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β-Hydroxy-β-methylbutyrate (HMB) prevents dexamethasone-induced myotube atrophy

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ABSTRACT

High levels of glucocorticoids result in muscle wasting and weakness. β-hydroxy-β-methylbutyrate (HMB) attenuates the loss of muscle mass in various catabolic conditions but the influence of HMB on glucocorticoid-induced muscle atrophy is not known. We tested the hypothesis that HMB prevents dexamethasone-induced atrophy in cultured myotubes. Treatment of cultured L6 myotubes with dexamethasone resulted in increased protein degradation and expression of atrogin-1 and MuRF1, decreased protein synthesis and reduced myotube size. All of these effects of dexamethasone were attenuated by HMB. Additional experiments provided evidence that the inhibitory effects of HMB on dexamethasone-induced increase in protein degradation and decrease in protein synthesis were regulated by p38/MAPK- and PI3K/Akt-dependent cell signaling, respectively. The present results suggest that glucocorticoid-induced muscle wasting can be prevented by HMB.

1. Introduction

High levels of glucocorticoids caused by cortisol producing adrenal tumors (Cushing's syndrome) or by treatment with steroids for inflammatory conditions, such as asthma and rheumatoid arthritis, are associated with muscle wasting and weakness [1–4]. The important role of glucocorticoids in the regulation of muscle mass is further illustrated by the fact that various catabolic conditions, including sepsis [5–7] and burn injury [8], cause muscle wasting at least in part through glucocorticoid-dependent mechanisms. Treatment of cultured muscle cells in vitro with dexamethasone results in increased protein degradation and expression of the ubiquitin ligases atrogin-1 and MuRF1 [9], metabolic changes that resemble the situation in skeletal muscle in multiple catabolic conditions [10]. In addition, treatment of cultured muscle cells with dexamethasone results in inhibited protein synthesis, further aggravating the catabolic effects of glucocorticoids [9,11]. Because of these effects of glucocorticoids, treatments that prevent glucocorticoid-induced muscle wasting have important clinical implications.

Previous studies suggest that β-hydroxy-β-methylbutyrate (HMB), a metabolite of the branched-chain amino acid leucine, may attenuate the loss of muscle mass caused by cancer cachexia [12–14], AIDS [15], endotoxemia [16,17], and aging [18] and that this effect of HMB reflects inhibited protein degradation and/or stimulated protein synthesis. Other reports suggest that the MAP kinase and PI3K/Akt signaling pathways are involved in the beneficial effects of HMB in skeletal muscle [17,19–21]. In contrast, the influence of HMB on glucocorticoid-regulated muscle wasting and the potential role of MAP kinase and PI3K/Akt signaling in this effect of HMB are not known. In addition, the influence of HMB on the expression of atrogin-1 and MuRF1 in glucocorticoid-induced muscle atrophy (or any other type of muscle wasting) has not been reported.

Here, we tested the hypothesis that HMB reduces protein degradation and stimulates protein synthesis in dexamethasone-treated myotubes through MAP kinase- and PI3K/Akt-dependent mechanisms. In addition, we examined the effect of HMB on the expression of atrogin-1 and MuRF1 in myotubes treated with dexamethasone.

2. Materials and methods

2.1. Cell culture

L6 muscle cells, a rat skeletal muscle cell line (American Type Culture Collection, Manassas, VA), were maintained and cultured as described in detail recently [9,22,23]. Differentiated myotubes were treated for 24 h with 1 μM dexamethasone (Sigma Aldrich,

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St. Louis, MO), 50 μM HMB (Alpha Aesar, Ward Hill, MA), or both drugs in combination. The concentrations of dexamethasone and HMB used here were based on previous studies [9,19,20,22–25]. Control myotubes were treated with the corresponding volume of vehicle (0.1% ethanol). In some experiments, myotubes were treated with 50 μM of the p38/MAPK inhibitor SB202190 (Sigma Aldrich), 50 μM of the Erk 1/2 inhibitor PD98059 (Calbiochem, EMD Chemicals Inc., San Diego, CA), 25 μM of the PI3K/Akt inhibitor LY29004 (Calbiochem, EMD Chemicals Inc.), or 100 nM of the mTOR inhibitor rapamycin (Calbiochem, EMD Chemicals Inc.). The inhibitors were added to the culture medium 1 h before the addition of dexamethasone and HMB.

