β-Hydroxy-β-Methylbutyrate (HMB) Supplementation in Humans Is Safe and May Decrease Cardiovascular Risk Factors

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ABSTRACT The leucine metabolite, β-hydroxy-β-methylbutyrate (HMB) enhances the effects of exercise on muscle size and strength. Although several reports in animals and humans indicate that HMB is safe, quantitative safety data in humans have not been reported definitively. The objective of this work was to summarize safety data collected in nine studies in which humans were fed 3 g HMB/d. The studies were from 3 to 8 wk in duration, included both males and females, young and old, exercising or nonexercising. Organ and tissue function was assessed by blood chemistry and hematology; subtle effects on emotional perception were measured with an emotional profile test (Circumplex), and tolerance of HMB was assessed with a battery of 32 health-related questions. HMB did not adversely affect any surrogate marker of tissue health and function. The Circumplex emotion profile indicated that HMB significantly decreased (improved) one indicator of negative mood (Unactivated Unpleasant Affect category, $P < 0.05$). No untoward effects of HMB were indicated. Compared with the placebo, HMB supplementation resulted in a net decrease in total cholesterol (5.8%, $P < 0.03$), a decrease in LDL cholesterol (7.3%, $P < 0.01$) and a decrease in systolic blood pressure (4.4 mm Hg, $P < 0.05$). These effects of HMB on surrogate markers of cardiovascular health could result in a decrease in the risk of heart attack and stroke. In conclusion, the objective data collected across nine experiments indicate that HMB can be taken safely as an ergogenic aid for exercise and that objective measures of health and perception of well-being are generally enhanced. J. Nutr. 130: 1937–1945, 2000.

KEY WORDS: • *leucine* • *cholesterol* • *blood pressure* • *toxicity* • *humans*

β-Hydroxy-β-methylbutyrate (HMB)[2] is a common dietary supplement used by many exercise enthusiasts and more recently used in a medically related nutritional product to reduce wasting of muscle tissue in AIDS (Clark et al. 2000, Nissen et al. 1996a, 1996b and 1997, Nissen and Abumrad 1997). The major benefit of HMB appears to be a reduction in muscle damage and/or reduced protein catabolism, which results in improved gains in muscle size and strength when combined with exercise (Nissen et al. 1996b). Although several animal studies have shown that HMB consumption does not cause adverse effects (Nissen et al. 1994a and 1994b, Peterson et al. 1999a and 1999b, Van Koevering et al. 1993 and 1994), until now there has not been a comprehensive analysis of safety data collected on HMB-fed humans.

HMB is a metabolite of the amino acid leucine and is produced endogenously in both animals and humans. The first step in the metabolism of leucine is transamination to α-ketoisocaproate (KIC). HMB is then produced from KIC by the cytosolic enzyme KIC-dioxygenase (Sabourin and Bieber 1983) and, at least in pigs, is produced exclusively from leucine (Van Koevering and Nissen 1992). Plasma concentrations of HMB normally range from 1 to 4 μmol/L, but can increase 5- to 10-fold after leucine is fed (Nissen and Abumrad 1997). The cytosolic dioxygenase enzyme has been characterized extensively and differs from the mitochondrial KIC-dehydrogenase enzyme in that the dioxygenase enzyme is a cytosolic enzyme, whereas the dehydrogenase enzyme is found exclusively in the mitochondrion (Sabourin and Bieber 1981 and 1983). Recently, the KIC-dioxygenase enzyme was found to be identical to the tyrosine dioxygenase enzyme (Janskiewiez et al. 1996). It has been calculated that, under normal conditions, ~5% of leucine oxidation proceeds via this pathway (Van Koevering and Nissen 1992).

Numerous biochemical studies have shown that HMB is a precursor of cholesterol (Bloch 1944, Rabinowitz 1955, Rudney 1957, Zabin and Bloch 1951). HMB in the cytosol of liver and muscle is first converted to cytosolic β-hydroxy-β-methylglutarate-Co-A (HMG-CoA), which can then be used for cholesterol synthesis (Rudney 1957). Thus HMB can serve as a precursor for cellular cholesterol synthesis especially in tissues such as muscle that rely on de novo synthesis of cholesterol. The working theory for HMB action is that stressed or damaged muscle cells may not be able to make sufficient HMG-CoA to support adequate cholesterol synthesis for cell functions, including proper functioning of cell membranes.

