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ß-Hydroxy-ß-methylbutyrate facilitates PI3K/Akt-dependent mammalian target of rapamycin and FoxO1/3a phosphorylations and alleviates tumor necrosis factor α/interferon γ–induced MuRF-1 expression in C2C12 cells

Kaoru Kimura[a], *Xian Wu Cheng*[a,*], *Aiko Inoue*[a], *Lina Hu*[a], *Teruhiko Koike*[b], *Masafumi Kuzuya*[a,*]

[a] *Department of Community Healthcare & Geriatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[b] *Department of Sports Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan*

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ABSTRACT

β-Hydroxy-β-methylbutyrate (HMB) prevents deleterious muscle responses under pathological conditions, including tumor- and chronic steroid therapy–related muscle losses. Here, we investigated the hypothesis that HMB may modulate the balance between protein synthesis and degradation in the PI3K/Akt-mediated mammalian target of rapamycin (mTOR) and FoxO1/FoxO3a-dependent mechanisms in differentiated C2C12 muscle cells. We also tested the effect of HMB on the expression of MuRF-1 and atrogin-1 in response to the inflammatory stress. β-Hydroxy-β-methylbutyrate up-regulated phosphorylation of Akt and mTOR, and these effects were completely abolished in the presence of PI3K inhibitor LY294002. β-Hydroxy-β-methylbutyrate also up-regulated FoxO1 and FoxO3a phosphorylation, and these changes were inhibited by LY294002. Although, unexpectedly, HMB failed to reduce the expressions of atrophy-related atrogin-1 messenger RNA and the protein response to the proinflammatory cytokines tumor necrosis factor α plus interferon γ, HMB did attenuate the MuRF-1 expression. Thus, HMB appears to restore the balance between intracellular protein synthesis and proteolysis, likely via activation of the PI3K/Akt-dependent mTOR and FoxO1/FoxO3a signaling pathway and the reduction of tumor necrosis factor α/interferon γ–induced MuRF-1 expression, thereby ameliorating aging-related muscle atrophy.

1. Introduction

Muscle atrophy is characterized by a decrease of protein synthesis and an increase of protein degradation and is a debilitating phenomenon found in a variety of catabolic conditions such as aging, pyaemia, diabetes, and starvation [1–4]. Accumulating evidence suggests that the phosphatidylinositol 3-kinase (PI3K)/Akt-dependent mammalian target of rapamycin (mTOR) signaling pathway plays an important role in protein synthesis [5,6]. Akt activation has also been shown

Abbreviations: FoxO, forkhead box class O; HMB, β-Hydroxy-β-methylbutyrate; IFN-γ, interferon γ; mTOR, mammalian target of rapamycin; MuRF-1, muscle RING-finger protein-1; PI3K, phosphatidylinositol 3-kinase; TNF-α, tumor necrosis factor-α.

* *Corresponding authors at:* Department of Community Healthcare & Geriatrics, Nagoya University Graduate School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya 466-8550, Japan. Tel.: +81 52 744 2364; fax: +81 52 744 2371.
E-mail addresses: xianwu@med.nagoya-u.ac.jp (X.W. Cheng), kuzuya@med.nagoya-u.ac.jp (M. Kuzuya).

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to result in the phosphorylation of FoxO transcription factors, leading to the exclusion of phosphorylated FoxO proteins from the nucleus and suppression of their transcriptional functions [7]. Previous studies have reported that branched-chain amino acids, especially leucine, provoke mTOR phosphorylation, which, in turn, enhances eukaryotic initiation factor 4E-binding protein 1 and ribosomal protein S6 kinase, a key molecule in protein synthesis, and ultimately increases protein synthesis [8]. On the basis of these findings and past reports that FoxO family members are implicated in proteasome-related intracellular protein ubiquitination by the regulation of target gene expressions in response to stress [6,9,10], we hypothesize that the administration of β-hydroxy-β-methylbutyrate (HMB), a major metabolite of leucine, restores the balance between intracellular protein synthesis and metabolism in the muscle system through activation of the PI3K/Akt-dependent mTOR and FoxO1/FoxO3a signaling transduction.

