

Post-exercise protein synthesis rates are only marginally higher in type I compared with type II muscle fibres following resistance-type exercise

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Abstract We examined the effect of an acute bout of resistance exercise on fractional muscle protein synthesis rates in human type I and type II muscle fibres. After a standardised breakfast (31 ± 1 kJ kg⁻¹ body weight, consisting of 52 Energy% (En%) carbohydrate, 34 En% protein and 14 En% fat), 9 untrained men completed a lower-limb resistance exercise bout (8 sets of 10 repetitions leg press and leg extension at 70% 1RM). A primed, continuous infusion of L-[ring-¹³C₆]phenylalanine was combined with muscle biopsies collected from both legs immediately after exercise and after 6 h of post-exercise recovery. Single muscle fibres were dissected from freeze-dried biopsies and stained for ATPase activity with pre-incubation at a pH of 4.3. Type I and II fibres were separated under a light microscope and analysed for protein-bound L-[ring-¹³C₆]phenylalanine

labelling. Baseline (post-exercise) L-[ring-¹³C₆]phenylalanine muscle tissue labelling, expressed as ($\delta^{13}\text{C}/^{12}\text{C}$), averaged -32.09 ± 0.28 , -32.53 ± 0.10 and -32.02 ± 0.16 in the type I and II muscle fibres and mixed muscle, respectively ($P = 0.14$). During post-exercise recovery, muscle protein synthesis rates were marginally ($8 \pm 2\%$) higher in the type I than type II muscle fibres, at 0.100 ± 0.005 versus $0.094 \pm 0.005\%/h$, respectively ($P < 0.05$), whereby rates of mixed muscle protein were $0.091 \pm 0.005\%/h$. Muscle protein synthesis rates following resistance-type exercise are only marginally higher in type I compared with type II muscle fibres.

Keywords Skeletal muscle · Muscle fibre type · ATPase · FSR · Stable isotopes

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Introduction

In humans, changes in muscle protein synthesis (MPS) and breakdown in response to food intake and/or exercise are normally assessed in *vastus lateralis* muscle (Rennie et al. 1982). In rats, mice, cats and dogs, lagomorphs rates of MPS are markedly different between different muscle groups (Garlick et al. 1989). However, it is generally assumed that the metabolic behaviour of skeletal muscle tissue is the same throughout the human body and that results obtained in quadriceps muscle can be extrapolated to total skeletal muscle and, as such, whole-body protein turnover (Mittendorfer et al. 2005). As no significant correlations have been observed between type I muscle fibre content and the measured muscle protein fractional synthetic rates of triceps, soleus and vastus lateralis, it was concluded that differences in basal, resting muscle protein synthesis rates between muscle groups are

relatively small (<15%) and of minor biological significance (Mittendorfer et al. 2005). However, interesting new data has emerged suggesting that resting muscle protein synthesis rates are substantially (~30%) higher in type I compared to type II muscle fibres (Dickinson et al. 2010). So far, almost no data are available on potential fibre type specific differences in the muscle protein synthetic response to anabolic stimuli such as food intake and/or exercise.

Rates of MPS increase rapidly following a single bout of resistance-type exercise (Phillips et al. 1997), and remain elevated for up to 24–48 h if the subjects are fed during post-exercise recovery (MacDougall et al. 1992; Phillips et al. 1997; Tang et al. 2008). It has been speculated that the muscle protein synthetic response to resistance-type exercise is fibre type specific (Koopman et al. 2006c; Mittendorfer et al. 2005; Trappe et al. 2004). In accordance with this hypothesis, we showed that muscle fibre hypertrophy after 12 weeks of resistance exercise training is restricted to the type II muscle fibres in human *vastus lateralis* tissue of elderly men (Verdijk et al. 2009). In addition, it has been reported that a predominantly oxidative fibred muscle (*soleus*) has a smaller hypertrophic response to resistance exercise than *vastus lateralis* muscle (Trappe et al. 2004). In agreement, the phosphorylation status of key-regulatory proteins involved in the activation of protein synthesis (i.e. PKB (or Akt), mTOR and S6K1) seems to be more pronounced in muscle tissue containing a greater proportion of type II muscle fibres following resistance-type exercise in rats (Baar and Esser 1999; Parkington et al. 2003; Sakamoto et al. 2003). The latter was confirmed recently by our observation that S6K1 (at the T⁴²¹/S⁴²⁴ phosphorylation sites) is phosphorylated in a fibre type dependent manner after a single bout of resistance exercise (Koopman et al. 2006c). These findings suggested that rates of MPS may be higher in type II than type I fibres after resistance exercise. However, so far no direct measurements of rates of MPS in human type I and II have been reported after exercise.