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[2] Abbreviations used: BUN, blood urea nitrogen; CPK, creatine phosphokinase; GGT, γ-glutamyl transpeptidase; HMB, β-hydroxy-β-methylbutyrate; HMG-Co-A, β-hydroxy-β-methylglutarate-Co-A; KIC, α-ketoisocaproate; LDH, lactate dehydrogenase; MCH, mean corpuscular hemoglobin; MCHC mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; SGOT, aspartate aminotransferase; SGPT, alanine aminotransferase.

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TABLE 1

Subject demographics and study descriptors

	Study number								
	1	2	3	4	5	6	7	8	9
Gender	Male	Male	Female	Male	Male/Female	Female	Male/Female	Male/Female	Male
Type of exercise	Weight lifting	Weight lifting	None	Weight lifting	Running	Weight lifting	Weight lifting	Weight lifting	Weight lifting
Age range, *y*	19–30	18–22	20–41	18–38	21–47	19–47	63–81	62–79	18–29
Placebo, *n*	13	15	19	18	6	18	18	16	11
HMB,[1] *n*	15	10	18	21	8	18	18	13	7
Study length, *wk*	3	7	4	4	5	4	8	8	8
Site[2]	ISU	ISU	ISU	ISU	ISU	ISU	ISU	WSU	BSU

[1] Abbreviation: HMB, β-hydroxy-β-methylbutyrate.
[2] ISU represents Iowa State University, Ames, IA. WSU represents Wichita State University, Wichita, KS. BSU represents Ball State University, Muncie, IN.

Therefore, supplemental HMB could be a convenient source of HMG-CoA in these cells to maintain adequate cholesterol synthesis and, in turn, plasma membrane function. This contention is supported by the observation that supplementation of HMB can markedly decrease muscle damage as evidenced by leaking of creatine phosphokinase (CPK) out of muscle cells (Cheng et al. 1998, Nissen et al. 1996b, Nissen and Abumrad 1997). Also supporting this concept are several studies showing that inhibition of cholesterol synthesis in muscle with drugs can result in muscle damage (Pierno et al. 1995), poor function (Bastiaanse et al. 1997, Yeagle 1991) and even muscle cell death (Mutoh et al. 1999).

Supplemental HMB is usually taken by humans at a dosage of ~3 g/d. Therefore, the objective of this study is to report the safety-related data collected on HMB given in a dose of 3 g/d over a series of nine experiments. These experiments encompassed the young, the old, men and women, exercising and nonexercising subjects, and were from 3 to 8 wk in duration. In each of these studies, three batteries of tests were used to determine safety. First, comprehensive blood work was completed at regular intervals during each study. Second, the Circumplex test of emotion (Russell 1980) was given periodically during each study; and third, an adverse events questionnaire was filled out at intervals during each study. Together, these measurements should indicate the safety and tolerance of HMB in the general population.

SUBJECTS AND METHODS

Subjects. Each study was approved by a local Institutional Review Board and signed informed consents were obtained from the subjects before participation in the respective study. Each study was conducted as a double-blind, placebo-controlled study. Exclusion criteria were ongoing chronic disease processes, smoking, illegal drug use, pregnancy and certain age and sex requirements, depending on the study.

Blood sampling. Blood samples were collected from a superficial forearm vein into Vacutainers (Becton Dickinson, Vacutainer Systems, Rutherford, NJ) after an overnight fast by the subjects. Both serum and whole blood were collected. A plasma sample was also collected, frozen and stored at −20°C until analyzed for HMB concentration by gas chromatography-mass spectrometry (Nissen et al. 1990b). The serum samples were processed the day of collection and analyzed for CPK by kinetic analysis (Integra 700 Analyzer, Roche Diagnostics, Indianapolis, IN). The following were determined using an automated blood chemistry analyzer (Olympus 5231, Olympus, Melville, NY): sodium, potassium and chloride electrolytes (ion selective electrode); lactate dehydrogenase, plasma creatinine, alkaline phosphatase, γ-glutamyl transpeptidase (GGT), aspartate aminotransferase (SGOT) and alanine aminotransferase (SGPT) by kinetic analysis; calcium, phosphorous, iron, bilirubin, protein and albumin by colorimetric methods; glucose, uric acid, blood urea nitrogen, total cholesterol, HDL cholesterol and triglycerides by enzymatic methods. LDL and VLDL were then calculated using the Friedewald formula (Friedewald et al. 1972) (Labcorp, St. Louis, MO). The whole-blood samples were also processed on the day of collection by Labcorp, and analyzed for hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, and red and white blood cell numbers using an automated cell counter (Coulter STKS, Coulter, Fullerton, CA).

