Amino Acid Concentrations and Protein Metabolism of Two Types of Rat Skeletal Muscle in Postprandial State and After Brief Starvation

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Summary

We have investigated amino acid concentrations and protein metabolism in musculus extensor digitorum longus (EDL, fast-twitch, white muscle) and musculus soleus (SOL, slow-twitch, red muscle) of rats sacrificed in the fed state or after one day of starvation. Fractional protein synthesis rates (FRPS) were measured using the flooding dose method (L-[3,4,5-³H]phenylalanine). Activities of two major proteolytic systems in muscle (the ubiquitin-proteasome and lysosomal) were examined by measurement of chymotrypsin like activity of proteasome (CTLA), expression of ubiquitin ligases atrogin-1 and muscle-ring-finger-1 (MuRF-1), and cathepsin B and L activities. Intramuscular concentrations of the most of non-essential amino acids, FRPS, CTLA and cathepsin B and L activities were in postprandial state higher in SOL when compared with EDL. The differences in atrogin-1 and MuRF-1 expression were insignificant. Starvation decreased concentrations of a number of amino acids and increased concentrations of valine, leucine, and isoleucine in blood plasma. Starvation also decreased intramuscular concentrations of a number of amino acids differently in EDL and SOL, decreased protein synthesis (by 31 % in SOL and 47 % in EDL), and increased expression of atrogin-1 and MuRF-1 in EDL. The effect of starvation on CTLA and cathepsin B and L activities was insignificant. It is concluded that slow-twitch (red) muscles have higher rates of protein turnover and may adapt better to brief starvation when compared to fast-twitch (white) muscles. This phenomenon may play a role in more pronounced atrophy of white muscles in aging and muscle wasting disorders.

Key words

Red and white muscle • Amino acids • Starvation • Atrogens • Branched-chain amino acids

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Introduction

There are two basic types of skeletal muscle fibers, which differ in both physical and biochemical properties (Gupta *et al.* 1989). In general, type I fibers have small diameter, more capillaries, contain higher levels of mitochondria and myoglobin, and their twitch rate is slower and more prolonged than type II fibers. Due to these properties, type I fibers are also called red or slow-twitch fibers. Among muscles predominantly composed by red fibers belong muscles of breathing and large back muscles, which are necessary for maintaining posture, and muscles used for walking, such as soleus muscle. Type II fibers, called also white, glycolytic, or fast-twitch fibers, are thicker. Their contraction is stronger and faster, muscle glycogen serves as the primary source of energy, and their resistance to fatigue is lower than that of fibers type I. High content of type II fibers is in muscles which ensure delicate and rapid movements, such as muscles of the eye and musculus extensor digitorum longus.

A number of reports demonstrate that muscles composed mostly by white fibers are more sensitive to catabolic stimuli, particularly to sepsis, when compared to muscles with high content of red fibers (Holecek *et al.* 2015, Holecek *et al.* 2014, Kovarik *et al.* 2012, Tiao *et al.* 1997, Safranek *et al.* 2006, Muthny *et al.* 2008). Also with advancing age there is a preferential loss and atrophy of white fibers with a preservation of red fibers (Evans 2010). However, the explanation of origin of these clinically important differences in response of red and white fibers to signals causing the loss of muscle is not available.

Food intake and a brief starvation are undoubtedly the most frequent conditions, which affect protein and amino acid metabolism in muscles. Food intake activates pathways of protein synthesis and inhibits proteolysis; brief starvation is associated with increased release of amino acids from muscles, which are used preferentially for gluconeogenesis. It may be hypothesized that differences to respond to food intake and to starvation play a role in differences in alterations in protein balance of white and red muscles during muscle wasting conditions. However, to the best of our knowledge, no study has so far been done on the effects of starvation in this context.

