The Effect of Beta-Hydroxy-Beta-Methyl Butyrate in Response to Exercise and High-Fat Diet on Body Weight, Body Composition and Characteristics of Metabolic Syndrome in Male Wistar Rats

Original Research

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Abstract

Introduction: The favorable effect of Beta-hydroxy-beta-methyl butyrate (HMB) on body weight, body composition, and food intake is reported. However, its effect in response to a Western diet and exercise was unclear.

Methods: Wistar rats (n=36) received a regular diet for 4 weeks. Thereafter, they were allocated to three groups and received a high-fat diet for 8 weeks. Group one received HMB (320mg/kg BW /d) and an exercise regimen; group two received HMB with no exercise and group three received a placebo with no exercise.

Bodyweight (BW), body composition, systolic (SBP) and diastolic (DBP) blood pressure, pulse rate, fasting blood glucose (FBG), and blood glucose (BG) response to a glucose load were measured.

Results: BW and food intake were not affected by either HMB supplement or exercise. Body fat was lower in HMB and HMB + exercise compared with the control group at week 12 (P<0.05). The glucose response to glucose preload was lower in HMB and HMB + exercise groups compared with control at week 12 (P<0.05).

Conclusion: The results of this study support the beneficial effects of HMB supplements on body composition and glucose metabolism. However, the HMB supplement did not have any additional effect when combined with an exercise regimen.

Keywords: food intake, glucose, blood pressure

Introduction:

Beta-hydroxy-beta-methyl butyrate (HMB) is a metabolite of leucine, one of three branched-chain amino acids, that has been used extensively by athletes as a dietary supplement. Approximately 5% of leucine is being converted to HMB.¹, ² It is suggested that the beneficial effects of leucine or alpha-ketoisocaproate (KIC) supplementation on performance, protein synthesis, and muscle hypertrophy can be partially attributed to HMB in both humans and rats.³, ⁴ HMB is a multifunctional metabolite involved in protein metabolism, muscle hypertrophy, and insulin activity. There is a wide range of metabolic and physiological properties attributed to HMB including...
promoting higher fat oxidation by activation of gamma co-activator 1-alpha (PGC-1α). HMB may also improve muscle glycogen synthesis by enhancing the insulin effect and amplifying phosphorylation. In addition, HMB may enhance muscle protein synthesis by upregulation of the mammalian target of rapamycin (mTOR). Other mechanisms have also been suggested to explain the ergogenic effects of HMB. Downregulation of protein degradation through the ubiquitin pathway, development of sarcolemma integrity, decreased cell apoptosis, stimulation of the testosterone and growth hormone, and insulin-like growth factor-1 (GH/IGF-1) axis are some of the well-known mechanisms employed by HMB. Although the beneficial effects of HMB on various aspects of metabolism have been shown, these effects may be influenced by other factors including age, gender, exercise regimen (type and duration of exercise), dietary factors (e.g., total calorie intake, macronutrient composition, and other dietary bioactive components), and HMB regimen (dose, timing, and frequency).

The effects of HMB on body weight and body composition are still unclear. The positive effects of HMB on body composition and/or performance during resistance exercise have been shown in previous studies in humans and in rats. Wilson et al. found that HMB-free acid (HMB-FA) ingestion for 12 weeks enhanced resistance training-induced increases in total body mass and fat-free mass and fat mass loss in humans. HMB resulted in increased muscle mass, muscle strength and anaerobic properties, fat-free mass, and peak and mean anaerobic power but had no effect on fatigue index and aerobic fitness or anabolic and catabolic and inflammatory mediators in elite adolescent volleyball players in humans. However, in another study, while HMB increased total body mass, it did not affect fat-free mass after resistance exercise in men.

Anti-catabolic effect of the HMB was found in studies on cancer patients. HMB supplementation reduced the downregulation of protein synthesis in skeletal muscle in response to cachectic stimuli. Moreover, HMB increased survival time and induced important metabolic changes in cancer-bearing rats.