Recent studies indicate that the ubiquitin-proteasome–mediated proteolysis pathway is a specific pathway for selective degradation of muscle intracellular proteins under all of those catabolic conditions [6,11,12]. It is well known that atrogin-1 and muscle RING-finger protein-1 (MuRF-1) are 2 key muscle-specific F-box type E3 ligases in protein ubiquitination [6]. Atrogin-1 has been shown to be highly expressed during muscle atrophy [13,14]. Atrogin-1 and MuRF-1 have been identified as likely targets of drug therapy for muscle atrophy [15]. Furthermore, genetic deletion of atrogin-1 ($MaFbx^{-/-}$) or MuRF-1 ($MuRF\text{-}1^{-/-}$) was shown to prevent muscle atrophy in mice [13]. In addition, proinflammatory cytokines such as tumor necrosis factor α (TNF-α) and interferon γ (IFN-γ) have been shown to play a critical role in muscle atrophy [11,12]. Previous studies reported that catabolic status with muscle loss increased circulating levels of TNF-α and IFN-γ [16,17]. Administration of TNF-α and IFN-γ has been shown to accelerate muscle loss [18,19]. Moreover, muscle atrophy has also been accelerated in TNF-α transgenic animals and animals bearing TNF-α–producing tumors [18,20,21]. Recent evidence highlighted the activation of inflammation-related proteasome-mediated protein ubiquitination in various muscle mass losses [12]. Previous studies tested the impact of HMB supplementation on aging-, tumor- and steroid therapy–related myotube atrophy [22–24]; however, the mechanisms by which HMB administration alleviates the loss of aging-related muscle mass remain largely undefined.

Here, we tested the hypothesis that HMB may modulate the balance between protein synthesis and degradation in the PI3K/Akt-mediated mTOR and FoxO1/FoxO3a-dependent mechanisms in differentiated C2C12 muscle cells. In addition, we examined the effect of HMB on the expression of MuRF-1 and atrogin-1 in response to the inflammatory stress.

2. Methods and materials

2.1. Cell culture

C2C12 mouse myoblasts obtained from the American Type Culture Collection (Manassas, VA, USA) were grown in Dulbecco modified Eagle medium (DMEM; GIBCO Life Technologies, Grand Island, NY, USA) containing 10% (vol/vol) fetal bovine serum and antibiotics at 37°C with 5% CO_2. At confluence, myoblasts were induced to fuse by changing the medium to medium containing 2% horse serum (GIBCO, Life Technologies, Auckland, New Zealand; hereinafter the *differentiation medium*), as described previously by Herningtyas et al [8]. After 4 to 5 days of differentiation, when the myoblasts had lengthened, fused, and become multinucleated myotubes, the differentiated C2C12 cells were cultured with serum-free DMEM for 12 hours, and then were treated with 50 μM HMB or the other indicated agents in serum-free basal medium. For the specific inhibitor experiments, after pretreatment with the PI3K inhibitor LY294002 (Calbiochem, Merk Bioscience, Darmstadt, Germany) at 25 μmol/L for 30 minutes, the cells were cultured in serum-free medium in the presence or absence of 50 μM HMB for the indicated time points. In addition, following culture in serum-free medium for 12 hours, differentiated C2C12 cells were also induced with TNF-α (20 ng/mL) plus IFN-γ (400 U/mL) in the presence or absence of HMB at the indicated concentrations.

2.2. Quantitation of gene expression

Total RNA was extracted from the cell extracts using an RNeasy Mini Kit (QIAGEN, Venlo, the Netherlands) according to the manufacturer's instructions. Messenger RNA (mRNA) was reverse transcribed to complementary DNA with a PrimeScript RT reagent Kit (TaKaRa, Tokyo, Japan) as described previously by Cheng et al [25]. Quantitative gene expression was studied by using an ABI 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with SYBRTM Green dye (Applied Biosystems), as previously described by Cheng et al [26]. All experiments were performed in triplicate. Targeted gene transcriptions were normalized to the corresponding control mRNA (18s). Specifically, the primer and probe sequences were 5′-ATGCACACTGGTGCAGAGAG-3′ (forward) and 5′-TGTAAGCACACAGGCAGGTC-3′ (reverse) for mouse atrogin-1, and 5′-TGAGGTGCCTACTTGCTCCT-3′ (forward) and 5′-TCACCTGGTGGCTATTCTCC-3′ (reverse) for mouse MuRF-1.

2.3. Western blotting

The protein concentration for each sample was determined using a protein assay system according to the manufacturer's instructions (Bio-Rad Dc; Bio-Rad Laboratories, Hercules, CA, USA). Equal amounts of total protein were separated on 10% sodium dodecyl sulfate–polyacrylamide gel and transferred to polyvinylidene difluoride membranes, and then blocked for 60 minutes with 5% skim milk, as previously described by Cheng et al [27]. After reaction with primary antibodies against Akt, phospho-Akt Ser473 (p-Akt), mTOR, phospho-mTOR (p-mTOR), FoxO1, phospho-FoxO1 Ser256 (p-FoxO1), FoxO3a, phospho-FoxO3a Ser294 (p-FoxO3a) (all from Cell Signaling, Beverly, MA, USA), atrogin-1, and MuRF-1 (Novus Biologicals, LLC, Littleton, CO, USA), the membranes were treated with peroxidase-linked antirabbit IgG (Cell Signaling), antimouse IgG (Amersham Biosciences, Buckinghamshire, UK), or rabbit antigoat IgG (Invitrogen, Frederick, MD, USA), respectively. Protein bands were developed by the chemiluminescence

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method (ECL Prime; Amersham) and detected using an LAS3000 digital imaging system (LAS3000 Imager; Fujifilm, Tokyo, Japan).