We hypothesised that MPS rates would be greater in type II than type I muscle fibres after a single session of resistance exercise. To test this, we conducted studies involving continuous infusions of L-[ring-¹³C₆]phenylalanine combined with muscle biopsies taken immediately after a single bout of resistance exercise and after 6 h of post-exercise recovery. A modified ATPase staining procedure for freeze-dried muscle fibres was applied to differentiate between type I and II muscle fibres, allowing us to assess MPS rates in mixed muscle and in separated type I and II muscle fibres during post-exercise recovery.

Methods

Subjects

Nine healthy, male volunteers (Table 1) who did not participate in any regular exercise program (nor had done previously) were recruited to participate in the present study. All subjects were informed about the nature and possible risks of the procedures, before written consent was obtained. This study was approved by the Medical Ethics Committee of the Academic Hospital Maastricht, according to the Helsinki Declaration. All volunteers were instructed to refrain from any exhausting physical activity and to consume a normal diet for 3 days prior to the study.

Pretesting

Body composition was assessed by hydrostatic weighing in the morning after an overnight fast as described previously (Koopman et al. 2006c). Thereafter, maximum leg strength was estimated using multiple repetitions of leg extensions. In an additional exercise session, at least 1 week before the first visit, the subjects' one-repetition maximum (1-RM) was determined (Kraemer and Fry 1995).

Protocol

On the evening before the study day, subjects consumed a standard meal (32 ± 2 kJ kg⁻¹ body weight, consisting of 55 Energy% (En%), carbohydrate, 25 En% protein and 30 En% fat). At 08.00, after an overnight fast, subjects arrived at the laboratory by car or public transportation. A Teflon cannula was inserted into an antecubital vein for stable isotope infusion. A second cannula was inserted into a dorsal vein of the contra-lateral hand, and the hand was placed in a hot-box (60°C) for arterialized blood sampling. After basal blood sample collection ($t = -125$ min), subjects consumed a standardised breakfast (31 ± 1 kJ kg⁻¹

Table 1 Subjects' characteristics

	Subjects
Age (years)	21.8 ± 0.8
Weight (kg)	80.4 ± 2.4
Height (m)	1.89 ± 0.02
BMI (kg m ⁻²)	22.6 ± 0.8
% Body fat (%)	13.7 ± 2.4
Leg volume (L)	9.8 ± 0.4
1RM leg press (kg)	242 ± 10
1RM leg extension (kg)	146 ± 5

Values are expressed as means ± SEM ($n = 9$). 1RM one-repetition maximum

body weight, consisting of 52 En% carbohydrate, 34 En% protein and 14 En% fat). Thereafter, at $t = -120$ min, a dose of L-[ring- $^{13}\text{C}_6$]phenylalanine ($2 \mu\text{mol kg}^{-1}$, Cambridge Isotope laboratories, Andover, MA, USA) was administered to prime the phenylalanine pool, whereupon, a continuous tracer infusion of $0.049 \pm 0.001 \mu\text{mol kg}^{-1} \cdot \text{min}^{-1}$ was started. Subjects rested in a supine position for 1 h after which another blood sample was collected ($t = -60$ min) just before subjects engaged in the resistance-type exercise bout, i.e. 1 h after ingestion of the breakfast. After a 5 min warm-up on a cycle ergometer (~ 75 W), the subjects completed a session of 8 sets of 10 repetitions each of leg press and extension (Technogym BV, Rotterdam, The Netherlands), at 70% of their individual 1-RM, with 2 min rest between sets. All subjects were encouraged during exercise and the entire exercise protocol required ~ 45 min. At the end of exercise ($t = 0$ min), subjects rested supine and an arterialized blood sample and muscle biopsies from the *vastus lateralis* of each leg were collected. Arterialized blood samples were collected at $t = 60, 120, 180, 240, 300,$ and 360 min with muscle biopsies taken from each leg at $t = 360$ min from a new incision. Biopsies (200 mg) were obtained from the middle region of the *vastus lateralis* muscle (~ 15 cm above the patella) and ~ 3 cm below the skin through the fascia using a percutaneous needle (Bergstrom 1975). Muscle samples were dissected, freed from any visible non-muscle material, immediately frozen in liquid nitrogen and stored at -80°C until analysis. Blood was collected in EDTA containing tubes and centrifuged at $1000 \times g$ for 5 min at 4°C . Aliquots of plasma were frozen in liquid nitrogen and stored at -80°C until analyses.

