Amino Acids (2011) 40:1015–1025
DOI 10.1007/s00726-010-0678-0

REVIEW ARTICLE

HMB supplementation: clinical and athletic performance-related effects and mechanisms of action

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Received: 15 March 2010 / Accepted: 25 June 2010 / Published online: 6 July 2010

Abstract Amino acids such as leucine and its metabolite α-ketoisocaproate (KIC), are returning to be the focus of studies, mainly because of their anti-catabolic properties, through inhibition of muscle proteolysis and enhancement of protein synthesis. It is clear that these effects may counteract catabolic conditions, as well as enhance skeletal muscle mass and strength in athletes. Moreover, beta-hydroxy-beta-methylbutyrate (HMB) has been shown to produce an important effect in reducing muscle damage induced by mechanical stimuli of skeletal muscle. This review aims to describe the general scientific evidence of KIC and HMB supplementation clinical relevance, as well as their effects (e.g., increases in skeletal muscle mass and/or strength), associated with resistance training or other sports. Moreover, the possible mechanisms of cell signaling regulation leading to increases and/or sparing (during catabolic conditions) of skeletal muscle mass are discussed in detail based on the recent literature.

Keywords HMB · Clinical effects · Performance-related effects · Mechanisms

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Introduction

The amino acid leucine and its metabolite α-ketoisocaproate (KIC) have been known to be potent anti-catabolic compounds for more than 35 years (Hider et al. 1960; Zanchi et al. 2008) and have recently been the focus of several studies. Nissen et al. (1996) demonstrated that a specific leucine-derived metabolite, beta-hydroxy-beta-methylbutyrate (HMB), is of special interest, due to its positive effects on sports performance and as a therapeutical supplement (mainly in atrophic conditions), since the use of inhibitors of leucine transamination (the process yielding HMB) suppresses the inhibition of protein degradation (Slater and Jenkins 2000). Other branched-chain amino acids (BCAAs), such as isoleucine and valine, are not able to trigger these effects, reinforcing the assumption that HMB or some other strictly related metabolite is the key element eliciting the mentioned anti-catabolic effect (Holecek et al. 2009). In addition, recent studies show that HMB supplementation in cachexia produces a dampening effect on skeletal muscle degradation and reinforces protein synthesis through multiple signaling pathways (Eley et al. 2007; Nunes et al. 2008; Caperuto et al. 2007). Moreover, HMB has been shown to

reduce muscle damage as induced by mechanical stimulation associated with increases in skeletal muscle mass (Nissen and Sharp 2003). These properties of HMB credit its use not only in pathological conditions in which loss of muscle mass is observed but also in sports. Therefore, this review aims to describe the effects of HMB supplementation from clinical to athletic performance. From this discussion, we hope to shed light on how this supplement is capable of exerting such divergent effects.

HMB metabolism

HMB is a metabolite of the amino acid leucine (Van Koverin and Nissen 1992), an essential amino acid. The first step in HMB metabolism is the reversible transamination of leucine to α-KIC that occurs mainly extrahepatically (Block and Buse 1990). Following this enzymatic reaction, α-KIC may follow one of two pathways. In the first, HMB is produced from α-KIC by the cytosolic enzyme KIC dioxygenase (Sabourin and Bieber 1983). The cytosolic dioxygenase has been characterized extensively and differs from the mitochondrial form in that the dioxygenase enzyme is a cytosolic enzyme, whereas the dehydrogenase enzyme is found exclusively in the mitochondrion (Sabourin and Bieber 1981, 1983). Importantly, this route of HMB formation is direct and completely dependent of liver KIC dioxygenase. Following this pathway, HMB in the cytosol is first converted to cytosolic β-hydroxy-β-methylglutaryl-CoA (HMG-CoA), which can then be directed for cholesterol synthesis (Rudney 1957) (Fig. 1). In fact, numerous biochemical studies have shown that HMB is a precursor of cholesterol (Zabin and Bloch 1951; Nissen et al. 2000).

In the second pathway, after transamination, α-KIC in liver generates isovaleryl-CoA through the enzymatic action of branched-chain ketoacid dehydrogenase (BCKD) and after several steps, there is production of HMG-CoA through the enzyme HMG-CoA synthase (Fig. 1). Under normal conditions the majority of KIC is converted into isovaleryl-CoA, in which approximately 5% of leucine is metabolized into HMB (Wilson et al. 2008; Van Koverin and Nissen 1992). However, Nissen and Abumrad (1997) provided evidence that the primary fate of HMB is probably conversion to HMG-CoA in the liver, for cholesterol biosynthesis. The purposed hypotheses underlying HMB action is that stressed or damaged muscle cells might not be able to produce enough HMG-CoA as warranting proper cellular function. One such pathway may be specially important in the muscle, that relies on de novo synthesis of cholesterol. However, as described here, this specific issue may depend on the degree of damage of the muscle cell provoked by exercise.

