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ORIGINAL ARTICLE

Glutamine deficiency in extracellular fluid exerts adverse effects on protein and amino acid metabolism in skeletal muscle of healthy, laparotomized, and septic rats

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Abstract Characteristic feature of critical illness, such as trauma and sepsis, is muscle wasting associated with activated oxidation of branched-chain amino acids (valine, leucine, isoleucine) and enhanced release of glutamine (GLN) to the blood. GLN consumption in visceral tissues frequently exceeds its release from muscle resulting in GLN deficiency that may exert adverse effects on the course of the disease. In the present study, we investigated the effects of GLN depletion in extracellular fluid on GLN production and protein and amino acid metabolism in skeletal muscle of healthy, laparotomized, and septic rats. Cecal ligation and puncture (CLP) was used as a model of sepsis. After 24 h, soleus muscle (SOL, slow-twitch, red muscle) and extensor digitorum longus (EDL, fast-twitch, white muscle) were isolated and incubated in a medium containing 0.5 mM GLN or without GLN. L-[1-^{14}C]leucine was used to estimate protein synthesis and leucine oxidation, 3-methylhistidine release was used to evaluate myofibrillar protein breakdown. CLP increased GLN release from muscle, protein breakdown and leucine oxidation, and decreased protein synthesis. The effects were more pronounced in EDL. Alterations induced by laparotomy were similar to those observed in sepsis, but of a lower extent. GLN deficiency in medium enhanced GLN release and decreased intramuscular GLN concentration, decreased protein synthesis in muscles of intact and laparotomized rats, and enhanced leucine oxidation in SOL of intact and protein breakdown in SOL of laparotomized rats. It is concluded that (1) fast-twitch fibers are more sensitive to septic stimuli than slow-twitch fibers, (2) extracellular GLN deficiency may exert adverse effects on protein and amino acid metabolism in skeletal muscle, and (3) muscles of healthy and laparotomized animals are more sensitive to GLN deficiency than muscles of septic animals.

Keywords Glutamine · Branched-chain amino acids · Sepsis · Skeletal muscle · Cachexia

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Introduction

Severe stress conditions, such as trauma, surgery, burn injury and sepsis, evoke systemic inflammatory response associated with activated protein breakdown, catabolism of branched-chain amino acids (BCAA; valine, leucine, and isoleucine), and synthesis of glutamine (GLN) in skeletal muscle (Ryan 1976; O'Donnel et al. 1976; Flores et al. 1989; Nawabi et al. 1990; Holecek 1996). The most of GLN released from the muscle is utilized in splanchnic area and the body's requirements for GLN frequently exceed the individual's ability to synthesize sufficient amounts of this amino acid (Fürst et al. 1987; Essen et al. 1992; Hardy and Hardy 2008; Wernerman 2011). Subsequent decrease of GLN concentration in plasma and tissues may exert adverse effects on the course of the disease, especially via suppression of immune functions and impaired gut integrity (O'Riordain et al. 1996; Buchman et al. 1999; Exner et al. 2002). In a number of studies favorable effects of dietary GLN supplementation on protein accretion, immune status, and intestinal function have been demonstrated (Wang et al. 2008; Wu et al. 2013; Ren et al. 2013).

Important donor of nitrogen for synthesis of GLN in skeletal muscle is the BCAA, essential amino acids that

