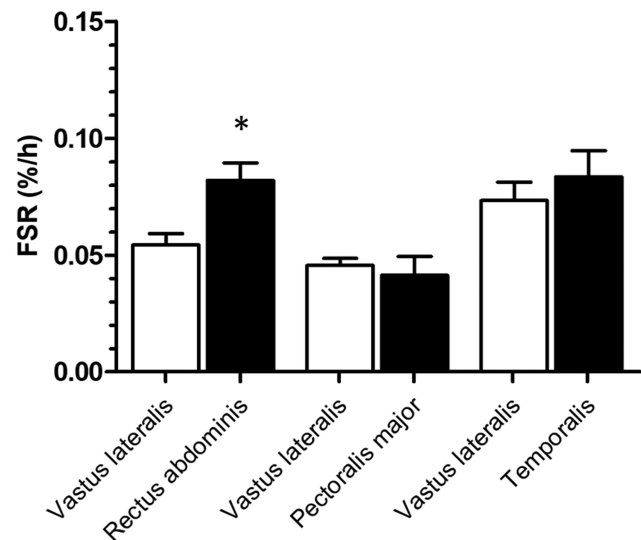


Figure 4 Muscle protein synthesis rates based upon muscle free L-[ring-¹³C₆]-phenylalanine enrichments as the precursor pool. Fractional mixed muscle protein synthesis rates (FSR) based on incorporation of L-[ring-¹³C₆]-phenylalanine in human skeletal muscle tissue protein with muscle free L-[ring-¹³C₆]-phenylalanine enrichments used as precursor pool. Pairwise comparisons were made between *vastus lateralis* muscle and *rectus abdominis* (n = 12), *pectoralis major* (n = 5), and *temporalis* (n = 6) muscle. Values represent means + SEM. Asterisk '*' denotes significantly different from *vastus lateralis* muscle protein synthesis rates (P < 0.01). Post-hoc power calculations for pairwise comparisons between *vastus lateralis* and *rectus abdominis*, *vastus lateralis* and *pectoralis major*, and *vastus lateralis* and *temporalis* muscle resulted in a power of 0.96, 0.07, and 0.19, respectively.

Muscle protein content and amino acid composition

Protein contents of the different skeletal muscle groups ranged between 39% and 81% of raw (dry) material (Table 3). *Vastus lateralis*, *rectus abdominis*, *pectoralis major*, and *temporalis* muscle protein contents averaged 81%, 76%, 39%, and 76% of dry weight, respectively. Whereas *rectus abdominis* and *temporalis* muscle did not differ from *vastus lateralis* muscle in their observed protein contents, *pectoralis major* muscle showed a significantly lower protein content

Table 3 Muscle tissue protein and amino acid contents

Variable	<i>Vastus lateralis</i> (n = 23)	<i>Rectus abdominis</i> (n = 12)	<i>Pectoralis major</i> (n = 5)	<i>Temporalis</i> (n = 6)
Total nitrogen content (% dry weight)	13 ± 0	12 ± 1	6 ± 1*	12 ± 1
Total protein content (% dry weight)	81 ± 2	76 ± 6	39 ± 9*	76 ± 6
Essential AA (% of total AA content)				
Histidine	2.5 ± 0.1	2.4 ± 0.1*	1.9 ± 0.1*	2.5 ± 0.1
Isoleucine	4.3 ± 0.2	4.5 ± 0.2*	3.3 ± 0.4*	2.4 ± 0.1*
Leucine	9.7 ± 0.2	9.7 ± 0.3	7.6 ± 0.4*	8.5 ± 0.3*
Lysine	9.2 ± 0.2	9.1 ± 0.3	7.4 ± 0.6	7.4 ± 0.2*
Methionine	1.6 ± 0.2	1.6 ± 0.3	0.4 ± 0.1	2.0 ± 0.2
Phenylalanine	3.8 ± 0.1	3.9 ± 0.1	3.3 ± 0.2	3.4 ± 0.1
Threonine	5.2 ± 0.1	5.3 ± 0.1	4.0 ± 0.2*	4.2 ± 0.1*
Valine	5.5 ± 0.3	6.2 ± 0.2	5.1 ± 0.1*	3.1 ± 0.6*
ΣEAA	41.8 ± 0.7	42.7 ± 0.8	33.0 ± 2.0*	33.6 ± 1.0*
Non-Essential AA (% of total AA content)				
Alanine	10.2 ± 0.3	10.4 ± 0.4*	13.3 ± 0.4*	10.6 ± 0.2
Arginine	5.2 ± 0.1	5.5 ± 0.2	5.8 ± 0.3	4.6 ± 0.1*
Aspartic acid	8.6 ± 0.3	7.7 ± 0.2	6.1 ± 0.3*	10.0 ± 0.3*
Cysteine	0.4 ± 0.0	0.5 ± 0.0	0.2 ± 0.0*	0.5 ± 0.0
Glutamic acid	12.9 ± 0.8	11.5 ± 0.9*	6.3 ± 1.4	14.5 ± 0.5*
Glycine	8.2 ± 0.2	8.6 ± 1.0	20.2 ± 2.2*	12.6 ± 1.4
Proline	5.1 ± 0.1	5.3 ± 0.2	9.7 ± 0.8*	6.3 ± 0.4
Serine	4.8 ± 0.2	4.9 ± 0.3	3.4 ± 0.3	4.9 ± 0.2*
Tyrosine	2.8 ± 0.1	2.9 ± 0.1	2.0 ± 0.2*	2.5 ± 0.1*
ΣNEAA	58.2 ± 0.7	57.3 ± 0.8	67.0 ± 2.0*	66.4 ± 1.0*

Values represent means ± SEM. Protein content is presented in % of raw material based on the determined nitrogen content multiplied by 6.25 as the standard conversion factor.³⁵ Amino acid content is presented in % of total AA content.

