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ORIGINAL PAPER

Effects of β-hydroxy-β-methylbutyrate treatment in different types of skeletal muscle of intact and septic rats

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Abstract β-Hydroxy-β-methylbutyrate (HMB) is a leucine metabolite that may have a positive effect in protein catabolic conditions. Therefore, we hypothesized that HMB treatment could attenuate the sepsis-induced protein catabolic state. The aims of our study were to elucidate the effect of HMB in healthy and septic animals and to evaluate the differences in the action of HMB in different muscle types. Intact and septic (5 mg endotoxin/kg i.p.) rats were administered with HMB (0.5 g/kg/day) or saline. After 24 h, extensor digitorum longus (EDL) and soleus (SOL) muscles were isolated and used for determination of total and myofibrillar proteolysis, protein synthesis, leucine oxidation, activity of cathepsins B and L, chymotrypsin-like activity, and expression of α-subunits of proteasome. Our results indicate that the catabolic state induced by the endotoxin treatment was caused both by increase in protein breakdown (due to activation of proteasome system) and by attenuation of protein synthesis. The EDL (muscle composed of white, fast-twitch fibers) was more susceptible to these changes than the SOL (muscle composed of red, slow-twitch fibers). The HMB treatment had no effect in healthy animals but counteracted the changes in septic animals. The action of HMB was mediated by attenuation of proteasome activity and protein breakdown, not by stimulation of protein synthesis. More pronounced effect of the HMB treatment on myofibrillar proteolysis was observed in the SOL.

Keywords Proteasome · Inflammation · Amino acid · Protein metabolism · Skeletal muscle

Abbreviations

3-MH	3-Methylhistidine
AMC	7-Amino-4-methylcoumarin
BCAA	Branched-chain amino acids (leucine isoleucine and valine)
CTLA	Chymotrypsin-like activity of proteasome
EDL	Musculus extensor digitorum longus
HMB	β-Hydroxy-β-methylbutyrate
MuRF-1	Muscle RING finger protein 1
NF-κB	Nuclear factor κB
SOL	Musculus soleus
Suc-LLVY-MCA	*N*-Succinyl-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin
Z-FA-MCA	Z-Phe-Arg 7-amido-4-methyl-coumarin hydrochloride
Z-FF-FMK	Z-Phe-Phe-fluoromethyl ketone

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Introduction

Sepsis is an important healthcare problem with a continuously increasing incidence. Despite the availability of potent antibiotics and refined supportive care, the mortality of septic patients remains high, with overall estimates of about 30% and increasing to 50% when associated with shock [1]. It is estimated that worldwide, about half a million people die of sepsis annually. In patients with severe and protracted sepsis, the catabolic response results in muscle wasting and fatigue which may adversely affect the outcome in these patients. The myofibrillar proteins actin and myosin are particularly sensitive to the effects of sepsis [14].

β-Hydroxy-β-methylbutyrate (HMB) is a naturally occurring metabolite of amino acid leucine, known as a regulator of protein metabolism and protein anabolic substance [6, 7]. Some of the effects of leucine (e.g.. attenuation of protein breakdown) are probably mediated by its metabolites, especially α-ketoisocaproate and HMB. For many years, it had been believed that HMB could serve as an additive source of β-hydroxy-β-methylglutaryl coenzyme A for the cholesterol synthesis (see Fig. 1), important for the synthesis of new and repair of the damaged cell membranes [27]. Recently, some papers described that HMB might be able to suppress the ubiquitin-proteasome proteolytic system due to an interference with nuclear factor κB (NF-κB) [36] or double-stranded RNA-dependent protein kinase signaling [9]. Others reported that HMB also induces stimulation of protein synthesis analogous to that of leucine [8].

In the last few years, it was reported that HMB prevents cachexia in animals bearing MAC-16 or Walker 256 tumor [29, 37]. Clinically, the effect of HMB was tested in combination with glutamine and arginine in cachectic patients. Positive results were observed in AIDS- and cancer-related cachexia [3, 25], not in case of rheumatoid cachexia [24]. Unfortunately, the common use of HMB is limited as a dietary supplement for sportsmen during resistance training because of its supposed increase of net lean mass and strength [28].

In our study, we tested the hypothesis that HMB treatment could attenuate the sepsis-induced protein catabolic state. Therefore, we elucidated the effects of the in vivo HMB treatment on protein metabolism in skeletal muscle of healthy and septic rats. We were interested in changes in protein synthesis and breakdown, especially the degradation of myofibrillar proteins and in the activity of main proteolytic systems—the ubiquitin proteasomal and lysosomal. In addition, considering the different responses of muscles varying in fibers' composition on many stimuli, we evaluated the differences in the action of HMB in different muscle types. Our study might be helpful to find new possibilities in treatment of muscle wasting in septic patients.