2.2. Preparation of cell lysates

Cell lysates were prepared by harvesting the myotubes in buffer (50 mM Tris–HCl, 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, and 1% Nonidet P-40) containing Protease Inhibitor Cocktail Tablets (Roche Applied Science, Indianapolis, IN). The myotubes were sonicated using a Sonic Dismembrator (Fisher Scientific, Model 100) followed by centrifugation at 14,000 × g for 10 min at 4 °C. Protein concentration was determined by using the Bradford Protein Assay Reagent Kit with bovine serum albumin as standard. Cell lysates were stored at −80 °C until analyzed.

2.3. Western blotting

Western blotting was performed as described in detail recently [9,22,23] using the following primary antibodies: a rabbit polyclonal anti-mouse atrogin-1 antibody (kindly supplied by Dr. Stewart Lecker, Harvard Medical School); a rabbit polyclonal anti-mouse MuRF1 antibody (kindly supplied by Regeneron Pharmaceuticals, NY). A mouse monoclonal anti-rat α-tubulin antibody (Sigma Aldrich) was used for loading control. Immunoreactive protein bands were detected by using the Western Lighting kit for enhanced chemiluminescence detection (Perkin-Elmer Inc, Waltham, MA) and analyzed using the public domain Image J program (http://rsb.info.nih.gov/ij/index.html). The bands were quantified by densitometry and normalized to loading controls.

2.4. Real-time PCR

mRNA levels for atrogin-1 and MuRF1 were determined by real-time PCR as described in detail recently [9,22,23]. The sequences of the forward, reverse, and double-labeled oligonucleotides for atrogin-1 and MuRF1 used here were described recently [9,22,23].

2.5. Protein degradation and synthesis

Protein degradation rates were determined by measuring the release of trichloroacetic acid-soluble radioactivity during 24 h from proteins prelabeled with [3H]-tyrosine as described in detail previously [9,22,23]. Protein degradation rates were expressed as %/24 h. Protein synthesis rates were determined by measuring the incorporation of [3H]-tyrosine into cellular proteins as

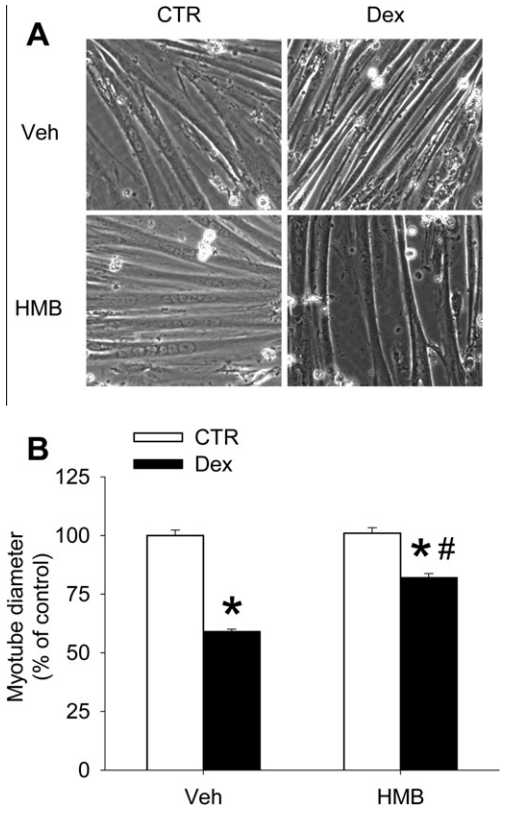

Fig. 1. HMB attenuates dexamethasone-induced atrophy of cultured myotubes. L6 myotubes were treated for 24 h with 1 μM dexamethasone in the absence or presence of 50 μM HMB. (A) Myotube morphology and (B) diameter were determined as described in Section 2. Results in (B) are means ± SEM with $n = 6$ per group. $^{*}p < 0.05$ vs corresponding control group; $^{\#}p < 0.05$ vs Vehicle/Dex by ANOVA.