Note: Tryptophan, Asparagine, and Glutamine were not measured. Pairwise comparisons were made between *vastus lateralis* muscle and *rectus abdominis* (n = 12), *pectoralis major* (n = 5), and *temporalis* (n = 6) muscle.

ΣEAA, sum of all essential amino acids; ΣNEAA, sum of all non-essential amino acids; AA, amino acid.

*Significantly different from *vastus lateralis* (P < 0.05).

when pairwise compared with *vastus lateralis* muscle (P < 0.01). Essential amino acid contents of all skeletal muscle groups ranged between 33% and 43% of total amino acid contents, whereas non-essential amino acid contents ranged between 57% and 67% of total amino acid content. Both essential and non-essential amino acid content was significantly lower in *pectoralis major* and *temporalis* muscle compared with *vastus lateralis* muscle. For the individual amino acid contents, the majority of the significant differences were observed between *vastus lateralis* muscle and *pectoralis major*, and *temporalis* muscle.

Discussion

In the present study, we observed that post-absorptive muscle protein synthesis rates, calculated based upon serum enrichments, did not differ between *vastus lateralis* and *rectus abdominis*, *vastus lateralis* and *pectoralis major*, or *vastus lateralis* and *temporalis* muscle. However, when fractional muscle protein synthesis rates were calculated based upon tissue free enrichments, muscle protein synthesis rates were significantly higher in *rectus abdominis* when compared with *vastus lateralis* muscle. No differences were observed between fractional muscle protein synthesis rates in

vastus lateralis and *pectoralis major* or *vastus lateralis* and *temporalis* muscle.

To date, most of our understanding of human muscle tissue protein metabolism has been obtained by measuring appendicular muscle protein synthesis rates in quadriceps muscle and, more specifically, *vastus lateralis* muscle. An important reason for this is the convenience and relative ease of accessing this muscle group for tissue samples using the Bergström percutaneous needle biopsy approach.²⁰ In the present study, we observed post-absorptive *vastus lateralis* muscle protein synthesis rates averaging 0.034 ± 0.003% per hour. Between groups, differences in *vastus lateralis* muscle protein synthesis rates may be attributed to differences in study protocol as well as differences in patient (group) characteristics. The observed rates are similar to post-absorptive muscle protein synthesis rates assessed previously in a wide variety of subjects studied in our lab^{6–8,10–12,14,33} as well as in many other laboratories.^{13,15–17} Although muscle protein synthesis rates of other skeletal muscle groups have been assessed previously,^{21–26,36–39} only few studies have directly compared protein synthesis rates between *vastus lateralis* muscle and other muscle groups by parallel sampling of different muscle tissues within the same individuals within the same study.

Assessing skeletal muscle protein synthesis rates using contemporary stable isotope methodology has yielded a vast amount of knowledge in muscle protein metabolism over the

past decades in both healthy and more clinically compromised populations. Skeletal muscle tissue has been shown to play an important role in increasingly prevalent clinical conditions such as obesity and diabetes, but also in critical illness, chronic diseases, and osteoporosis.⁴⁰ However, obtaining additional skeletal muscle tissue from the *vastus lateralis* muscle in clinical populations is often complicated by logistical and medical ethical restraints. Therefore, it is of important clinical relevance to evaluate whether protein synthesis rates in muscle tissue that becomes accessible during surgery can serve as a reference for muscle protein synthesis rates that are typically assessed in *vastus lateralis* tissue.