Materials and methods

Materials

HMB (calcium salt) was kindly donated by Metabolic Technologies, Inc. (Ames, IA, USA). L-[1-^{14}C]leucine was purchased from GE Healthcare Life Sciences (Buckinghamshire, UK); lipopolysaccharides from *Escherichia coli* 0127:B8 (endotoxin), amino acids, cycloheximide, Folin-Ciocalteu's phenol reagent, *N*-succinyl-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin (Suc-LLVY-MCA), Z-Phe-Arg 7-amido-4-methylcoumarin hydrochloride (Z-FA-MCA), Z-Phe-Phe-fluoromethyl ketone (Z-FF-FMK), and albumin were purchased from Sigma Chemicals (St. Louis, MO, USA); Aminoplasmal 15 from B. Braun Medicals (Melsungen, DE); MG132 from Biomol (Hamburg, DE); osmotic pumps 2001D from Alzet Osmotic Pumps (Cupertino, CA, USA); mouse monoclonal antibodies to 20S proteasome subunits α1+2+3+5+6+7 (clone MCP231) and goat anti-mouse antibodies from Abcam (Cambridge, UK). The remaining chemicals were obtained from Sigma Chemicals (St. Louis, MO, USA), Bio-Rad (Hercules, CA, USA), Amersham Biosciences (Little Chalfont, UK), Waters (Milford, MA, USA), and Lachema (Brno, CZ).

Animals

Male Wistar rats (body weight 40–60 g) obtained from BioTest (Konarovice, CZ) were used in this study. It is necessary to use such young animals for muscle isolation and incubation to ensure the sufficient exchange of respiratory gases, nutrients, and catabolites between muscle cells and incubation medium. The rats were housed under controlled

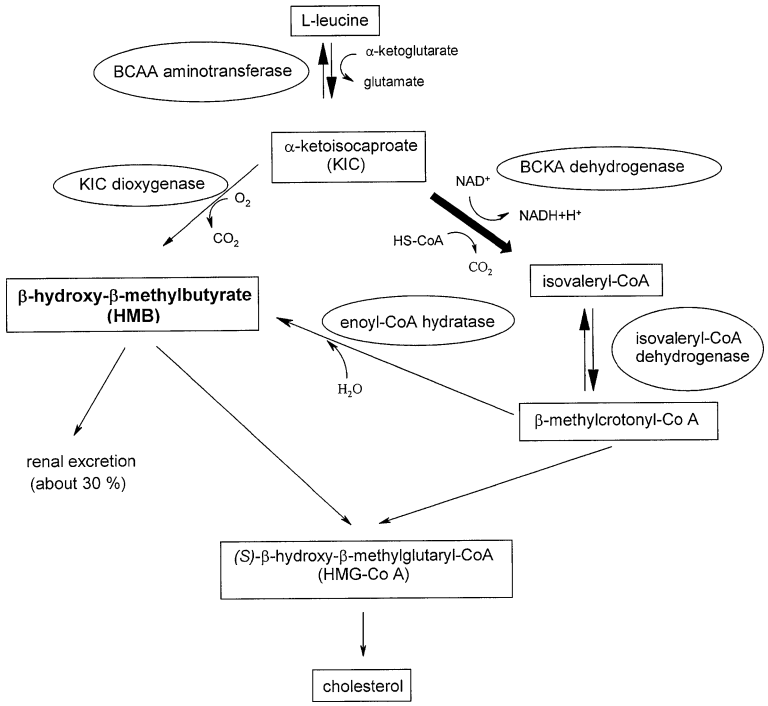

Fig. 1 Scheme of leucine and β-hydroxy-β-methylbutyrate metabolism. *BCAA* branched-chain amino acids (leucine, isoleucine, and valine); *BCKA* branched-chain keto acids; *KIC* α-ketoisocaproate; *CoA* coenzyme A; *HMG-CoA* β-hydroxy-β-methylglutaryl-coenzyme A. The leucine metabolism starts with transamination to KIC and subsequent decarboxylation to isovalerylcoenzyme A, which is further metabolized to HMG-CoA, important precursor of cholesterol synthesis. The alternate way of KIC metabolism (5–10%) is oxidation to HMB, which is eliminated by kidneys or further metabolized to HMG-CoA

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 and approved by the local scientific commitee for animal welfare.