The main objective of the present study was to examine the differences in parameters of protein metabolism and amino acid levels in musculus extensor digitorum longus (EDL, fast-twitch, white muscle) and musculus soleus (SOL, slow-twitch, red muscle) of rats in postprandial state and after a brief starvation. We measured fractional rates of protein synthesis and the activities of two major proteolytic systems within the cells (the ubiquitin-proteasome and lysosomal pathways). In addition, we examined expression of mRNAs of atrogin-1 and muscle-ring-finger-1 (MuRF-1), two E3 ubiquitin ligases (termed atrogenes) that are important regulators of ubiquitin-mediated protein degradation, which is recognized as the main proteolytic pathway for degradation of structural proteins of muscle tissue (Wing 2005).

Materials and Methods

Animals and material

Male Wistar rats (BioTest, Konarovice, Czech Republic) were housed in standardized cages in quarters with controlled temperature and a 12-h light-dark cycle. All rats received the standard laboratory diet ST-1 (Velaz,

Czech Republic) and drinking water *ad libitum*. All procedures involving animals were performed according to the guidelines set by the Institutional Animal Care and Use Committee of Charles University. Animal Care and Use Committee of Charles University, Faculty of Medicine in Hradec Kralove specifically approved this study. L-[3,4,5-³H]phenylalanine was purchased from American Radiolabeled Chemical, Inc. (St. Louis, MO, USA). Chemicals were obtained from Sigma Chemical (St. Louis, MO, USA), Lachema (Brno, Czech Republic), and Waters (Milford, MA, USA).

Experimental design

A total of 40 male Wistar rats weighing approximately 200 g each were randomly divided into two groups. Half of the animals in each group were sacrificed in pentobarbital narcosis (6 mg/100 g body weight, intraperitoneally) by exsanguination from the abdominal aorta in the fed state, and the other half after one day of starvation. Afterwards, small pieces (approximately 0.1 g) of soleus (SOL) and extensor digitorum longus (EDL) muscles were quickly removed and frozen in liquid nitrogen.

Two separate studies were performed. Alterations in amino acid concentrations in blood plasma, SOL, and EDL muscles, and various parameters of protein breakdown were examined in the first study. Tissue protein synthesis rates were measured using the flooding dose method (L-[3,4,5-³H]phenylalanine) in the second study.

Amino acid concentrations in blood plasma and muscles

Amino acid concentrations were determined in the supernatants of deproteinized blood plasma and tissue samples using high-performance liquid chromatography (Alliance 2695, Waters, Milford, MA, USA) after derivatization with 6-aminoquinolyl-N-hydroxy-succinimidyl carbamate. The intracellular concentration of each amino acid in samples of muscle tissues was calculated by subtracting the free extracellular portion from the total amount, assuming the plasma concentration to be equal to the concentration in the interstitial fluid as described by Bergström *et al.* (1974). Total tissue water was measured from the tissue weight obtained after drying for 24 h at 90 °C. The determination of extra- and intracellular water was based on the chloride method according to Graham *et al.* (1967).

Chymotrypsin-like activity (CTLA)

The chymotrypsin-like activity of proteasomes was determined using the fluorogenic substrate Suc-LLVY-MCA (Gomes-Marcondes and Tisdale 2002) as follows. The muscles were homogenized in 0.4 ml of ice-cold 20 mM Tris buffer, pH 7.5, containing 2 mM ATP, 5 mM MgCl₂ and 1 mM dithiothreitol. The homogenates were centrifuged for 10 min at 18,000 × g at 4 °C. Cellular supernatants (0.1 ml) were incubated with 0.1 ml of substrate Suc-LLVY-MCA (0.1 mM) with or without inhibitor MG132 (0.02 mM) for 1 h on ice. A volume of 0.4 ml of 100 mM sodium acetate buffer (pH 4.3) was added to stop the reaction. Sample fluorescence was immediately determined at an excitation wavelength of 340 nm and emission wavelength of 440 nm (Tecan InfiniteTM 200). The standard curve was established for 7-amino-4-methylcoumarin (AMC), which permitted the expression of CTLA as nmol of AMC/g protein/h. Differences after the subtraction of inhibited from non-inhibited activities were used for calculations. The activity was adjusted for the protein concentration of the supernatant.