Emotional profile. Weekly emotional profiles were measured by administration of the Circumplex test of emotion (Russell 1980). This test consisted of 48 words that describe various emotions. Each word was scored by the subject from 1 to 5 with the degree of feeling that the word evoked: 1-very slightly or not at all; 2-a little; 3-moderately; 4-quite a bit; and 5-extremely. Groups of 6 words that characterized an emotional category were then summed into the following categories: *1*) High Activation (aroused, astonished, stimulated, surprised, active and intense); *2*) Activated Pleasant Affect (enthusiastic, elated, excited, euphoric, lively and peppy); *3*) Unactivated Pleasant Affect (relaxed, content, at rest, calm, serene and at ease); *4*) Pleasant Affect (happy, delighted, glad, cheerful, warmhearted and pleased); 5) Low Activation (quiet, tranquil, still, inactive, idle and passive); 6) Unactivated Unpleasant Affect (dull, tranquil, still, inactive, idle and passive); *7*) Unpleasant (unhappy, miserable, sad, grouchy, gloomy and blue); 8) Activated Unpleasant Affect (distressed, annoyed, fearful, nervous, jittery and anxious). A range of values between 6 and 30 were possible for each category.

Adverse events. Weekly adverse events were measured by filling out a questionnaire. This questionnaire examined common complaints related to major organ systems. Subjects were asked if they experienced any of these symptoms over the last 3 d. In most studies, the questionnaires were given weekly.

General study protocols. **Table 1** summarizes the basic subject characteristics and protocols used during the individual studies. Studies are referred to by study numbers one through nine. All of the studies were conducted as randomized double-blind, placebo-controlled studies in which neither the researchers nor the subjects knew which treatment contained the HMB. The subjects were informed as to the purpose of each study in the informed consent statement. In all but Study 3, the purpose of the study was to assess the effects of HMB on exercising subjects. In Study 3, the purpose was only to collect safety data and no exercise was included. In each study, HMB was administered as the calcium salt of HMB, $Ca(C_5H_{10}O_3)_2 \cdot H_2O$. This compound contained ~13% calcium; when consumed at 3 g/d, this amounted to ~400 mg/d of additional calcium in the HMB-supplemented subjects. In Studies 1–3, HMB was administered as the

calcium salt mixed in either orange juice or a protein shake. In Studies 4–9, capsules containing the HMB or placebo (rice flour) were used. Potassium phosphate (KH_2PO_4) was added to the HMB supplement capsules to partially maintain the Ca/P ratio. This amounted to ~135 mg of P and ~170 mg of K per day. The RDA for Ca, P and K are 1000, 1000 and 3500 mg/d, respectively (NRC 1989). The placebo treatments were not balanced for any of these minerals.

Compliance with the treatment regimens was assessed by the use of plasma HMB levels and by written questionnaires. In all studies, samples of plasma were analyzed for HMB, which increases several fold after HMB is taken (Nissen and Abumrad 1997). In addition, written questionnaires were given at the end of Studies 1 and 2, asking the subjects to report their compliance in taking the treatments. In Study 3, subjects were given weekly questionnaires and asked to report any noncompliance to the treatments; in Studies 5 and 7, the subjects were asked to record each time a dosage of either the placebo or HMB treatment was taken. In the other studies, compliance was assessed by plasma HMB only.

Study 1. This study was conducted at Iowa State University. Subjects were assigned randomly to either a placebo (0 g HMB/d) or HMB (3.0 g HMB/d) treatment. Neither the subjects nor the researchers knew which treatment contained HMB; two equal dosages per day in 0.47 L of orange juice (one 16-oz bottle) were administered. The subjects were instructed to drink one bottle of juice in the morning and another bottle of juice in the evening. The subjects then underwent a supervised, resistance-exercise program 3 times/wk for 3 wk and alternated exercising either the upper or lower body during each exercise session. A blood sample was drawn from fasting subjects on Thursday and Friday of each week. The Circumplex survey and the adverse events questionnaire were given weekly.

Study 2. This study was conducted at Iowa State University in healthy male athletes who were subjected to stringent exercise schedules over the 7-wk study. Subjects were assigned randomly to either a placebo or HMB treatment. The HMB treatment consisted of 3 servings/d of a protein drink mix, each containing 1 g of HMB for a total of 3 g HMB/d. Similarly, the placebo treatment consisted of 3 servings of an isocaloric carbohydrate drink mix without HMB. Subjects were instructed to drink one serving with each meal and neither the subjects nor the researchers knew which drink contained the HMB supplement. Subjects exercised a total of 6 d/wk with resistance exercise on 5 d/wk. Blood samples were obtained from fasting subjects on d 0, 17, 31 and 49 of the study. The Circumplex survey and the adverse events questionnaire were given on the blood sampling day.