While the beneficial effects of HMB on body weight, body composition, and fat metabolism have received considerable studies, only a few studies examined its effect in response to a high fat, high carbohydrate diet as a typical western diet. In one study, HMB reduced body weight and improved insulin resistance in mice fed a high-fat diet (HFD). Authors suggested that this effect is mediated at least partially by gut microflora. Moreover, as previously mentioned, the exercise regimen determines metabolic response to HMB supplementation. However, to the best of our knowledge, no study examined the interactive effects of HMB, high fat, high carbohydrate diet, and exercise in one single study. Therefore, this study aimed to examine the interactive effects of HMB, diet, and exercise on body weight, body composition, glucose metabolism, and blood pressure in Wistar rats. We hypothesized that HMB supplementation improves body composition, blood pressure and glucose metabolism in rats fed a high-fat diet. Exercise enhances the effect of HMB on body weight, body composition, and glucose metabolism.

Methods

Animals and Diets

Male Wistar Rats (BW: 194.33 ± 4.44 g) were housed individually in ventilated plastic cages with bedding at 22 ± 1 °C and a 12-h light-dark cycle (lights off at 0900 to 2100 h). The diets were provided ad libitum in glass jars. All rats had free access to water throughout the experiment. The regular (AIN-93G) and high-fat (60% FDC) diets were purchased from Dyets (Dyets Inc. Bethlehem, Pa, USA). The composition (per kilogram diet) of the diets is illustrated in Table 1. The protocol was approved by the University of North Florida Institutional Animal Care and Use Committee.

HMB Supplement

Powdered Ca-β-hydroxy-β-methylbutyrate was supplied by Metabolic Technologies, Inc. (Ames, IA, USA). Dosage and delivery methods were determined based on previous studies. A dose of 320 mg/kg BW/d was administered to rats via combination with a food pellet. The calcium salt form of HMB was selected to be used in this study based on previous studies. The dosage of HMB supplementation for each rat was recalculated on a weekly basis (as described earlier, the dosage was based on the body weight). The food pellets containing the HMB were separated into plastic bags labeled with the corresponding rat’s number to ensure the proper dosage was administered. The weekly dosages were stored in the refrigerator.
Experimental design
Newly weaned Wistar rats (n=36) received a regular diet for 4 weeks to become more mature. Thereafter, rats were allocated to three groups (n = 12/group). All groups received a high-fat diet for 8 weeks. Group one received HMB (320mg/kg BW /d) and an exercise regimen; group two received HMB with no exercise and group three received a placebo with no exercise (control group) for 8 weeks. To ensure all the supplements are consumed, the HMB was given by mixing the daily dose with a pellet of food before introducing the rest of the food. Food and water were ad libitum. Bodyweight (BW) was measured weekly for 12 weeks. Systolic (SBP) and diastolic (DBP) blood pressure, pulse rate, fasting blood glucose (FBG), and glucose tolerance tests were measured at weeks 4, 8, and 12. Fat pad mass was measured at killing at week 12. Plasma glucose and insulin were measured at baseline and week 12.

Exercise Protocol
A 5-line rat’s treadmill (Columbus Instrument, 950 North Hague Ave, Columbus, Ohio. Model: EXER-4 Treadmill) was used. The adaptation process to the procedure was implemented for two weeks. Repeated bouts of running sessions (5 minutes/day) with a lower speed (15 m/min) were used when introducing rats to a treadmill for the first time, then the speed was slightly increased throughout training to reach the final speed and time (20 m/min for 30 min). An electrical stimulus was applied to ensure continual exercise (the amp was less than 0.5 amps and was limited to a max of 15 voltage). However, no individual rat received no more than two electrical stimuli in total since they were all good runners. During the intervention, the room temperature was kept around 21 degrees Celsius. The exercise regimen was 30 minutes/day, 5 days per week. The total exercise time (30 minutes) was split into 2 sets of active running for 15 minutes with a 30-minute interval time for rest.