Cathepsin B and L activities

The activities of cathepsin B and L were determined using the fluorogenic substrate Z-FA-MCA (Koohmaraie and Kretchmar 1990, Tardy *et al.* 2004) as follows. Tissue samples (approximately 20 mg) were homogenized in 0.6 ml of ice-cold 300 mM sodium acetate buffer, pH 5.0, containing 4 mM EDTA, 8 mM dithiothreitol and 0.2 % Triton X-100 (v/v). The homogenates were allowed to stand for 30 min on ice and centrifuged for 30 min at 18,000 × g at 4 °C. Cellular supernatant (0.01 ml) were incubated with 0.19 ml of substrate Z-FA-MCA (0.1 mM) with or without the

inhibitor Z-FF-FMK (0.04 mM) for 30 min at 37 °C. The reaction was stopped by the addition of 1 ml of 100 mM sodium acetate buffer, pH 4.3, and the activities of cathepsin B and L were determined as described above for CTLA.

Real time RT-PCR analysis

Expression of atrogen-1 (Fbxo32), and MuRF-1 mRNA was examined using qRT-PCR on 7500HT Fast Real-Time PCR System (Applied Biosystems, Foster City, USA). Total RNA was isolated from rat skeletal muscles using TRIzol reagent (Invitrogen, USA) and converted into cDNA *via* High Capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, USA). Reaction mixture contained 30 ng of analyzed cDNA. The amplification of each sample was performed in triplicate using TaqMan[®] Fast Universal PCR Master Mix (Applied Biosystems, Foster City, USA). Atrogen-1 and MuRF-1 quantitative PCR (qPCR) assays were designed and optimized in GENERI BIOTECH s.r.o. (Hradec Kralove, Czech Republic) as shown in Table 1. The time-temperature profile used in the „fast“ mode was: 95 °C for 3 min; 40 cycles: 95 °C for 7 s, 60 °C for 25 s. For normalization, two reference genes were selected using the geNorm according to Vandesompele *et al.* (2002), GAPDH (4352338E, Applied Biosystems, Foster City, USA), and Ywhaz (GENERI BIOTECH s.r.o., Hradec Kralove, Czech Republic), as shown in Table 1. Expression values of each sample were obtained as described previously (Radilova *et al.* 2009). Briefly, the expression data were normalized by the geometric mean of GAPDH, and Ywhaz expressions. Finally, the relative expression between control and affected tissue was determined by comparison of normalized data.

Table 1. Quantitative PCR assays for reference gene and target genes provided by GENERI BIOTECH.

Gene symbol	qPCR assay	Reference sequence	Exons spanned
<i>Ywhaz</i> (reference gene)	rYwhaz_Q1	NM_013011	exon5/exon6
<i>Fbxo32</i> (Atrogen-1)	rFbxo32_Q2	NM_133521	exon8/exon9
<i>Trim63</i> (MuRF-1)	rTrim63_Q3	NM_080903	exon8/exon9

Protein synthesis

The rats were injected intravenously with a flooding dose of L-[3,4,5-³H]phenylalanine (50 µCi/100 g b.w.) combined with unlabelled L-phenylalanine

(150 µmol/100 g b.w.) 10 min before the sacrifice by exsanguination *via* the abdominal aorta (Garlick *et al.* 1980). Tissue samples were homogenized in 6 % (v/v) perchloric acid, and the precipitated proteins were

collected *via* centrifugation for 5 min at $12,000 \times g$. The supernatant was used for the measurement of L-[3,4,5- 3H]phenylalanine specific activity. The pellet was washed three times and hydrolyzed in 2 N NaOH. Aliquots were taken for protein content (Lowry *et al.* 1951) and radioactivity measurements. The fractional rate of protein synthesis (FRPS) was calculated according the formula derived by McNurlan *et al.* (1979):

$$\text{FRPS (\% per day)} = (S_b \cdot 100)/(t \cdot S_a)$$

where S_b and S_a are the specific activities (dpm/nanomole) of protein-bound phenylalanine and tissue-free phenylalanine in the acid-soluble fraction of tissue homogenates, respectively, and t is the time (days) between isotope injection and tissue immersion into liquid nitrogen. The value of 274 μmol phenylalanine/g protein was used for the calculation of protein-bound phenylalanine specific activity (Welle 1999). Sample radioactivity was measured using a liquid scintillation radioactivity counter LS 6000 (Beckman Instruments, Fullerton, CA, USA).

