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c0023 HMB Supplementation: Clinical and Performance-Related Effects and Mechanisms of Action

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s0010 Introduction

p0010 β-Hydroxy-β-methylbutyrate (HMB) is a leucine derivative compound with anabolic and anticatabolic properties that may improve both clinical and performance outcomes. Clinically, HMB supplementation has been shown to decrease muscle proteolysis and spare muscle mass during catabolic states associated with muscle wasting syndromes (Smith et al., 2005). These results are clinically significant because preventing muscle loss during catabolic diseases also decreases rates of mortality and comorbidities in several conditions. Thus, besides being a nutrient-derived molecule, HMB may be considered a physio-pharmacological entity owing to its potency and specificity of action (Eley et al., 2007; Nunes et al., 2008). The mechanisms by which HMB prevents muscle loss during catabolic conditions seem to be associated with an inhibition of several proteolytic systems, including the ubiquitin–proteasome system and the lysosomal system (Smith et al., 2005; Lecker et al., 2006). Of the many proposed mechanisms leading to decreased muscle proteolysis, proteasome inhibition has been the most investigated. However, there is a certain degree of uncertainty related to which proteolytic mechanism is more inhibited by HMB, because each catabolic state leads to a specific proteolytic system activation (Dehoux et al., 2004; Latres et al., 2005). In addition, HMB may be converted to β-hydroxy-β-methylglutaryl-coenzyme A (HMG-CoA) (Rudney, 1957; Zabin and Bloch, 1951; Nissen et al., 2000), a substrate for cholesterol synthesis. Decreased cholesterol synthesis in muscle tissue can lead to an increased risk for myopathies as a result of enhanced muscle damage and loss. In this context, HMB

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may be useful to prevent muscle loss not only via proteolytic inhibition but also by enhancing the activity of cytosolic HMG-CoA reductase (Nissen et al., 2000; Pierno et al., 1995; Zabin and Bloch, 1951).

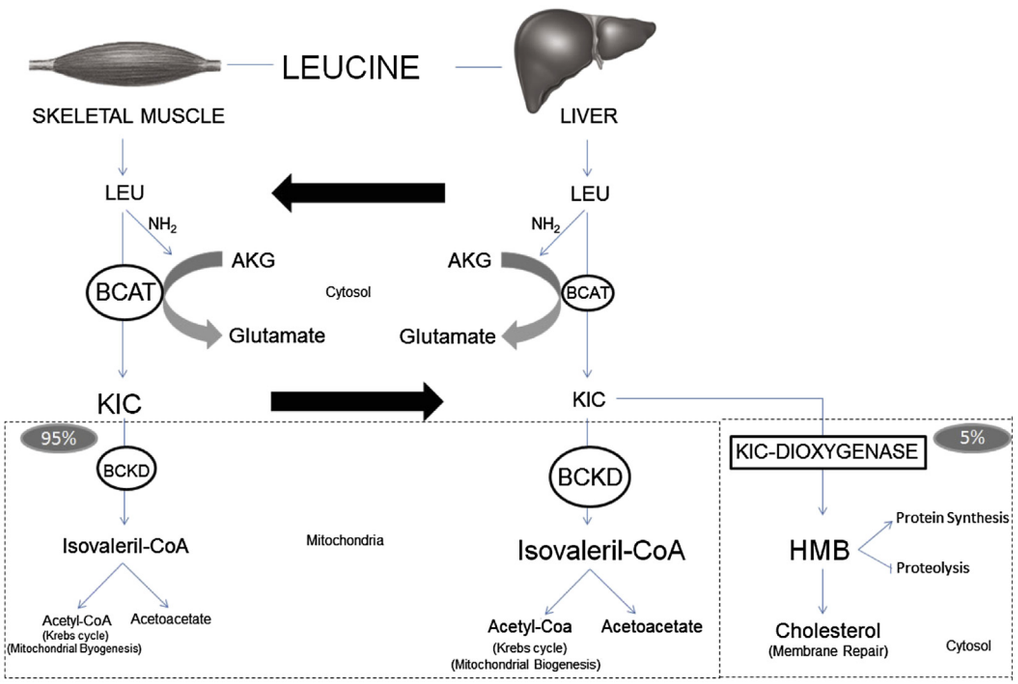
p0015 HMB may also improve muscle wasting conditions via increased skeletal muscle anabolism. HMB has been shown to activate the mammalian target of rapamycin (mTOR) pathway in skeletal muscle in vivo, and in vitro in rodents and humans. Collectively, this information strongly suggests that the anabolic actions of HMB are at least partially due to increased messenger RNA (mRNA) translation coding for myofibrillar proteins, because mTOR mechanisms of action on muscle hypertrophy seem to be strongly correlated to increased mRNA translational efficiency (Zanchi and Lancha, 2008; Proud, 2007a,b).