Long-Term Food Intake
Long-term food intake was measured on a weekly basis by weighing the food at the beginning and at the end of each week. Spillage was collected, measured, and subtracted to obtain the actual intake.25

|                        | Regular Diet (AIN-93 G Diet) | High Fat Diet (Diet # 112252) |
|------------------------|------------------------------|-------------------------------|
| Cal                    | 3597 g/kg                    | 3963 g/kg                     |
| Casein                 | 200                          | 200                           |
| Sucrose                | 92.7                         | 68.8                          |
| Soybean oil            | 40                           | 25                            |
| Lard                   | 0                            | 245                           |
| t-Butyhydroquinone     | 0                            | 0.005                         |
| Cornstarch             | 426.79                       | 0                             |
| Dycetrose              | 140                          | 125                           |
| Cellulose              | 50                           | 50                            |
| Mineral Mix            | 35                           | 10                            |
| Calcium Carbonate      | 0                            | 5.5                           |
| Potassium Citrate      | 0                            | 16.5                          |
| Vitamin Mix            | 10                           | 10                            |
| Choline Bitartrate     | 2.5                          | 2                             |
| L-Cystine              | 3                            | 3                             |

Table 1. Composition of the Normal and High-fat Diets
Glucose tolerance test
After 12 h fasting overnight, blood samples were drawn from the tail vein at fasting and 15, 30, and 60 min after a glucose administration (0.375 g glucose per mL, 5 g glucose per kg BW).23

Blood pressure
Systolic (SBP) and diastolic blood pressure (DBP) were measured by a non-invasive tail-cuff method (optical plethysmography) using a tail manometer tachometer system (BP-2000, Visitech system; Apex, NC, USA). After an adaptation process (5-day adaptation period), rats were restrained in holders on a constant warm platform (30 °C). On the day of measurement, five mock measurements preceded a series of ten measurements that were used to calculate the average as reported previously.25

Blood glucose
Tail vein glucose concentration was assessed using test strips using a handheld commercial glucometer (Contour ® Next Blood Glucose Meter, Bayer Healthcare LLC, Mishawaka, IN, USA). The accuracy and variance of the glucometer and test strips were examined by applying control solutions (levels 1 and 2) provided by the manufacturer (Bayer, Bayer Healthcare LLC, Mishawaka, IN, USA).25

Body composition
As previously described25, fat mass and lean mass (fat-free mass) were measured right after killing at the end of the study. Fat mass was measured by dissection of extracted abdominal, epididymal, and perirenal fat. Lean mass was calculated by subtracting body weight from the fat-mass weight.

Statistical analysis
The effect of the HMB supplement and exercise and their interactions on BW, glucose response, SBP, and DBP were analyzed by two-way analysis of variance. When repeated measures were made over time on BW, food intake, SBP, DBP, pulse, BG response, and FBG, the PROC MIXED procedure was used with HMB supplement, exercise, and time as the main factors. When interactions were statistically significant, a one-way analysis of variance followed by a post hoc Tukey’s test was conducted to evaluate treatment effects. The effects of HMB supplementation on plasma measures were compared using a Student's unpaired t-test. BG response was calculated as the total incremental area under the curve (tAUC) of the BG concentration over 1 h after receiving glucose administered for the glucose tolerance test. The homeostasis model assessment of insulin resistance index was calculated as fasting glucose multiplied by fasting insulin divided by 22. Data are expressed as means with standard errors. Statistical significance was defined at P < 0.05. All analyses were conducted using SAS (version 9.4; SAS Institute, Cary, NC, USA).

Results
BW was not affected by either HMB supplement or exercise. However, there was an interactive effect of the treatment and time (p<0.05) (Fig 1).
Similarly, food intake was not affected by either HMB supplement or exercise. However, there was an interactive effect of the treatment and time ($p<0.05$) (Fig 2).

**Fig 1.** Effect of HMB supplement and exercise on body weight (n = 12/group). Data are means ± SEM; BW was analyzed by one-way ANOVA. **HMB**: HMB supplement; **HEX**: HMB + Exercise; **CON**: Control group; **TRT**: Treatment

**Fig 2.** Effect of HMB supplement and exercise on Food Intake (n = 12/group). Data are means ± SEM; BW was analyzed by one-way ANOVA. **HMB**: HMB supplement; **HEX**: HMB + Exercise; **CON**: Control group; **TRT**: Treatment
Body composition was altered by HMB and exercise: Body fat (as total and as a percentage) was lower in HMB and HMB + exercise compared with the control group at week 12 (P<0.05) (Table 2).