Study 3. This 4-wk study was conducted at Iowa State University and was designed to document the safety of HMB when consumed by women. The subjects enrolled into the study were instructed to maintain any exercise program they were currently involved in and not to start any new exercise programs during the study. Subjects were assigned randomly to receive either a placebo (0 g HMB/d) or HMB (3 g HMB/d) treatment. The placebo and HMB were administered twice daily in equal dosages mixed in ~180 mL of orange juice. Neither the subjects nor the researchers knew which drink contained HMB. The subjects were instructed to consume one dosage of the juice in the morning and one dosage in the evening. Blood samples were taken after an overnight fast on d 0, 5, 10, 25 and 28. Subjects also filled out Circumplex and adverse event questionnaires on the blood sampling day.

Study 4. In this study, conducted at Iowa State University, the effect of prior training on the effects of HMB supplementation was examined in young males. Study participants were classified as trained if they currently participated in a regular resistance exercise program at least 3 times/wk. Subjects were classified as untrained if they had not participated in any regular weight lifting for at least 6 mo before the study. Subjects were assigned randomly to receive either a placebo (0 g HMB/d) or HMB (3 g HMB/d) treatment administered in capsules. Again, neither the subjects nor the researchers knew which capsules contained HMB. The placebo capsules (0 g HMB/d) contained rice flour, whereas each HMB capsule contained 250 mg of calcium HMB and 50 mg of KH_2PO_4. The subjects were instructed to take either four placebo or four HMB capsules 3 times/d with meals, which supplied either 0 or 3 g HMB/d. All subjects then underwent a supervised, resistance-training regimen 3 times/wk during the 4-wk study. Blood samples were obtained from fasting subjects at the beginning and again at the end of the study. Circumplex and adverse events questionnaires were given weekly during the study.

Study 5. This study was conducted at Iowa State University with male and female runners. Subjects were paired according to their best two-mile run time and past running experience and then were assigned randomly to either a placebo (0 g HMB/d) or HMB (3 g HMB/d) treatment administered in capsules as described for Study 4. Neither the subjects nor the researchers knew which capsules contained HMB. Subjects continued to train and were instructed to take four capsules, either placebo or HMB, 3 times/d with their meals. The supplements were taken for 5 wk and all subjects were given a log to record the day and the times they took their supplement. These logs were used to verify compliance in taking the supplement. Blood samples were obtained from fasting subjects at wk 0 and 5 of the study. The Circumplex survey and the adverse events questionnaire were given weekly.

Study 6. This 4-wk study was conducted at Iowa State University and was similar to Study 4 except that women subjects were tested. As in Study 4, similar criteria were used to classify the subjects as either trained or untrained. Subjects were then randomly administered either a placebo (0 g HMB/d) or HMB (3 g HMB/d). Treatment was as described for Study 4 and neither the subjects nor the researchers knew which capsules contained HMB. Fasting blood samples were obtained at wk 0, 2 and 4 of the study. The Circumplex survey and the adverse events questionnaires were given on d 0, 7, 15, 20, 23 and 28 of the study.

Study 7. This study was conducted at Iowa State University in elderly subjects who were assigned randomly to either a placebo (0 g HMB/d) or HMB (3 g HMB/d) treatment for 8 wk. Neither subjects nor researchers knew which capsules contained HMB; treatments were administered as described for Study 4. All subjects underwent strength training 3 times/wk for the duration of the study. Blood samples were obtained from fasting subjects at wk 0, 4 and 8 of the study. The Circumplex and the adverse events questionnaires were again given weekly.

Study 8. This study was similar to Study 7 but was conducted at Wichita State University (Wichita, KS). Elderly subjects were assigned randomly to either a placebo (0 g HMB/d) or HMB (3 g HMB/d) treatment for 8 wk. Neither subjects now researchers knew which capsules contained HMB; treatments were administered as described for Study 4. Subjects participating in this study had no contraindications to exercise and had their physicians approval to participate. Before the initiation of the study, subjects had no experience with resistance training. Subjects underwent strength training 2 times/wk on nonconsecutive days. On the three other days during the week, subjects reported to an indoor track for walking and stretching, which consisted of 10 min of warm-up stretching, 40 min of self-paced walking and then another 10 min of stretching for a cool-down period. Blood samples were obtained from fasting subjects at wk 0, 2, 4, 6 and 8 of the study. The Circumplex and the adverse events questionnaires were given weekly.