[**AU3**]

p0020 Of interest to athletes and coaches is the potential for HMB supplementation to act synergistically with mechanical loading to promote hypertrophy and strength. It is not clear, however, whether this effect results from mechanisms related solely to increased muscle protein synthesis or whether accelerated recovery and muscle regeneration owing to satellite cell activation after periods of intense muscle-damaging training are also contributors (Kormasio et al., 2009). There is some evidence to support both hypotheses, but research has indicated that high-intensity resistance exercise (potentially leading to muscle damage) is necessary to optimize gains in muscle mass induced by HMB (Wilson et al., 2014). In this chapter, HMB metabolism, kinetics, and mechanisms of action leading to clinical or athletic performance-related effects are discussed in detail.

s0015 Metabolism

p0025 HMB is a metabolite of the amino acid leucine (Van Koverin and Nissen, 1992a,b). After leucine ingestion, the first step in HMB synthesis is the reversible transamination of leucine to α-ketoisocaproate (KIC), which occurs mainly extrahepatically, through branched chain transaminase enzyme present in various tissues, including skeletal muscles (Block and Buse, 1990). After this enzymatic reaction, KIC is converted into HMB by the cytosolic enzyme KIC dioxygenase (Sabourin and Bieber, 1983) or isovaleryl-CoA, which is catalyzed predominantly by the hepatic mitochondrial branched-chain ketoacid dehydrogenase enzyme tissue (Sabourin and Bieber, 1981, 1983). Once formed, isovaleryl-CoA can generate the Krebs cycle intermediates acetyl-CoA and acetoacetate. Under normal conditions, 95% of KIC is converted into isovaleryl-CoA. On the other hand, approximately 5% of ingested leucine is metabolized into HMB (reviewed by Zanchi et al., 2011) (Fig. 23.1). Endogenous HMB production is about 0.2−0.4 g HMB per day, depending on the content of leucine in the diet. Because L-leucine is an essential amino acid not synthesized in humans, HMB production is a reflection of dietary protein intake. However, to exert ergogenic or clinical effects, a dose of 3 g/day HMB must be consumed (Wilson et al., 2008), which means that an individual would need to consume 60 g leucine, or approximately 750 g protein per day (assuming leucine makes up 8% of consumed protein sources).



f0010 **FIGURE 23.1** β-Hydroxy-β-methylbutyrate (HMB) metabolism. *AKG*, alpha ketoglutarate; *BCAT*, branched chain amino acid transferase; *BCKD*, branched-chain ketoacid dehydrogenase; *CoA*, coenzyme A; *KIC*, alpha-ketoisocaproate; *LEU*, leucine. *Adapted from Nissen, S.L., Abumrad, N.N., 1997. Nutritional role of the leucine metabolite b-hydroxy-b-methylbutyrate (HMB). J. Nutr. Biochem. 8, 300–311; Zanchi, N.E., Gerlinger-Romero, F., Guimarães-Ferreira, L., de Siqueira Filho, M.A., Felitti, V., Lira, F.S., Seelaender, M., Lancha, Jr., A.H., April 2011. HMB supplementation: clinical and athletic performance-related effects and mechanisms of action. Amino Acids 40 (4), 1015–1025; Zanchi, N.E., Filho, M.A., Felitti, V., Nicastro, H., Lorenzeti, F.M., Lancha, Jr., A.H., August 2010. Glucocorticoids: extensive physiological actions modulated through multiple mechanisms of gene regulation. J. Cell. Physiol. 224 (2), 311–315.*

s0020 Pharmacokinetics

p0030 As a dietary supplement, HMB has been commercially available either as a monohydrated calcium salt (HMB-Ca) or free acid. The magnitude and rate of appearance of HMB after ingestion depend on the dose, co-nutrient consumption, and form of HMB (i.e., calcium or free acid conjugated). Higher doses of HMB promote an increased rate of absorption and greater plasma concentrations, because 1 g of HMB-Ca was shown to result in a peak HMB level of 120 nmol/ml in the blood after 2 h, whereas 3 g resulted in peak HMB levels just after 60 minutes after ingestion, reaching 487 nmol/ml (Vukovich et al., 2001a,b). Glucose co-ingestion with HMB assessed with an insulinemic clamp demonstrated no major differences in insulin kinetics between glucose and HMB versus HMB supplementation alone (Vukovich et al., 2001a,b). However, a difference was detected in the interval required for HMB concentrations to peak, which was significantly longer when HMB was consumed with glucose.