HMB supplementation: effects on metabolism and immune responses

Based on the contention that HMB can serve as a precursor for cellular cholesterol synthesis, an important question that arises is: Is this cholesterol pathway activated through HMB supplementation capable of modulating circulating cholesterol levels? In order to respond such important issue, Nissen et al. (2000) analyzed data from nine studies in which humans had been supplemented with 3 g/day of HMB for three to 8 weeks. The results revealed an LDL cholesterol reduction of 7.3% in individuals with hypercholesterolemia (with no changes in HDL cholesterol), especially when total cholesterol was increased compared with the placebo group. In the same study, liver function was unchanged compared to the endpoint function. In summary, the authors concluded that the only definitive effects of HMB were positive in nature, particularly relating to lowering plasma cholesterol. These data suggested that the popular use of supplemental HMB at 3 g/day as an ergogenic aid for exercise is well tolerated and safe.

On the same line, Peterson et al. (1999) isolated macrophages from an avian macrophage cell line (MQ-NCSU) and supplemented those cells with increasing concentrations of HMB, exposing cells to 0, 10, 20, 40, 80, and 100 μg of HMB per 5×10^4 cells in a 96-well culture plate. When exposed to 40 μg of HMB, the phagocytic potential of MQ-NCSU macrophages was significantly higher (31.7%) than that of the controls. These data demonstrate that HMB exposure induces proliferation of macrophages in culture in addition to enhancing macrophage effector functions, such as nitrite production and phagocytosis. The findings of this study imply that HMB might be used as a possible dietary immunomodulator.

HMB kinetics and urinary excretion

Vukovich et al. (2001) determined the time course kinetics of HMB in humans, and the influence of oral glucose ingestion upon this parameter. Because Green et al. (1996) have reported that carbohydrate intake alters creatine retention, probably through the action of insulin, the kinetics of HMB could also be affected by macronutrient intake. In Study 1, eight male subjects (32 ± 10 years) participated in two randomized trials: (1) oral ingestion of 1 g of HMB in capsule form with water (HMB), and (2) placebo. Blood samples were obtained prior to ingestion of the capsules and at 30, 60, 90, 120, 150, and 180 min in order to measure plasma HMB. Two hours after consumption of 1 g of HMB with 75 g of

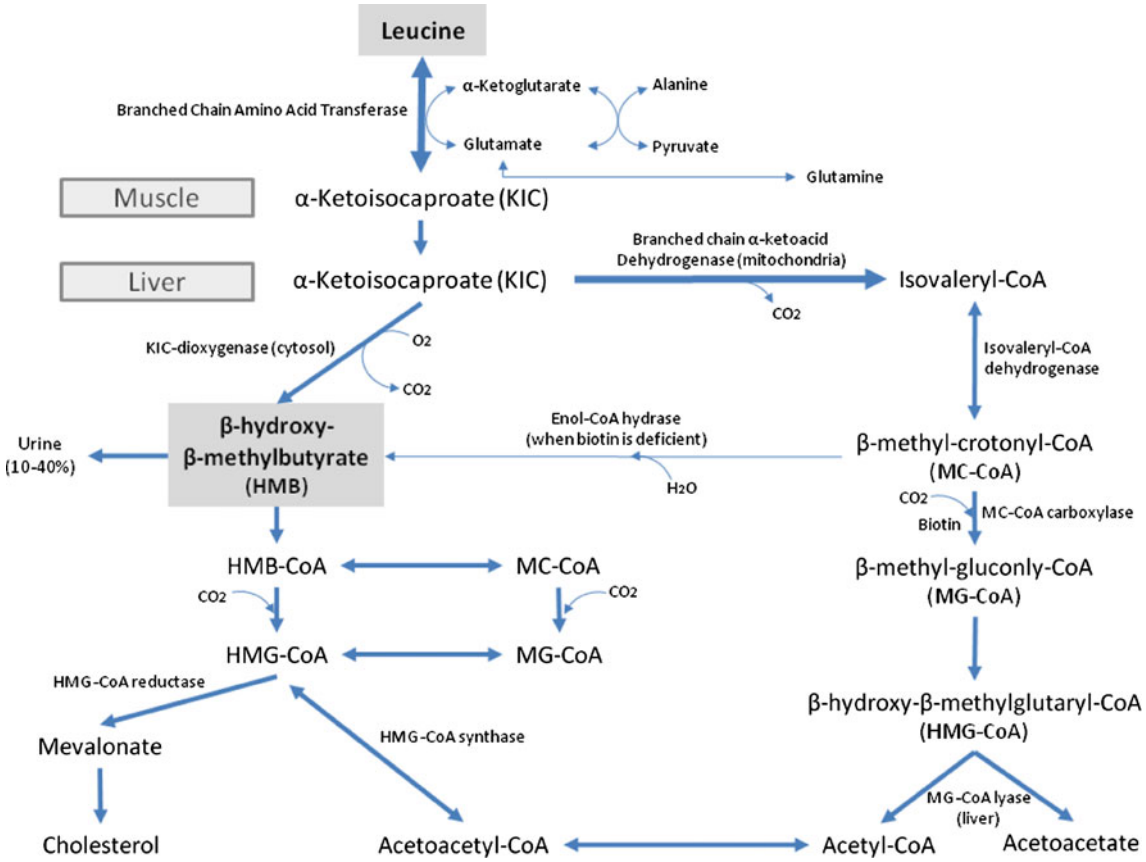

Fig. 1 HMB metabolism. Adapted from Nissen and Abumrad (1997) and Van Koverin and Nissen (1992)