Statistics

The results are expressed as the means $\pm$ SE. F-test followed by paired t-test (to estimate the differences between EDL and SOL obtained from the same animal) and unpaired t-test (to estimate the effects of starvation on the specific muscle type) have been used for the analysis of the data. Differences were considered significant at $P < 0.05$. NCSS 2001 statistical software (Kaysville, UT, USA) was used for the analyses.

Results

Amino acid concentrations in blood plasma

Starvation for 24 h decreased blood plasma concentrations of histidine, methionine, alanine, arginine, ornithine, and proline, whereas concentrations of all three branched-chain amino acids (BCAA; valine, isoleucine, and leucine) increased (Table 2).

Amino acid concentrations in muscles

Table 3 demonstrates that intramuscular concentrations of histidine and lysine and of the most of non-essential amino acids were in postprandial state higher in SOL when compared with EDL. More than

double were the concentrations of aspartate, asparagine, and glutamate. Lower concentrations in SOL than in EDL exhibited glycine and threonine.

Starvation decreased intramuscular concentrations of a number of essential (histidine, lysine, and threonine in EDL; the BCAA and methionine in SOL) and non-essential (alanine, arginine, glycine, ornithine, proline, and serine in EDL; alanine, ornithine, and proline in SOL) amino acids. The exceptions observed in both muscle types were increased concentrations of aspartate and glutamate.

Table 2. Effect of a brief starvation on amino acid concentrations in blood plasma.

	Control (n=10)	Starvation (n=10)
EAA		
<i>Histidine</i>	70 $\pm$ 1	58 $\pm$ 4*
<i>Isoleucine</i>	84 $\pm$ 3	112 $\pm$ 2*
<i>Leucine</i>	134 $\pm$ 6	160 $\pm$ 3*
<i>Lysine</i>	283 $\pm$ 9	356 $\pm$ 15
<i>Methionine</i>	49 $\pm$ 1	44 $\pm$ 1*
<i>Phenylalanine</i>	65 $\pm$ 1	64 $\pm$ 1
<i>Threonine</i>	256 $\pm$ 6	255 $\pm$ 10
<i>Valine</i>	174 $\pm$ 7	200 $\pm$ 4*
Σ BCAA	392 $\pm$ 16	472 $\pm$ 9*
Σ EAA	1,115 $\pm$ 19	1,250 $\pm$ 22*
NEAA		
<i>Alanine</i>	528 $\pm$ 20	309 $\pm$ 12*
<i>Arginine</i>	127 $\pm$ 5	113 $\pm$ 2*
<i>Asparagine</i>	62 $\pm$ 3	54 $\pm$ 2
<i>Aspartate</i>	18 $\pm$ 2	16 $\pm$ 2
<i>Citrulline</i>	83 $\pm$ 2	77 $\pm$ 2
<i>Glutamate</i>	87 $\pm$ 4	97 $\pm$ 3
<i>Glutamine</i>	642 $\pm$ 16	665 $\pm$ 13
<i>Glycine</i>	310 $\pm$ 17	423 $\pm$ 18
<i>Ornithine</i>	51 $\pm$ 1	43 $\pm$ 2*
<i>Proline</i>	226 $\pm$ 8	112 $\pm$ 3*
<i>Serine</i>	237 $\pm$ 9	243 $\pm$ 9
<i>Taurine</i>	274 $\pm$ 29	285 $\pm$ 33
<i>Tyrosine</i>	87 $\pm$ 4	81 $\pm$ 6
Σ NEAA	2,816 $\pm$ 71	2,604 $\pm$ 66*
Σ Amino acids	3,932 $\pm$ 66	3,853 $\pm$ 80

Values are in $\mu\text{mol/l}$. Means $\pm$ SE. * $P < 0.05$. BCAA, branched-chain amino acids; EAA, essential amino acids; NEAA, non-essential amino acids.