[**AU4**]

p0035 Both the calcium form and free acid form (β-hydroxy-β-methylbutyric acid; HMB-FA) have been investigated. Commercially, HMB was first available in HMB-Ca as a powder; it was generally supplemented in capsule form. Currently, HMB−FA is available with a buffering gel (K_2CO_3) that raises the pH to 4.5 (Wilson et al., 2014). It was previously thought that because calcium dissociates relatively easily from HMB-Ca (10−15 minutes in the gut), there would be no difference in digestion kinetics between HMB-Ca and HMB-FA (Baxter et al., 2011). However, Fuller et al. (2011) observed that an HMB-FA supplement absorbed more quickly (peaked after 30 minutes) and had a shorter clearance time than HMB-Ca, which thus shortened the time needed between supplementation and exercise. In that study, 0.8 g HMB-FA compared with 1.0 g HMB-Ca (providing equivalent amounts of HMB) resulted in a doubling of peak plasma levels in one-fourth the time (30 versus 120 minutes). Moreover, area under the curve analysis of HMB concentrations over 180 minutes after ingestion was 91%−97% greater in the HMB-FA than the HMB-Ca form. Interestingly, even with greater peak plasma concentrations of HMB, urinary losses were not different between the two HMB forms and HMB-FA presented 25% greater clearance, indicating improved tissue uptake. This general information was confirmed in a subsequent study also performed by Fuller et al. (2015), which compared both forms (HMB-Ca versus HMB-FA) when administered in a gel form or dissolved in water. In contrast, Shreeram et al. (2014) observed the opposite results in rats, in which HMB-Ca demonstrated higher relative bioavailability compared with HMB-FA. The increased bioavailability of HMB-Ca may have resulted from decreased systemic clearance of HMB-Ca compared with HMB-FA; however, this discrepancy may also have been related to different intraspecies effects. To date, it is unclear whether HMB-FA is superior to HMB-Ca, because most studies were conducted using HMB-Ca. Therefore, more research is necessary to answer this question.

s0025 Clinically Related Effects

p0040 Loss of muscle mass and force output is an important predictor of morbidity and mortality in many catabolic conditions, including aging, cancer, and heart failure (Argilés et al., 2016). It has been estimated that in the general population, 10% loss of lean mass is associated with decreased immunity and increased risk of infection. A 20% loss of lean mass results in impaired wound healing, increased muscle weakness, and further risk of infection. Loss of lean mass on the order of 30%−40% results in locomotion dysfunction (loss of autonomy), pneumonia, pressure ulcers, and increased mortality (Argilés et al., 2016). This information suggests that the maintenance of muscle mass or the attenuation of muscle loss during catabolic states diminishes the risk of comorbidities and mortality. Loss of muscle mass associated with disease, disuse, or aging has been reported to account for 1.5% of annual total health care costs in the United States (estimated to be $45 billion in 2014), and a 10% reduction in the prevalence of sarcopenia has been estimated to reduce this figure by approximately 5%−6%

(Ian Janssen et al., 2004). With this concept in mind, the development of strategies aimed to reduce muscle catabolism are of high importance.

p0045 After reports that HMB supplementation increased markers of protein synthesis and reduced markers of proteolysis in healthy subjects, investigators began studying the effects of HMB supplementation in pathological conditions and experimental models characterized by high rates of muscle proteolysis. HMB treatment or supplementation has been shown to reduce muscle loss during several muscle-wasting disorders (ranging from cancer to immobilization) (Smith et al., 2005; Aversa et al., 2011). In cancer-inducing cachexia, for example, HMB supplementation has been shown to reduce the proliferation and size of tumor cells and diminish muscle proteolysis while increasing protein synthesis. Nunes et al. (2008) demonstrated that supplementation with 76 mg/kg body weight per day HMB in adult Walker 256 tumor-bearing Wistar rats for 8 weeks induced a lower tumor weight and lower tumor cell proliferation with suppression of the nuclear factor-κB signaling pathway. In addition, using the same animal model, Caperuto et al. (2007) reported that 320 mg/kg body weight per day of HMB supplementation for 4 weeks increased the survival time of tumor-bearing animals. Also, studies have demonstrated that HMB supplementation in cachexia produces a dampening effect on skeletal muscle degradation and enhances protein synthesis by activating multiple signaling pathways (Eley et al., 2007; Nunes et al., 2008; Caperuto et al., 2007). In an interesting study by Smith et al. (2005) in mice implanted with the MAC16 tumor, HMB supplementation was found to attenuate weight loss induced by cancer.