**Table 2. Effect of HMB and Exercise on body composition at week 12.**

|                    | HMB          | HMB + Exercise | Control       |
|--------------------|--------------|----------------|---------------|
| Body Wt (g)        | 523.45 ± 15.98 | 511.22 ± 10.21 | 515.26 ± 14.92 |
| Fat (g)            | 19.99 ± 1.73b | 18.90 ± 2.34b  | 25.91 ± 1.89a |
| Fat/Body Wt (%)    | 3.78 ± 0.26b  | 3.71 ± 0.45b   | 5.02 ± 0.27a   |

Data are means ± SEM; (n = 12/group). Different letters in a row are significantly different (p<0.05). The effect of HMB and exercise was analyzed by two-way ANOVA.

While fasting plasma glucose was not affected by either HMB or HMB + exercise, the glucose response to glucose preload was lower in HMB and HMB + exercise groups compared with control at week 12 (P<0.05) (Table 3). The glucose response to administered insulin was not affected by either HMB or exercise. No effect of either HMB or exercise on systolic and diastolic blood pressure and pulse was observed (Table 4).

**Table 3. Effect of HMB and Exercise on fasting blood glucose, glucose response to glucose preload, and glucose response to insulin administration at week 12.**

|                 | HMB | HMB + Exercise | Control       |
|-----------------|-----|----------------|---------------|
| Glucose, mM     | 5.65 ± 0.16 | 5.93 ± 0.19    | 6.04 ± 0.18   |
| OGTT            | 468.75 ± 17.99b | 484.75 ± 15.40b | 564.00 ± 82.38a |
| ITT             | 213.40 ± 13.07 | 230.42 ± 5.98  | 220.83 ± 7.50 |

Data are means ± SEM; (n = 12/group). Different letters in a row are significantly different (p<0.05). The effect of HMB and exercise was analyzed by two-way ANOVA. **OGTT:** Oral glucose tolerance test; **ITT:** Insulin tolerance test.

**Table 4. Effect of HMB and Exercise on systolic and diastolic blood pressure and pulse rate at week 12.**

|               | HMB          | HMB + Exercise | Control       |
|---------------|--------------|----------------|---------------|
| Systolic BP   | 120.5 ± 6.56 | 137.60 ± 5.86  | 127.38 ± 7.61 |
| Diastolic BP  | 73.16 ± 15.58| 92.1 ± 6.69    | 70.65 ± 7.57  |
| Pulse         | 470.16 ± 17.86 | 466.8 ± 6.56  | 444.19 ± 16.02 |

Data are means ± SEM; (n = 12/group). Different letters in a row are significantly different (p<0.05). The effect of HMB and exercise was analyzed by two-way ANOVA. **BP:** Blood pressure.
Discussion

Body weight and body composition:
The results of this study support our hypothesis that HMB supplementation improves body composition as illustrated by enhanced lean mass observed in this study. Moreover, HMB reduced fat mass (as an absolute content and as a percentage of body weight) in this study. The results are consistent with previous studies: In athletes, HMB supplements resulted in an increased lean body mass, muscle mass, and muscle strength. In elderly men, supplementation of HMB combined with a 12-week resistance training program resulted in a decrease in abdominal adiposity. HMB supplementation was also associated with the preservation of muscle mass during a 10-day bed rest period in elderly patients in a randomized control trial. In cancer and AIDS-related wasting syndromes, HMB improved body composition by increasing fat-free mass through slowing protein breakdown and improving protein synthesis. However, these studies included the supplementation of arginine and glutamine along with HMB. Finally, a meta-analysis, concluded that HMB supplementation contributed to the preservation of muscle mass and may be beneficial in the prevention of muscle atrophy related to bed rest. In contrast, a meta-analysis found no beneficial effects of HMB as a supplement on body composition in athletes. This discrepancy can be explained by the differences in study designs implemented in these studies including the length of the study, type of population, exercise regimen, and the dose of the HMB applied in these studies. The majority of studies on HMB were in clinical settings examining its effect on body composition in patients with wasting syndromes which is based on the fact that HMB downregulates glucocorticoid-induced muscle loss. Moreover, a great number of studies tested its effect on muscle synthesis in athletes.