Table 3. Amino acid concentrations in EDL and SOL of rats in postprandial state and after brief starvation.

	Postprandial state		Starvation for 24 h	
	EDL (n=10)	SOL (n=10)	EDL (n=10)	SOL (n=10)
EAA				
<i>Histidine</i>	430 ± 19	781 ± 71*	323 ± 13#	606 ± 45*
<i>Isoleucine</i>	127 ± 6	124 ± 5	146 ± 8	119 ± 10*
<i>Leucine</i>	224 ± 11	231 ± 9	223 ± 14	193 ± 17*#
<i>Lysine</i>	1,103 ± 54	1,323 ± 88*	857 ± 58#	1,318 ± 116*
<i>Methionine</i>	75 ± 7	82 ± 5	78 ± 5	62 ± 5*#
<i>Phenylalanine</i>	110 ± 5	108 ± 4	111 ± 5	95 ± 7*
<i>Threonine</i>	1,235 ± 33	1,107 ± 4*	1,116 ± 40#	1,052 ± 42
<i>Valine</i>	311 ± 10	300 ± 14	277 ± 15	223 ± 19*#
Σ BCAA	662 ± 27	656 ± 27	646 ± 37	534 ± 45*#
Σ EAA	3,615 ± 67	4,057 ± 179*	3,130 ± 137#	3,668 ± 218*
NEAA				
<i>Alanine</i>	3,732 ± 199	4,019 ± 228	2,919 ± 163#	3,403 ± 196*#
<i>Arginine</i>	583 ± 34	691 ± 59*	367 ± 27#	543 ± 60*
<i>Asparagine</i>	367 ± 19	773 ± 67*	407 ± 11	847 ± 52*
<i>Aspartate</i>	461 ± 25	2,094 ± 202*	583 ± 62#	4,659 ± 284*#
<i>Citrulline</i>	571 ± 27	736 ± 48*	521 ± 23	807 ± 46*
<i>Glutamate</i>	2,255 ± 157	5,606 ± 326*	3,609 ± 180#	6,430 ± 303*
<i>Glutamine</i>	9,225 ± 484	12,005 ± 702*	8,256 ± 343	12,333 ± 467*
<i>Glycine</i>	7,195 ± 374	4,176 ± 322*	6,009 ± 397#	4,329 ± 155*#
<i>Ornithine</i>	111 ± 4	143 ± 10*	54 ± 3#	105 ± 10*#
<i>Proline</i>	758 ± 18	856 ± 65	429 ± 20#	403 ± 24#
<i>Serine</i>	1,932 ± 95	3,563 ± 262*	1,457 ± 37#	3,241 ± 145*
<i>Taurine</i>	25,278 ± 811	34,124 ± 1,198*	26,887 ± 1,014	35,500 ± 1,300*
<i>Tyrosine</i>	204 ± 8	189 ± 7	232 ± 9#	191 ± 12*
Σ NEAA	52,672 ± 1,416	68,976 ± 2,627*	51,729 ± 1,323	72,791 ± 2,609*
Σ Amino acids	56,287 ± 1,454	73,034 ± 2,771*	54,860 ± 1,413	76,459 ± 2,739*

Values are in $\mu\text{mol/l}$ of intracellular fluid. Means $\pm$ SE. $P < 0.05$. * Effects of muscle type (paired-t test, compared muscles of the same animals); # effect of starvation (unpaired t-test, comparison to corresponding type of muscle of fed animals). BCAA, branched-chain amino acids; EAA, essential amino acids; NEAA, non-essential amino acids.