2.4. Statistical analyses

All data included in this article were considered to be normally distributed and are presented as means ± SD, unless indicated otherwise. One-way analysis of variance (ANOVA) followed by the post hoc test was used for the statistical analysis. A P value less than .05 was considered to indicate statistical significance.

3. Results

3.1. HMB-induced phosphorylation of PI3K-dependent Akt and mTOR in C2C12 myotubes

After differentiated C2C12 cells were cultured in serum-free DMEM for 12 hours, the cells were treated with 50 μM of HMB at various time points (0, 10, 30, and 60 minutes and 24 hours) for the Western blotting assay of targeted protein phosphorylations. β-Hydroxy-β-methylbutyrate treatment resulted in a marked increase in Akt phosphorylation in the extracts of the cultured C2C12 cells (Fig. 1). Changes in cellular Akt phosphorylation appeared as early as 10 minutes and reached a 3-fold peak at 30 minutes, but had fallen by 60 minutes after the intervention. PI3K inhibition by LY294002 completely abolished this HMB-induced phosphorylation at the indicated time points (Figs. 1 and 2A). In the same way, HMB also upregulated the phosphorylation of mTOR, a downstream molecule of Akt, and LY294002 blocked it with or without HMB induction (Fig. 2B). We also observed that LY294002 had effects on the levels of both p-Akt and p-mTOR at baseline, and there was no difference in p-Akt or p-mTOR between the treated and nontreated C2C12 cells.

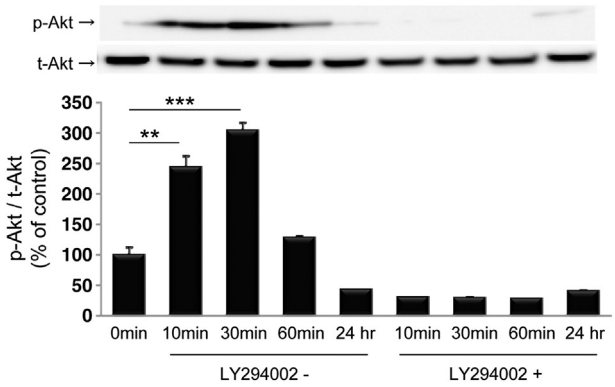

Fig. 1 – Time course of HMB-induced Akt phosphorylation in cultured C2C12 cells. After differentiation for 5 days, the C2C12 cells were cultured with serum-free basal medium for 12 hours. Then, the cells were treated with 50 μM HMB in the presence or absence of a PI3K inhibitor and were collected 10, 30, and 60 minutes and 24 hours later for the analyses of Akt phosphorylation at Ser473 by Western blotting. The Western blotting results are shown using representative images, and the ratio of the p-Akt to the t-Akt levels at various time points is shown using combined quantitative data. Results are means ± SD (n = 4 for each group). *P < 0.05, **P < 0.01, and ***P < 0.001 by ANOVA and Tukey post hoc tests.

3.2. HMB stimulated FoxO1 and FoxO3a phosphorylation via the PI3K/Akt signaling pathway

The FoxO transcription factor has been shown to be a key regulator for protein degradation [7]. To determine whether HMB affects FoxO transcription factor phosphorylation, cells were stimulated with HMB at 50 μM for 30 minutes and were evaluated for the levels of p-FoxO1 and p-FoxO3a in the extracts. Phosphorylation of both FoxO1 and FoxO3a was induced by treatment with HMB (Fig. 3). We used an inhibitor of PI3K to explore the cross-talk between FoxO transcription factor phosphorylation and the PI3K/Akt signaling pathway. As anticipated, LY294002 significantly impaired both phosphorylations induced by HMB (Fig. 3), indicating that the HMB-mediated FoxO1 and FoxO3a phosphorylations may be due to PI3K/Akt signaling pathway activation. In addition, LY294002 affected the levels of both p-FoxO1 and p-FoxO3a at baseline. There was also no difference in the p-FoxO1 or p-FoxO3a levels between treated and nontreated C2C12 cells.

3.3. Effects of HMB on atrogin-1 and MuRF-1 expressions

To further explore the possible mechanism of the beneficial effects mediated by HMB, we evaluated the effects of HMB on the expressions of ubiquitin ligases such as atrogin-1 and MuRF-1 in C2C12 cells. Quantitative data showed that HMB had no effects on the expression of atrogin-1 and MuRF-1 at either the gene level or the protein level (Figs. 4 and 5). PI3K inhibition enhanced the levels of both the genes and proteins, and these effects were not influenced by treatment with HMB at 50 μM for 12 hours.

3.4. HMB attenuated the MuRF-1 protein expression induced by TNF-α/IFN-γ

To investigate whether HMB affected the atrogin-1 and MuRF-1 expression induced by proinflammatory cytokines, C2C12 cells were treated with the combination of TNF-α and IFN-γ. As expected, quantitative data revealed increased levels of atrogin-1 and MuRF-1 proteins after 12 hours of treatment (Fig. 6). β-Hydroxy-β-methylbutyrate had a significant inhibitory effect on the increased expression of MuRF-1 protein in response to TNF-α/IFN-γ, but not on the increased atrogin-1 protein expression.