glucose, an HMB peak of 120 nmol/mL was observed. An increased plasma HMB response was observed after consuming a mixture of 3 g HMB plus 75 g of glucose, peaking at 352 nmol/mL 2 h after HMB consumption. Moreover, further analysis using the insulinemic clamp technique indicated no major differences between concomitant glucose/HMB and HMB supplementation alone (Vukovich et al. 2001). The only difference concerned the interval required for the HMB concentration to peak, which was significantly longer when HMB was consumed with glucose; nevertheless, plasma half-life was enhanced. When examining urinary excretion, it was observed that when following the protocol of low doses (1 g of HMB), approximately 14% (0.14 g) of the HMB was excreted in the urine. In contrast, after the high dose protocol (3 g/day) approximately 29% (0.87 g) of the ingested HMB excreted in the urine following the ingestion of HMB and glucose or glucose alone. At 1 g, HMB levels peaked faster than when consumed at 3 g/day, as it was being retained in a way independent of glucose consumption. Similar data about safety of renal function was observed by Gallagher et al. (2000b).

Leucine and HMB supplementation: similarities and differences

As with HMB, it is not clear if leucine per se or one of its metabolites signals the activation of its anti-catabolic effects (e.g., inhibition of protein degradation or activation of protein synthesis), because no receptors for leucine (Lynch et al. 2002) or HMB have been identified to date. When supplemented, the first step in leucine metabolism is the reversible transamination of leucine to α-KIC, which is catalyzed by the branched-chain amino transferase isoenzymes [mitochondrial (BCATm) and cytosolic (BCATc)] (Lynch et al. 2002). The former enzyme is expressed mainly in neural tissue (Hutson et al. 1995). Thus, among BCAT enzymes, BCATm is of special interest because of its specific expression in tissues where these supplements present abundant effects. As previously mentioned, α-KIC can follow distinct routes, because HMB synthesis through BCAA transamination is responsible for endogenously synthesized HMB. On the other hand, after leucine transamination, branched-chain ketoacid dehydrogenase (BCKA dehydrogenase) appears to be the enzyme involved

in completing the leucine signal (Lynch et al. 2002). The implication of this hypothesis is that a single step in leucine-KIC metabolism provides both the transduction of the leucine signal and its termination, also controlling the production of other products such as HMB (Xu et al. 2001). This hypothesis may help to elucidate the mechanisms leading to the finishing signals provided by HMB and leucine supplementation. Unfortunately, little is known about the action of BCKD on HMB in the liver and muscle. Another limiting factor is associated with the lack of specific inhibitors of the first reversible step in leucine metabolism, which produces α-KIC (Lynch et al. 2003). The commonly adopted inhibitors, such as aminooxyacetic acid, are non-specific and inhibit a number of transaminases in the cell in addition to the one of interest, the mitochondrial BCAA transaminase (Lynch et al. 2003). Thus, conclusions based on such inhibition need to be carefully evaluated.

On the other hand, Nissen et al. (1996) suggested that HMB or some other metabolite (since there is no specific inhibitor to BCAT) is the main component responsible for the anti-catabolic effects of HMB because when adopting inhibitors of BCAA transamination, the only BCAA capable of anti-proteolytic effects is leucine, which undergoes a process capable of generating HMB (Slater and Jenkins 2000). Such effects were not observed when other BCAAs were tested (isoleucine and valine), suggesting that HMB or some metabolite may be the key element in promoting the effects mentioned above (Holecek et al. 2009).

Corroborating that study, the effects of leucine supplementation in low doses (3 g/day) were compared by Wilson, Wilson and Manninen (2008) to the effects of a protocol of high leucine supplementation (15 g/day) in a similarly designed study. The authors concluded that the highest leucine dose was more effective in generating some metabolite (possibly HMB) that optimized leucine's ergogenic effects. In addition, Tischler et al. (1982) observed that the same concentrations of leucine and α-KIC showed different anti-proteolytic effects in incubated diaphragm muscles. At the same concentration (0.5 mM), α-KIC was unable to stimulate protein synthesis when compared to administration of leucine alone, while protein degradation was inhibited by 7% compared to leucine. Moreover, plasma concentrations of HMB normally range from 1 to 4 mmol/L but can increase five to tenfold after leucine is taken orally (Nissen and Abumrad 1997). Although a teleological relationship is still unknown, it is possible that leucine supplementation acts as a precursor for α-KIC favoring the route of metabolism; however, as mentioned above, it remains unknown if an extracellular or intracellular leucine receptor is responsible for such effects (Hundal and Taylor 2009).

