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Review Article

Effects of Leucine Metabolite (B-Hydroxy-B-Methylbutyrate) Supplementation and Resistance Training on Cardiovascular Risk Factors, Oxidative Stress, and Inflammatory Markers: a Review on Recent Literature

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Abstract: β-hydroxy β-methylbutyrate (HMB) is a bioactive metabolite formed from breakdown of the branched-chain amino acid leucine. Given the popularity of HMB supplements among different athletes, specifically, those who engage in regular resistance training, this review was performed to summarize current literature on some aspects of HMB supplementation that have received less attention. Because of the small number of published studies, it has not been possible to conclude the exact effects of HMB on cardiovascular parameters, oxidative stress and inflammatory markers. Thus, the interpretation of outcomes should be taken cautiously. However, the data presented here suggest that acute HMB supplementation may attenuate pro-inflammatory response following an intense resistance exercise in athletes. Also, the available findings collectively indicate that chronic HMB consumption in conjunction with resistance training has no more adaptive advantages associated with decreasing cardiovascular risk factors and oxidative stress markers. Taken together, there is clearly a need for further well-designed, longer duration studies to support these findings and determine whether HMB supplementation affects the adaptations induced by resistance training associated with body's inflammatory condition, antioxidative defense system, and cardiovascular risk factors in humans.

Keywords: HMB, Branched-chain amino acid, Strength training, Sports nutrition, Inflammation.

1. Introduction

Some athletes believe that most of the normal diets do not provide sufficient demands for an optimum performance during intensive training and competitions. Dietary supplements are a commonly used strategy to improve exercise performance and recovery and many athletes use them as a part of their regular training or competition routine [1]. Over the last decades, numerous studies have been conducted to identify anabolic nutrients for skeletal muscles.

β-Hydroxy β-methylbutyrate (HMB) is one of the newest and most popular nutritional supplements on the market [2], as one study showed that HMB was the sixth most commonly used supplement in a group of 263 male college football players in the US [3]. HMB is added to many training protocols with hopes of enhanced lean muscle mass and sports performance [4]. Scientific researches during the past 20 years demonstrate that HMB supplementation in conjunction with resistance training may improve body

composition [5-10], muscle strength [5, 7-12] and power [5, 8, 11]. It has also been reported that supplying HMB promotes favorable changes in aerobic [13-15] and anaerobic [9, 13, 16] capacity, and muscle recovery after exercise [10, 17-20] in different athletes. To date, several systematic reviews and meta-analyses have investigated the aforementioned actions of HMB supplementation in a variety of populations [21-27]. However, although recent studies are proposed some other actions of HMB with regard to effects on cardiovascular, oxidative stress, and inflammatory markers, these new aspects have received less attention. Therefore, we aimed to review recent findings in these contexts in a systematic manner.

2. A brief overview of HMB metabolism

HMB is a metabolic by-product of the essential branched-chain amino acid (BCAA) leucine, which has key roles in protein metabolism [28]. Figure 1 shows the different steps in the production of HMB [26, 29]. Following the reversible transamination of leucine to α-ketoisocaproate (KIC) through the enzymatic action of BCAA transferase [27], KIC in the liver can either produce isovaleryl-CoA by the enzyme branched-chain ketoacid dehydrogenase [29] or generate HMB by the cytosolic enzyme KIC dioxygenase [30]. Most amounts of produced KIC are metabolized into isovaleryl-CoA and it has been estimated that only approximately 2-10% of leucine is oxidized into HMB [26, 31]. Normal plasma range of HMB concentrations is 1 to 4 μmol/L, but can increase 5-to 10-fold following leucine administration [32]. Although some foods including citrus fruits, some fish, and breast milk have a mount of HMB [29], it is impractical to provide the typical 3 g daily dosage of HMB used in most previous human studies that demonstrate improvement of body composition [5, 6, 8-10], and muscle strength [5, 8-12]. Therefore, HMB supplementation is a reasonable way for different athletes, specifically, those who participate in resistance training programs to obviate the recommended daily amount of HMB.