Protein synthesis and proteolysis

Fractional rates of protein synthesis, CTLA and cathepsin B and L activities in muscles of fed animals were higher in SOL compared to EDL muscles. Starvation decreased protein synthesis both in SOL and EDL (by 31 % and 47 %, respectively). The effect of starvation on CTLA and cathepsin B and L activities was insignificant (Figs 1, 2 and 3).

Atrogenes

There were no differences between SOL and EDL in mRNA expression of atrogen-1 and MuRF-1 in muscles obtained from fed animals. Starvation increased expression of both ubiquitin ligases in EDL, whereas the effect on SOL was insignificant (Figs 4 and 5).

Fig. 1. Fractional rates of protein synthesis (FRPS) in EDL and SOL of fed and one day starving rats. Means $\pm$ SE (n=10). $P < 0.05$. * Effects of muscle type (paired-t test, compared muscles of the same animals); # effect of starvation (unpaired t-test, comparison to corresponding type of muscle of fed animals).

Fig. 2. Chymotrypsin like activity (CTLA) in EDL and SOL of fed and one day starving rats. Means $\pm$ SE (n=10). $P < 0.05$. * Effects of muscle type (paired-t test, compared muscles of the same animals); # effect of starvation (unpaired t-test, comparison to corresponding type of muscle of fed animals).

Fig. 3. Cathepsin B and L activities in EDL and SOL of fed and one day starving rats. Means $\pm$ SE (n=10). $P < 0.05$. * Effects of muscle type (paired-t test, compared muscles of the same animals); # effect of starvation (unpaired t-test, comparison to corresponding type of muscle of fed animals).

Discussion

The differences in amino acid concentrations and protein metabolism in postprandial state

Higher concentrations of histidine and lysine and of the most of non-essential amino acids in SOL (slow-twitch) compared with EDL (fast-twitch) muscle are in agreement with our previous study (Holecek and Sispera 2016) and with Turinsky and Long (1990), who reported higher concentrations of a number of amino acids in SOL when compared with other types of fast twitch muscles (plantaris and gastrocnemius muscles). Also the observations of higher FRPS, CTLA and cathepsin B and L activities in SOL when compared to EDL is in agreement with other studies (Holecek and Sispera 2014, Garlick *et al.* 1989). Greater CTLA activities in SOL are consistent with higher release of 3-methylhistidine

Fig. 4. Expression of mRNA of atrogin-1 in EDL and SOL of fed and one day starving rats. Means $\pm$ SE (n=10). $P < 0.05$. * Effects of muscle type (paired-t test, compared muscles of the same animals); # effect of starvation (unpaired t-test, comparison to corresponding type of muscle of fed animals).

Fig. 5. Expression of mRNA of MuRF-1 in EDL and SOL of fed and one day starving rats. Means $\pm$ SE (n=10). $P < 0.05$. * Effects of muscle type (paired-t test, compared muscles of the same animals); # effect of starvation (unpaired t-test, comparison to corresponding type of muscle of fed animals).

(marker of degradation of myofibrillar proteins) from isolated SOL to incubation medium when compared with EDL (Holecek and Sispera 2014, Muthny *et al.* 2009, Muthny *et al.* 2008).

We assume that higher intracellular levels of amino acids and rates of protein synthesis and proteolysis in SOL than EDL indicate more appropriate conditions of red muscles for adaptation to various physiological and pathological conditions affecting muscle protein balance, such as starvation and muscle wasting disorders.

Effects of brief starvation on amino acid concentrations in blood plasma and muscles

Characteristic features of a brief starvation are accelerated release of amino acids from muscles and augmentation of gluconeogenesis in the liver, which are caused mostly by reduced insulin and increased glucagon

levels. Activated gluconeogenesis is undoubtedly the main cause of the decreased concentration of a number of amino acids in blood plasma of starved rats. The cause of the unique increase in all three BCAA (valine, leucine, and isoleucine) is not clear. In our opinion, a role have their enhanced release from muscles, reduced uptake due to decreased insulin production, and increased BCAA synthesis from branched-chain keto acids in the liver, which may be activated in various catabolic conditions (Holecek *et al.* 2001, Holecek 2001).