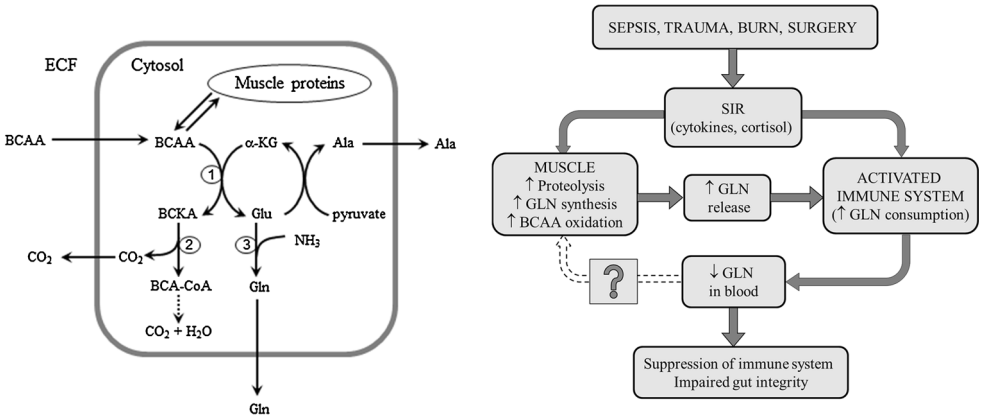

Fig. 1 The main metabolic pathways involved in GLN synthesis in skeletal muscle (on the *left*) and alterations in GLN metabolism associated with systemic inflammatory response (on the *right*). Stress conditions enhanced GLN consumption in splanchnic tissues may lead to GLN deficiency in extracellular fluid that in turn may exert adverse effects on the gut integrity and immune functions. The influence of GLN deficiency on protein and amino acid metabolism in skeletal muscle is obscure. *1* branched-chain aminotransferase, *2* branched-chain α-ketoacid dehydrogenase, *3* glutamine synthetase

exert unique regulatory role in protein metabolism including activation of mTOR (mammalian target of rapamycin) signaling pathway and protein translation (Tischler et al. 1982; Nair and Short 2005). Data from the literature collectively suggest that alterations in BCAA catabolism that occur in stress conditions contribute to lean body mass loss (Nawabi et al. 1990; Holecek et al. 1997; O'Connell 2013). The pathways of GLN synthesis and the relationship of the BCAA to the pathways of GLN synthesis in skeletal muscle are shown in Fig. 1 (left side).

Both the BCAA catabolism and GLN synthesis are responsive to substrate and product regulation (Smith et al. 1984). Therefore, it may be supposed that the lack of GLN in extracellular fluid occurring in severe illness acts as a stimulus enhancing GLN production and BCAA catabolism and exerts adverse effects on protein balance in muscle (Fig. 1, right side). Unfortunately, the existence of this potentially important feedback has been neither confirmed nor invalidated although there are a number of studies demonstrating that intramuscular GLN depletion may directly contribute to lean body mass wasting (Feng et al. 1990; Jepson et al. 1988; Wu 2009).

The main objective of the present study was to estimate if there is a direct effect of GLN deficiency in extracellular fluid on GLN production, BCAA catabolism, and protein turnover in skeletal muscle in subjects undergoing surgery (laparotomy) and in sepsis (ligation and puncture of cecum). Several studies have reported different responses to septic stimuli (Hasselgren et al. 1986; Muthny et al. 2008, 2009) and different GLN synthetase activities in fast-twitch and slow-twitch muscle fibers (Hickson et al. 1996). Therefore, the experiments were performed in both types of skeletal muscle—in soleus muscle (SOL, slow-twitch, red muscle) and extensor digitorum longus (EDL, fast-twitch, red muscle).

Materials and methods

Animals

Male Wistar rats (body weight 40–60 g) obtained from BioTest, Konarovice, CZ, were used in this study. The rats were housed under controlled conditions (12-h light–dark cycle, 22 °C, 55–65 % relative humidity) with free access to standard laboratory chow and water. All procedures involving animal manipulation were performed in accordance with guidelines set by the Institutional Animal Use and Care Committee of Charles University, Prague, CZ.

Materials

L-[1-^{14}C]leucine was purchased from GE Healthcare Life Sciences (Buckinghamshire, UK), hyamine hydroxide was obtained from Packard Instrument (Meriden, CT). The remaining chemicals were obtained from Sigma Chemicals (St Louis, MO), Waters (Milford, MA), and Lachema (Brno, CZ).

Experimental design

Sepsis was induced in ether anesthesia by cecal ligation and puncture (CLP), a frequent and clinically relevant model of sepsis associated with proteocatabolic state. In brief, 80 % of the cecum was ligated through a 1.5-cm abdominal midline incision. One through-and-through puncture was made using an 18-gauge needle, and a small amount of stool was expelled from the puncture to ensure leakage of the intestinal content. After reposition of the bowel, the abdomen was closed in two layers (Singleton and Wischmeyer 2003). Laparotomized (LAP) rats

underwent the same procedure as CLP animals without ligation and puncture of cecum. Intact (INT) animals served as controls. All rats were allowed water ad libitum, but were fasted for 24 h before killing to avoid any influence of differences in food intake.