Interactive effects of HMB and exercise on body weight and body composition:
Only a few studies cross-examined the interaction between HMB and exercise. Results from 10 randomized controlled trials (RCTs) examining the interactive effect of HMB supplementation and physical activity in elderly adults showed that there is no additional effect of HMB supplementation over physical exercise on body composition, muscle strength, or physical performance compared to exercise alone. HMB had minimal effect on total body mass gain when combined with an exercise regimen in the elderly, and this effect was not reflected in greater increases in fat-free mass, strength, or decreases in fat mass. Authors suggested the HMB has no beneficial effect on the improvement of body composition or strength when added to the resistance training exercise regimen. These results are consistent with our observation indicating that HMB and exercise had no interactive effect on body composition: Exercise did not have any robust effect on body composition in addition to the effect of the HMB supplementation and vice versa. However, in another clinical study, HMB but not exercise enhanced protein synthesis in older adults only in the first two weeks of the study.

Food intake:
The effect of HMB on food intake is studied. HMB supplementation for 10 days significantly decreased food intake and body weight in mice. The authors suggested that these results can be attributed to an increase in β-hydroxybutyrate since it suppresses food intake and reduces skeletal muscle catabolism. The results of this study did not support any main effect of either HMB supplement or exercise on food intake in rats. However, there was an interactive effect of HMB supplement and time. In another word, the effect of HMB has become more significant over time. It can be suggested the length of this study was not enough long to determine the effect of HMB on food intake.

Glucose metabolism:
HMB altered glucose metabolism in this study. While no effect on plasma fasting glucose was observed, the glucose total AUC was lower in the HMB and HMB + exercise groups compared with the control. However, the results from previous studies are not consistent which can be explained by various study designs: In a clinical study, in younger subjects, coadministration of HMB and insulin resulted in a smaller insulin AUC compared with insulin alone. It did not affect glucose total AUC. In mice, the HMB supplement increased fasting blood glucose and insulin resistance compared with the non-supplemented trained group.

Blood pressure:
Only a few studies examined the role of HMB in the prevention of cardiovascular diseases in general and blood pressure in particular. HMB supplementation reduced total cholesterol and systolic blood pressure but did not affect diastolic blood pressure in hypertensive subjects. The results of this study did not support any effect of HMB on either systolic, diastolic blood pressure, or pulse. A longer study with a higher dosage of HMB supplement might be a better approach for future studies.
**HMB and high-fat diet:**

It is notable that the significant effect of the HMB on body composition and glucose response to glucose preload observed in this study was in rats fed a high-fat diet throughout the study. Similarly, HMB reduced body weight and improved insulin resistance in mice fed a high-fat diet (HFD).\(^{20}\)

**Dose of HMB supplement:**

It has been shown that HMB affects body composition in a dose-dependent manner.\(^{15}\) The dosage of the HMB supplementation implemented in this study (320mg/kg BW /d) and the implementation was based on previous studies.\(^{19-22}\) The recommended dose of HMB in humans is around 3 g per day.\(^{5,34,42-43}\) However, the metabolic rate in rodents is at least 6 times higher than in humans.\(^{6}\) Therefore, the suggested dose in rodents is up to 500 mg/kg BW. However, even 125 mg/kg BW caused a significant reduction in BW loss in mice.\(^{15}\)

**Conclusion:**

These results support our hypothesis that HMB has a favorable effect on body composition and glucose metabolism when rats were on a high-fat diet. However, no effect of either HMB, exercise, or their combination on body weight and blood pressure was observed. Although there is no direct implementation of these results in humans, it may help to have a better understanding of the interactive effects of HMB, exercise, and diet on food intake and characteristics of Metabolic syndrome. A clinical study with a similar study design can be considered as a further step.

As a future direction, studies examining the effect of sex (male vs. female) may help to understand the sex-specific parameters related to the effect of HMB on body weight and body composition in response to a high-fat diet. Moreover, comparing normal-weight subjects vs. obese subjects can help to understand the interactive effect of HMB and obesity on metabolic and physiologic responses to exercise.

The main potential limitation was the duration of the study. A longer study may help to have a better understanding of the physiological properties of HMB in the long term.

This study was reviewed and approved by the University of North Florida Institutional Animal Care and Use Committee (IA 16-007).