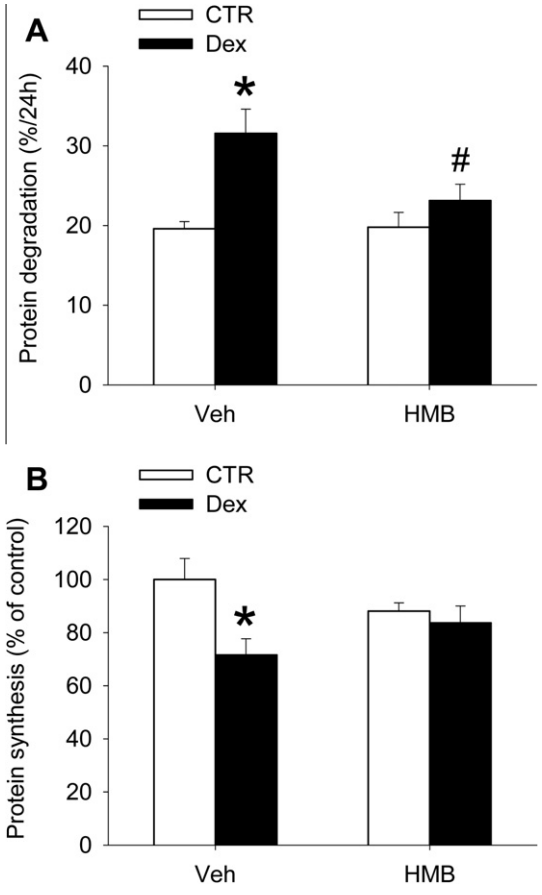

Fig. 2. HMB prevents dexamethasone-induced increase in protein degradation and decrease in protein synthesis in cultured myotubes. L6 myotubes were treated with dexamethasone in the absence or presence of HMB as described in Fig. 1. (A) Protein degradation and (B) protein synthesis rates were measured as described in Section 2. Results are means ± SEM with $n = 6$ per group. $^{*}p < 0.05$ vs control; $^{\#}p < 0.05$ vs Vehicle/Dex by ANOVA.