There are two commercially available forms of HMB supplement including i) calcium HMB (HMB-Ca), a mono-hydrated calcium salt, and ii) free acid form of HMB (HMB-FA), beta-hydroxy-beta-methylbutyric acid [23, 33]. Depending upon the dose and its ingestion with other additional nutrients, the magnitude and rate of appearance, and clearance rate of HMB following consumption are different. In this context, Vukovich et al. [34] compared two doses of HMB-Ca and found that a 3 g-dose can cause to peak plasma concentrations of HMB 1-h after consumption, while a peak HMB level occurred 2-h after the ingestion of 1 g-dose. Plasma concentrations of HMB and urinary losses with the 3 g-dose were also significantly higher than 1 g-dose (300%, and 14%, respectively). The authors also reported that adding 75 g of glucose to the HMB-Ca dosage may delay peak HMB concentrations by an hour and decrease its magnitude because of slow gastric emptying, or improvement in HMB clearance [34]. However, compared to 1 g of HMB-Ca, the absorption rate of 0.8 g of HMB-FA was higher and it only took 36 min to reach peak plasma concentrations following ingestion [33]. Furthermore, these increases in peak plasma levels were along with a higher plasma clearance rate (25%) compared to HMB-Ca indicating more tissue uptake and utilization [23, 26, 35]. Although these benefits provided more effective and practical effects on muscle recovery after exercise through the greater intra-muscular HMB bioavailability [23, 33, 36], another study reported higher bioavailability of HMB after HMB-Ca intake when compared with an equivalent dosage of HMB-FA [37]. Taken together, the majority of published studies have administered HMB-Ca and further researches are needed in this area.

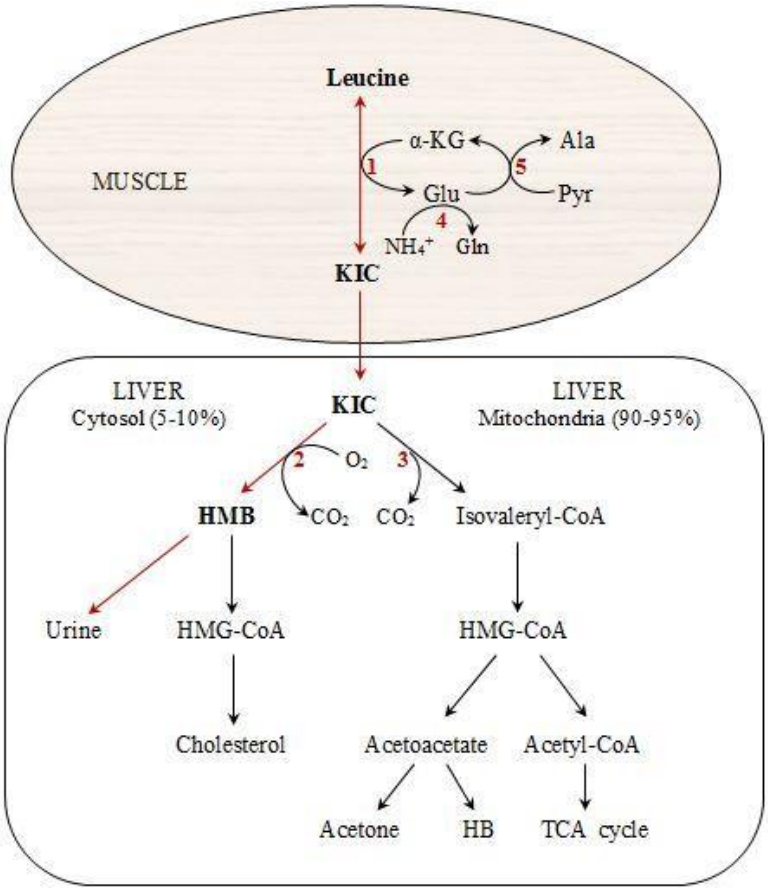

Figure 1. Pathways of HMB metabolism from amino acid leucine. Modified from Nissen and Abumrad [32]. HMB: beta-hydroxy-beta-methylbutyrate, KIC: alpha-ketoisocaproic acid, HB: beta-hydroxybutyrate, HMG-CoA: 3-hydroxy-3-methyl-glutaryl-CoA, Ala: alanine, Pyr: pyruvate, 1: branched-chain amino acid (BCAA) aminotransferase, 2: KIC dioxygenase, 3: branched-chain alpha-keto acid dehydrogenase (BCKAD), 4: glutamine synthetase, 5: alanine aminotransferase.

3. An overview of different HMB effects and its potential mechanisms

We have summarized a number of the most important and beneficial effects of HMB supplementation and its suggested mechanisms of action (Table 1).