p0050 In human patients with HIV-related muscle wasting, 8 weeks of supplementation with a mixture of HMB, arginine, and glutamine (3 g HMB plus 14 g arginine plus 14 g glutamine) divided into two daily doses resulted in a significant increase in lean body mass and improved immune status (Clark et al., 2000). Employing the same mixture for a 24-week period, May et al. (2002) reported increases in lean mass in advanced (stage IV) cancer patients. Importantly, there was no negative effect of treatment on the incidence of adverse effects or quality of life measures. In contrast, Berk et al. (2008) reported a nonsignificant trend ($P = .08$) for increased lean mass after 8 weeks of HMB, arginine, and glutamine supplementation in patients with cancer cachexia. It is plausible that had the duration been extended, this trend might have reached statistical significance. Although HMB has not been tested as an individual ingredient in patients with cancer, these studies suggest that HMB may be an efficient anticatabolic compound. Although hypercortisolemia is present in these atrophy-related conditions, HMB has been shown not to reduce or rescue muscle losses owing to treatment with the potent synthetic glucocorticoid dexamethasone (Wilson et al., 2014). Whether increased lean mass as a result of HMB supplementation is related indirectly to increased immune function or directly to changes in the muscle proteolytic machinery is unknown. Both an increase in immune function and a reduction in proteolysis can potentially lead to a decreased number of comorbidities and possibly increased life span in this population; however, more investigations are necessary to assess the potential benefits of HMB fully on cancer cachexia, especially in humans.

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p0055 HMB may also attenuate and/or prevent muscle loss during periods of immobilization or in the development of sarcopenia. Using an immobilization model in rats, Soares et al. (2001) observed that HMB supplementation resulted in increased muscle fiber diameter (+6.9%) compared with the nonsupplemented group. In older mice, HMB treatment was shown to enhance recovery from injury in vitro, and a combination of HMB and β-alanine was shown to enhance force production in vitro (Vallejo et al., 2016). In humans, Deutz et al. (2013) demonstrated that HMB prevented a decline in muscle mass in elderly subjects after 10 days of bed rest. On the contrary, employing two different atrophic models (dexamethasone treatment and hind limb immobilization), Baptista et al. (2013) demonstrated that HMB supplementation was inefficacious in attenuating muscle loss during these catabolic conditions. Why HMB presents different specific anticatabolic effects is not known, but it seems that glucose intolerance induced by glucocorticoid excess is further impaired by co-administration of HMB in rats (Nunes et al., 2013). We observed similar results with glucocorticoid treatment and leucine supplementation, in that both treatments resulted in a robust atrophic state (Zanchi et al., 2012). It is well known that insulin resistance activates important proteolytic systems in the muscle cell, including the ubiquitin−proteasome system (Wang et al., 2006). However, in healthy individuals, and contrary to the potent insulin secretagogue effects of leucine, acute HMB supplementation does not increase plasma insulin (Wilkinson et al., 2013). Thus, the specific inefficacy of HMB in reverse glucocorticoid-induced muscle atrophy may be linked to the induction of insulin resistance. In favor of this hypothesis, a study performed by Noh et al. (2014) observed positive effects of HMB by reducing muscle degradation and preventing muscle (and protein concentration) decreases in soleus muscle. Discrepancies among the studies of Noh et al. (2014), Nunes et al. (2013), and Zanchi et al. (2012) may be linked to differences in the dexamethasone dose employed in each study: 5, 1, and 0.6 mg/kg, respectively. Interestingly, HMB also demonstrated direct antiatrophy effects in dexamethasone-treated myotubes (Aversa et al., 2012). This result suggests that antiatrophy effects of HMB can be reversed by negative effects of HMB on glucose homeostasis, but more research is necessary to validate this hypothesis.

p0060 It is difficult to assess the effects of HMB supplementation fully as a potential treatment for sarcopenia because most longitudinal studies conducted in older or elderly adults administered HMB either in conjunction with other amino acids or in addition to a resistance exercise program. A combination of HMB, arginine, and lysine supplementation for 8 weeks was shown to enhance protein synthesis, increase lean mass, and improve strength outcomes in elderly women (Flakoll et al., 2014). Vukovich et al. (2001a,b) reported that HMB supplementation in conjunction with resistance training 5 days/week resulted in improvements in body composition in older adults similar to [**AU5**] those observed in younger adults. On the contrary, Stout et al. (2013) reported that HMB supplementation did not enhance lean mass accretion during 24 weeks of resistance training. The reason for the contrasting results is unknown; however, trends toward improvements greater than placebo were reported at 12 weeks but not 24 weeks in the

research by Stout et al. This suggests that there is a limit to the duration at which HMB supplementation enhances protein synthesis, or that subjects adapted to the demands of the exercise protocol and experienced less muscle damage, thus reducing the effectiveness of HMB, which has been postulated to be most effective when combined with a damaging exercise protocol (Wilson et al., 2014); however, this remains speculative and requires further investigation.

p0065 To our knowledge only two studies have investigated the effects of HMB supplementation on lean mass accretion or muscular strength in otherwise healthy elderly subjects not engaged in resistance exercise. Berton et al. (2015) assessed the effects of 8 weeks of 1.5 g/day HMB supplementation on markers of physical performance and muscle quality in 80 healthy women aged over 65 years. HMB supplementation increased lower limb peak force, hand grip strength, walking speed, and muscle density compared with placebo. Stout et al. (2013) reported similar results after 12 and 24 weeks of 3 g/day HMB supplementation in healthy adults aged over 65 years. Given the limitations to prescribing intense resistance training in certain elderly subpopulations, including low adherence and discontinuation, and in frail elderly individuals an inability to exercise at high enough intensities to attenuate or reverse the loss of muscle mass and function, HMB may be a potential nutritive intervention that warrants further research.