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**References**

1. Pinheiro CH, Gerlinger-Romero F, Guimarães-Ferreira L, et al. Metabolic and functional effects of beta-hydroxy-beta-methylbutyrate (HMB) supplementation in skeletal muscle. Eur J Appl Physiol. 2012;112(7):2531-2537. doi:10.1007/s00421-011-2224-5
2. Van Koevering M, Nissen S. Oxidation of leucine and alpha-ketoisocaprate to beta-hydroxy-beta-methylbutyrate in vivo. Am J Physiol. 1992;262(1 Pt 1):E27-E31. doi:10.1152/ajpendo.1992.262.1.E27
3. Wilson GJ, Wilson JM, Manninen AH. Effects of beta-hydroxy-beta-methylbutyrate (HMB) on exercise performance and body composition across varying levels of age, sex, and training experience: A review. Nutr Metab (Lond). 2008;5:1. Published 2008 Jan 3. doi:10.1186/1743-7075-5-1
4. Slater GJ, Jenkins D. Beta-hydroxy-beta-methylbutyrate (HMB) supplementation and the promotion of muscle growth and strength. Sports Med. 2000;30(2):105-116. doi:10.2165/00007256-200030020-00004
5. He X, Duan Y, Yao K, et al. β-Hydroxy-β-methylbutyrate, mitochondrial biogenesis, and skeletal muscle health. Amino Acids. 2016;48(3):653-664. doi:10.1007/s00726-015-2126-7
6. Wilson JM, Fitschen PJ, Campbell B, et al. International Society of Sports Nutrition Position Stand: beta-hydroxy-beta-methylbutyrate (HMB). J Int Soc Sports Nutr. 2013;10(1):6. Published 2013 Feb 2. doi:10.1186/1550-2783-10-6
7. Zanchi NE, Gerlinger-Romero F, Guimarães-Ferreira L, et al. HMB supplementation: clinical and athletic performance-related effects and mechanisms of action. Amino Acids. 2011;40(4):1015-1025. doi:10.1007/s00726-010-0678-0
8. Holeček M. Beta-hydroxy-beta-methylbutyrate supplementation and muscle health in healthy and muscle-wasting conditions. J Cachexia Sarcopenia Muscle. 2017;8(4):529-541. doi:10.1002/jcem.12208
9. Gepner Y, Varanoske AN, Boffey D, Hoffman JR. Benefits of β-hydroxy-β-methylbutyrate supplementation in trained and untrained individuals. Res Sports Med. 2019;27(2):204-218. doi:10.1080/15438627.2018.1533470

10. Portal S, Eliakim A, Nemet D, Halevy O, Zadik Z. Effect of HMB supplementation on body composition, fitness, hormonal profile and muscle damage indices. J Pediatr Endocrinol Metab. 2010;23(7):641-650. doi:10.1515/jpem.2010.23.7.641

11. Wilson JM, Lowery RP, Joy JM, Andersen JC, Wilson SM, Stout JR, et al. The effects of 12 weeks of beta-hydroxy-beta-methylbutyrate free acid supplementation on muscle mass, strength, and power in resistance-trained individuals: a randomized, double-blind, placebo-controlled study. Eur J Appl Physiol 2014;114:1217–1227.

12. Portal S, Zadik Z, Nemet D, et al. The effect of HMB supplementation on body composition, fitness, hormonal and inflammatory mediators in elite adolescent volleyball players: a prospective randomized, double-blind, placebo-controlled study. European Journal Of Applied Physiology. August 2011;111(9):2261-2269.

13. Townsend J, Hoffman J, Gonzalez A et al. Effects of B-Hydroxy-B-Methylbutyrates Free Acid Ingestion and Resistance Exercise on the Acute Endocrine Response. International Journal of Endocrinology 2015; 2015: 1 – 7. DOI: 10.1155/2015/856708.

14. Nissen SL, Sharp RL. Effect of dietary supplements on lean mass and strength gains with resistance exercise: a meta-analysis. J Appl Physiol (1985). 2003;94(2):651-659. doi:10.1152/japplphysiol.00755.2002

15. Smith HJ, Mukerji P, Tisdale MJ. Attenuation of proteasome-induced proteolysis in skeletal muscle by [beta]-hydroxy- [beta]-methylbutyrate in cancer-induced muscle loss. Cancer Res. 2005;65(1):277-283.