4. Discussion

Several important observations can be made on the basis of these results. First, the administration of HMB to the differentiated C2C12 muscle cells promoted phosphorylations of FoxO1 and FoxO3a, a subgroup of the forkhead family of transcription factors, and mTOR, concomitant with an increase in PI3K/Akt signaling activation in the protein synthetic

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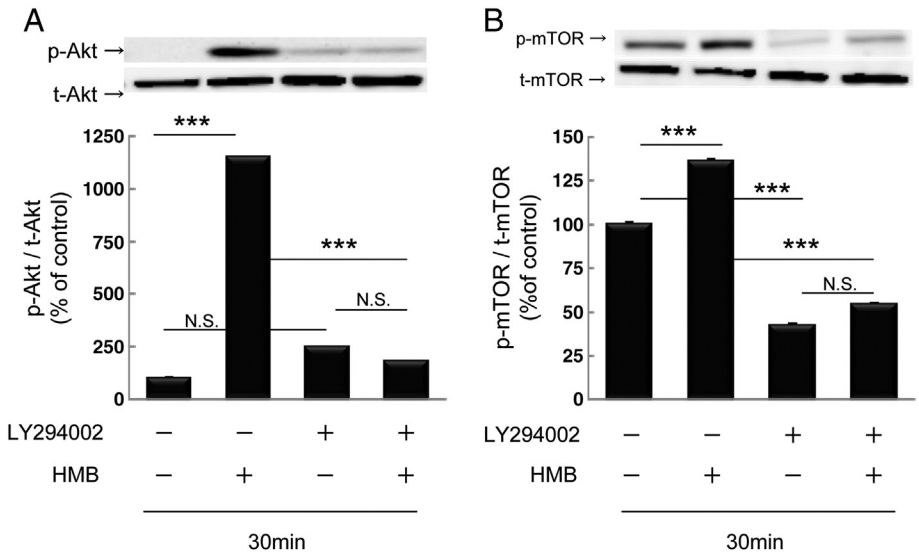

Fig. 2 – Effect of HMB on the Akt (A) and mTOR (B) phosphorylations in cultured C2C12 cells. After culturing in serum-free basal medium for 12 hours, the cells were incubated in the presence of 50 μM HMB or LY294002 or both. LY294002 was added 30 minutes prior to the HMB addition. The lysates were collected 30 minutes after the addition of HMB for Western blotting analysis. The Western blotting results are shown using representative images, and the ratios of the p-Akt to the t-Akt and the p-mTOR to the t-mTOR levels are shown using combined quantitative data. Results are means ± SD (n = 4 for each group). *P < 0.05, **P < 0.01, and ***P < 0.001 by ANOVA and Tukey post hoc tests.

process. Second, HMB suppressed the protein ubiquitination-related MuRF-1 ligase expression induced by inflammatory cytokines (TNF-α and IFN-γ) in differentiated C2C12 myotubes. Thus, the present findings suggest that HMB supplementation may be beneficial for aging-related muscle wasting and muscle atrophy through amelioration of the imbalance between protein synthesis and ubiquitin-proteasome–mediated proteolysis.

It is well established that the PI3K/Akt signaling pathway is involved in translation initiation and protein synthesis in skeletal muscle [5,6]. Activation of Akt promotes smooth muscle hypertrophy through an enhancement of mTOR