Why use HMB as a supplement?

A person weighing 70 kg produces about 0.2–0.4 g of HMB per day, depending on the content of leucine in the diet. Based on the fact that all essential amino acids, such as L-leucine (the precursor of HMB), are not synthesized by the human body, this quantity is reached through the dietary intake of protein. The main dietary sources of L-leucine are protein sources, for example, meat. In a recent review, Wilson et al. (2008) pointed out that an individual would need to consume about 60 g of L-leucine to produce 3 g of HMB, the usual supplemented dose in studies concerning the effects of HMB (Wilson et al. 2008).

To achieve these levels, an individual would have to consume larger quantities of all amino acids, including leucine. Translating such quantities to the "real world" (through food intake alone), achieving the recommended amounts proposed by the studies cited above may be feasible but is certainly beyond the dietary principles of a balanced diet.

Main aims of HMB supplementation

The dietary supplement HMB has been shown to decrease muscle proteolysis during the stress of exercise and disease (Vukovich et al. 2001). Besides this direct effect, HMB can markedly decrease muscle damage, which is evidenced by the diminished leakage of phosphorylcreatine phosphokinase (CPK) out of muscle cells (Cheng et al. 1998; Nissen et al. 1996; Nissen and Abumrad 1997). Also supporting this concept, there are several studies showing that drugs inhibiting cholesterol synthesis in muscle carry a potential risk for myopathy because HMG-CoA reductase inhibitors affect the electrical properties of the rat skeletal muscle fiber membrane, leading to increased muscle damage (Pierno et al. 1995) and even muscle cell death (Mutoh et al. 1999). For these reasons, HMB supplementation has been adopted as an alternative by those practicing resistance exercise or weight training, by individuals under extreme muscular stress, by elderly individuals and by patients with diseases associated with muscle wasting syndromes, such as cachexia (Smith et al. 2005).

Effects of HMB on strength and muscle mass

Although there is an intuitive idea that the increase in protein intake associated with resistance training is necessary to ensure maximum gains in strength and muscle mass, there are many important questions to be answered regarding the consumption of food supplements. In the

recent years, the growing interest in HMB supplementation has arisen from previous demonstrations of its anti-catabolic properties (Nissen and Sharp 2003; Smith et al. 2005) and speculations related to its mechanisms of action. In fact, HMB supplementation may affect cellular receptors and hormones, such as cortisol, testosterone, and insulin, or the modulation of enzymes responsible for muscle catabolism (Slater and Jenkins 2000). However, recent studies have generated a more concrete basis regarding HMB mechanisms of action (Eley et al. 2007).

The first studies addressing the effect of oral supplementation with different doses of HMB on the regulation of muscle mass in humans were carried out by Nissen et al. (1996) in a resistance training study. 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 doing 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. They also found a reduction in muscle damage indicators during the third week, as indicated by the measurement of CPK and lactate dehydrogenase (LDH) activity. When these authors performed supplementation at doses of 0 and 3.0 g of HMB per day associated with resistance training for 7 weeks, they reported a significant increase in fat-free mass and strength (Jowko et al. 2001). However, controversial results have been found in studies with humans previously trained or untrained when assessing the effects of oral supplementation of HMB in conjunction with resistance training (Nissen et al. 1996; Gallagher et al. 2000a; Slater et al. 2001; Jowko et al. 2001; Vukovich et al. 2001; Ransone et al. 2003; Hoffman et al. 2004).

There is evidence that adults new to physical training exhibit lower levels of muscle damage markers induced by resistance training, as assessed by CPK activity in blood, when supplemented with 3.0 g/day of HMB (Panton et al. 2000; Gallagher et al. 2000a) or when supplemented with creatine (Jowko et al. 2001). In distance runners, reductions in muscle damage markers represented by serum CPK and LDH were also found (Knitter et al. 2000). Other effects involve significant increases in muscle strength during resistance training along with HMB supplementation in a manner that is independent of gender or training status (Panton et al. 2000), or in conjunction with creatine supplementation (Jowko et al. 2001). In addition, there are data indicating that 8 weeks after consumption of 3.0 g of HMB per day during resistance training there is an increase of muscle mass (Gallagher et al. 2000a). However, in this study, the authors found that doses of 6 g of HMB per day did not elicit the same effect, and that doses higher than 3 g did not exacerbate the effects in individuals who are starting resistance training.

On the other hand, a number of studies failed to confirm the results presented above. In adult beginners, resistance training with HMB supplementation (3.0 g HMB per day) did not change body composition, muscular strength levels and biochemical markers of protein turnover and muscle damage (Slater et al. 2001), elicit increased muscle mass (Jowko et al. 2001) or alter the strength gain and fat-free mass gain in elderly subjects (Vukovich et al. 2001). In addition, in athletes conditioned to resistance training, the same dose of HMB was unable to change the strength and body composition of athletes involved in water polo, rowing (Slater et al. 2001) or football (Ransone et al. 2003; Kreider et al. 2000), and did not present effects on several markers of muscle damage (CPK and LDH) as well as speed between the groups (Kreider et al. 2000).