Plasma sample analyses

For plasma phenylalanine labelling measurements, plasma phenylalanine was derivatized using a t-butyldimethylsilyl (TBDMS) whose ^{13}C labelling was determined by electron ionisation gas chromatography–mass spectrometry (GC–MS, Agilent 6890 N GC/5973 N MSD Little Falls, USA) using selected ion monitoring of masses 336 and 342 for unlabelled and labelled L-[ring- $^{13}\text{C}_6$]phenylalanine, respectively (Koopman et al. 2005). We applied standard regression curves in all isotopic enrichment analysis to assess linearity of the mass spectrometer and to control for loss of tracer.

Muscle sample analyses

Selection of type I and II muscle fibres using modified ATPase staining

For measurement of protein-bound L-[ring- $^{13}\text{C}_6$]phenylalanine labelling in the type I and II muscle fibres, ~ 200 mg

wet muscle tissue was freeze-dried. Collagen, blood and other non-muscle fibre material were removed from the muscle, and individual muscle fibres were separated under a light microscope. The isolated muscle fibres were stained for ATPase activity using a modified staining procedure for histochemical applications (Mabuchi and Sreter 1980a, b). Briefly, isolated muscle fibres were placed in an Eppendorf tube and pre-incubated at room temperature (RT) at pH 4.3, washed with Tris-based buffer (pH 7.8) and distilled water, and subsequently incubated in ATP containing glycine buffer (pH 9.4) for 25 min at RT. Thereafter, fibres were washed with 1% calcium chloride, distilled water, and 1% cobalt chloride, and type I and II fibres were stained black and white, respectively, using an ammonium sulphide solution in the Eppendorf tubes at RT. Type I and II muscle fibres were separated under a light microscope (Fig. 1) and transferred to separate vials. We separated 3.3 ± 0.2 mg type I fibres (range: 1.9–4.3 mg wet weight), and 3.5 ± 0.2 mg type II fibres (range: 2.0–4.6 mg wet weight). After adding ice-cold 2% perchloric acid (PCA) the muscle fibres were homogenised and centrifuged. Protein pellets were further processed to determine protein-bound L-[ring- $^{13}\text{C}_6$]phenylalanine labelling as described below.

Measurement of free and protein-bound L-[ring- $^{13}\text{C}_6$]phenylalanine enrichment

For measurement of precursor (tissue free amino acid pool) and protein-bound (mixed muscle protein) L-[ring- $^{13}\text{C}_6$]phenylalanine labelling, 50 mg of muscle was freeze-dried and non-muscle fibre material removed under a light microscope. The isolated muscle fibre mass (~ 10 mg) was weighed and eight volumes ($\sim 8 \times$ dry weight of isolated muscle fibres \times wet/dry ratio) of PCA was added. After



Fig. 1 Separation of type I and II muscle fibres using a modified ATPase staining procedure to allow muscle fibre type specific assessment of L-[ring- $^{13}\text{C}_6$]phenylalanine incorporation into muscle protein. Collagen, blood and other non-muscle fibre material were removed from the muscle fibres, and individual muscle fibres were separated. Following the modified ATPase staining, type I (black) and II (white/grey) muscle fibres were separated under a light microscope

homogenisation and centrifugation the supernatant was collected and processed in the same manner as plasma so that tissue free L-[ring- $^{13}\text{C}_6$]phenylalanine labelling could be measured (Koopman et al. 2005). We did not differentiate between intra- and extracellular free L-[ring- $^{13}\text{C}_6$]phenylalanine labelling and as free phenylalanine concentrations did not differ between type I and II muscle fibres (Blomstrand and Essen-Gustavsson 2009), it was assumed that free L-[ring- $^{13}\text{C}_6$]phenylalanine labelling is also similar in both fibre types.