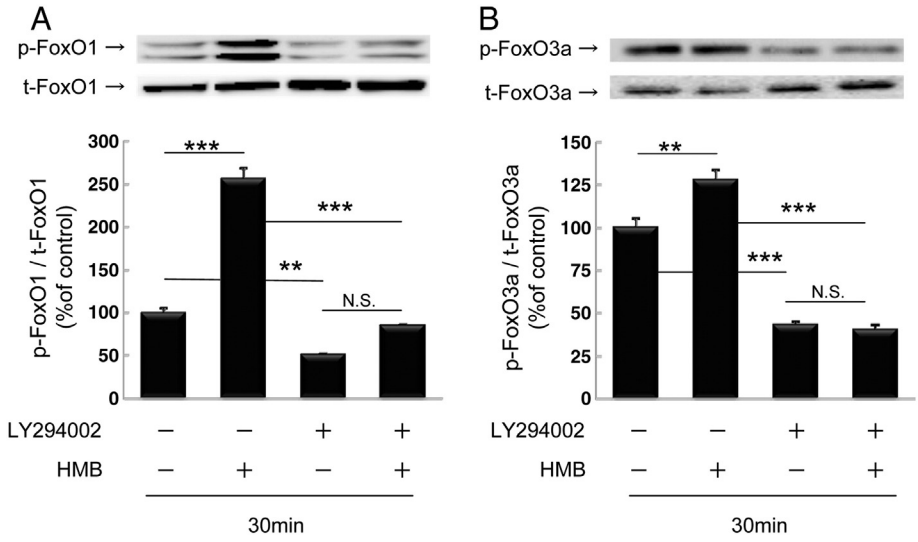

Fig. 3 – Effect of HMB on the FoxO1 (A) and FoxO3a (B) phosphorylations in cultured C2C12 cells. C2C12 cells were incubated in the presence of 50 μM HMB or LY294002 or both. LY294002 was added 30 minutes prior to addition of HMB. The lysates were collected 30 minutes after the addition of HMB for Western blotting analysis. The Western blotting results are shown using representative images, and the ratios of the p-FoxO1 to the t-FoxO1 and the p-FoxO3a to the t-FoxO3a levels are shown using combined quantitative data. Results are means ± SD of 4 experiments. *P < 0.05, **P < 0.01, and ***P < 0.001 by ANOVA and Tukey post hoc tests.

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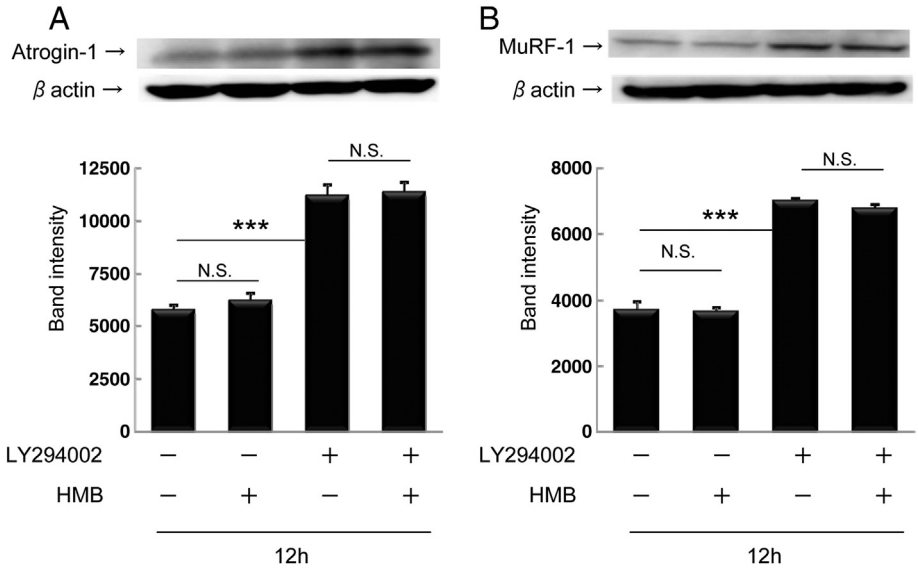

Fig. 4 – The effects of HMB on atrogin-1 (A) and MuRF-1 (B) protein expressions in cultured C2C12 cells. C2C12 cells were incubated in the presence of 50 μM HMB or LY294002 or both. LY294002 was added 30 minutes prior to addition of HMB. Samples were collected at 12 hours after addition of HMB for Western blotting analysis. The Western blotting results are shown using representative images, and the levels of the atrogin-1 and MuRF-1 protein are shown using combined quantitative data. Results are means ± SD of 4 experiments.*P < 0.05, **P < 0.01, and ***P < 0.001 by ANOVA and Tukey post hoc tests.

signaling [6]. We have shown that HMB enhanced the phosphorylation of Akt and mTOR and that these effects were significantly abolished by PI3K inhibition with LY294002. A previous study demonstrated that branched-chain amino acids induced Akt/mTOR signaling pathway activation in the C2C12 cell line [8]. Aversa et al [22] reported that HMB preserved protein synthesis in a dexamethasone-induced myotube atrophy model via the mTOR pathway. Thus, HMB appears to promote protein synthesis in muscle through activation of the PI3K/Akt-mediated mTOR signaling pathway.

In addition, activation of the PI3K/Akt pathway also results in phosphorylation of the FoxO proteins [28]. FoxO family members have been implicated in the regulation of target genes involved in metabolism, apoptosis, and cell cycle progression [6,9,10,28]. As early as 1999, Brunet et al [29] clearly demonstrated that Akt phosphorylates FoxO transcription factors at multiple sites, leading to the exclusion of phosphorylated FoxO proteins from the nucleus and the suppression of their transcriptional functions. Akt-dependent regulation of the FoxO protein has been shown to play a critical role in ubiquitin-proteasome–related proteolysis [30]. Our observations here show that HMB induced phosphorylation of both FoxO1 and FoxO3a and that these effects were suppressed by PI3K inhibition. Collectively, these results indicate that the