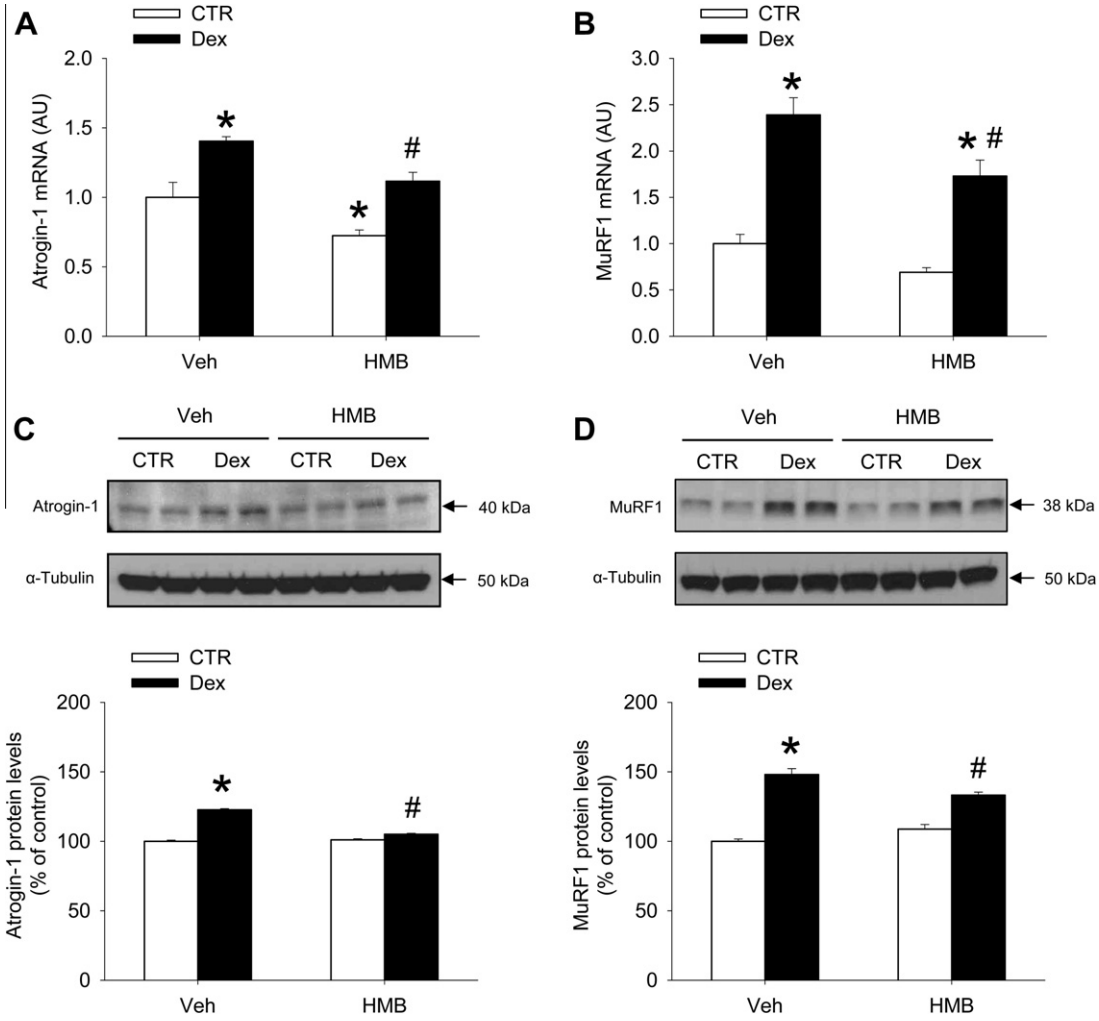

Fig. 3. HMB attenuates dexamethasone-induced expression of atrogin-1 and MuRF1 in cultured myotubes. L6 myotubes were treated with dexamethasone in the absence or presence of HMB as described in Fig. 1. mRNA levels for (A) atrogin-1 and (B) MuRF1 and protein levels for (C) atrogin-1 and (D) MuRF1 were determined as described in Section 2. Representative Western blots are shown in the upper panels and quantifications of the Western blots are shown in the lower panels of C and D. α-tubulin levels were determined for loading control. Results are means ± SEM with $n = 6$ per group. $^{*}p < 0.05$ vs control; $^{\#}p < 0.05$ vs Vehicle/Dex by ANOVA.

described previously [9,22,23,26] and were expressed as per cent of control.

2.6. Myotube diameter

For measurement of myotube diameter, myotube cultures were photographed under a phase contrast microscope at 100X magnification. The diameters were measured as described in detail previously [22,23] and with the investigator being unaware of from which experimental group the myotubes originated. Results were expressed as per cent of control.

2.7. Statistics

Results are reported as means ± SEM. Statistical analysis was performed by using one-way ANOVA followed by Tukey's post hoc test. $p < 0.05$ was considered statistically significant.

3. Results

In previous reports, treatment of cultured muscle cells with dexamethasone resulted in myotube atrophy [9,22,23]. A similar response was seen in the present study with an approximately 40% reduction of myotube diameter during dexamethasone treatment (Fig. 1A and B). The dexamethasone-induced myotube atrophy was significantly attenuated, although not completely prevented, by HMB.

Myotube atrophy may reflect increased protein degradation and/or reduced protein synthesis. Previous reports suggest that HMB protects skeletal muscle by inhibiting protein degradation and by stimulating protein synthesis during muscle wasting caused by cancer, endotoxin, TNFα, or angiotensin II [13,17,19,20,24,25]. In contrast, the influence of HMB on glucocorticoid-induced changes in muscle protein turnover rates is not known. Here, we found that treatment of myotubes with HMB prevented both the dexamethasone-induced increase in protein degradation and the dexamethasone-induced inhibition of protein synthesis (Fig. 2A and B). Together, the results in Fig. 1 and 2 suggest that HMB can prevent glucocorticoid-induced muscle atrophy by inhibiting protein degradation and stimulating protein synthesis.