On the contrary, oral supplementation of HMB in untrained individuals undergoing routine resistance training may contribute to the ergogenic effects of aspects of strength and muscle mass because these effects appear to be more prominent among those who are in the initial phase of training. Furthermore, HMB consumption seems to pose no risk because there are no records of negative responses in, for instance, parameters of renal function, hepatic and hematologic parameters after the intake of up to 6 g of HMB per day (Gallagher et al. 2000a, b).

Some explanations for these conflicting results are noteworthy. From the standpoint of gaining muscle mass, it is believed that supplementation with HMB exerts significant effects in conditions in which muscle proteolysis is more pronounced, such as in sedentary individuals acutely exposed to mechanical stimulation. In addition, the type of contraction is also a factor to be considered, as eccentric contractions promote more muscle tension, maximizing muscle damage (Spiering et al. 2008; Zanchi and Lancha 2008). Table 1 summarizes some data presented in this section and in the section below.

Therapeutic effects of supplementation

As said before, HMB has been adopted as an alternative supplement by individuals under extreme muscular stress, by elderly individuals, and by patients with diseases associated with muscle wasting syndromes, such as cachexia (Smith et al. 2005).

Although cachexia involves progressive wasting of muscle and adipose tissue and is associated with increased morbidity and mortality in cancer, chronic obstructive pulmonary disease (COPD) and congestive heart failure (CHF) patients, no single nutritional therapy is able to revert it. The BCAA leucine, the leucine metabolite HMB, arginine, glutamine, omega-3 long-chain fatty acids, conjugated linoleic acid, and polyphenols have demonstrated

Table 1 Effects of supplementation of HMB in different protocols and different populations regarding muscle strength, muscle mass and muscle damage

References	Samples	Methodology		Results
Clark et al. (2000)	Humans	3 g of HMB + 14 g arginine + 14 g glutamine/day	8 weeks	In individuals with HIV, increased lean mass and improved immune system function
Flakoll et al. (2004)	Humans	2.0 g HMB + 5.0 g arginine + 1.5 g lysine/day	12 weeks	Gain in maximum strength and muscle mass
Gallagher et al. (2000a)	Humans	3.0 g HMB/day	8 weeks with RT	Induced increases in muscle mass
Gallagher et al. (2000a)	Humans	6.0 g HMB/day	8 weeks with RT	No response to supplementation
Gallagher et al. (2000a)	Humans	6.0 g HMB/day	8 weeks with RT	No risk regarding renal and liver function or hematological parameters
Hoffman et al. (2004)	Humans	3.0 g HMB/day	10 days[a]	No gain in strength nor in muscle mass
Jowko et al. (2001)	Humans	3.0 g HMB/day	3 weeks with RT	No changes in muscle mass
Kreider et al. (1999)	Humans	3.0 or 6.0 g HMB/day	4 weeks with RT	No gain in strength, nor in muscle mass
Lamboley et al. (2007)	Humans	3.0 g HMB/day	5 weeks with aerobic exercise program	Aerobic capacity enhancement with no alterations in body composition
Nissen et al. (1996)	Humans	1.5 or 3.0 g HMB/day	3 and 7 weeks with RT	Significant decrease in muscle proteolysis induced by exercise
O'Connor and Crowe (2007)	Humans	3.0 g HMB/day	6 weeks[b]	No gain in strength, nor in anthropometrical parameters
Panton et al. (2000)	Humans	3.0 g HMB/day	4 weeks with RT	Lower levels of muscle damage markers
Ransone et al. (2003)	Humans	3.0 g HMB/day	4 weeks[a]	No gain in strength nor in muscle mass
Slater et al. (2001)	Humans	3.0 g HMB/day	6 weeks with RT	No changes in body composition, muscular strength and biochemical markers
Soares et al. (2001)	Rats	0.002 g HMB/day	7 days	Decreased muscle damage and increased muscle fiber diameter under hindlimb immobilization procedures
Thomson et al. (2009)	Humans	3.0 g HMB/day	9 weeks with RT	Increased lower-body strength with no effects on body composition
Van Someren et al. (2005)	Humans	3.0 g HMB + 3.0 g KIC/day	2 weeks with RT	Gain in maximum strength and increased skeletal muscle repair
Vukovich et al. (2001)	Humans	3.0 g HMB/day	8 weeks with RT	No effect on strength gain and fat-free mass gain in elderly subjects

RT Resistance training, *HMB* beta-hydroxy-beta-methylbutyrate, *KIC* alpha-keto isocaproate

[a] Associated with specific exercise program for football players

[b] Associated with specific exercise program for rugby players

some efficacy in animal and human studies. Optimal treatment for cachexia is aimed at maximizing muscle and adipose synthesis while minimizing degradation (Siddiqui et al. 1996).