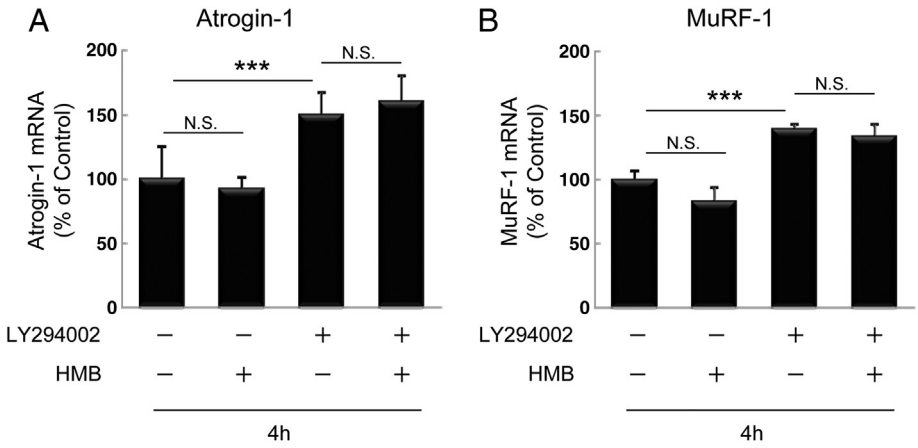

Fig. 5 – The effects of HMB on the expressions of atrogin-1 (A) and MuRF-1 (B) mRNA in cultured C2C12 cells. C2C12 cells were treated with 50 μM HMB for 4 hours with or without LY294002, which was added 30 minutes prior to the HMB addition. Total cellular RNA was analyzed by quantitative real-time reverse transcriptase polymerase chain reaction. Data are expressed as a percentage of the corresponding control mRNA (18s) levels. Results are means ± SD of 3 experiments performed in duplicate. *P < 0.05, **P < 0.01, and ***P < 0.001 by ANOVA and Tukey post hoc tests.

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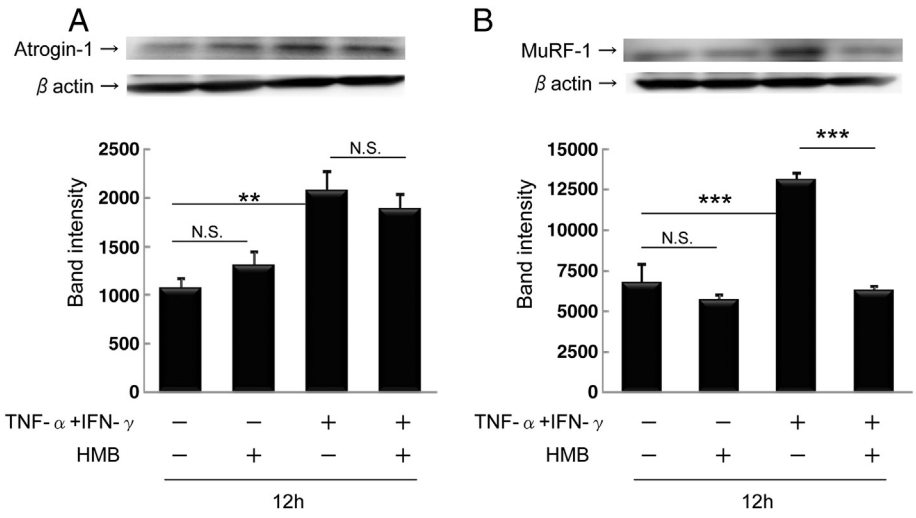

Fig. 6 – The effects of HMB on the TNF-α/IFN-γ–induced expressions of atrogin-1 (A) and MuRF-1 protein (B) in cultured C2C12 cells. C2C12 cells were incubated in the presence of 50 μM HMB or TNF-α/IFN-γ, or both. β-Hydroxy-β-methylbutyrate was added 30 minutes prior to the TNF-α/IFN-γ addition. The lysates were collected at 12 hours after the addition of HMB for Western blotting analysis. The Western blotting results are shown using representative images, and the levels of the atrogin-1 and MuRF-1 protein are shown using combined quantitative data. Results are means ± SD of 4 experiments. *P < 0.05, **P < 0.01, and ***P < 0.001 by ANOVA and Tukey post hoc tests.

beneficial effects of HMB may include not only the activation of mTOR and the regulation of PI3K/Akt-dependent FoxO1/FoxO3a phosphorylations, but also an antiproteolysis effect that could prevent muscle atrophy. However, it should be noted that HMB failed to inhibit the expression of FoxO downstream molecules such as the MuRF-1 and atrogin-1 gene and protein regardless of PI3K inhibition. This observation makes it unlikely that the HMB-mediated protection of the myotubes was due to attenuations of MuRF-1 and atrogin-1 expression-induced PI3K/Akt-dependent FoxO1/FoxO3a phosphorylation under our experimental conditions. Further studies will be needed to investigate this issue.