In multiple previous reports, the expression of the muscle atrophy-related ubiquitin ligases atrogin-1 and MuRF1 was upregulated in catabolic muscle [10,27,28]. Here, we found that both mRNA and protein levels of atrogin-1 and MuRF1 were increased in dexamethasone-treated myotubes (Fig. 3). Importantly, the

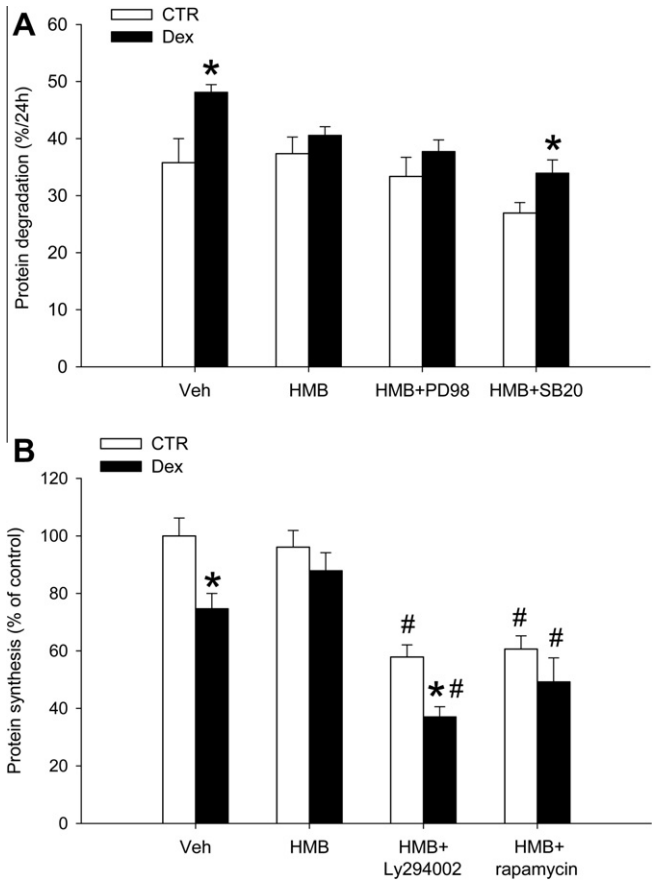

Fig. 4. HMB prevents dexamethasone-induced increase in protein degradation and dexamethasone-induced decrease in protein synthesis through p38/MAPK- and PI3K/Akt-dependent mechanisms, respectively. Cultured L6 myotubes were treated with dexamethasone in the absence or presence of HMB as described in Fig. 1. In some of the experiments, myotubes were exposed to PD98059 (PD98), SB202190 (SB20), LY294002, or rapamycin as described in Section 2. (A) Protein degradation and (B) synthesis rates were determined as described in Section 2. Results are means ± SEM with $n = 6$ per group. $^{*}p < 0.05$ vs control; $^{\#}p < 0.05$ vs corresponding vehicle group by ANOVA.

dexamethasone-induced increase in atrogin-1 and MuRF1 expression was attenuated by HMB (Fig. 3). Interestingly, HMB reduced basal atrogin-1 (but not MuRF1) mRNA levels but did not influence the corresponding protein levels, suggesting that HMB may regulate atrogin-1 protein levels by both transcriptional and post-transcriptional mechanisms.

In a recent study, inhibition of protein degradation by HMB was suggested to reflect a p38/MAPK-dependent mechanism [17]. We tested whether a similar mechanism may be involved in the protective effects of HMB in dexamethasone-treated myotubes. The inhibition of dexamethasone-induced protein degradation by HMB was abolished by the p38/MAPK-specific inhibitor SB202190 (Fig. 4A), suggesting that the protective effect of HMB was p38/MAPK-dependent. In contrast, the protective effect of HMB on protein degradation in dexamethasone-treated myotubes was maintained in the presence of the p42/44 MAPK inhibitor PD98 059, suggesting that the effects of HMB on protein degradation in dexamethasone-treated myotubes was not related to p42/44 MAPK signaling.

Other studies suggest that PI3K/Akt and/or mTOR signaling may be involved in the HMB-mediated stimulation of protein synthesis in catabolic muscle [20,21]. Here, we tested the involvement of these pathways in HMB-induced stimulation of protein synthesis by treating the myotubes with the PI3K/Akt inhibitor LY29004 or the mTOR inhibitor rapamycin. As expected, both drugs reduced basal protein synthesis rates. Importantly, the prevention by HMB of the dexamethasone-induced decrease in protein synthesis was abolished by LY29004 but not by rapamycin (Fig. 4B). Taken together, the results in Fig. 4 suggest that HMB prevents dexamethasone-induced increase in protein degradation and dexamethasone-induced inhibition of protein synthesis through p38/MAPK- and PI3K/Akt-dependent mechanisms, respectively.