Study 9. This 8-wk study was conducted at Ball State University (Muncie, IN) with untrained college-aged males. Subjects were matched on the basis of body weight and then assigned randomly to receive either a placebo [0 mg HMB/(kg body weight · d)] or HMB [38 mg HMB/(kg body weight · d)] treatment. These dosages were chosen because they are equivalent to either 0 or 3 g HMB/d in a person of average body weight. Subjects and researchers did not know which treatment contained HMB. Subjects underwent a resistance training program 3 times/wk during the study. Blood samples were taken at wk 0, 1, 2, 4 and 8 after an overnight fast. The Circumplex and the adverse events questionnaires were again given weekly.

Statistical analysis. For blood, hematology and Circumplex data, for which multiple samples or questionnaires were taken over the course of the study, the net change in variables was estimated by regressing the measured values vs. week of study and calculating the net change over the study for each subject. Because day-to-day variations in measurement occur, this linear estimate of change better reflects the overall change due to treatment. For the Circumplex data, the differences (in slopes) were analyzed by using a Student's *t*

TABLE 2

Effect of β-hydroxy-β-methylbutyrate (HMB) on the emotional profile of humans in a summary of 9 studies[1]

Emotional category	Descriptive words/category		Screen	Delta	Net change due to HMB
High activation	Aroused, astonished, stimulated, surprised, active, intense	Control	12.3	−1.34	−0.25
		HMB	12.1	−1.59	
Activated pleasant affect	Enthusiastic, elated, excited, euphoric, lively, peppy	Control	14.9	−2.20	0.11
		HMB	14.2	−2.10	
Unactivated pleasant affect	Relaxed, content, at rest, calm, serene, at ease	Control	18.8	−3.28	−0.36
		HMB	18.5	−3.64	
Pleasant affect	Happy, delighted, glad, cheerful, warmhearted, pleased	Control	18.5	−2.83	0.28
		HMB	17.5	−2.55	
Low activation	Quiet, tranquil, still, inactive, idle, passive	Control	13.5	−1.72	−0.62
		HMB	14.0	−2.34	
Unactivated, unpleasant affect	Dull, tired, drowsy, sluggish, bored, droopy	Control	10.3	−0.23	−1.04*
		HMB	11.0	−1.27	
Unpleasant	Unhappy, miserable, sad, grouchy, gloomy, blue	Control	7.5	0.18	0.05
		HMB	7.7	0.23	
Activated unpleasant affect	Distressed, annoyed, fearful, nervous, jittery, anxious	Control	8.7	0.61	−0.12
		HMB	8.9	0.74	

[1] Results presented are means of the screening and change over the studies to the Circumplex emotional profile ($n = 127$ placebo and $n = 129$ HMB). Each word of the profile was scored from 1 (very slightly or not at all) to 5 (extremely) according to how the subject felt towards that word. Category scores were then calculated by adding the scores of the 6 words in each category (6–30 points possible in each category).

* Indicates a significant effect ($P < 0.05$) across all experiments as determined by a Student's t test.

test. For the blood and hematology data, the changes were analyzed using ANOVA (SAS, Cary, NC) with the main effects of experiment, treatment and experiment × treatment interaction in the model. The adverse events data were analyzed as categorical data (Agresti 1990). For statistical analysis, the subjects were categorized by before (yes or no) and after treatment response (any yes = yes, all no = no) and statistical significance between treatments determined using the Cochran-Mantel-Haenszel test (Agresti 1990). In the case of Study 8, "before" treatment questionnaires were not given; therefore, the subjects were divided into groups of either a "Yes" or "No" answer during the treatment period and statistical significance was determined by Fisher's Exact test (Agresti 1990). Statistical significance was determined for $P \leq 0.05$. A trend was determined to be $0.05 \leq P \leq 0.10$ and no statistical significance was determined for $P > 0.10$.

RESULTS

Compliance. Supplementation compliance was checked by measuring plasma levels of HMB after supplementation. In addition, in Studies 1, 2, 3, 5 and 7, a self-reported record of compliance was also obtained from the subjects. Basal plasma levels of HMB were between 1 and 4 μmol/L and rose to between 17 and 25 μmol/L after an overnight fast in subjects consuming 3 g CaHMB/d (Nissen and Abumrad 1997). Although this is indicative of compliance only immediately before the sampling time, this method does give some indication of overall compliance during the studies. On the basis of both the written responses from the subjects and plasma HMB levels measures, no subjects were dropped from the analyses for noncompliance.

Circumplex emotional profile. The Circumplex Model of Affect questionnaire was administered in all of the studies, and the summary of the results is shown in **Table 2**. The only significant change measured indicated a decrease ($P < 0.05$) in the category "Unactivated Unpleasant Affect," which is described by the words dull, tired, drowsy, sluggish, bored and droopy.