4. Effects of HMB on inflammation

HMB improves immune function, especially under stressful conditions. *In vitro*, HMB has been shown to increase lymphocyte blastogenesis in a dose-dependent fashion [32]. In an animal study, Peterson et al. [50] found that HMB enhances nitrite production in macrophages and also antibody production. The favorable effects of HMB supplementation on the number of CD3 and CD8 cells, and human immunodeficiency virus (HIV) load have also been reported [51]. These data were supported by Hsieh et al. [52] that concluded supplementation of HMB at 3 g/day for 7 days may have an anti-inflammatory effect in a group of elderly patients with chronic obstructive pulmonary disease (COPD).

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Table 1. A summary of potential mechanisms proposed for the different beneficial effects of HMB supplementation on skeletal muscle.

Effect	Mechanisms of action
Increasing protein synthesis	• Stimulation of the mTOR signalling pathway: inhibition of MuRF-1 expression, and phosphorylation of FoxO1 and FoxO3a through activation of the PI3K/Akt signalling pathway [38]; increasing the expression of MyoD and MEF2, activation of the MAPK/ERK and PI3K/Akt pathways leading to myogenic cell proliferation [39]. • Stimulation of the GH/IGF-1 axis: increasing the mRNA expression of pituitary GH and serum concentrations of IGF-1 [40].
Decreasing proteolysis (protein breakdown)	• Down-regulation of catabolic signalling pathways including ubiquitin-proteasome and autophagy-lysosome systems: inhibiting proteasome expression, reducing activities of proteasome enzyme, down-regulation of caspases, decreasing the apoptosis of myonuclei [41-44]. • Increasing GH and IGF-1 [45].
Enhancing tissue repair	• Increasing proliferation of satellite cells [39]. • Increasing cholesterol synthesis: HMB acts as a substantial precursor of cell membranes and improves the repair of sarcolemma after contractile activity [46].
Improving excitation-contraction coupling in muscle cells	• Increasing calcium release from sarcoplasmic reticulum (SR) [47].
Improving aerobic capacity	• Increasing the mitochondrial biogenesis and fat oxidation [48]. Increasing the hormone-sensitive lipase (HSL) gene and protein expression in white adipose tissue because of increased GH levels leading to increased lipolysis and thus, more lipid availability [29, 40].
Delaying acute muscle fatigue	• Increasing the content of mitochondrial acetyl-CoA through the conversion of HMG-CoA into acetoacetyl-CoA [31, 32].
Increasing ATP and glycogen content in skeletal muscle	• Accelerating the TCA cycle, increasing malate-aspartate shuttle, and providing needed carbon for glycogen synthesis [49].

It has been well documented that acute exercise, specifically high-intensity exercise, results in increased inflammatory markers [53]. However, regular exercise training exerts anti-inflammatory effects on the blood [54]. The investigation of new strategies for decreasing the body's inflammatory reactions after exercise may provide further insight into improved recovery and subsequent performance. It has been suggested that some pro-inflammatory factors such as interleukin (IL) 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) increase proteolysis and may modulate protein turnover [55]. Because HMB is associated with less proteolysis [41, 43], to date, several researches have focused on unraveling whether or not HMB supplementation affects the inflammatory responses following exercise training. Beneficial effects of acute HMB supplementation on attenuating pro-inflammatory mediators have been reported in trained athletes [56] and untrained individuals [57]. Townsend et al. [56] showed that circulating pro-inflammatory markers TNF-α and monocyte TNF-α receptor 1 (TNFR1) expression on monocytes was elevated during an acute heavy resistance exercise and subsequent recovery in healthy, resistance-trained men. However, HMB-FA supplementation (acute ingestion before and/or after exercise) decreased these mediators immediately after resistance exercise. Supporting these findings, Vulcan [57] investigated the effect of acute supplementation of HMB (before, or before and after exercise) on inflammatory responses following three

sets of 50 eccentric leg extensions on each leg. It was observed a drop in serum concentration of IL-1 receptor antagonist (IL-1ra) and TNF-α for the placebo group, which was attenuated by HMB at 48-h and 72-h post exercise. Although these data provide evidence for a potential blunted or delayed inflammatory response following an acute, intense exercise protocol with acute HMB supplementation, anti-inflammatory effects of HMB were not confirmed by one study that examined if a longer period of HMB administration can influence inflammatory mediators in elite, national team level adolescent volleyball players. In this study, Portal et al. [16] found that 7 weeks consumption of HMB (3 g/day) did not change serum concentrations of IL-6 and IL-1ra during the early phase of the volleyball season. It should be taken into account that the training stimulus in this research was different from that performed by two aforementioned studies [56, 57]. Therefore, more studies with a longer period of HMB supplementation (at least 10 to 12 weeks) are needed to determine the impact of this dietary supplement on inflammatory properties.