Bearing this is mind, two main strategies have been followed: (1) to reduce the proliferation and the size of tumor cells and (2) to diminish muscle proteolysis while increasing protein synthesis with nutritional supplementation of HMB and leucine (this topic will be discussed in more detail in the section on mechanisms of action). To exemplify the first, the recent work of Nunes et al. (2008), using adult Walker 256 tumor-bearing Wistar rats, demonstrated that supplementation with 76 mg/kg body weight/day of HMB for 8 weeks induced a lower tumor weight and lower tumor cell proliferation with suppression of the NF-κB signaling pathway. The anti-tumor and anti-cachectic effects of HMB supplementation were accompanied by significant NF-κB inhibition (NF-κB alpha

content) by 100% and suppression of NF-κB p65 subunit content by 17% over untreated tumor-bearing animals. Additionally, Caperuto et al. (2007), using the same animal model, have shown that 320 mg/kg body weight/day of HMB supplementation for 4 weeks increased the survival time of tumor-bearing animals. Finally, in an elegant study by Smith et al. (2005) in mice implanted with the MAC16 tumor, supplementation of HMB was found to be effective in reducing weight loss induced by cancer, but only at doses >0.125 g/kg body weight, which clearly indicates that HMB effects appear to be dose-dependent.

Due to the encouraging results of HMB supplementation in sports, HMB supplementation has been widely adopted in clinical/pathological conditions and in experimental models characterized by high muscle proteolytic rates.

For example, using the suspension model characterized by robust muscle atrophy, Soares et al. (2001) showed that supplementation of HMB during immobilization of hindquarters of adult rats resulted in decreased muscle damage and increased muscle fiber diameter (+6.9%), compared with the non-supplemented group. Additionally, Clark et al. (2000) observed that in individuals infected with the HIV virus, after 8 weeks of supplementation with a mixture of HMB, arginine and glutamine (3 g of HMB + 14 g arginine + 14 g glutamine) divided into two daily doses, the subjects showed a significant gain in lean body mass and an improvement of immune status. This same mixture was tested in patients with cancer for a period of 24 weeks. Within 4 weeks of supplementation, individuals showed a significant gain in lean body mass, which was sustained until the end of the study (May et al. 2002).

Thus, we conclude in accordance with Alon et al. (2002) "the continuation of investigations of supplemental HMB treatment of patients with advanced-stage can potentially lead to discovery of methods to increase the resistance and immunity and thus improve the chances of survival."

Mechanisms of action: possible signaling pathways involved in the control of skeletal muscle mass

Studies investigating the mechanisms by which HMB promotes increases in strength and muscle hypertrophy are relatively scarce and new. Based on these studies, it is postulated that such supplementation would involve the following mechanisms: (1) upregulation of IGF-I gene expression in skeletal muscles, (2) stimulation of protein synthesis by increasing the mTOR signaling pathway, and (3) suppression of proteolysis by the inhibition of the ubiquitin–proteasome system (Fig. 2). Moreover, some studies have pointed out other possible mechanisms that could be involved in HMB actions, as discussed below.

IGF-1 expression in skeletal muscles

In a recent chicken and human myoblasts cell culture study, Kormasio et al. (2009) demonstrated that incubation with HMB (25–100 μg/mL) promoted an increase in thymidine incorporation (an indicator of DNA synthesis) that was 2.5 times greater than in controls. In parallel, the mRNA expression of MyoD (a marker of cell proliferation), which is increased in activated satellite cells, was also augmented, following a dose–response curve. Moreover, the addition of various concentrations of HMB (25–100 μg/mL) to the culture medium for 24 h resulted in a marked increase of myogenin and MEF2 expression (markers of cell differentiation) in these cells, which was also in a dose-dependent fashion. These are secondary myogenic regulatory factors (MRFs) and are related to the differentiation of satellite cells. As a result, there was a significant increase in the number of cells, suggesting a direct action of HMB upon the proliferation and differentiation of myoblasts. The relevance of this action in the whole animal (i.e., in vivo), however, remains to be determined, but in the study of Kormasio et al. (2009), the increase in IGF-1 expression was nearly twofold compared to control untreated cells. In this context, several explanations are possible. For example, transgenic mice overexpressing IGF-1 in skeletal muscles show pronounced muscle hypertrophy, attenuation of age-related atrophy and improvement in strength (Barton-Davis et al. 1998; Fiorotto et al. 2003). Satellite cells were also augmented, and consequently the number of myonuclei, as evidenced by the increase in total DNA content (Fiorotto et al. 2003).