16. Eley HI., Russell ST, Baxter JH, Mukerji P, Tisdale MJ. Signaling pathways initiated by beta-hydroxy-beta-methylbutyrate to attenuate the depression of protein synthesis in skeletal muscle in response to cachectic stimuli. Am J Physiol Endocrinol Metab. 2007;293(4):E923-E931. doi:10.1152/ajpendo.00314.2007

17. Baxter JH, Carlos JL, Thurmond J, Rehani RN, Bultman J, Frost D. Dietary toxicity of calcium beta-hydroxy-beta-methyl butyrate (CaHMB). Food Chem Toxicol. 2005;43(12):1731-1741. doi:10.1016/j.fct.2005.05.016

18. Duan Y, Zhong Y, Xiao H, et al. Gut microbiota mediates the protective effects of dietary β-hydroxy-β-methyl butyrate (HMB) against obesity induced by high-fat diets. FASEB J. 2019;33(9):10019-10033. doi:10.1096/fj.201900665RR

19. Kougias D, Nolan S, Juraska J, et al. Regular article: Beta-hydroxy-beta-methylbutyrate ameliorates aging effects in the dendritic tree of pyramidal neurons in the medial prefrontal cortex of both male and female rats. Neurobiology Of Aging. April 1, 2016;40:78-85.

20. Wilson JM, Grant SC, Lee SR, et al. Beta-hydroxy-beta-methyl-butyrate blunts negative age-related changes in body composition, functionality and myofiber dimensions in rats. J Int Soc Sports Nutr. 2012;9(1):18. Published 2012 Apr 18. doi:10.1186/1550-2783-9-18.

21. Vallejo J, Spence M, Brotto M, et al. Cellular and Physiological Effects of Dietary Supplementation with β-Hydroxy-β-Methylbutyrate (HMB) and β-Alanine in Late Middle-Aged Mice. Plos ONE. March 8, 2016;11(3):1-19.

22. Dare L, Dias D, Andreo J, et al. Effect of beta-hydroxy-beta-methylbutyrate in masticatory muscles of rats. Journal Of Anatomy. n.d.;226(1):40-46.

23. Yonamine C, Teixeira S, Nunes M, et al. Beta hydroxy beta methylbutyrate supplementation impairs peripheral insulin sensitivity in healthy sedentary Wistar rats. Acta Physiologica. n.d.;212(1):62-74.

24. Gerlinger-Romero F, Guimarães-Ferreira L, Giannocero G, Nunes M. Chronic supplementation of beta-hydroxy-beta methylbutyrate (HMβ) increases the activity of the GH/IGF-I axis and induces hyperinsulinemia in rats. Growth Hormone & IGF Research. January 1, 2011;21:57-62.

25. Jahan-Mihan A, Labyak CA, Arikawa AY. The effect of characteristics of proteins fed during gestation and lactation on development of metabolic syndrome in dams and male offspring of Wistar rats. Obes Sci Pract. 2017;3(2):224-232. Published 2017 Mar 10. doi:10.1002/osp4.95

26. Portal S, Zadik Z, Nemet D, et al. The effect of HMB supplementation on body composition, fitness, hormonal and inflammatory mediators in elite adolescent volleyball players: a prospective randomized, double-blind, placebo-controlled study. European Journal Of Applied Physiology. August 2011;111(9):2261-2269.
27. Ribeiro Ferreira H, Gill P, Fernandes Filho J, Cláudio Fernandes L. Effects of 12-Weeks of Supplementation with β-Hydroxy-β-Methylbutyrate-Ca (HMB-Ca) on Athletic Performance. Journal Of Exercise Physiology Online. April 2015;18(2):85-94.

28. Stout JR, Fukuda DH, Kendall KL. β-Hydroxy-β-methylbutyrate (HMB) supplementation and resistance exercise significantly reduce abdominal adiposity in healthy elderly men. Experimental Gerontology. 2015 Apr; 64:33-4. DOI: 10.1016/j.exger.2015.02.012.

29. Deutz N, Pereira S, Wolfe R, et al. Randomized control trials: Effect of β-hydroxy-β-methylbutyrate (HMB) on lean body mass during 10 days of bed rest in older adults. Clinical Nutrition. October 1, 2013;32:704-712.

30. May PE, Barber A, D'Olimpio JT et al. Reversal of cancer-related wasting using oral supplementation with a combination of beta-hydroxy-beta-methylbutyrate, arginine, and glutamine. American Journal of Surgery. 2002 Apr;183(4):471-9.