s0030 Ergogenic-Related Effects

p0070 In performance settings, HMB demonstrated synergistic effects when supplemented during a resistance training regimen in sedentary and trained individuals. The seminal study addressing the effects of HMB supplementation with different doses in regulating muscle mass in humans was carried out by Nissen et al. (1996). In this study, the authors adopted two experimental designs in which subjects were supplemented with 0, 1.5, and 3.0 g/day of HMB while performing resistance training for 3 weeks. They observed a significant decrease in exercise-associated muscle proteolysis during the first 2 weeks, as assessed by measurement of urinary excretion of 3-methyl-histidine. A reduction in markers of muscle damage was also found during the third week, notably reductions in creatine phosphoryl-kinase and lactate dehydrogenase. Whereas HMB has been shown to promote important anticatabolic effects, it is not clear whether HMB's anabolic effects occurs via increases in protein synthesis (or in muscle regeneration) or decreases in protein degradation.

p0075 There appear to be differences in the response to HMB supplementation based on training status. Conflicting results have been reported with regard to the hypertrophic effects of HMB supplementation in trained subjects. It seems that highly trained individuals supplemented with HMB respond more favorably to high-intensity resistance training than low- or moderate-intensity resistance training (Hoffman et al., 2004; Kreider et al., 1999; Slater et al., 2001). As an example, Slater et al. (2001) demonstrated in

highly trained subjects supplemented with HMB the inefficacy of both resistance training and HMB supplementation in increasing muscle mass. It was not clear from that study whether the period of training was of too short a duration or whether the exercise intensity was below the threshold necessary to induce muscle gains. More recently, HMB in conjunction with a periodized, high-intensity resistance training regimen in highly trained subjects was capable of inducing significant hypertrophic effects (Lowery et al., 2016). Collectively these results suggest that a prolonged period of resistance training (greater than 6 weeks) with a higher intensity and higher volume is necessary to induce increases in muscle mass in trained subjects. Table 23.1 presents data from the literature detailing the effects of HMB supplementation on fat-free mass and strength, obtained in a literature search conducted in November, 2016.

s0035 Mechanisms of Action

p0080 Several mechanisms have been suggested to explain the ergogenic and hypertrophic effects of HMB supplementation. These effects appear to be achieved through the following mechanisms of action: (1) increased sarcolemmal integrity, (2) increased metabolic efficiency, (3) upregulation of insulin-like growth factor-I (IGF-I) expression, (4) increased activation of satellite cells and myogenic factors, (5) stimulation of protein synthesis, and (6) suppression of proteolysis by inhibition of proteolytic systems. The following mechanisms of action are discussed in detail subsequently.

s0040 Increased Sarcolemmal Integrity

p0085 The protective effect of HMB against contractile activity–induced damage may be associated with increased stability of the muscle plasma membrane. The proposed hypothesis underlying HMB action is that stressed or damaged muscle cells might not be able to produce enough HMG-CoA to maintain proper cellular function (Mutoh et al., 1999; Pierno et al., 1995). One such pathway that relies on de novo synthesis of cholesterol may be especially important in the muscle. As example, inhibition of HMG-CoA reductase (the enzyme responsible for conversion of HMB to β-methylglutaryl-CoA-HMG-CoA) for cholesterol synthesis profoundly affects the electrical properties of the cell membrane in skeletal muscle (Zanchi et al., 2010 for review). It is interesting that trained subjects seem to show fewer hypertrophy effects compared with sedentary ones (Slater et al., 2001), but it is not completely known whether this effect depends on the shorter duration of training (especially in trained individuals) or the absence of muscle damage and subsequent recovery. Wilson et al. (2014) employed a high-intensity resistance training program designed to induce muscle damage and reported robust increases in the muscle mass of HMB-supplemented subjects. Interpretation of these data is still open to question, but they suggest that HMB may optimize the repair of muscle membrane, which can be linked to cholesterol biosynthesis.

t0010 **Table 23.1** Effects of Supplementation of β-Hydroxy-β-methylbutyrate in Different Protocols and Different Populations Regarding Fat-Free Mass and Muscle Strength