The protein pellet, of type I, II and mixed muscle fibres, was washed with 3 additional 1.5 ml washes of 2% PCA, dried and the proteins hydrolysed in 6 M HCl at 120°C for 15–18 h. The hydrolysed protein fraction was dried under a nitrogen stream while heated to 120°C, then dissolved in a 50% acetic acid solution, and passed over a Dowex resin (AG 50 W-X8, 100-200 mesh H^+ form, Biorad, Hercules, CA, USA) using 2 M NH_4OH . Thereafter, the eluate was dried and the purified amino acids were derivatized as their *N*(*O,S*)-ethoxycarbonyl ethyl esters for the determination of $^{13}\text{C}/^{12}\text{C}$ ratios of muscle protein-bound phenylalanine (Koopman et al. 2007a), by gas chromatography-isotope ratio mass spectrometry (GC-IRMS, Finnigan MAT 252, Bremen, Germany) using HP Ultra I GC-column (#19091A-112, Hewlett-Packard, Palo Alto, CA), combustion interface II and monitoring of ion masses 44, 45 and 46. By establishing the relationship between the labelling of a series of L-[ring- $^{13}\text{C}_6$]phenylalanine standards of variable enrichment and the enrichment of the *N*(*O,S*)-ethoxycarbonyl ethyl esters of these standards, the muscle protein-bound labelling of phenylalanine was determined. We applied standard regression curves to assess linearity of the mass spectrometer and to control for the loss of tracer. The coefficient of variance (CV) for the measurement of L-[ring- $^{13}\text{C}_6$]phenylalanine enrichment in mixed muscle protein averaged $1.0 \pm 0.1\%$.

Calculations

The plasma, tissue free and protein-bound L-[ring- $^{13}\text{C}_6$]phenylalanine labelling is presented as tracer (L-[ring- $^{13}\text{C}_6$]phenylalanine)/tracee (unlabelled phenylalanine) ratio (TTR). The fractional rate of mixed muscle protein synthesis (FSR) during post-exercise recovery was calculated by dividing the increment in enrichment in the product, i.e. protein-bound L-[ring- $^{13}\text{C}_6$]phenylalanine in type I and type II muscle fibres and in mixed muscle protein, by the enrichment of the precursor, [i.e. plasma or tissue free L-[ring- $^{13}\text{C}_6$]phenylalanine labelling (Koopman et al. 2005)]. As it has been recently shown that free phenylalanine concentrations do not differ between type I and II muscle fibres (Blomstrand and Essen-Gustavsson 2009), it seems reasonable to assume that free L-[ring- $^{13}\text{C}_6$]

phenylalanine labelling is also similar in both fibre types. As such, we have used plasma and tissue free L-[ring- $^{13}\text{C}_6$]phenylalanine labelling as precursor for the determination of muscle fibre type specific protein synthesis rates, as described previously (Dickinson et al. 2010).

Statistics

The present study was designed to assess muscle fibre type specific protein synthesis rates during post-exercise recovery. All data are expressed as means \pm SEM. Calculation of the required sample size was based on effect size and variance in previous studies from our laboratory (Koopman et al. 2005, 2006b, 2007a, 2008, 2009). We calculated sample size using the following parameters: difference in FSR 15%, SD of 10%, with type I error of 5% and type II error of 10%. Power calculations showed that at least 8 participants were needed and we therefore included nine men in this study. A Student paired *t* test was applied to test for differences in protein synthesis rates between type I and II fibres types. A one-way ANOVA was applied to test whether there were any differences in protein-bound labelling and protein synthesis between mixed muscle and the different fibre types. Statistical significance was set at $P < 0.05$. All calculations were performed using StatView 5.0 (SAS Institute inc., Cary, NC, USA).

Results

Resistance exercise

Individual one-repetition maximum strength (1RM) measured during the pre-test averaged 242 ± 10 kg (horizontal leg press) and 146 ± 5 kg (leg extension). Therefore, average weight lifted during the resistance exercise session was set at 181 ± 7 and 110 ± 4 kg for the leg press and leg extension, respectively. All subjects completed 8 sets with 10 repetitions on the leg press and the leg extension.

Plasma and tissue free phenylalanine labelling

Plasma L-[ring- $^{13}\text{C}_6$]phenylalanine labelling increased over time and remained stable during the last 3 h of post-exercise recovery (Fig. 2). During 6 h of post-exercise recovery, plasma L-[ring- $^{13}\text{C}_6$]phenylalanine labelling averaged 0.070 ± 0.002 (TTR). Muscle tissue free L-[ring- $^{13}\text{C}_6$]phenylalanine labelling increased over time, averaging 0.046 ± 0.002 and 0.061 ± 0.001 (TTR) in the biopsy samples taken immediately after exercise and after 6 h of post-exercise recovery, respectively ($P < 0.05$).