Recent data also point out a stimulatory effect of HMB on the production of hepatic IGF-1. Animals treated with 320 mg/kg body weight/day HMB for 4 weeks showed a significant increase in IGF-1 mRNA expression and, consequently, in serum levels of this hormone, which appears to occur through GH action in the liver (F. Gerlinger-Romero, L. Guimarães-Ferreira, G. Giannocco, M. T. Nunes, unpublished data). IGF-1 produced by both the liver and muscle in response to treatment with HMB would then act on the skeletal muscle in an endocrine, paracrine and autocrine fashion. It is well established that IGF-1 exerts an anabolic action in skeletal muscle, leading to hypertrophy of muscle fibers (Barton-Davis et al. 1998; Fiorotto et al. 2003).

mTOR pathway

The enzyme known as mTOR (mammalian target of rapamycin) is a protein kinase responsive to mechanical, hormonal and nutritional stimuli, with a central role in the

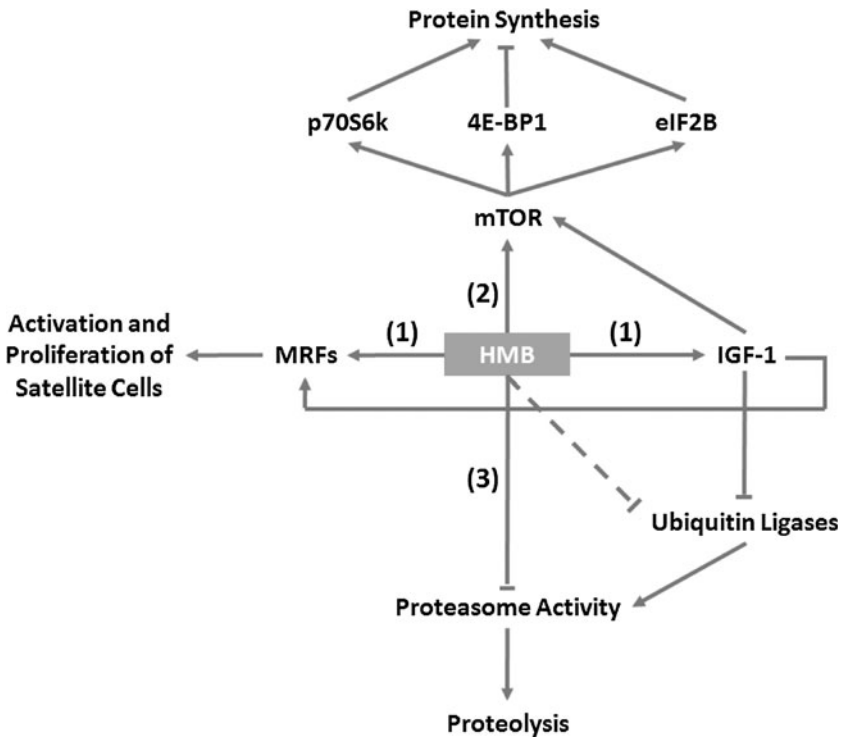

Fig. 2 Mechanisms of action of HMB in the processes of muscle protein synthesis and degradation. Data from: (1) Kormasio et al. (2009); (2) Eley et al. (2007); and (3) Smith et al. (2005)

control of cell growth, primarily by controlling mRNA translation efficiency (Zanchi and Lancha 2008). In this anabolic context, HMB appears to act upon the mTOR pathway by yet unknown mechanisms, increasing the phosphorylation of its protein substrates (4EBP-1 and p70S6K) and resulting in increased myofibrillar protein synthesis. In support of this argument, Eley et al. (2007) observed that in cultures of skeletal muscle cells (C2C12 lineage), incubation with 50 mM of HMB significantly stimulated muscle protein synthesis. This response was positively correlated with an increase in phosphorylation of mTOR and two important substrates of mTOR (4EBP-1 and p70S6K), proteins involved in the increased translation of mRNA and protein synthesis in muscle. Importantly, this stimulating effect was completely abolished in the presence of rapamycin, an mTOR inhibitor. In practical terms, this effect could contribute to the preservation of muscle mass in catabolic situations. Moreover, when used in combination with strength training, HMB supplementation enhances the gain of muscle mass (Nissen et al. 1996). This effect could be mediated through a direct action on the mTOR pathway, and/or by a reduction in the appearance of signs and symptoms associated with chronic muscle damage (Van Someren et al. 2005).

Bodine et al. (2001) demonstrated that hypertrophy of myotubes in vitro induced by IGF-1 was dependent on the pathway initiated by PI3K and Akt, which leads to the activation of mTOR. The targets of this protein are p70S6K and 4E-BP1. Thus, this hormone could promote activation of protein synthesis by stimulating the process of initiation of mRNA translation. Rapamycin, a selective inhibitor of mTOR, blocked the hypertrophy in all experimental models tested, without causing atrophy in the control muscles (Bodine et al. 2001). Thus, further studies are needed to determine if activation of this signaling pathway by HMB occurs as a result of increased expression of IGF-1, direct stimulation of mTOR, leucine or other metabolites (Zanchi et al. 2008).

Ubiquitin–proteasome system

The ubiquitin–proteasome system is a proteolytic system dependent on energy (ATP) and degradation of intracellular proteins whose activity are increased in conditions of exacerbated muscle catabolism, such as fasting, hypogravity, immobilization, bed rest and others (Lecker et al. 2006). Thus, inhibition of this proteolytic system, especially in atrophic situations, could explain the attenuation of muscle protein losses observed during treatment with HMB. In line with this hypothesis, Smith et al. (2005) observed in mice implanted with the MAC16 tumor that HMB supplementation was effective in reducing the muscle proteolysis observed in cancer cachexia, which was reflected in the attenuation of muscle mass loss (as measured in the soleus muscle). Importantly, this effect was positively correlated with a decrease in the catalytic activity of the proteasome.