Animals were killed in pentobarbital narcosis (6 mg/100 g body weight, intraperitoneally) by exsanguination via abdominal aorta. Soleus (SOL) and extensor digitorum longus (EDL) muscles of both legs were dissected according to Maizels et al. (1977) and fixed via the tendons to stainless steel clips to provide slight tension (at approximately resting length), and immediately transferred into 2.5 ml of modified Krebs–Henseleit bicarbonate buffer with 6 mM glucose and 2 mU/ml insulin (pH 7.4, 37 °C). The medium was saturated with O_2/CO_2 (19:1).

The muscles were preincubated for 30 min in a thermostatically controlled bath (37 °C) with a shaking device (70 cycles/min). After the preincubation, the muscles were quickly rinsed in 0.9 % NaCl, blotted and transferred to a second set of vials and incubated in a medium containing 0.5 mM GLN or in the same medium without GLN. Other amino acids were in approximately physiological concentrations. Muscles of the left leg were used for determination of the effect of GLN deficiency, and muscles of the right leg served as paired controls. Other components present in the medium were dependent on the parameter measured as described below. The viability of the incubated muscles was previously confirmed in our laboratory (Safranek et al. 2003). Two separate studies were performed.

Study 1: Effect of GLN deficiency on release of GLN and 3-methylhistidine (3-MH) into incubation medium and intramuscular amino acid concentrations

At the end of incubation, the muscles were quickly removed, rinsed and homogenized in 0.6 ml of 2 % (v/v) perchloric acid and then centrifuged for 5 min at 12,000*g*. The supernatant was used for the determination of free amino acid concentration in muscle with liquid chromatograph (Waters, Milford, MA). Results were expressed in μmol/g wet muscle. The release of GLN and of 3-MH, a characteristic product of the myofibrillar breakdown, was calculated on the basis of their concentrations in medium at the beginning and after 2 h of the incubation, the volume of the medium, and the weight of the muscle. Results were expressed as nmol/g wet muscle/h.

Study 2: Leucine oxidation and protein synthesis

Leucine oxidation and protein synthesis were measured after 1-h incubation of the muscle in 2.5 ml of medium containing 0 or 0.5 mM GLN and L-[1-^{14}C]leucine (0.6 μCi/ml) (Safranek et al. 2003). At the end of the incubation period, 0.4 ml of hydroxide of hyamine was added into the well hanging above the incubation medium, the reaction was stopped by the addition of 35 % (v/v) perchloric acid solution (0.2 ml) into the incubation medium, and the flasks were shaken for 1 h to ensure complete absorption of $^{14}CO_2$ into the hyamine hydroxide. The hanging wells containing hyamine were placed into counting vials containing 10 ml of scintillation mixture and counted in the liquid scintillation radioactivity counter LS 6000 (Beckman Instruments, CA, USA). The muscles were removed from the incubation flasks, quickly rinsed in cold 6 % (v/v) $HClO_4$, blotted, and homogenized in 0.6 ml of 6 % (v/v) $HClO_4$. The homogenate was centrifuged for 5 min at 12,000*g*. The pellet was washed three times and then hydrolyzed in 1 M NaOH. Aliquots were taken for measurements of protein content (Lowry et al. 1951) and L-[1-^{14}C]leucine radioactivity.

Leucine oxidation rates were calculated using the radioactivity of released $^{14}CO_2$, efficiency of $^{14}CO_2$ recovery, and leucine-specific activity in the incubation medium by the following formulas:

$$\mathrm{SA_{Leu}(dpm/nmol)} = \frac{\text{Leu radioactivity} * (\mathrm{dpm/ml})}{\text{Leu concentration } (\mathrm{nmol/ml})}$$

$$\text{Leucine oxidation } (\mathrm{nmol/g \text{ wet muscle/h}}) = \frac{\text{radioactivity of released} ^{14}CO_2 * (\mathrm{dpm/h})}{\mathrm{SA_{Leu}} * (\delta\pi\mu/\nu\mu\omega\lambda) \times \mu\upsilon\sigma\psi\lambda\varepsilon\ \chi\varepsilon\iota\gamma\dagger\hbar\tau\ (\gamma) \times * \Phi\varrho}$$

The efficiency of $^{14}CO_2$ recovery (FR) was tested by adding sodium[^{14}C]bicarbonate to the medium. There was 96 % recovery in 1 h after addition of the acid to the incubation medium. The correction factor 0.96 was used for calculation of leucine oxidation. The results were expressed as nmol of oxidized leucine/g wet weight/h.