Adverse effects. None of the questions asked had responses that led to a significant treatment effect (**Table 3**). Analysis of the data showed no significant differences in either the pretreatment or treatment period categories. A trend was indicated for a decrease in the incidence of loss of appetite ($P < 0.07$); however, this difference was < 1.5%. A trend was also indicated for a difference in stiff joints reported ($P < 0.08$). However, because the statistical model also took into consideration starting values, the indication was that a difference in incidence in the starting populations existed rather than a treatment effect.

Blood chemistry, hematology and pressure. Blood lipid profiles of all subjects and those subjects with total cholesterol levels either >5.17 or <5.17 mmol/L (200 mg/dL) are shown in **Table 4**. No significant differences existed in any of the starting values except for triglycerides in the subjects whose total cholesterol was > 5.17 mmol/L. Significant decreases of 3.7% ($P < 0.03$) for total cholesterol and 5.7% ($P < 0.05$) for LDL cholesterol were observed in the subjects consuming 3 g HMB/d. Because of the health risk of high levels of cholesterol, the subjects were subclassified into two groups, those subjects having a total cholesterol level < 5.17 mmol/L (200 mg/dL) and those subjects having a total cholesterol level > 5.17 mmol/L. The average starting values for these groups were 4.27 mmol/L in the low cholesterol group and 5.72 mmol/L in the high cholesterol group. Although significant decreases in total cholesterol were observed in both groups, the difference was only 2.5% ($P < 0.04$) in the low cholesterol group, whereas in the high cholesterol group, there was a 5.8% decrease ($P < 0.03$) or −0.334 mmol/L (12.9 mg/dL). The decrease in total cholesterol can be attributed to a decrease in LDL cholesterol, which declined by 4.2% ($P < 0.09$) and 7.3% ($P < 0.01$) in the low and high cholesterol groups, respectively.

HDL cholesterol did not change in the HMB-fed subjects but increased ~4% in the placebo group ($P < 0.04$ placebo vs. HMB). The high risk subgroup had no significant change in HDL cholesterol.

Resting blood pressures were measured in seven of the nine studies and the combined results are also shown in Table 4. In the studies, supplementation with HMB resulted in a significant decrease in systolic blood pressure of 4.4 mm Hg (P

TABLE 3

Summary of adverse events questionnaires given during 9 studies in which subjects consumed 3 g/d of β-hydroxy-β-methylbutyrate (HMB)[1]

Characteristic	Control		HMB		Treatment effect[2]
	Initial incidence, %	Treatment period incidence, %	Initial incidence, %	Treatment period incidence, %	
Stomachache	2.7	3.7	4.6	3.3	0.54
Nausea/Vomit	1.4	2.9	1.3	2.3	0.54
Dizziness	1.4	6.0	1.3	4.1	0.42
Coughing	12.8	8.3	13.9	11.0	0.66
Wheezing	2.0	1.6	4.0	5.2	0.51
Chest pain	0.7	1.0	2.0	1.1	0.47
Weakness	4.7	4.4	4.0	4.1	0.27
Increased headache	10.1	6.4	9.3	8.0	0.71
Negative mood[3]	7.0	2.4	1.8	3.6	0.38
Rash	1.4	2.1	2.0	0.8	0.33
Dry scalp/Hair[3]	4.0	3.5	2.8	6.4	0.67
Dry skin	4.7	3.6	6.0	8.5	0.24
Nail changes	0.7	0.7	0.7	1.5	0.50
Ear pain	0.7	1.5	1.3	1.6	0.46
Decreased memory[3]	1.0	1.3	0.9	1.3	0.36
Itching[4]	3.3	2.8	3.2	4.3	0.63
Swelling[4]	0.8	1.7	1.6	2.1	0.82
Diarrhea[4]	5.8	4.7	4.8	2.4	0.15
Stiff joints[4]	11.7	12.4	4.0	9.3	0.08
Nose bleeds[4]	0.8	0.7	1.6	0.9	0.81
Heart burn[4]	3.3	4.1	4.0	2.2	0.85
Numbness[4]	0.8	2.2	0.0	1.9	0.42
Nasal congestion[4]	20.8	14.8	10.3	14.9	0.26
Ringing in ears[4]	4.2	7.7	6.3	9.1	0.80
Increased stress[3]	11.9	6.9	3.7	4.9	0.19
Decreased libido[5]	0.0	1.7	0.0	0.6	0.93
Constipation[4]	1.7	2.2	2.4	3.5	0.88
Shortness of breath[4]	2.5	1.7	2.4	3.9	0.79
Loss of appetite[4]	3.3	3.4	4.0	1.9	0.07
Loss of energy[4]	7.5	5.6	4.8	5.8	0.61
Blood in urine[4]	0.8	0.2	0.8	0.2	1.00
Blood in stool[4]	0.0	0.2	0.0	0.2	0.98