Experiment	Sample	Dose	Duration	Additional Supplements	Outcomes
Gallagher et al. (2000)	Humans	3.0 versus 6.0 g HMB/day	8 weeks with RT	No	FFM: +3.0%; strength: +2.0% −3.5%; no differences (3.0 vs. 6.0 g)
Jowko et al. (2001)	Humans	3.0 g HMB/day	3 weeks with RT	HMB + creatine	FFM: no effects; strength: +14.0%
Kim et al. (2012)	Rats	0.48 g/kg per day HMB	10 weeks with RT	No	FFM: +20.0%
Kraemer et al. (2009)	Humans	3.0 g HMB/day	12 weeks with RT	HMB + arginine + glutamine	FFM: +40.0%; strength: 50.0%
Kreider et al. (1999)	Humans	3.0 versus 6.0 g HMB/day	4 weeks with RT	No	FFM: no effects; strength: no effects; no differences (3.0 vs. 6.0 g)
Lowery et al. (2016)	Humans	3.0 g HMB/day	12 weeks with RT	HMB + ATP	FFM: +12.7%
Nissen et al. (1996)	Humans	1.5 versus 3.0 g HMB/day	3 and 7 weeks with RT	No	FFM: +1.9%; strength: +2.3% average; results for 3.0 g
Panton et al. (2000)	Humans	3.0 g HMB/day	4 weeks with RT	No	FFM: +0.5 kg; strength: +3.0%−15.0%
[AU6] Ransone et al. (2003)	Humans	3.0 g HMB/day	4 weeks with RT	No	FFM: +0.3; strength: +1.7%
Thomson et al. (2009)	Humans	3.0 g HMB/day	9 weeks with RT	No	FFM: +0.4%; strength: 1.1%−9.0%

ATP, adenosine triphosphate; *HMB*, β-hydroxy-β-methylbutyrate; *FFM*, fat-free mass; *RT*, resistance training.

s0045 Increased Metabolic Efficiency

p0090 HMB supplementation may increase energy production in the muscle cell directly or indirectly. In relation to direct energy production, HMB consumption is capable of promoting increases in acetyl-CoA content by converting HMG-CoA into acetoacetyl-CoA by HMG-CoA synthase, which can be used by skeletal muscles to produce adenosine triphosphate (ATP) (van Koverin and Nissen, 1992a,b; Nissen and Abumrad, 1997). Although directly providing intermediates of the Krebs cycle (and then energy), the endogenous production of HMB is not thought to produce larger amounts of energy compared with other energetic substrates. For example, endogenous daily HMB production in humans is approximately 0.2−0.4 g/day, and such an amount of HMB being converted into energy is far less than the energy production of leucine oxidation (Duan et al., 2016). Interestingly, although increases in energy metabolism are not predicted to occur via HMB supplementation, increases in several muscle strength and physical performance parameters including endurance hand grip and isokinetic peak torque were observed in old woman supplemented with HMB (Berton et al., 2015). How these muscle adaptations are linked to enhanced energetic metabolism is unknown, but they may be the result of increased mitochondrial biogenesis. In this regard, Stancliffe (2012) demonstrated that HMB increases mitochondrial biogenesis by 50% in C_2C_{12}-treated myotubes. Such effects seemed to be linked to a stimulatory effect of HMB in increasing the expression of regulatory mitochondrial genes (peroxisome proliferator-activated receptor-γ co-activator 1-α) and component genes such as mitochondrial uncoupling protein 3 (Stancliffe, 2012). Interestingly, it has been demonstrated that inhibition of KIC dioxygenase enzyme in skeletal muscle cells (through silencing RNA) leads to the inhibition of mitochondrial biogenesis stimulated by HMB, which suggests that HMB metabolites have a role in these activations (Verdin et al., 2010; Hardie et al., 2006). Thus, increased energy metabolism mediated by HMB consumption can occur through increased intermediary metabolites for the Krebs cycle (generating ATP) or via increased mitochondrial biogenesis. The individual roles of both parameters in muscle performance are unknown.

s0050 Upregulation of Insulin-like Growth Factor-I

p0095 Another possible mechanism underlying the effects of HMB supplementation is the increased expression of IGF-I in the liver and skeletal muscles. In this respect, Kornasio et al. (2009) demonstrated in myoblasts that HMB could stimulate IGF-I mRNA expression as well myogenic regulatory factors and increase thymidine incorporation (an indicator of DNA synthesis). These direct effects of HMB on myoblast differentiation and survival (including starving conditions) resemble those of the IGF-I pathway, which suggests that at least in in vitro conditions, increased HMB-induced IGF-I expression may positively influence enhanced muscle anabolism. In rodents, Gerlinger-Romero

et al. (2011) demonstrated that HMB supplementation promoted increased growth hormone (GH) and IGF-I expression in the pituitary and liver, respectively. Considering that hypophysectomized rats (which present decreased circulating GH and IGF-I) are able to hypertrophy to the same extent as controls (DeVol et al., 1990), the general contribution of IGF-I expression to muscle growth is unknown. However, considering the consistency of HMB in increasing IGF-I expression and secretion, more research is warranted to investigate the role of HMB and the resultant IGF-I response on skeletal muscle hypertrophy.