4. Discussion

The present study provides evidence that HMB can prevent glucocorticoid-induced muscle atrophy. The observation is important from a clinical standpoint because high glucocorticoid levels, as seen for example in patients with Cushing's syndrome and in patients treated with corticosteroids for asthma and rheumatoid arthritis, are associated with muscle wasting and weakness [1–4]. In addition, loss of muscle mass in different catabolic conditions, including sepsis and burn injury, is mediated by glucocorticoids [5–8]. Thus, the present results suggest that multiple patient groups suffering from glucocorticoid-dependent muscle wasting may benefit from treatment with HMB.

Although HMB may protect skeletal muscle from the catabolic effects of cancer cachexia [12–14], AIDS [15], aging [18], muscular dystrophy [29], endotoxemia [16,17], and glucocorticoids (present study), the protective effects of HMB are not universal. For example, in a controlled clinical trial, HMB (in combination with glutamine and arginine) did not prevent muscle wasting in patients with rheumatoid arthritis [30]. Therefore, it is important to specifically test the influence of HMB on muscle protein balance during muscle wasting caused by different conditions and mechanisms.

In several studies, HMB attenuated muscle wasting by inhibiting protein breakdown and stimulating protein synthesis [13,17,19,20,24,25]. In endotoxemic rats, however, only the increase in protein degradation was improved by HMB whereas the reduction of protein synthesis was not influenced [16]. In light of the present study and previous reports suggesting that sepsis- and endotoxemia-induced muscle wasting is at least in part mediated by glucocorticoids [5–7], the results reported in endotoxemic rats [16] may seem surprising. It is possible, however, that the role of glucocorticoids in the regulation of muscle protein synthesis during endotoxemia is less prominent than their role in the regulation of protein degradation. Furthermore, other mechanisms (not sensitive to HMB) may regulate muscle protein synthesis during endotoxemia.

Previous observations [17,19–21] and the present study suggest that the p38/MAPK and PI3K/Akt signaling pathways are involved in the beneficial effects of HMB in skeletal muscle. In addition to these mechanisms, multiple other mechanisms have been proposed to be involved in the protective effects of HMB in skeletal muscle, including activation of RNA-dependent protein kinase [17,19,20], inhibition of NF–kB activity [23], stimulation of muscle cell proliferation and differentiation [21], inhibition of ubiquitin–proteasome-dependent protein degradation [13], inhibition of caspase 3 and 8 [17,19], prevention of p38-dependent production of oxygen radicals [19], and stabilization of the sarcolemma [31,32]. It will be important in future studies to determine if any of these mechanisms is involved in the protective effects of HMB in glucocorticoid-induced muscle wasting.

A limitation of the present study is the fact that experiments were performed in vitro in dexamethasone-treated myotubes and it is not known if treatment with HMB in vivo can prevent glucocorticoid-induced muscle atrophy. Previous reports suggest, however, that dexamethasone-induced changes in protein turnover rates are similar in cultured muscle cells and in vivo [5–7,9,26] and that HMB can exert beneficial effects both in cultured muscle

cells and in skeletal muscle in vivo [12–14,19,20,24,25]. It is therefore reasonable to assume that the present results are predictive of a protective effect of HMB on glucocorticoid-induced muscle atrophy in vivo.