[1] Means of the percentage of reported incidents during all studies in which the question was asked (n = 136 placebo and n = 133 HMB). Subjects were asked to fill out the questionnaires on a weekly basis. All studies have initial starting questionnaire values except for Study 8 in which case only the treatment period incidence is included in the means.
[2] Cochran-Mantel-Haenszel statistic for probable differences between the treatment groups.
[3] Question asked only in Studies 4 through 9 (n = 89 placebo and n = 91 HMB).
[4] Question asked only in Studies 3 through 9 (n = 108 placebo and n = 108 HMB).
[5] Question asked in all but Study 3 (n = 117 placebo and n = 116 HMB).

< 0.05 placebo vs. HMB). When subjects were stratified into high and low risk, the subjects with a systolic pressure of > 130 mm Hg had an even greater decrease in systolic pressure, but the net difference between the HMB and placebo groups remained at 4.9 mm Hg. Diastolic blood pressures were not affected by HMB supplementation.

Blood indicators of liver function are summarized in **Table 5**. There were no significant differences between the placebo and HMB groups for any of the measured indicators [bilirubin, alkaline phosphatase, lactate dehydrogenase (LDH), SGOT, SGPT, GGT, and iron]. There were significant experiment by treatment interactions for LDH, GGT, and iron. In the case of LDH, this effect was likely due to the variation in response to the exercise component in the different studies because plasma LDH can also be derived from damaged muscle.

General blood chemistries are shown in **Table 6**. No significant differences between the placebo and HMB groups were seen for CPK, glucose, uric acid, blood urea nitrogen (BUN), creatinine, BUN/creatinine, sodium, chloride, phosphorous, protein, albumin and globulin. A small but significant decrease (1.9%, $P < 0.003$) in potassium, the primary intracellular electrolyte, was seen. Additionally, there was a 5% increase ($P < 0.03$) in the albumin/globulin ratio in the HMB group. A significant experiment × treatment effect was also seen for the albumin/globulin ratio because a response did not occur in all experiments. Similarly, significant experiment × treatment effects were observed for BUN, BUN/creatinine, protein and globulin, which resulted from the fact that the responses between the placebo and treatment groups differed from experiment to experiment with no particular pattern.

The only significant effect of HMB on blood hematology was a 0.48% relative decrease ($P < 0.05$) in hematocrit across all studies (**Table 7**). The initial values and final values for hematocrit, however, were not significantly different between the placebo and HMB groups. Final values for hematocrit were 0.43 for both placebo and HMB groups. There were no significant experiment × treatment interactions for any of the hematology variables measured.

TABLE 4

Effect of 3 g/d supplemental β-hydroxy-β-methylbutyrate (HMB) on blood lipid profiles and blood pressure (BP) in a summary in humans in 9 clinical studies[1]

	All subjects					Lower risk subjects					Higher risk subjects				
	Screen	Delta	Change %	HMB effect[2]	Exp × HMB[2]	Screen	Delta	Change %	HMB effect[2]	Exp × HMB[2]	Screen	Delta	Change %	HMB effect[2]	Exp × HMB[2]
						Subjects with cholesterol < 5.17 mmol/L					*Subjects with cholesterol ≥ 5.17 mmol/L*				
Total cholesterol, *mmol/L*															
Control	4.68	−0.034	−0.7			4.24	0.057	1.3			5.72	−0.036	−0.6		
HMB	4.76	−0.176	−3.7	0.03	0.92	4.29	−0.106	−2.5	0.04	0.46	5.72	−0.334	−5.8	0.03	0.21
HDL cholesterol, *mmol/L*															
Control	1.14	0.049	4.3			1.09	0.052	4.7			1.26	0.028	2.3		
HMB	1.17	0.008	0.7	0.04	0.57	1.13	0.003	0.2	0.06	0.77	1.30	0.003	0.2	0.53	0.59
VLDL cholesterol, *mmol/L*															
Control	0.53	−0.009	−1.7			0.50	0.011	2.3			0.64	−0.070	−10.9		
HMB	0.54	−0.016	−3.0	0.79	0.36	0.51	0.009	1.7	0.93	0.30	0.58	−0.054	−9.4	0.78	0.09
LDL cholesterol, *mmol/L*															
Control	2.97	−0.062	−2.1			2.66	−0.008	−0.3			3.83	0.034	0.9		
HMB	3.05	−0.173	−5.7	0.05	1.00	2.69	−0.114	−4.2	0.09	0.60	3.90	−0.284	−7.3	0.01	0.33
Total/HDL cholesterol															
Control	4.31	−0.16	−3.7			4.09	−0.12	−2.9			4.96	−0.12	−2.4		
HMB	4.24	−0.17	−4.0	0.95	0.49	3.96	−0.07	−1.8	0.63	0.32	4.64	−0.31	−6.7	0.22	0.42
Triglycerides, *mmol/L*															
Control	1.22	−0.019	−1.6			1.13	0.034	3.0			1.64	−0.207	−12.6		
HMB	1.23	−0.066	−5.3	0.46	0.08	1.16	−0.028	−2.4	0.46	0.19	1.29	−0.114	−8.8	0.41	0.10
						Subjects with systolic BP < 130					*Subjects with systolic BP ≥ 130*				
Systolic BP, *mm Hg*															
Control	133	−0.8	−0.6			121	2.0	1.7			143	−4.4	−3.1		
HMB	134	−4.4	−3.3	0.05	0.16	121	0.9	0.7	0.76	0.18	144	−9.3	−6.5	0.05	0.39
Diastolic BP, *mm Hg*															
Control	73.0	−3.6	−4.9			68.7	−2.5	−3.6			77.7	−5.4	−6.9		
HMB	73.7	−3.7	−5.0	0.96	0.52	67.9	−3.3	−4.9	0.70	0.42	78.3	−5.0	−6.4	0.83	0.30