Experimental design

The animals were divided into four groups (nine to 10 animals in each): (I) intact animals, (II) animals treated with HMB, (III) septic animals, and (IV) septic animals treated with HMB. Sepsis was induced by intraperitoneal administration of endotoxin (dissolved in 0.9% saline solution in a dose of 5 mg/kg body weight). The dose of endotoxin was determined according to the Slota's study [35] and our preliminary experiments. This dose caused a marked increase in protein breakdown but was not lethal. Control animals received a corresponding volume of the saline solution. HMB was administered continuously using osmotic pump, which was implanted subcutaneously in dorsolumbal area immediately after endotoxin or saline solution injection under ether narcosis (animals were placed in digestor during narcosis). The dose of HMB (dissolved in 0.9% saline solution in a dose of 0.5 g of HMB/kg body weight/day; total volume was approximately 220 μl) was determined according to the studies of Smith [37], Holeček [17] and our preliminary experiments. Twenty-four hours later, the 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. [23].

Muscle incubation

Freshly isolated SOL and EDL were fixed via the tendons to stainless steel clips to provide slight tension (at approximately resting length) and immediately transferred to 2.5 ml of modified Krebs–

Heinseleit bicarbonate buffer with 6 mM glucose and 2 mU/ml insulin (pH 7.4, 37°C). Other components present in the medium were dependent on the parameter measured—proteolysis and chymotrypsin-like activity (CTLA) of proteasome or protein synthesis and leucine oxidation. The medium was saturated with O_2/CO_2 (19:1). Always muscles from left leg were used for determination of protein synthesis and leucine oxidation and muscles from right leg for determination of proteolysis and CTLA of proteasome. The muscles were preincubated for 30 min in a thermostatically controlled bath (37°C) with a shaking device (70 cycles/min) to ensure stable intramuscular concentrations of components present in the medium. After the preincubation, the muscles were quickly rinsed in 0.9% NaCl, blotted, and transferred to a second set of vials containing fresh incubation media identical in composition and volume with the preincubation one. The viability of incubated muscles was previously confirmed in our laboratory [33] as well as in others [10].

Protein synthesis and leucine oxidation

Protein synthesis and leucine oxidation were measured after 1-h incubation of the muscle in 2.5 ml of medium enriched with amino acids in approximately physiological concentrations (Aminoplasmal 15 with added glutamine, tyrosine, threonine, serine, lysine, cysteine, and asparagine in total concentration of amino acids 2.8 mM) and L-[1-^{14}C] leucine (0.6 μCi/ml). The final concentration of labeled and unlabeled leucine was 150 μM/l to equilibrate intracellular specific activity to the specific activity of the incubation medium [20]. The 1-h (shortened) incubation was performed to minimize the bias of results via degradation of proteins. At the end of the incubation period, 0.4 ml of hydroxide of hyamine was added to 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 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*, and the pellet was used for the determination of protein synthesis and protein content of samples. The L-[1-^{14}C] leucine incorporation into the precipitated proteins was estimated after their hydrolysis in 1 M NaOH. Protein synthesis and leucine oxidation were calculated using leucine specific activity in the incubation medium and expressed as nmol of incorporated leucine/g protein/hour and nmol of oxidized leucine/g wet weight/hour, respectively. The radioactivity of the samples was measured with the liquid scintillation radioactivity counter LS 6000 (Beckman Instruments, CA, USA). The protein content of the samples was estimated according to Lowry et al. [22].

Total and myofibrillar proteolysis

The total and myofibrillar protein breakdown were estimated after a 2-h incubation of the muscle in a medium enriched with cycloheximide (0.5 mM), which prevented the reincorporation of the released amino acids into the muscle proteins. Since tyrosine is neither synthesized nor degraded in the skeletal muscle and since the intracellular pool of tyrosine remains constant during incubation [12], its amount released into the medium reflects total proteolysis in the muscle. The amount of 3-methylhistidine (3-MH), a characteristic product of the myofibrillar breakdown, released into the medium, served for the myofibrillar proteolysis determination. The rates of amino acid release were calculated on the basis of their respective concentrations in the medium and the weight of the muscle. 3-Methylhistidine was quantified using a high-performance liquid chromatography method (Waters, MA, USA) based on the reaction with fluorescamine [21, 40]; concentration of tyrosine was determined by a high-performance liquid chromatography method after a precolumn derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate [5, 32].