Acknowledgments

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References

[1] T.B. Carroll, J.W. Findling, The diagnosis of Cushing's syndrome, Rev. Endocr. Metab. Disord. 11 (2010) 147–153.
[2] M.A. Minetto, F. Lanfranco, A. Botter, G. Motta, G. Mengozzi, R. Giordano, A. Picu, E. Ghigo, E. Arvat, Do muscle fiber conduction slowing and decreased levels of circulating muscle proteins represent sensitive markers of steroid myopathy? A pilot study in Cushing's disease, Eur. J. Endocrinol. 164 (2011) 985–993.
[3] S.L. Bowyer, M.P. La Mothe, J.R. Hollister, Steroid myopathy: incidence and detection in a population with asthma, J. Allergy Clin. Immunol. 76 (1985) 234–242.
[4] J.P. Seale, M.R. Compton, Side-effects of corticosteroid agents, Med. J. Aust. 144 (1986) 139–142.
[5] G. Tiao, J. Fagan, V. Roegner, M. Lieberman, J.J. Wang, J.E. Fischer, P.O. Hasselgren, Energy-ubiquitin-dependent muscle proteolysis during sepsis in rats is regulated by glucocorticoids, J. Clin. Invest. 97 (1996) 339–348.
[6] P.O. Hasselgren, N. Alamdari, Z. Aversa, P. Gonnella, I.J. Smith, S. Tizio, Corticosteroids and muscle wasting – role of transcription factors, nuclear cofactors, and hyperacetylation, Curr. Opin. Clin. Nutr. Metab. Care 13 (2010) 423–428.
[7] O. Schakman, H. Gilson, J.P. Thiessen, Mechanisms of glucocorticoid-induced myopathy, J. Endocrinol. 197 (2008) 1–10.
[8] C.H. Fang, J.H. James, C. Ogle, J.E. Fischer, P.O. Hasselgren, Influence of burn injury on protein metabolism in different types of skeletal muscle and the role of glucocorticoids, J. Am. Coll. Surg. 180 (1995) 33–42.
[9] M. Menconi, P. Gonnella, V. Petkova, S. Lecker, P.O. Hasselgren, Dexamethasone and corticosterone induce similar, but not identical, muscle wasting responses in cultured L6 and C2C12 myotubes, J. Cell. Biochem. 105 (2008) 353–364.
[10] Z. Aversa, N. Alamdari, P.O. Hasselgren, Molecules modulating gene transcription during muscle wasting in cancer, sepsis, and other critical illness, Crit. Rev. Clin. Lab. Sci. 48 (2011) 71–86.
[11] M.M. Desler, S.J. Jones, C.W. Smith, T.C. Woods, Effects of dexamethasone and anabolic agents on proliferation and protein synthesis and degradation in C2C12 myogenic cells, J. Anim. Sci. 74 (1996) 1265–1273.
[12] P.E. May, A. Barber, J.T. D'Olimpio, A. Hourichane, N.N. Abumrad, Reversal of cancer-related wasting using oral supplementation with a combination of β-hydroxy-β-methylbutyrate, arginine, and glutamine, Am. J. Surg. 183 (2002) 471–479.
[13] H.J. Smith, P. Mukeji, M.J. Tisdale, Attenuation of proteasome-induced proteolysis in skeletal muscle by β-hydroxy-β-methylbutyrate in cancer-induced muscle loss, Cancer Res. 65 (2005) 277–283.
[14] Z. Aversa, A. Bonetto, P. Costelli, V.G. Minero, F. Penna, F.M. Baccino, S. Lucia, F. Rossi Fanelli, M. Muscaritoli, β -hydroxy-β-methylbutyrate (HMB) attenuates muscle and body weight loss in experimental cancer cachexia, Int. J. Oncol. 38 (2011) 713–720.
[15] R.H. Clark, G. Feleke, M. Din, T. Yasmin, G. Singh, F.A. Khan, J.A. Rathmacher, Nutritional treatment for acquired immunodeficiency virus-associated wasting using β-hydroxy-β-methylbutyrate, glutamine, and arginine: a randomized, double-blind, placebo-controlled study, J. Parenter. Enteral Nutr. 24 (2000) 133–139.
[16] M. Kovarik, T. Muthny, L. Sispera, M. Holecek, Effects of β-hydroxy-β-methylbutyrate treatment in different types of skeletal muscle of intact and septic rats, J. Physiol. Biochem. 66 (2010) 311–319.
[17] S.T. Russell, M.J. Tisdale, Mechanism of attenuation by β-hydroxy-β-methylbutyrate of muscle protein degradation induced by lipopolysaccharide, Mol. Cell. Biochem. 330 (2009) 171–179.