Muscle protein-bound labelling

Basal (post-exercise) muscle protein-bound L-[ring- $^{13}\text{C}_6$]phenylalanine enrichment ($\delta^{13}\text{C}/^{12}\text{C}$) in mixed muscle averaged -32.02 ± 0.16 , whereas basal protein-bound L-[ring- $^{13}\text{C}_6$]phenylalanine labelling averaged -32.09 ± 0.28 and -32.53 ± 0.10 in type I and II muscle fibres, respectively. No differences were observed between mixed muscle and the different fibre types ($P = 0.14$). During 6 h of post-exercise recovery, the increase in L-[ring- $^{13}\text{C}_6$]phenylalanine labelling in mixed muscle protein averaged 0.000291 ± 0.000011 (TTR). The increase in protein-bound L-[ring- $^{13}\text{C}_6$]phenylalanine labelling was marginally, albeit statistically higher in the type I versus type II muscle fibres (0.000308 ± 0.000007 vs. 0.000286 ± 0.000010 (TTR), respectively: $P < 0.01$).

Muscle protein synthesis

Rates of mixed MPS during post-exercise recovery averaged $0.069 \pm 0.0038\%/h$ when calculated using plasma free L-[ring- $^{13}\text{C}_6$]phenylalanine labelling as precursor. Muscle protein FSR after exercise was significantly higher in type I compared with type II fibres and averaged 0.070 ± 0.003 and $0.068 \pm 0.003\%/h$, respectively ($P < 0.01$). When muscle protein FSR was calculated with the average muscle tissue free L-[ring- $^{13}\text{C}_6$]phenylalanine labelling as the precursor pool, similar results were obtained. Mixed muscle protein synthetic rates during the post-exercise recovery averaged $0.091 \pm 0.005\%/h$. Muscle protein synthesis rates were $8 \pm 2\%$ higher in

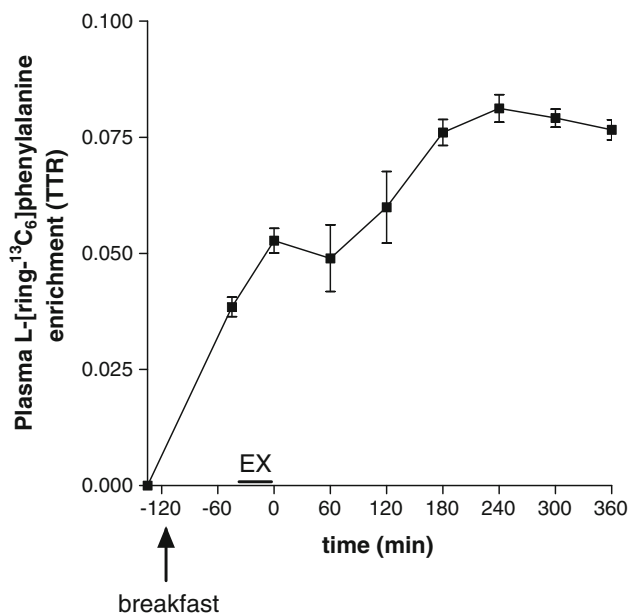


Fig. 2 Plasma L-[ring- $^{13}\text{C}_6$]phenylalanine enrichments at rest, during exercise and subsequent recovery. Data provided are means \pm SEM

type I than type II muscle fibres (Fig. 3, $P < 0.05$), at 0.100 ± 0.005 and $0.093 \pm 0.005\%/h$, respectively ($P < 0.01$).

Discussion

We have previously shown that the phosphorylation status of S6K1 ($\text{T}^{421}/\text{S}^{424}$) increases to a greater (30%) extent in the type II versus type I muscle fibres after resistance-type exercise (Koopman et al. 2006c). In addition, we have reported that muscle hypertrophy after a 12 week RET program is specific in increasing type II muscle fibre size in elderly men (Verdijk et al. 2009). Therefore, we hypothesised that muscle protein synthesis rates would be higher in type II than in type I muscle fibres during the acute stages of post-exercise recovery. We measured muscle protein synthetic rates after resistance exercise in type I and II muscle fibres from human *vastus lateralis* muscle. To the best of our knowledge, this study is the first to compare MPS rates in type I and II muscle fibres following resistance-type exercise within a single muscle. Fractional muscle protein synthetic rates during post-exercise recovery were 8% higher in the type I compared with the type II muscle fibres.