The observed decrease of a number of amino acids in muscles is mostly due to their enhanced release, activated catabolism, and decreased uptake from the blood. Increased levels of glutamate and aspartate in muscles of starving animals indicate draining of alpha-ketoglutarate and oxaloacetate from tricarboxylic acid cycle (cataplerosis) to act as the main acceptor of amino nitrogen released in amino acid catabolism, notably of the BCAA. Glutamate synthesized from alpha-ketoglutarate may be used for ammonia detoxification to glutamine or as the donor of nitrogen for synthesis of alanine, the amino acid released in exceptionally high amounts from the muscles during a brief starvation. Aspartate formed from oxaloacetate may be used for synthesis of nucleotides.

The main differences in amino acid concentrations induced by brief starvation in EDL and SOL were a decrease in a larger number of non-essential amino acids in EDL and the decrease in the BCAA in SOL. Unfortunately, we do not have explanation for origin of these differences. A role may have lower amino acid concentrations in white muscles, different sensitivity of white and red fibers to decreased ratio of insulin to glucagon, etc.

Effects of brief starvation on protein metabolism

Increased release of amino acids from skeletal muscle during starvation is associated with protein loss, which can be caused by decreased protein synthesis, increased breakdown or both. An early event in starvation is a decline in muscle protein synthesis, whereas increased rates of protein breakdown may be observed when starvation is prolonged (Stirewalt *et al.* 1985, Jepson *et al.* 1986, Goodman *et al.* 1981).

More pronounced decrease in protein synthesis in EDL compared to SOL observed in our study indicates that a brief starvation impairs protein balance more in white than in red fibers. Higher suppression of protein synthesis in EDL compared to SOL was found also under

in vitro conditions in muscles of animals with sepsis induced by cecal ligation and puncture (Holecek *et al.* 2015), endotoxin treatment (Kovarik *et al.* 2010), and with turpentine-induced inflammation (Muthny *et al.* 2008).

We did not see effect of a one-day starvation on CTLA and activities of B and L cathepsins in any of the two muscles. However, a great increase in expression of atrogin-1 and MuRF-1 genes was found in EDL. Atrogin-1 and MuRF-1 are upregulated in various experimental models of muscle atrophy, including starvation, and their substrate targets are regulatory and contractile muscle proteins (Foletta *et al.* 2011, Bodine and Baehr 2014). Overexpression of atrogin-1 in myotubes produced atrophy, whereas mice deficient in either atrogin-1 or MuRF-1 were found to be resistant to atrophy (Bodine *et al.* 2001). We assume that the transcriptional differences of EDL and SOL muscles induced by a brief starvation indicate a presence of a complex adaptive program responsible for more pronounced acceleration of protein degradation in white muscles observed in response to various catabolic stimuli.

Conclusions

According to our knowledge, this study is the first that examined the effects of a brief starvation on amino acid levels and main parameters of protein metabolism in red (slow-twitch) and white (fast-twitch) muscles. The results demonstrate that red muscles have higher rates of protein turnover and may adapt better to a brief starvation when compared to white muscles. This phenomenon may play a role in more pronounced atrophy of muscles composed mostly by white fibers in aging and various muscle wasting disorders. Further studies are needed to examine the effects of prolonged starvation, in which changes in protein and amino acid metabolism are different when compared to short-term starvation.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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Abbreviations

AMC, 7-amino-4-methylcoumarin; BCAA, branched-chain amino acids; CTLA, chymotrypsin like activity of proteasome; EAA, essential amino acids; EDL, musculus

extensor digitorum longus; SOL, musculus soleus; FRPS, fractional rate of protein synthesis; MuRF-1, muscle-ring-finger-1; NEAA, non-essential amino acids.

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