Protein synthesis rates were calculated using the radioactivity of leucine incorporated in muscle protein and leucine-specific activity in the incubation medium:

$$\text{Protein synthesis } (\mathrm{nmol\ Leu/g\ protein/h}) = \frac{\text{Leu radioactivity in protein } (\mathrm{dpm/g\ protein/h})}{\mathrm{SA_{Leu*}(dpm/nmol)}}$$

Statistical analysis

The results are expressed as the mean ± SEM. An analysis of variance (ANOVA) followed by Bonferroni multiple comparison procedure or *F* test followed by paired *t* test (to estimate the effect of GLN deficiency) has been used for analysis of the data. Differences were considered significant at $P < 0.05$. Statistical software NCSS 2001 (Kaysville, UT) was applied for analysis.

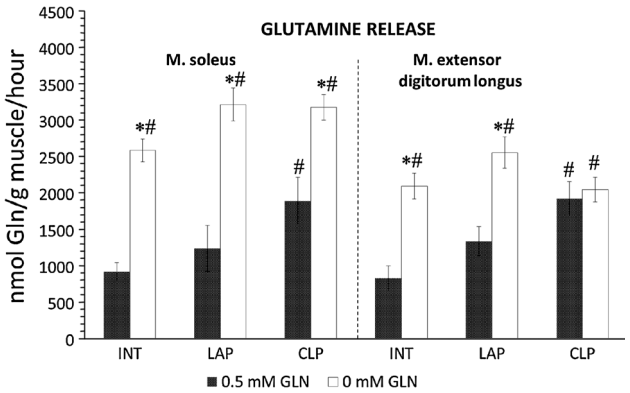

Fig. 2 Effect of GLN deficiency in incubation medium on GLN release from muscles of healthy (INT), laparotomized (LAP), and septic (CLP) animals. Mean ± SE; $P < 0.05$. *Asterisk* indicates the effect of GLN deficiency (paired *t* test); *hash* indicates comparison vs. INT 0.5 mM GLN (ANOVA and Bonferroni)

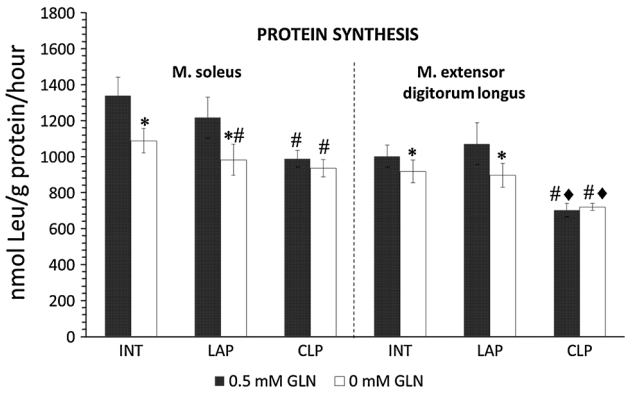

Fig. 3 Effect of GLN deficiency in incubation medium on protein synthesis in muscles of healthy (INT), laparotomized (LAP), and septic (CLP) animals. Mean ± SE; $P < 0.05$. *Asterisk* indicates the effect of GLN deficiency (paired *t* test); *hash* indicates comparison vs. INT 0.5 mM GLN (ANOVA and Bonferroni); *filled diamond* indicates the comparison of the effect of CLP groups to LAP 0.5 mM (ANOVA and Bonferroni)

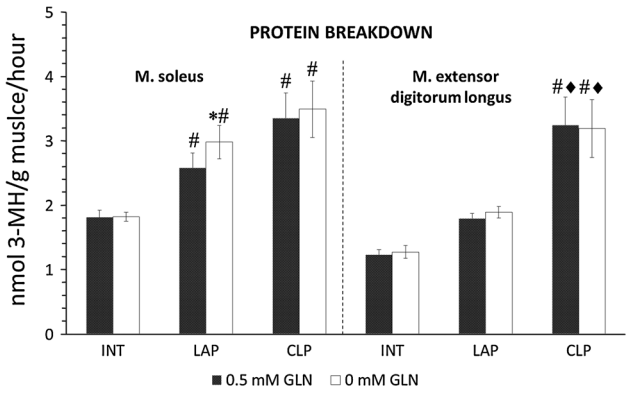

Fig. 4 Effect of GLN deficiency in incubation medium on protein breakdown in muscles of healthy (INT), laparotomized (LAP), and septic (CLP) animals. Mean ± SE; $P < 0.05$. *Asterisk* indicates the effect of GLN deficiency (paired *t* test); *hash* indicates comparison vs. INT 0.5 mM GLN (ANOVA and Bonferroni); *filled diamond* indicates the comparison of the effect of CLP groups to LAP 0.5 mM (ANOVA and Bonferroni)