Recently published data suggest that MPS in human *vastus lateralis* muscle is markedly (33%) higher in type I compared with type IIa muscle fibres following an

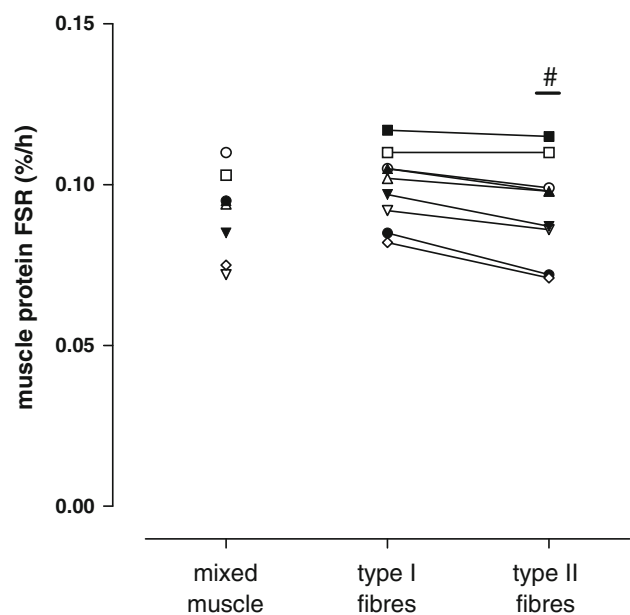


Fig. 3 Individual muscle protein fractional synthetic rates (FSR) during 6 h of post-exercise recovery in mixed muscle and in separated type I and II muscle fibres. #Significantly different between the type I versus type II muscle fibres, $P \leq 0.05$. No differences between mixed muscle FSR and type I or II muscle fibre FSR

overnight fast (Dickinson et al. 2010). We extend on these data by assessing muscle fibre type specific protein synthesis rates following resistance-type exercise. We measured protein-bound L-[ring- $^{13}\text{C}_6$]phenylalanine labelling in type I and II muscle fibres dissected (and stained for ATPase activity) from freeze-dried muscle biopsies collected immediately after cessation of exercise and again after 6 h of post-exercise recovery. We did not observe any differences in protein-bound L-[ring- $^{13}\text{C}_6$]phenylalanine labelling between the different muscle fibre types immediately after cessation of exercise. Although we did not aim to assess basal/resting rates of fibre type specific MPS, the lack of difference in protein-bound enrichment between muscle fibre types in biopsies taken immediately after cessation of exercise strongly suggests that MPS rates in the fed state (at rest and during exercise) do not differ between the type I and type II muscle fibres, as suggested by previous work on muscles composed of predominantly one of the fibre types (Mittendorfer et al. 2005). During the 6 h post-exercise recovery period, protein-bound L-[ring- $^{13}\text{C}_6$]phenylalanine enrichments increased to a greater extent in the type I compared with the type II muscle fibres ($P < 0.01$). Muscle protein synthesis rates were $8 \pm 2\%$ higher in type I compared with type II muscle fibres following resistance-type exercise (Fig. 3, $P < 0.01$). Consequently, our data indicate that muscle protein synthesis rates are only marginally higher in type I compared with type II muscle fibres during recovery following resistance-type exercise.

To be able to determine muscle fibre type specific muscle protein synthesis rates, we collected as much as 200–250 mg muscle tissue to have sufficient tissue available to adequately detect protein-bound L-[ring- $^{13}\text{C}_6$]phenylalanine enrichment both in mixed muscle and in specifically prepared type I and II muscle fibres. This required us to take biopsies from both legs at each time-point, restricting the total number of time points where biopsies could be collected. Furthermore, as muscle fibre type specific protein synthesis rates following an overnight fast have been reported previously (Dickinson et al. 2010), we did not assess basal MPS rates. Muscle sample preparation requires separation of each individual muscle fibre, followed by ATPase staining, and subsequent separation of the type I (black) and II (white/grey) muscle fibres (Fig. 1) until sufficient (2–4 mg dry weight) fibres are separated to allow accurate detection of protein-bound L-[ring- $^{13}\text{C}_6$]phenylalanine labelling using GC-IRMS. The coefficient of variance (CV) of the measurements of protein-bound L-[ring- $^{13}\text{C}_6$]phenylalanine labelling in the type I and type II muscle fibres was similar to the CV ($\sim 1\%$) we normally observe for mixed muscle tissue samples. We must, however, recognise that it is theoretically possible that there are small (short lived) differences in MPS rates within the 6 h