s0055 Increased Activation of Satellite Cells and Myogenic Factors

p0100 Although increased IGF-I secretion can lead to the activation of satellite cells (and myogenic factors) involved in the muscle hypertrophy process. The ability of HMB to induce the activation of satellite cells or myogenic factors directly may also influence muscle hypertrophy (Kormasio et al., 2009). Szcześniak et al. (2016) investigated the direct effects of HMB in equine satellite cells. Upon isolation from the semitendinosus muscle, equine satellite cells were cultured until the second day of differentiation, and differentiating cells were incubated with HMB for 24 h. Employing microarray analyses, these investigators were able to detect a robust effect of HMB in inducing the expression of genes highly involved in satellite cell activation and muscle growth, including cell proliferation and cell differentiation genes. These genes are also highly correlated with "muscle organ development," which suggests that HMB may be involved in improving skeletal muscle growth and regeneration, at least in satellite cells extracted from equine muscles. In isolated murine C_2C_{12} myoblasts, HMB treatment enhanced myoblast proliferation and viability, even under the unfavorable conditions of serum reduction (Vallejo et al., 2016). In senescent rats, after a period of disuse (muscle unloading), HMB supplementation increased the activation of satellite cells (and markers of satellite cell activation), resulting in an increase in differentiated stem cells relative to total myonuclei in reloaded muscles (Alway et al., 2013). In another study, the effects of HMB supplementation during gestation on the reproductive performance of sows (and the mRNA expression of myogenic markers in skeletal muscle) of neonatal pigs were tested (Wan et al., 2016). HMB supplementation in sows significantly improved pregnancy outcomes and increased the expression of myogenic genes in the skeletal muscle of neonatal piglets. Interestingly, increased myogenic gene expression was correlated with increased muscle weight and the overall weight of the piglets at birth (Wan et al., 2016). Because increased satellite cell activation is part of the remodeling process, muscle growth, muscle repair, and muscle regeneration, it is interesting to speculate that the effects of HMB on muscle hypertrophy or in muscle atrophy (especially in atrophy conditions related to loss of myonuclei) are related to satellite cell activation (Kormasio et al., 2009); however, future studies addressing these questions are needed to validate this hypothesis.

s0060 Stimulation of Protein Synthesis Through Mammalian Target of Rapamycin Signaling Pathway Activation

p0105 In relation to the protein synthetic process, a major signaling pathway controlling when and where the muscle growth occurs is the mTOR pathway. mTOR is a protein kinase responsive to mechanical stimuli, hormones, and nutrients that controls the cellular growth of cells, including skeletal muscle. The main mechanism involved in muscle hypertrophy stimulated by mTOR is through increased mRNA translational efficiency (Proud, 2007a,b; Zanchi and Lancha, 2008). In this context, HMB seems to act on the mTOR pathway via several different mechanisms, mainly activating its effectors 4EBP-1 and P70S6K, culminating with increased translation of mRNA, specially coding for myofibrillar proteins (Bodine et al., 2001). In support of this hypothesis, Eley et al. (2007) observed that C_2C_{12} myoblasts incubated with 50 μM of HMB significantly increased muscle protein synthesis. Importantly, this stimulating effect was completely abolished in the presence of rapamycin, an mTOR inhibitor. In rodents, a similar effect was observed, in which HMB supplementation induced increased phosphorylation of P70S6K in the extensor digitorum longus and soleus muscles (Pimentel et al., 2011). In a study performed in humans, acute HMB supplementation increased muscle protein synthesis in quadriceps muscle to a similar degree of leucine by enhancing activation of the mTOR pathway (Wilkinson et al., 2013). Interestingly, although HMB and resistance training are capable of acting synergistically, studies are lacking in humans investigating the role of HMB and resistance training on mTOR (or related cell signaling pathways) and muscle hypertrophy.

s0065 Suppression of Proteolysis by Inhibition of Proteolytic Systems

p0110 The ubiquitin–proteasome system is an ATP-dependent proteolytic system that contributes to the degradation of aging, malformed, and damaged proteins. Importantly, if overactivated, the ubiquitin–proteasome system can lead to robust muscle degradation and atrophy, as observed during several catabolic conditions (Lecker et al., 2006). Thus, inhibition of this proteolytic system, especially under atrophic conditions, helps explain attenuation of muscle protein losses observed during HMB treatment. In this regard, HMB has been shown to decrease proteasome activity both in vitro (Eley et al., 2007; Smith et al., 2005) and in vivo (Baptista et al., 2013; Holecek et al., 2009). In line with this, Smith et al. (2005) observed in mice implanted with the MAC16 tumor that HMB supplementation was effective in reducing muscle proteolysis observed in cancer cachexia, which reflected the attenuation of muscle loss. Importantly, this effect was positively correlated with a decrease in the catalytic activity of the proteasome. As previously described, HMB potentiates IGF-I secretion, which decreases muscle proteasomal-induced proteolysis both directly and indirectly via inhibition of E3 ligase expression, namely atrogin-1 or MuRF-1 (Hoffman and Nader, 2004; Dehoux et al., 2004; Latres et al., 2005; Zanchi et al., 2010). In another study, Girón et al. (2015) observed that in rats

treated with dexamethasone, HMB supplementation ameliorated the loss of body weight and lean mass and the reduction in the muscle fiber cross-sectional area in gastrocnemius muscle. In addition, these changes resulted in decreased atrogin-1 and MuRF-1 gene expression.