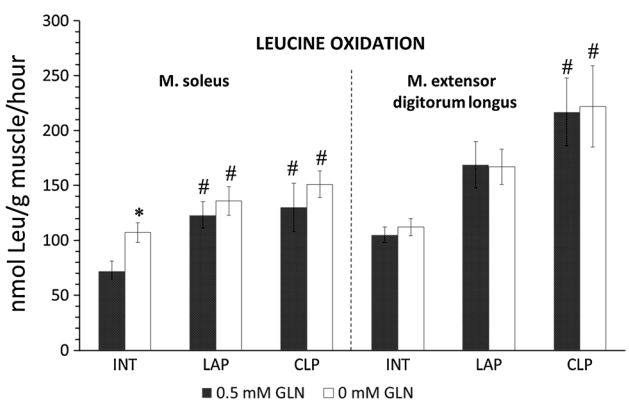

Fig. 5 Effect of GLN deficiency in incubation medium on leucine oxidation in muscles of healthy (INT), laparotomized (LAP), and septic (CLP) animals. Mean ± SE; $P < 0.05$. *Asterisk* indicates the effect of GLN deficiency (paired *t* test); *hash* indicates comparison vs. INT 0.5 mM GLN (ANOVA and Bonferroni)

Results

CLP induced significant increase in GLN release to incubation medium from both types of skeletal muscle. GLN deficiency in medium enhanced, with exception of EDL of CLP-treated animals, GLN release from muscle (Fig. 2).

Significant decrease in protein synthesis both in SOL (27 %) and EDL (30 %) was found after CLP. GLN deficiency in incubation medium suppressed protein synthesis in healthy (19 % in SOL; 8 % in EDL) and LAP (19 % in SOL; 16 % in EDL) animals. The effect of GLN deficiency on protein synthesis in CLP animals was not found (Fig. 3).

CLP enhanced the release of 3-MH from both SOL (87 %) and EDL (163 %). In LAP animals, the rise in 3-MH release was significant only in SOL. GLN deficiency in medium enhanced 3-MH release only from SOL of LAP animals (Fig. 4).

Significant increase in leucine oxidation was found in SOL (80 %) and EDL (107 %) of CLP-treated animals and in SOL of LAP animals. GLN deficiency in incubation medium enhanced leucine oxidation in SOL of healthy animals (Fig. 5).

The most impressive effect of CLP on amino acid concentration in skeletal muscle was significant a decrease in the number of amino acids including GLN in EDL, while its effect in SOL was insignificant. After LAP, we found

Table 1 Effect of GLN deficiency in incubation medium on amino acid concentrations in SOL