IGF-1 administration in fasted animals also stimulates the phosphorylation of Akt/PKB, inhibiting the shuttling of FOXO transcription factors to the cell nucleus. Such inactivation inhibits genomic programs activated through FOXO transcription factors in the nucleus, which include decreased of E3 ligases, such as atrogin-1 protein expression (Zanchi et al. 2010). Such enzymes constitute an important class of enzymes involved in the activation and ubiquitination of proteins to be degraded by the proteasome (Hoffman and Nader 2004; Dehoux et al. 2004; Latres et al. 2005). Additionally, the effects of E3 ligases activation was markedly increased in non-treated animals when compared to IGF-1-treated animals. Moreover, the addition of this hormone also inhibited the expression of the ubiquitin-ligase in culture muscle cells incubated with high doses of glucocorticoids (Dehoux et al. 2004). However, again, more studies are needed to determine whether the actions of HMB are direct or mediated by an increased expression of IGF-1. Summarizing, in addition to its anabolic effects, this hormone also seems to play a role in the inhibition of the process of protein degradation via the ubiquitin–proteasome system.

Parallel mechanisms controlling skeletal muscle mass mediated through HMB actions

A recent work by Eley et al. (2008) described a possible new mechanism of action of HMB supplementation. HMB could attenuate protein synthesis depression through modulation of lipopolysaccharides, tumor necrosis factor alpha (TNF-alpha) and angiotensin 2. These agents inhibited protein synthesis through the activation of the protein kinase PKR with subsequent phosphorylation of eukaryotic initiation factor 2 (eIF2) in its alpha subunit, leading to phosphorylation of eukaryotic elongation factor 2 (eEF2). HMB attenuates phosphorylation of eEF2, possibly by increasing the phosphorylation of mTOR as well by suppressing the phosphorylation of eIF2-alpha by preventing activation of PKR. Thus, HMB supplementation could prevent phosphorylation of the protein kinases responsible for inhibiting the elongation step of mRNA translation.

Conclusions

1. Although a meta-analysis by Nissen and Sharp (2003) has indicated that the effects of HMB on strength and lean mass are statistically significant, this review has received some criticism that must be taken into account. The fact that it analyzed only nine studies with HMB supplementation, which are conducted by three distinct groups of researchers, may present a significant bias to the conclusions proposed. Subsequent studies should investigate the reason for the heterogeneity observed in the literature.
2. Another crucial point to be considered is that the proposed mechanisms of action of HMB involves increased sarcolemmal integrity. The inconsistent findings in athletes may be partly explained by the lower degree of muscle damage caused by the exercise stimulus in this population. Thus, considering this aspect, training protocols that are sufficiently intense and accrued should be established to test the effectiveness of HMB on this variable.
3. The vast majority of studies have employed 3 g/day of HMB, grounded in evidence that this dose produces better results than 1.5 g/day and is equivalent to 6 g/day. Further studies are required to validate this hypothesis by varying the dose given, the supplement and the degree of catabolism and adopting different training intensities.
4. The mechanisms of action of HMB must also be explored. Although experiments in vitro and in animal models provided a major breakthrough in this field, human studies are lacking. Considering the great spectrum of action of HMB in modifying the activity and expression of various genes and proteins, deeper use of molecular biology tools could provide new insights into the mechanisms underlying the effects of supplementation.
5. Finally, the therapeutic use of this supplement is quite promising. Additional clinically controlled studies, with important primary endpoints, must be conducted in patients with AIDS, trauma, cancer, malnutrition, and inflammation, among other frameworks of severe catabolism. Likewise, studies should examine the possibility that athletes could benefit from HMB in periods of rehabilitation, during which the loss of lean mass and strength are inevitable.

Studies addressing the efficacy of the combination of supplementation of HMB with other nutrients, leading to potentially increased strength and lean mass, are of great value. In this context, Jowko et al. (2001) submitted healthy volunteers to creatine supplementation, HMB, a combination of these or a placebo. All subjects performed strength training throughout the 3-week trial. The most important findings were related to the higher gain in strength and mass observed in the lean group supplemented with creatine and HMB compared to the other groups. Indicators of muscle damage and protein degradation, such as CPK in serum and urinary urea, were reduced only in the groups supplemented with HMB. These findings led the authors to conclude that the combination of creatine supplementation and HMB results in additive effects, with

different mechanisms of action. From these data, we can suggest that the employment of HMB in combination with creatine is a promising strategy for allowing gains in lean mass and strength. Future studies should examine the effects of this combination in athletes and patients during catabolic settings, to identify the best conditions and the populations that would benefit from this strategy.

Acknowledgments Brazilian Funding Agency (FAPESP—Fundação de Amparo à Pesquisa do Estado de São Paulo); Grant Number: 08/51090-1.

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