CTLA of proteasome

The CTLA of proteasome was determined using the fluorogenic substrate Suc-LLVY-MCA [13] as follows. After the incubation, the muscles were homogenized in 0.4 ml of ice-cold 20 mM Tris buffer (pH 7.5), containing 2 mM ATP, 5 mM $MgCl_2$, and 1 mM dithiothreitol. The homogenates were centrifuged for 10 min at 18,000×*g* at 4°C. The cellular supernatant (0.1 ml) was incubated with 0.1 ml of

substrate Suc-LLVY-MCA (0.05 mM), with and without inhibitor MG132 (0.02 mM), for 1 h on ice. Adding 1 ml of 100 mM sodium acetate buffer (pH 4.3) stopped the reaction. The fluorescence of the samples was immediately determined at the excitation wavelength of 340 nm and the emission wavelength of 440 nm (Tecan Infinite^{TM} 200). The standard curve was established for 7-amino-4-methylcoumarin (AMC), which permitted the expression of CTLA as nmol of AMC/g protein/hour. The activity was adjusted for the protein concentration of the supernatant estimating according to Lowry et al. [22]. The differences after the subtraction of inhibited from non-inhibited activities were used for the calculations.

Activity of cathepsin B and L

The activity of cathepsin B and L was determined in tibialis muscle using the fluorogenic substrate Z-FA-MCA [19, 38] as follows. This muscle was used because it has similar composition of fibers as EDL (predominantly composed of white, fast-twich fibers), and unfortunately, there was not enough material left from EDL or SOL after determining the protein synthesis, proteolysis, and CTLA. Samples (approximately 20 mg) were homogenized in 0.6 ml of ice-cold 300 mM acetate buffer, pH 5.0, containing 4 mM EDTA, 8 mM dithiothreitol, and 0.2% (*v*/*v*) Triton X-100. The homogenates were allowed to stand for 30 min on ice and then centrifuged for 30 min at 18,000×*g* at 4°C. The cellular supernatant (10 μl) was incubated with 190 μl of substrate Z-FA-MCA (0.1 mM), with and without inhibitor Z-FF-FMK (0.04 mM), for 15 min in a thermostatically controlled bath (37°C) with a shaking device (70 cycles/min). Adding 1 ml of 100 mM sodium acetate buffer (pH 4.3) stopped the reaction. Fluorescence determination was the same as in CTLA of proteasome. The differences after the subtraction of inhibited from non-inhibited activities were used for the calculations.

Expression of α-subunits of proteasome

Samples containing 15 μg of cytosolic protein, obtained for the CTLA assay, were diluted by Laemli Sample Buffer and separated electrophoretically on 12% sodium dodecylsulphate polyacrylamide gel. Subsequently, proteins were transferred to 0.45 μm pore size nitrocellulose membrane, which was then blocked with 5% nonfat dried milk in phosphate buffer saline at 4°C overnight. The primary antibodies (mouse monoclonal to 20S proteasome subunits α1+2+3+5+6+7) were used at dilution 1:1,000, secondary peroxidase-conjugated antibodies (goat antimouse IgG) at dilution 1:4,000. Incubation was carried out for 1 h at room temperature, and proteins were visualized using chemiluminescence detection system. The density of the proteins was quantified using GS-800 Calibrated Densitometer with Quantity One software v. 4.2.2 (BioRad Laboratories, Hercules, CA, USA).

Statistical analysis

Results are expressed as the mean ± SD. *F* test and ANOVA followed by Tukey–Kramer multiple-comparison test (statistical software ncss 2001; NCSS Raysville, UT, USA) were used for analysis of the data. Differences were considered significant at $P < 0.05$.

Results

Table 1 indicates that endotoxin treatment inhibited the protein synthesis in EDL by about 31%; while changes in SOL were insignificant. HMB had no effect on protein synthesis in both muscle types of healthy and septic animals.

Sepsis enhanced markedly the total and myofibrillar protein breakdown in both types of muscles, more susceptible to the effect of endotoxin was EDL. HMB administration to septic rats reduced significantly the total proteolysis in EDL (by about 24%) and myofibrillar proteolysis in SOL (by about 38%). The treatment of healthy rats with HMB had an insignificant effect on the protein breakdown; however, we observed some tendency of decreased myofibrillar proteolysis (by about 13% in SOL and 26% in EDL).

The endotoxin treatment stimulated the leucine oxidation in both muscle types (44% rise of oxidation in SOL and 173% in EDL). The HMB administration reduced the leucine oxidation in septic animals to the values comparable with the control group; however, it did not affect significantly this parameter in intact animals (a tendency of reduced leucin oxidation observed only in SOL by about 27%).