Interventions with TNF-α and IFN-γ have been shown to accelerate the loss of muscle mass [18,19]. Animals with TNF-α overexpression exhibit severe cardiac muscle atrophy [20]. In vitro experiments have shown that TNF-α and IFN-γ induced early activation of atrogin-1 gene expression in C2C12 myotubes [12]. We have observed that C2C12 myotubes treated with a mixture of TNF-α and IFN-γ resulted in increased MuRF-1 protein expression, and this change was improved by HMB treatment. Recently, Aversa and colleagues reported that HMB prevented dexamethasone-induced myotube atrophy via a reduction of atrogin-1 and MuRF-1 expression [22]. Thus, the attenuation of MuRF-1 expression by HMB could represent a common mechanism in the protection of muscle tissue against inflammatory stress. However, it should be pointed out that, in our study, HMB failed to reverse the expression of atrogin-1 caused by TNF-α/IFN-γ. It has been reported that dexamethasone-induced MuRF-1 and atrogin-1 expressions were targeted by HMB treatment [22]. This discrepancy might be due to many factors, especially the differences in cell species (C2C12 for the former, L6 for the latter) and stimulating factors (inflammatory factor for the former, steroid hormone for the latter). Further studies will also be needed to explore this issue.

Several limitations of the present work should be pointed out. First, this study was not designed to examine whether there is a transporter or receptor for HMB on the plasma membrane to regulate intracellular signaling pathway activation in muscle cells. Second, we also presented no evidence regarding whether HMB influences intracellular protein synthesis and proteolysis in vivo and ex vivo. Further studies will be needed to investigate these issues as well.

The results of the present study may have clinical relevance from 2 perspectives. First and most directly, our findings have implications in terms of the molecular mechanism underlying the ability of HMB supplementation to prevent muscle atrophy in humans [24]. The ability of HMB to regulate PI3K/Akt-dependent mTOR and FoxO1/FoxO3a signaling pathway activation in the protein synthesis of C2C12 myotubes suggests that it may be effective in countering the loss of muscle mass. Second, when considered together with other research regarding antitumor growth and antisteroid therapy [22,23], the present study has implications for our general understanding of HMB-mediated anti-inflammatory effects on muscle wasting and weakness. Our results using the present cell inflammation model show that the inhibitory effect of HMB on MuRF-1 expression may be responsible for the prevention of muscle loss due to inflammatory stress. It would be of great interest to examine in prospective clinical studies whether HMB supplementation protects skeletal muscle from the catabolic effects of cancer cachexia, acquired immune deficiency syndrome, aging, muscular dystrophy, endotoxemia, and glucocorticoids.

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REFERENCES

[1] Suetta C, Frandsen U, Jensen L, Jensen MM, Jespersen JG, Hvid LG, et al. Aging affects the transcriptional regulation of human skeletal muscle disuse atrophy. PLoS One 2012;7:e51238.

[2] Voisin L, Breuille D, Combaret L, Pouyet C, Taillandier D, Aurousseau E, et al. Muscle wasting in a rat model of long-lasting sepsis results from the activation of lysosomal, Ca2+ –activated, and ubiquitin-proteasome proteolytic pathways. J Clin Invest 1996;97:1610–7.

[3] Medina R, Wing SS, Haas A, Goldberg AL. Activation of the ubiquitin-ATP–dependent proteolytic system in skeletal muscle during fasting and denervation atrophy. Biomed Biochim Acta 1991;50:347–56.

[4] Lecker SH, Solomon V, Mitch WE, Goldberg AL. Muscle protein breakdown and the critical role of the ubiquitin-proteasome pathway in normal and disease states. J Nutr 1999;129:227S–37S.

[5] Sharp ZD, Bartke A. Evidence for down-regulation of phosphoinositide 3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR)-dependent translation regulatory signaling pathways in Ames dwarf mice. J Gerontol A Biol Sci Med Sci 2005;60:293–300.

[6] Goodman CA, Mayhew DL, Hornberger TA. Recent progress toward understanding the molecular mechanisms that regulate skeletal muscle mass. Cell Signal 2011;23:1896–906.

[7] Daitoku H, Fukamizu A. FOXO transcription factors in the regulatory networks of longevity. J Biochem 2007;141:769–74.

[8] Herningtyas EH, Okimura Y, Handayaningsih AE, Yamamoto D, Maki T, Iida K, et al. Branched-chain amino acids and arginine suppress MaFbx/atrogin-1 mRNA expression via mTOR pathway in C2C12 cell line. Biochim Biophys Acta 2008;1780:1115–20.

[9] Carlsson P, Mahlapuu M. Forkhead transcription factors: key players in development and metabolism. Dev Biol 2002;250:1–23.

[10] Birkenkamp KU, Coffer PJ. Regulation of cell survival and proliferation by the FOXO (Forkhead box, class O) subfamily of Forkhead transcription factors. Biochem Soc Trans 2003;31:292–7.