[18] M.D. Vukovich, N.B. Stubbs, R.M. Bohicken, Body composition in 70 year old adults responds to dietary β-hydroxy-β-methylbutyrate similarly to that of young adults, J. Nutr. 131 (2001) 2049–2052.
[19] H.L. Eley, S.T. Russell, M.J. Tisdale, Mechanism of attenuation of muscle protein degradation induced by tumor necrosis factor-α and angiotensin II by β-hydroxy-β-methylbutyrate, Am. J. Physiol. Endocrinol. Metab. 295 (2008) E1417–E1426.
[20] H.L. Eley, S.T. Russell, M.J. Tisdale, Attenuation of depression of muscle protein synthesis induced by lipopolysaccharide, tumor necrosis factor, and angiotensin II by β-hydroxy-β-methylbutyrate, Am. J. Physiol. Endocrinol. Metab. 295 (2008) E1409–E1416.
[21] R. Kornasio, I. Riederer, G. Butler-Browne, V. Mouly, Z. Uni, O. Halevy, β -hydroxy-β-methylbutyrate (HMB) stimulates myogenic cell proliferation, differentiation, and survival via the MAPK/ERK and PI3K/Akt pathways, Biochim. Biophys. Acta 1793 (2009) 755–763.
[22] P. Gonnella, N. Alamdari, S. Tizio, Z. Aversa, V. Petkova, P.O. Hasselgren, C/EBPβ regulates dexamethasone-induced muscle cell atrophy and expression of atrogin-1 and MuRF1, J. Cell. Biochem. 112 (2011) 1737–1748.
[23] N. Alamdari, Z. Aversa, E. Castillero, A. Gurav, V. Petkova, S. Tizio, P.O. Hasselgren, Resveratrol prevents dexamethasone-induced expression of the muscle atrophy-related ubiquitin ligases atrogin-1 and MuRF1 in cultured myotubes through a SIRT1-dependent mechanism, Biochem. Biophys. Res. Commun. 417 (2012) 528–533.
[24] H.J. Smith, S.M. Wyke, M.J. Tisdale, Mechanism of the attenuation of proteolysis-inducing factor stimulated protein degradation in muscle by β-hydroxy-β-methylbutyrate, Cancer Res. 64 (2004) 8731–8735.
[25] H.L. Eley, S.T. Russell, J.H. Baxter, P. Mukerji, M.J. Tisdale, Signaling pathways initiated by β-hydroxy-β-methylbutyrate to attenuate the depression of protein synthesis in skeletal muscle in response to catabolic stimuli, Am. J. Physiol. Endocrinol. Metab. 293 (2007) E923–E931.
[26] L. Wang, G.J. Luo, J.J. Wang, P.O. Hasselgren, Dexamethasone stimulates proteasome- and calcium-dependent proteolysis in cultured L6 myotubes, Shock 10 (1998) 298–306.
[27] M.D. Gomes, S.H. Lecker, R.T. Jagoe, A. Navon, A.L. Goldberg, Atrogin-1, a muscle specific F-box protein highly expressed during muscle atrophy, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 14440–14445.
[28] S.C. Bodine, E. Latres, S. Baumheuter, V.K. Lai, L. Nunez, B.A. Clarke, W.T. Poueymirou, F.J. Panaro, E. Na, K. Dharmarajan, Z.Q. Pan, D.M. Valenzuel, T.M. DeChiara, T.N. Stitt, G.D. Yancopoulos, D.J. Glass, Identification of ubiquitin ligases required for muscle atrophy, Science 294 (2001) 1704–1708.
[29] E.T. Payne, N. Yasuda, J.M. Bourgeois, M.C. Devries, M.C. Rodriguez, J. Yousuf, M.A. Tarnopolsky, Nutritional therapy improves function and complements corticosteroid intervention in mdx mice, Muscle Nerve 33 (2006) 66–77.
[30] S. Marcora, A. Lemmey, P. Maddison, Dietary treatment of rheumatoid cachexia with β-hydroxy-β-methylbutyrate, glutamine, and arginine: a randomized controlled trial, Clin. Nutr. 24 (2005) 442–454.
[31] S. Nissen, R. Sharp, M. May, J.A. Rathmacher, D. Rice, J.C. Fuller, A.S. Connelly, N. Abumrad, Effect of leucine metabolite β-hydroxy-β-methylbutyrate on muscle metabolism during resistance-exercise training, J. Appl. Physiol. 81 (1996) 2095–2104.
[32] S. Nissen, R.L. Sharp, L. Panton, M. Vukovich, S. Trappe, J.C. Fuller, β-hydroxy-β-methylbutyrate (HMB) supplementation in humans is safe and may decrease cardiovascular risk factors, J. Nutr. 130 (2000) 1937–1945.