p0115 In addition to the ubiquitin–proteasome system, HMB also seems to attenuate upregulation of other proteolytic systems. For example, caspases are proteases involved in skeletal muscle proteolysis, mainly inducing myonuclei apoptosis, and are commonly upregulated in catabolic states. HMB supplementation seems to attenuate the upregulation of caspases (also reducing myonuclear apoptosis) during catabolic states, such as unloading (Hao et al., 2011) or the presence of large concentrations of inflammatory cytokines (Eley et al., 2008). Another proteolytic system that can be attenuated during catabolic conditions is the lysosomal system inducing autophagic process. Autophagy is a physiological process used by skeletal muscle to sequester cytoplasmic proteins and organelles into vacuoles known as autophagosomes, and is vital for remodeling the cellular architecture (Komatsu and Ichimura, 2010). In rat L6 myotubes pretreated with HMB, significant attenuation of lysosomal proteolysis induced by dexamethasone was observed. This effect was mediated by normalizing changes observed in autophagosome formation, via expression of proteins involved in the process. Thus, in addition to its effects on the ubiquitin–proteasome pathway, HMB may attenuate skeletal muscle protein degradation by inhibiting caspase and lysosomal activity (Girón et al., 2015). As discussed previously, the potential for HMB to induce antiproteolytic effects varies according to the predetermined catabolic state. Presumably, such effects will vary according to which proteolytic system is more or less activated. Future studies will help in describing the HMB mechanisms of action in each catabolic state.

s0070 Conclusions

p0120 HMB is a nutraceutical compound with clinical and ergogenic effects that influence both catabolic and anabolic conditions. During catabolic conditions, HMB spares lean mass, in part by decreasing the activity of several muscle proteolytic systems. It is not clear, however, exactly how HMB supplementation should be manipulated to increase its potency or efficacy. In healthy subjects, it is well established that 3 g/day promotes anabolic effects compared with lower doses with no increased effects at higher doses (6.0 g). It is unknown whether these dosages are equally efficacious in reducing muscle protein breakdown during several catabolic conditions of different etiologies where distinct proteolytical systems are activated. With regard to the composition of HMB, free acid form has been shown to reduce muscle protein breakdown after high-volume resistance training. However, studies comparing HMB-Ca and HMB-FA are lacking. In a seminal work, Nissen et al. (1996) demonstrated that HMB increases the muscle mass of sedentary subjects submitted to a resistance training program. In certain aspects, these effects are similar to leucine, because HMB is a leucine-derived metabolite.

However, it was demonstrated that leucine and HMB-FA have distinct effects in the human muscle. For example, the anabolic or anticatabolic actions of HMB seems to occur independently of insulin secretion, and the magnitude of activation in the muscle signaling pathways leading to muscle hypertrophy was distinctly different from that presented by leucine. Overall, HMB appears to possess anabolic qualities capable of enhancing muscle protein accretion in response to resistance training in healthy individuals and strong anticatabolic qualities that appear capable of preserving or even increasing muscle mass in atrophic or muscle wasting conditions. Several mechanisms of action have been proposed to explain HMB's effects at the cellular and molecular level. However, more research is needed to translate this information fully into practical applications before HMB can be employed as a treatment for sarcopenia or muscle wasting disease. Further research is also required to elucidate fully the specific mechanisms under which these anticatabolic properties are exerted.

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Further Reading [**AU8**]

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Abstract

β-Hydroxy-β-methylbutyrate (HMB) is a leucine-derived compound with anabolic and anticatabolic properties that may improve both clinical and performance outcomes. Clinically, HMB supplementation has been shown to decrease muscle proteolysis and spare muscle mass during catabolic states associated with muscle wasting syndromes. In resistance training studies, HMB may promote enhanced gains in muscle mass and strength. When evaluating the potential effects of HMB, dosage, form (calcium and the free acid form), and subject status (atrophic or anabolic states) must be taken into consideration. This chapter aims to describe general scientific evidence on HMB supplementation with regard to clinical outcomes, as well as their effects associated with resistance training and other sports. Possible mechanisms of action are also discussed in detail, based on the physiological, biochemical, and cellular/molecular literature.

Keywords:

Atrophy; Hypertrophy; Leucine; Protein synthesis; Proteolysis; Skeletal muscle.