Table 1 Parameters of protein and leucine metabolism in skeletal muscle

	Muscle	Intact (n=10)	HMB (n=10)	Septic (n=9)	Septic + HMB (n=9)
Muscle wet weight	EDL	19±1	19±2	16±4	17±3
(mg)	SOL	18±1	18±2	16±4	18±2
Protein synthesis	EDL	1,321±268	1,570±224	911±293[a]	860±111[a]
(nmol Leu/g protein/h)	SOL	1,522±330	1,625±281	1,296±404	1,348±187
Total proteolysis	EDL	133±16	150±13	297±98[a]	225±26[a,b]
(nmol Tyr/g wet weight/h)	SOL	274±62	278±62	364±74[a]	319±66
Myofibrillar proteolysis	EDL	0.905±0.220	0.667±0.071	2,894±0.788[a]	2,529±0.788[a]
(nmol 3-MH/g wet weight/h)	SOL	1,645±0.294	1,432±0.290	4,144±1.768[a]	2,571±0.339[b]
Leucine oxidation	EDL	46±12	48±21	127±74[a]	56±21[b]
(nmol Leu/g wet weight/h)	SOL	91±22	66±15	130±40[a]	79±13[b]

Values are presented as Mean ± SD

HMB β-hydroxy-β-methylbutyrate (0.5 g/kg/day), *EDL* m. extensor digitorum longus, *SOL* m. soleus, *Leu* leucine, *Tyr* tyrosine, *3-MH* 3-methylhistidine

[a] Significantly different from Intact at $P<0.05$

[b] Significantly different from Septic at $P<0.05$ (ANOVA + Tukey–Kramer multiple-comparison test)

Table 2 shows that the predominant proteolytic activity of proteasome, CTLA, increased in both muscle types of septic animals (by about 78% in SOL and 233% in EDL). HMB administration to septic animals reduced the CTLA in SOL significantly, while in EDL, the effect of HMB was on the borders of statistical significance ($P\leq0.056$). The HMB treatment of healthy animals did not influence the CTLA.

Changes in expression of α subunits of 20S proteasome were not detected in either EDL or in SOL groups (see Fig. 2 for a representative example of a blot).

We have not found significant changes in proteolytic activity of cathepsin B and L in tibialis muscle in any groups.

Discussion

Our results indicate that in our model of sepsis, the protein catabolic state in the skeletal muscle is

Table 2 Activity of proteolytic enzymes in skeletal muscle

	Muscle	Intact (n=10)	HMB (n=10)	Septic (n=9)	Septic + HMB (n=9)
Chymotrypsin-like activity of proteasome	EDL	1,830±441	1,929±432	6,103±2,777[a]	3,615±1,440[a]
(nmol AMC/mg protein/h)	SOL	1,924±569	1,392±268	3,426±559[a]	2,685±591[a,b]
Expression of α subunits of 20S proteasome	EDL	1,037±121	1,046±138	1,047±121	1,041±121
(density of blots in AU)	SOL	879±100	843±129	879±117	873±103
Activity of cathepsin B and L (nmol AMC/mg protein/h)	TIB	84±23	85±25	109±26	109±62

Values are presented as Mean ± SD

HMB β-hydroxy-β-methylbutyrate (0.5 g/kg/day), *EDL* m. extensor digitorum longus, *SOL* m. soleus, *TIB* m. tibialis, *AMC* 7-amino-4-methylcoumarin, *AU* arbitrary units

[a] Significantly different from Intact at $P<0.05$

[b] Significantly different from Septic at $P<0.05$ (ANOVA + Tukey–Kramer multiple-comparison test)

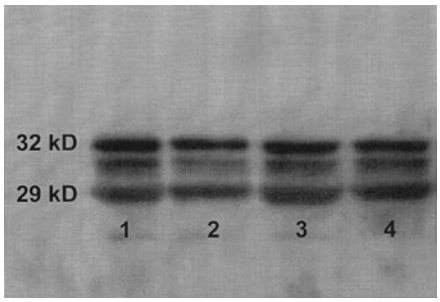

Fig. 2 Expression of α subunits of 20S proteasome in m. soleus—example of a blot. The α subunits of 20S proteasome are detected as two bands at approximately 29 and 32 kD. Lanes: *1* Intact; *2* Intact + HMB; *3* Septic; *4* Septic + HMB; *HMB* β-hydroxy-β-methylbutyrate (0.5 g/kg/day)