SOL	INT ($n = 10$)	INT (−GLN) ($n = 10$)	LAP ($n = 10$)	LAP (−GLN) ($n = 10$)	CLP ($n = 10$)	CLP (−GLN) ($n = 10$)
Asp	0.99 ± 0.10	1.03 ± 0.07	0.97 ± 0.09	1.13 ± 0.09[a]	0.87 ± 0.06	0.89 ± 0.06
Glu	5.71 ± 0.41	6.17 ± 0.31	5.42 ± 0.36	6.18 ± 0.37[a]	4.89 ± 0.45	4.66 ± 0.44
Ser	2.74 ± 0.25	2.92 ± 0.19	2.49 ± 0.23	3.00 ± 0.30[a]	2.00 ± 0.17	2.23 ± 0.20
Asn	0.53 ± 0.06	0.59 ± 0.06[a]	0.36 ± 0.03	0.44 ± 0.04[a]	0.39 ± 0.04	0.36 ± 0.03
Gly	3.95 ± 0.26	3.90 ± 0.14	3.70 ± 0.25	4.33 ± 0.28[a]	3.76 ± 0.17	3.91 ± 0.24
GLN	8.18 ± 0.42	5.79 ± 0.36[a,b]	8.03 ± 0.49	6.15 ± 0.38[a,b]	8.13 ± 0.65	5.48 ± 0.66[a,b,c]
His	0.57 ± 0.04	0.71 ± 0.05[a]	0.59 ± 0.03	0.72 ± 0.05[a]	0.51 ± 0.04	0.60 ± 0.06[a]
Tau	10.3 ± 0.6	11.1 ± 0.5	10.7 ± 0.7	12.5 ± 0.9[a]	13.2 ± 0.9	13.0 ± 1.1
Ctr	0.73 ± 0.07	0.75 ± 0.06	0.67 ± 0.07	0.78 ± 0.08[a]	0.54 ± 0.07	0.52 ± 0.07
Ala	3.27 ± 0.29	3.50 ± 0.14	3.03 ± 0.22	4.09 ± 0.37[a]	3.07 ± 0.25	3.64 ± 0.28[a]
Arg	0.81 ± 0.07	0.87 ± 0.06	0.79 ± 0.06	0.96 ± 0.05[a]	0.83 ± 0.04	0.87 ± 0.03
Pro	0.55 ± 0.04	0.56 ± 0.02	0.50 ± 0.03	0.62 ± 0.05[a]	0.45 ± 0.02	0.54 ± 0.02[a]
Tyr	0.24 ± 0.01	0.26 ± 0.02[a]	0.23 ± 0.01	0.29 ± 0.02	0.23 ± 0.01	0.29 ± 0.02[a]
Val	0.24 ± 0.02	0.25 ± 0.02	0.24 ± 0.02	0.31 ± 0.03	0.22 ± 0.01	0.28 ± 0.01[a]
Met	0.08 ± 0.01	0.10 ± 0.00[a]	0.12 ± 0.01[b]	0.14 ± 0.01[b]	0.09 ± 0.01[c]	0.13 ± 0.01[a,b]
Ile	0.15 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.20 ± 0.02	0.14 ± 0.01	0.17 ± 0.01[a]
Leu	0.31 ± 0.02	0.33 ± 0.02	0.30 ± 0.03	0.40 ± 0.03[a]	0.28 ± 0.02	0.34 ± 0.01[a]
Lys	1.56 ± 0.15	1.62 ± 0.14	1.40 ± 0.08	1.72 ± 0.11[a]	1.58 ± 0.08	1.66 ± 0.09
Phe	0.16 ± 0.01	0.18 ± 0.01[a]	0.16 ± 0.01	0.21 ± 0.02	0.15 ± 0.01	0.19 ± 0.01[a]
BCAA	0.71 ± 0.04	0.74 ± 0.05	0.69 ± 0.06	0.92 ± 0.08	0.64 ± 0.04	0.78 ± 0.03[a]
Σ	41.1 ± 2.5	40.9 ± 1.1	39.8 ± 2.2	44.3 ± 2.6[a]	41.4 ± 2.6	39.7 ± 2.9
Σ-GLN	32.9 ± 2.1	35.16 ± 1.0	31.8 ± 1.7	38.1 ± 2.3[a]	33.2 ± 2.0	34.2 ± 2.3

Values are mean ± SE in μmol/g; $P < 0.05$. (−GLN): incubation in medium without GLN

[a] Effect of GLN deficiency (paired t test)

[b] Comparison vs. INT (ANOVA and Bonferroni)

[c] Comparison of the effect of CLP groups to LAP (ANOVA and Bonferroni)

increased concentrations of Met in SOL and of Gly in EDL. With exception of EDL of CLP-treated animals, incubation of muscles in medium without GLN reduced GLN and increased concentrations of a number of other amino acids. Marked increase was observed especially in LAP animals—serine, glycine, histidine, taurine, citrulline, arginine, proline, and lysine rose in both muscle types (Tables 1, 2).

Discussion

Integrated activation of proteolysis, BCAA oxidation and GLN synthesis in skeletal muscle can be seen as a characteristic feature of inflammatory response that may simultaneously provide energy fuel for skeletal muscle and amino acids for needs of other tissues. The present study demonstrates that muscle protein catabolism in rat models of sepsis (CLP) and of surgical stress (LAP) is due to both inhibition of protein synthesis and enhanced protein breakdown. Enhanced rates of leucine oxidation indicate activated catabolism of the BCAA that acts in skeletal muscle as an important source of energy and amino nitrogen for synthesis of GLN. All these findings are in a good agreement with our previous studies (Safranek et al. 2006; Kadlcikova et al. 2004; Kovarik et al. 2010) and with others (Hobler et al. 1998; Fischer et al. 2000; Fareed et al. 2006).