31. Clark RH, Feleke G, Din M et al. Nutritional treatment for acquired immunodeficiency virus-associated wasting using beta-hydroxy beta-methylbutyrate, glutamine, and arginine: a randomized, double-blind, placebo-controlled study. Journal of Parenteral and Enteral Nutrition. 2000 May-Jun;24(3):133-9.

32. Wu H, Xia Y, Niu K, et al. Effect of beta-hydroxy-beta-methylbutyrate supplementation on muscle loss in older adults: A systematic review and meta-analysis. Archives Of Gerontology And Geriatrics. September 1, 2015;61:168-175.

33. Holland BM, Roberts BM, Krieger JW, Schoenfeld BJ. Does HMB Enhance Body Composition in Athletes? A Systematic Review and Meta-analysis. J Strength Cond Res. 2022;36(2):585-592. doi:10.1519/JSC.0000000000003641

34. Szczesniak KA, Ostaszewski P, Fuller JC Jr, Ciecierska A, Sadkowski T. Dietary supplementation of β-hydroxy-β-methylbutyrate in animals - a review. J Anim Physiol Anim Nutr (Berl). 2015;99(3):405-417. doi:10.1111/jpn.12234

35. Cournel-Ibáñez J, Vetrovsky T, Dadova K, Pallarés JG, Steffl M. Health Benefits of β-Hydroxy-β-Methylbutyrate (HMB) Supplementation in Addition to Physical Exercise in Older Adults: A Systematic Review and Meta-Analysis. Nutrients. 2019;11(9):2082. Published 2019 Sep 3. doi:10.3390/nu11092082

36. Jakubowski JS, Nunes EA, Teixeira FJ, et al. Supplementation with the Leucine Metabolite β-hydroxy-β-methyl butyrate (HMB) does not Improve Resistance Exercise-Induced Changes in Body Composition or Strength in Young Subjects: A Systematic Review and Meta-Analysis. Nutrients. 2020;12(5):1523. Published 2020 May 23. doi:10.3390/nu12051523

37. Din USU, Brook MS, Selby A, et al. A double-blind placebo-controlled trial into the impacts of HMB supplementation and exercise on free-living muscle protein synthesis, muscle mass, and function, in older adults. Clin Nutr. 2019;38(5):2071-2078. doi:10.1016/j.clnu.2018.09.025

38. Ikeda K, Takahashi M, Aburaya S, et al. Produced β-hydroxybutyrate after β-hydroxy-β-methyl butyrate (HMB) administration may contribute HMB function in mice. Biochem Biophys Rep. 2021;27:101097. Published 2021 Aug 8. doi:10.1016/j.bbrep.2021.101097

39. Herrod PJJ, Gharahdaghi N, Rudrappa SS, et al. The impact of acute beta-hydroxy-beta-methyl butyrate (HMB) ingestion on glucose and insulin kinetics in young and older men. J Funct Foods. 2020;73:104163. doi:10.1016/j.jff.2020.104163

40. Schadock I, Freitas BG, Moreira IL, et al. Supplementation with beta-hydroxy-beta-methyl butyrate impacts glucose homeostasis and increases liver size in trained mice. Int J Vitam Nutr Res. 2020;90(1-2):113-123. doi:10.1024/0300-9831/a000445

41. Nissen S, Sharp RL, Panton L, Vukovich M, Trappe S, Fuller JC Jr. beta-hydroxy-beta-methylbutyrate (HMB) supplementation in humans is safe and may decrease cardiovascular risk factors. J Nutr. 2000;130(8):1937-1945. doi:10.1093/jn/130.8.1937

42. Gallagher PM, Carrithers JA, Godard MP, Schulze KE, Trappe SW. Beta-hydroxy-beta-methylbutyrate ingestion, Part I: effects on strength and fat free mass. Med Sci Sports Exerc. 2000;32(12):2109-2115. doi:10.1097/00005768-200012000-00022

43. Gallagher PM, Carrithers JA, Godard MP, Schulze KE, Trappe SW. Beta-hydroxy-beta-methylbutyrate ingestion, part II: effects on hematology, hepatic and renal function. Med Sci Sports Exerc. 2000;32(12):2116-2119. doi:10.1097/00005768-200012000-00023