induced both by attenuation of protein synthesis (in EDL only) and by the activation of protein breakdown. An increase both in total and myofibrillar proteolysis was observed. The finding that the endotoxin treatment-activated degradation of muscle proteins is in agreement with an increase in leucine oxidation rate, which is the characteristic finding in protein wasting illnesses [16, 42]. The effect of endotoxin was more expressed in EDL (muscle composed mostly of white, fast-twitch fibers) than in SOL (muscle composed mostly of red, slow-twitch fibers). In EDL, more pronounced activation of protein breakdown, leucine oxidation, and inhibition of protein synthesis was observed. Higher sensitivity of white fibers composed muscles was described also in other models of inflammation, e.g., after turpentine oil administration [26] or caecal ligation and puncture [34, 39].

Determining the activity of main proteolytic systems, the ubiquitin-proteasome and lysosomal, proved that protein catabolic state in the skeletal muscle induced by endotoxin treatment is mediated by the activation of the proteasome system. The increase of the CTLA, the predominant proteolytic activity of proteasome, was observed in both muscle types. As the changes in the activity of proteasome were not accompanied by the changes in expression of its α subunits, a predictor of proteasome expression as a complex, we suggest that these are due to alteration of proteasome proteolytic activity rather than a variation in the proteasome expression. This is in agreement with observations that changes in proteolytic activity and transcription of mRNA for various parts of the ubiquitin-proteasome proteolytic system do not necessarily have to correspond with changes in expression on protein level [15]. Our findings correspond with a common opinion that proteasome system plays the key role in activation of intracellular proteolysis in various catabolic conditions like sepsis [11], cancer [18], burn injury [2], chronic kidney disease [31], or denervation atrophy [41].

The treatment of healthy rats with HMB had no significant effect on protein metabolism in the skeletal muscle. In contrast to healthy animals, the HMB administration to septic rats was effective and counteracted the changes in protein metabolism induced by sepsis. This is in agreement with positive effects of HMB on protein metabolism described in other protein catabolic conditions [29, 37]. Our findings indicate that HMB treatment of septic rats suppressed protein degradation enhanced by endotoxin administration without affecting the protein synthesis. Total proteolysis was decreased in EDL and the myofibrillar proteolysis in SOL of septic rats. In agreement with the reduction of myofibrillar proteolysis (estimated as 3-methylhistidine release), we observed the inhibition of proteasome activity in SOL muscle. Attenuation of proteasome activity without effect on lysosomal activity indicate that HMB affects protein breakdown mainly due to inhibition of activated proteasome system, as also observed in gastrocnemius muscle of mice bearing the MAC16 tumor [37]. We did not study the precise mechanism of HMB-induced attenuation of proteasome activity and thus protein breakdown. Other works suggest that HMB could suppress some signaling pathways leading to activation of proteasome activity, e.g., reduction of double-stranded RNA-dependent protein kinase [9] and NF-κB [36] activation. NF-κB regulates the expression of proteasome subunits and ubiquitin ligase MuRF-1 (muscle RING finger protein 1), which is often overexpressed in many proteocatabolic states. In addition, MuRF-1 acts as ubiquitin ligase for myosin heavy chain and, therefore, is involved in myofibrillar proteolysis [4].

Although the HMB treatment of septic rats caused more intense attenuation of most parameters of protein breakdown in EDL (total proteolysis, CTLA, and leucine oxidation), more significant decrease in myofibrillar proteolysis was observed in SOL. With regard to the fact that myofibrillar proteins are particularly sensitive to degradation induced by sepsis [14], the effect of the HMB treatment on septic muscles would be more

beneficial in the muscles containing higher amount of red, slow-twitch fibers than those containing higher amount of white, fast-twitch fibers. Considering that red fibers are the major component of respiratory muscles [30], which are of vital importance in serious diseases, this finding may be of a practical impact. However, this hypothesis should be verified by further studies, because conclusions based on studies on laboratory animals could differ from clinical investigations.

In conclusion, our data demonstrate that short-term HMB treatment is able to reverse changes in protein metabolism in rat skeletal muscle induced by sepsis; however, it has no significant effect on intact muscles. The HMB decreases the rate of protein breakdown, which is caused by attenuation of proteolytic activity of proteasome, while the lysosomal activity is not affected. The HMB effect on myofibrillar proteolysis in sepsis is more effective in muscles composed from slow-twitch fibers. Further investigation is needed to prove whether the use of HMB in septic conditions is beneficial in the whole-body context and in a long-term use.

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