More pronounced decrease in protein synthesis and higher rates of leucine oxidation and 3-MH release from EDL than from SOL in CLP-treated animals demonstrate that muscles composed mostly by fast-twitch fibers (white muscles) are more sensitive to septic stimuli than muscles composed mostly by slow-twitch fibers (red muscles). Considering that the proportions between white and red fibers differ due to genetic factors and that red fibers are the major component of respiratory muscles (Polla et al. 2004), this finding may be of practical importance.

Qualitatively similar effects of LAP and CLP on a number of parameters of amino acid and protein metabolism observed in this study are opening a question which controls are optimal—sham operated, or intact animals? As a matter of fact, CLP is a model of sepsis complicated by surgical stress and therefore animals with LAP may be considered more suitable. However, both after LAP and CLP systemic inflammatory response is activated and it may be supposed that some significant effects of sepsis induced by CLP may be obscured when animals with LAP are used as only controls.

Effects of GLN deficiency in extracellular fluid

According to our knowledge, this is the first study clearly demonstrating that GLN depletion in extracellular fluid may

Table 2 Effect of GLN deficiency in incubation medium on amino acid concentrations in EDL

	INT ($n = 10$)	INT (−GLN) ($n = 10$)	LAP ($n = 10$)	LAP (−GLN) ($n = 10$)	CLP ($n = 10$)	CLP (−GLN) ($n = 10$)
Asp	0.78 ± 0.06	0.73 ± 0.07	0.75 ± 0.04	0.82 ± 0.06	0.72 ± 0.05	0.81 ± 0.08
Glu	3.41 ± 0.26	3.47 ± 0.27	3.55 ± 0.16	3.92 ± 0.24	2.38 ± 0.14[b,c]	2.63 ± 0.16[c]
Ser	1.98 ± 0.16	2.04 ± 0.16	1.97 ± 0.10	2.15 ± 0.1[a]	1.29 ± 0.11[b,c]	1.36 ± 0.11[b]
Asn	0.21 ± 0.02	0.25 ± 0.02[a]	0.16 ± 0.02	0.18 ± 0.02	0.19 ± 0.01	0.20 ± 0.01
Gly	5.28 ± 0.20	5.28 ± 0.23	8.03 ± 0.44[b]	8.99 ± 0.49[a,b]	5.84 ± 0.57[c]	6.35 ± 0.65
GLN	8.90 ± 0.48	6.79 ± 0.52[a,b]	8.72 ± 0.49	7.6 ± 0.37[a]	5.05 ± 0.68[b,c]	3.62 ± 0.63[a,b,c]
His	0.53 ± 0.04	0.60 ± 0.05[a]	0.51 ± 0.02	0.61 ± 0.03[a]	0.32 ± 0.02[b,c]	0.34 ± 0.02[b,c]
Tau	9.78 ± 0.31	9.21 ± 0.41	9.63 ± 0.37	10.2 ± 0.34[a]	9.70 ± 0.50	9.89 ± 0.53
Ctr	0.69 ± 0.07	0.72 ± 0.09	0.59 ± 0.03	0.66 ± 0.04[a]	0.25 ± 0.04[b,c]	0.25 ± 0.04[b,c]
Ala	3.17 ± 0.14	3.35 ± 0.23	3.75 ± 0.34	4.15 ± 0.18[b]	3.67 ± 0.20	3.86 ± 0.30
Arg	0.73 ± 0.06	0.86 ± 0.07[a]	0.84 ± 0.05	0.95 ± 0.07[a]	0.68 ± 0.07	0.72 ± 0.05
Pro	0.70 ± 0.02	0.79 ± 0.07	0.62 ± 0.03	0.68 ± 0.04[a]	0.50 ± 0.02[b]	0.55 ± 0.03[a,b]
Tyr	0.18 ± 0.01	0.20 ± 0.02[a]	0.18 ± 0.01	0.21 ± 0.03	0.20 ± 0.01	0.21 ± 0.01
Val	0.16 ± 0.01	0.19 ± 0.02	0.17 ± 0.01	0.18 ± 0.01	0.17 ± 0.00	0.18 ± 0.01
Met	0.07 ± 0.00	0.09 ± 0.01	0.08 ± 0.00	0.08 ± 0.0	0.09 ± 0.00	0.10 ± 0.01[a,b]
Ile	0.10 ± 0.01	0.12 ± 0.02	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.00	0.12 ± 0.00
Leu	0.19 ± 0.01	0.23 ± 0.03	0.20 ± 0.01	0.21 ± 0.01	0.18 ± 0.01	0.20 ± 0.01
Lys	1.88 ± 0.25	2.18 ± 0.26[a]	2.03 ± 0.15	2.28 ± 0.19[a]	1.76 ± 0.16	1.95 ± 0.13
Phe	0.11 ± 0.01	0.13 ± 0.01	0.12 ± 0.00	0.12 ± 0.01	0.13 ± 0.01	0.14 ± 0.01[b]
BCAA	0.45 ± 0.02	0.53 ± 0.07	0.49 ± 0.02	0.51 ± 0.03	0.45 ± 0.02	0.49 ± 0.01
Σ	38.8 ± 1.4	37.2 ± 1.8	42.0 ± 0.8	44.1 ± 0.87[a]	33.2 ± 2.0[c]	33.5 ± 1.6[a,c]
Σ-GLN	29.9 ± 1.0	30.4 ± 1.4	33.3 ± 0.8	36.5 ± 0.62[a,b]	28.2 ± 1.4[c]	29.9 ± 1.4[a]