recovery period from exercise, which might not have been detected by the applied protocol. Short-lived responses of feeding and resistance exercise on MPS have previously been observed (Rennie et al. 2002; Kumar et al. 2009). However, given the lack of evidence of significant differences in the feeding responses of MPS in muscles containing predominantly type 1 or type 2 muscle fibres (Mittendorfer et al. 2005) made over a 3 h period, this seems unlikely.

The observation that type II muscle fibres do not show a greater post-exercise muscle protein synthesis rates when compared with the type I muscle fibres seems to be at odds with previous findings from our laboratory (Koopman et al. 2006c) as well as others (Tannerstedt et al. 2009). Tannerstedt et al. (2009) have recently shown that the phosphorylation status of S6K1 (T 389) and S6 (but not Akt and mTOR) are increased to a greater extent in type II compared with type I fibres following maximal lengthening contractions. These data are in line with our previous findings that S6K1 phosphorylation at T 421 /S 424 increases to a greater extent in type II compared with type I fibres following resistance exercise (when exercise is performed in the fasted state) (Koopman et al. 2006c). The latter suggests that the anabolic response to resistance-type exercise is greater in the type II versus type I muscle fibres. We will highlight a few mechanisms that may explain the described discrepancy between our protein synthesis rates and previously published signalling data.

Glycogen utilisation during resistance-type exercise is higher in type II than in type I muscle fibres (Koopman et al. 2006c). AMP-activated protein kinase (AMPK) has a glycogen-binding domain (Sakamoto et al. 2004), and has been shown to be substantially activated when muscle glycogen concentrations are low (Wojtaszewski et al. 2003). Therefore, it could be speculated that AMPK activity is increased to a greater extent in type II than in type I fibres during/following resistance exercise. As a result, the rise in 4E-BP1 phosphorylation (Dreyer et al. 2006; Drummond et al. 2008; Koopman et al. 2006c), and MPS could be more attenuated specifically in type II muscle fibres following exercise. In addition, synthesis rates of myofibrillar and mitochondrial protein are increased following a single session of resistance-type exercise (Wilkinson et al. 2008). As muscle fibre mitochondrial content is up to 2-fold higher in type I versus II muscle fibres (Koopman et al. 2006a) and mitochondrial protein synthesis rates are $\sim 50\%$ higher than that of myofibrillar protein (Wilkinson et al. 2008) it can be speculated that the observed small differences in post-exercise mixed muscle protein FSR between fibre types are due to differences in mitochondrial protein content/turnover. Alternatively, the fact that we observed only marginal differences between fibre types may also be attributed to

the fact that food was ingested prior to exercise. Free phenylalanine concentrations do not differ between type I and II muscle fibres prior to exercise and during post-exercise recovery (Blomstrand and Essen-Gustavsson 2009), suggesting that amino acid-induced changes in muscle protein synthesis are similar between fibre types. In line with these observations, we have shown that S6 phosphorylation increases to a similar extent in both type I and II muscle fibres during post-exercise recovery when food is ingested prior to and/or after exercise (Koopman et al. 2007b).

As recent studies have highlighted the discrepancies between anabolic signalling and muscle protein synthesis (Greenhaff et al. 2008), it is apparent that studies are warranted that investigate fibre type specific muscle protein synthesis at rest and following exercise with and without food intake using contemporary stable isotope methodology (i.e. using ^{13}C -labelled phenylalanine). To the best of our knowledge, our study is the first to compare muscle protein synthesis rates in type I and II muscle fibres within one specific muscle group, showing that post-exercise muscle protein synthesis rates are only marginally higher in type I compared with type II muscle fibres. Based on the minor differences between muscle protein synthesis rates in the type I versus type II muscle fibres, it seems reasonable to rely on the measurement of mixed muscle protein synthesis rates when aiming to study the combined effects of physical activity and food intake on muscle protein synthesis.

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