[11] Sheriff S, Kadeer N, Joshi R, Friend LA, James JH, Balasubramaniam A. Des-acyl ghrelin exhibits pro-anabolic and anti-catabolic effects on C2C12 myotubes exposed to cytokines and reduces burn-induced muscle proteolysis in rats. Mol Cell Endocrinol 2012;351:286–95.

[12] Li YP, Chen Y, John J, Moylan J, Jin B, Mann L, et al. TNF-alpha acts via p38 MAPK to stimulate expression of the ubiquitin ligase atrogin1/MAFbx in skeletal muscle. FASEB J 2005;19:362–70.

[13] Bodine SC, Latres E, Baumhueter S, Lai VK, Nunez L, Clarke BA, et al. Identification of ubiquitin ligases required for skeletal muscle atrophy. Science 2001;294:1704–8.

[14] Gomes MD, Lecker SH, Jagoe RT, Navon A, Goldberg AL. Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. Proc Natl Acad Sci U S A 2001;98:14440–5.

[15] Foletta VC, White LJ, Larsen AE, Leger B, Russell AP. The role and regulation of MAFbx/atrogin-1 and MuRF1 in skeletal muscle atrophy. Pflugers Arch 2011;461:325–35.

[16] Argiles JM, Lopez-Soriano J, Busquets S, Lopez-Soriano FJ. Journey from cachexia to obesity by TNF. FASEB J 1997;11:743–51.

[17] Esper DH, Harb WA. The cancer cachexia syndrome: a review of metabolic and clinical manifestations. Nutr Clin Pract 2005;20:369–76.

[18] Langstein HN, Doherty GM, Fraker DL, Buresh CM, Norton JA. The roles of gamma-interferon and tumor necrosis factor alpha in an experimental rat model of cancer cachexia. Cancer Res 1991;51:2302–6.

[19] Garcia-Martinez C, Lopez-Soriano FJ, Argiles JM. Acute treatment with tumour necrosis factor-alpha induces changes in protein metabolism in rat skeletal muscle. Mol Cell Biochem 1993;125:11–8.

[20] Kubota T, McTiernan CF, Frye CS, Slawson SE, Lemster BH, Koretsky AP, et al. Dilated cardiomyopathy in transgenic mice with cardiac-specific overexpression of tumor necrosis factor-alpha. Circ Res 1997;81:627–35.

[21] Tracey KJ, Morgello S, Koplin B, Fahey III TJ, Fox J, Aledo A, et al. Metabolic effects of cachectin/tumor necrosis factor are modified by site of production. Cachectin/tumor necrosis factor–secreting tumor in skeletal muscle induces chronic cachexia, while implantation in brain induces predominantly acute anorexia. J Clin Invest 1990;86:2014–24.

[22] Aversa Z, Alamdari N, Castillero E, Muscaritoli M, Rossi Fanelli F, Hasselgren PO. beta-Hydroxy-beta-methylbutyrate (HMB) prevents dexamethasone-induced myotube atrophy. Biochem Biophys Res Commun 2012;423:739–43.

[23] Nunes EA, Kuczera D, Brito GA, Bonatto SJ, Yamazaki RK, Tanhoffer RA, et al. Beta-hydroxy-beta-methylbutyrate supplementation reduces tumor growth and tumor cell proliferation ex vivo and prevents cachexia in Walker 256 tumor-bearing rats by modifying nuclear factor–kappaB expression. Nutr Res 2008;28:487–93.

[24] Flakoll P, Sharp R, Baier S, Levenhagen D, Carr C, Nissen S. Effect of beta-hydroxy-beta-methylbutyrate, arginine, and lysine supplementation on strength, functionality, body composition, and protein metabolism in elderly women. Nutrition 2004;20:445–51.

[25] Cheng XW, Kuzuya M, Nakamura K, Maeda K, Tsuzuki M, Kim W, et al. Mechanisms underlying the impairment of ischemia-induced neovascularization in matrix metalloproteinase 2–deficient mice. Circ Res 2007;100:904–13.

[26] Cheng XW, Song H, Sasaki T, Hu L, Inoue A, Bando YK, et al. Angiotensin type 1 receptor blocker reduces intimal neovascularization and plaque growth in apolipoprotein E–deficient mice. Hypertension 2011;57:981–9.

[27] Cheng XW, Kuzuya M, Kim W, Song H, Hu L, Inoue A, et al. Exercise training stimulates ischemia-induced neovascularization via phosphatidylinositol 3-kinase/Akt–dependent hypoxia-induced factor-1 alpha reactivation in mice of advanced age. Circulation 2010;122:707–16.

[28] Stitt TN, Drujan D, Clarke BA, Panaro F, Timofeyva Y, Kline WO, et al. The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy–induced ubiquitin ligases by inhibiting FOXO transcription factors. Mol Cell 2004;14:395–403.

[29] Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 1999;96:857–68.

[30] Bois PR, Grosveld GC. FKHR (FOXO1a) is required for myotube fusion of primary mouse myoblasts. EMBO J 2003;22:1147–57.

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