Values are mean ± SE in μmol/g; $P < 0.05$. (−GLN): incubation in medium without GLN

[a] Effect of GLN deficiency (paired *t* test)

[b] Comparison vs. INT (ANOVA and Bonferroni)

[c] Comparison of the effect of CLP groups to LAP (ANOVA and Bonferroni)

act as a stimulus leading to enhanced release of GLN from muscle and to intramuscular GLN deficiency. The increase in leucine oxidation in SOL of INT animals and marked decrease of protein synthesis in muscles of INT and LAP animals indicate that GLN deficiency may activate BCAA catabolism and exert adverse effects on muscle protein balance.

The obtained findings are in agreement with reports demonstrating the existence of the feedback regulation of GLN production and BCAA oxidation in skeletal muscle by reactant availability. A number of studies have demonstrated stimulatory effect of the BCAA on GLN synthesis (Ruderman and Berger 1974; Odessey et al. 1974; Holecek 2013), inhibitory effect of GLN on BCAA catabolism (Holecek et al. 2000, 2002), and/or activated GLN production and BCAA catabolism by hyperammonemia (Clemmesen et al. 2000; Holecek et al. 2011; Holecek 2014; Rodney and Boneh 2013).

Insignificant effects of GLN deficiency on protein and BCAA oxidation in muscles of septic animals are probably due to a marked decrease in protein synthesis and increase in BCAA oxidation induced by mediators of systemic inflammatory response. Limitations in the capacity of skeletal muscle to respond to the influence of GLN deficiency indicates also insignificant effect of GLN depletion on GLN release from EDL of septic animals, in which alterations induced by CLP were of the greatest extent.

We suppose that increased concentrations of a number of amino acids in muscles incubated in medium without GLN are mostly due to alterations in their transport across the cell membrane. GLN shares various cellular transporters with other amino acids, such as L-transporter, which couples the uptake of a number of neutral amino acids from extracellular space with efflux of amino acids from cytosol (Meier et al. 2002). Thus, enhanced efflux of GLN may be coupled with enhanced influx of various amino acids, including the BCAA, histidine, methionine, phenylalanine, and tyrosine. To the increase in intracellular concentrations of a number of amino acids in muscles incubated in medium without GLN contributes undoubtedly a decrease in protein synthesis observed in INT and LAP animals. A role may play also various alterations in amino acid metabolism. Wu and Thompson (1989) found that the BCAA strongly inhibit methionine transamination in rat muscle homogenates. Thus, increased concentrations of methionine in muscles incubated in GLN deficient medium may be related to its decreased catabolism due to increased BCAA concentrations in cytosol.

Conclusions

The results demonstrate that fast-twitch fibers are more sensitive to septic stimuli than slow-twitch fibers, and that

GLN deficiency in extracellular fluid may enhance GLN release from skeletal muscle, affect distribution of amino acids between intracellular and extracellular space, enhance BCAA oxidation, and exert negative effect on protein balance in skeletal muscle. Probably due to enormous alterations induced by the mediators of systemic inflammatory response, the negative effects of GLN deficiency on muscle are in severe stress conditions less significant than in mild injury or in healthy state.

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