5. Effects of HMB on oxidative stress

To the best of our knowledge, the available scientific literature on the effect of HMB supplementation on oxidative stress in humans is still preliminary in nature and it should be taken into more accurate consideration. Although HMB may improve immune function in humans [56, 57], unfortunately, there is only one study [58] that has directly examined the effects of HMB supplementation on oxidative stress responses to exercise training. In a randomized, double-blind, placebo-controlled trial, we investigated the effects of 6-week HMB-FA supplementation on oxidative stress markers in sixteen healthy young males. 8-hydroxy-2-deoxyguanosine (8-OHdG), malondialdehyde (MDA), and protein carbonyl (PC) were measured 48 h before and after resistance training. A significant decrease in MDA and PC was observed in both placebo and HMB groups. However, 8-OHdG did not change after resistance training in any of the groups. Thus, it seems that adding HMB supplement to resistance training had no further adaptive benefits related to oxidative stress markers [58]. Given the limited available data about HMB effects on oxidative stress mediators, more researches examining its effects are warranted. After more well-designed trials of HMB have been performed and its effects on oxidative stress profile have been better defined will it be possible to comment on the effectiveness of HMB as a dietary agent.

6. Cardiovascular effects of HMB

As shown in Figure 1, produced cytosolic β-hydroxy-β-methylglutarate-Co-A (HMG-CoA) from HMB in the cytosol of the liver can then be used for cholesterol synthesis [59]. Thus, HMB action as a precursor for cellular cholesterol synthesis can be important for membrane production during the periods of high muscular stress especially during exercise training and subsequent recovery period. This is known as the Cholesterol Synthesis Hypothesis (CSH) [60]. There are controversial findings about the effect of HMB on some cardiovascular risk factors. For example, different authors have found no change [61], an increase [62], or a decrease [60] in low-density lipoprotein (LDL) or total cholesterol (TC). In a comprehensive study, Nissen et al. [60] summarized data from nine studies in which humans were fed 3 g HMB/day for 3 to 8 weeks and reported that the findings collectively indicate that HMB supplementation has resulted in a net decrease in TC (5.8%), a decrease in LDL cholesterol (7.3%) and a decrease in systolic blood pressure (4.4 mmHg). However, Hsieh et al. [61] concluded that HMB supplementation (2 g/day) for 2 or 4 weeks have no significant effect on serum lipids in bed-ridden elderly men and women. More recently, an animal study examined the effectiveness of a 12-week HMB administration on insulin resistance induced by a high fructose diet in rats. Compared to the control group, HMB significantly enhanced insulin tolerance and decreased fasting insulin, insulin resistance index (HOMA-IR), glycosylated hemoglobin (Hb_{A1c}), hepatic glycogen content, and serum triglycerides (TG), LDL and very low-density lipoprotein (VLDL) [63]. It has also been demonstrated that 2-week consumption of HMB reduces side effects of induced ischemia in rats

through arrhythmias reduction [64]. Generally, these data suggest that HMB supplementation may result in a decrease in the risk of heart attack and stroke.

However, only one study investigated the cardiovascular effects of HMB supplementation in conjunction with exercise training. Arazi et al. [65] examined the effect of HMB-Ca (3 g/day) on cardiovascular risk factors after 4 weeks of resistance training (3 sessions per week) in athletes. After the training period, TC, LDL, and TG were significantly decreased in both groups and diastolic blood pressure was reduced only in the HMB group. However, no significant differences were found between HMB and placebo groups. Because of limited data regarding this topic, it's difficult to declare the certain and exact cardiovascular effects of HMB supplementation when combined with exercise training. Thus, further prolonged investigations are needed to determine these effects.

7. Conclusion

The available data collectively indicate that acute ingestion of HMB before and after resistance exercise can attenuate some circulating pro-inflammatory mediators, which improves the subsequent recovery process. However, more research is needed to support these effects and verify if chronic HMB consumption in conjunction with resistance training has more favorable effects on pro- and anti-inflammatory markers. Although the number of studies examining the interaction effects of HMB and exercise training on inflammation, oxidative stress and cardiovascular parameters are limited, it seems that adding HMB supplement to a resistance exercise protocol did not produce further adaptive advantages. Generally, future researches should be performed to specify the effectiveness of HMB supplementation on inflammatory profile, body's antioxidative defense system and oxidative stress markers, and cardiovascular risk factors, when combined with exercise training.

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