In the current study, we were able to simultaneously sample *rectus abdominis* muscle tissue with *vastus lateralis* muscle tissue during abdominal surgery. Muscle protein synthesis rates in *rectus abdominis* muscle did not differ from *vastus lateralis* muscle when calculated using serum L-[ring-¹³C₆]-phenylalanine enrichments as precursor pool (Figure 3). However, when using muscle free L-[ring-¹³C₆]-phenylalanine enrichments as the preferred precursor pool, *rectus abdominis* muscle protein synthesis rates were significantly higher when compared with *vastus lateralis* muscle protein synthesis rates (0.089 ± 0.008 vs. $0.054 \pm 0.005\%/h$, respectively; $P < 0.01$; Figure 4). The higher muscle protein synthesis rates of *rectus abdominis* muscle were attributed to the lower muscle tissue free L-[ring-¹³C₆]-phenylalanine enrichments, which are likely secondary to a higher tissue protein turnover in the *rectus abdominis* compared with the *vastus lateralis* muscle. The greater turnover may be attributed to the continuous activity of *rectus abdominis* muscle due to posture and breathing, when compared with the *vastus lateralis* muscle that had remained in relative rest for at least 12 h following hospital admission. Therefore, data may differ when muscle protein synthesis rates are assessed after a bout of physical activity or when assessed over a more extensive intervention period. In agreement, similar or higher muscle protein synthesis rates have been observed in *quadriceps* when compared with *rectus abdominis* muscle when assessed in patients with upper gastro-intestinal cancer during 1 week following labelled water ingestion.³⁹ Although protein content and amino acid composition did not differ between *rectus abdominis* and *vastus lateralis* muscle tissue (Table 3), it seems evident that basal muscle protein synthesis rates assessed in *rectus abdominis* muscle obtained during surgery may not always provide a good proxy for appendicular skeletal muscle mass (Figure 4).

In addition, we collected *pectoralis major* muscle tissue together with *vastus lateralis* muscle tissue in patients undergoing cardiothoracic surgery. Protein synthesis rates of *pectoralis major* muscle did not differ from *vastus lateralis* muscle protein synthesis rates (Figures 3–4). Interestingly, protein content was significantly lower in *pectoralis major* muscle tissue when compared with *vastus lateralis* muscle

tissue (39 ± 9 vs. $81 \pm 2\%$ dry weight; Table 3). Access to the pectoral muscle was gained through the sternotomy, and sampling was done close to the sternum, which in theory may have caused the muscle samples to contain more connective tissue. Although this does not explain the lower protein content *per se*, the substantially higher content of proline and glycine in *pectoralis major* muscle tissue compared with *vastus lateralis* muscle tissue could support this (Table 3). Finally, we also sampled *temporalis* muscle tissue simultaneously with *vastus lateralis* muscle tissue in patients undergoing brain surgery. *Temporalis* muscle tissue protein synthesis rates did not differ from *vastus lateralis* muscle protein synthesis rates (Figures 3–4). Whereas protein content did not differ between *temporalis* muscle and *vastus lateralis* muscle, the amino acid composition differed between both muscle groups (Table 3).

Protein synthesis rates of human skeletal muscle tissue other than *vastus lateralis* have been assessed previously.^{21–26,36–39} Although the methods applied in these studies vary considerably, muscle protein synthesis rates seem to be in line with the rates measured here. The present study is the first to compare muscle tissue protein synthesis rates of distinct muscle groups such as *rectus abdominis*, *pectoralis major*, and *temporalis* with *vastus lateralis* muscle. Despite great differences in anatomical location, locomotor function, and involvement in weight bearing of these muscle groups, muscle protein synthesis rates seemed to be similar for *pectoralis major* and *temporalis* muscle when compared with protein synthesis rates in *vastus lateralis* muscle. However, this did not seem to apply to *rectus abdominis* muscle tissue. The present study is limited to the measurement of muscle protein synthesis rates in the basal, post-absorptive state. Changes in skeletal muscle mass can be modulated by changes in basal muscle protein synthesis and breakdown rates but are also driven by changes in the responsiveness to anabolic and catabolic stimuli. It could be speculated that although differences in post-absorptive muscle protein synthesis rates between *vastus lateralis* and *pectoralis major* or *temporalis* muscle are negligible, greater differences may be evident in their responsiveness to anabolic or catabolic stimuli in either health or disease. Furthermore, although basal, post-absorptive muscle protein synthesis rates do not seem to differ substantially between muscle groups, we cannot rule out that differences may exist in protein synthesis rates of specific myofibrillar, sarcolemmal, or mitochondrial subfractions.^{22,23,26} Finally, the present study design was exploratory, and statistical power may not have been high enough to detect small, but physiologically relevant, differences in basal muscle protein synthesis rates. However, it should be noted that post-absorptive muscle protein synthesis rates are quite homogenous between various muscle groups when considering the much higher muscle protein synthesis rates assessed using the same approach in various organ tissues.^{33,34}

Nevertheless, these data support the use of *pectoralis major* and *temporalis* muscle tissue sampling in the surgical area to assess post-absorptive muscle protein synthesis rates, making the additional sampling of muscle tissue in the leg using the percutaneous Bergström needle for this specific purpose less important. This may strongly facilitate research in the area of sarcopenia and cachexia and may help to gain more insight in abnormalities in muscle protein metabolism in various patient populations.³² However, muscle protein synthesis rates in the *rectus abdominis* muscle may not necessarily represent a good proxy for most appendicular skeletal muscle tissue.

In conclusion, post-absorptive muscle protein synthesis rates are higher in *rectus abdominis* compared with *vastus lateralis* muscle. Post-absorptive muscle protein synthesis rates do not differ between *vastus lateralis* and *pectoralis major* and *vastus lateralis* and *temporalis* muscle. Clearly, protein synthesis rates in muscle tissue samples obtained during surgery do not necessarily represent a good proxy for appendicular skeletal muscle protein synthesis rates.

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Conflict of interest

The authors declare no conflicts of interest.

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