[1] Results presented are least-square means from the combined analysis of the nine studies ($n = 134$ placebo and $n = 128$ HMB for blood lipids and $n = 112$ placebo and $n = 110$ HMB for blood pressure). Screen values are the initial values of the participating subjects and delta values are the change over the study. Blood pressures were not measured in Studies 5 and 9.

[2] *P*-values for HMB effect and Experiment by HMB interaction as determined by ANOVA.

DISCUSSION

Measurement of the safety of nutritional products in humans is usually accomplished using surrogate markers of health and pathology. In the studies outlined here, three general areas of well-being were assessed. Blood chemistry, hematology and blood pressure were measured as indicators of tissue and organ functions. Emotional profiles were measured as an indication of subtle changes in metabolism/chemistry that might be manifested in emotional changes. Last, negative changes in perception of body functions were assessed by looking for possible symptoms. Together, these data across both men and women, in young and old and in exercising and sedentary people give a good indication of the safety of HMB in the general human population.

Physiologic and biochemical measurements indicated that HMB affects primarily cholesterol metabolism. Across all experiments, decreases in total cholesterol and LDL cholesterol of 3.7 and 5.7%, respectively, were seen. If a subset of subjects whose average starting cholesterol was > 5.17 mmol/L (200 mg/dL) is examined, the decrease was even greater, and HMB resulted in a 5.8 and 7.3% decrease in total and LDL cholesterol, respectively. This amounted to a drop of ~13 points (mg/dL) in total and a drop of 11 points (mg/dL) in LDL cholesterol. In contrast, subjects with cholesterol values < 5.17 mmol/L (200 mg/dL) did not have a significant decrease in LDL cholesterol when HMB was given. This suggests that HMB is more effective in lowering cholesterol when blood levels are elevated above the current standard associated with increased risk of heart disease.

There was also a difference in HDL cholesterol response with HMB supplementation compared with placebo although this difference appeared to disappear when total cholesterol was elevated. The increase in HDL cholesterol in the placebo group is likely related to the exercise-induced rise in HDL cholesterol seen in previous studies (Halle et al. 1999).

A second cardiovascular risk factor affected by HMB was

TABLE 5

Effect of 3 g/d supplemental β-hydroxy-β-methylbutyrate (HMB) on blood indicators of liver function in humans in a summary of 9 clinical studies[1]

	Screen	Delta	% Change	HMB effect[2]	Exp × HMB[2]
Bilirubin, *μmol/L*					
Control	12.7	−1.7	−13.5		
HMB	12.1	−1.9	−15.5	0.76	0.18
Alkaline phosphatase, *IU/L*					
Control	80	0.9	1.1		
HMB	83	0.5	0.6	0.81	0.76
Lactate dehydrogenase, *IU/L*					
Control	184	−8.9	−4.8		
HMB	176	−5.0	−2.8	0.44	0.03
SGOT,[3] *IU/L*					
Control	35.8	−4.7	−13.1		
HMB	31.6	−2.8	−8.9	0.42	0.56
SGPT, *IU/L*					
Control	21.3	0.15	0.7		
HMB	20.2	0.37	1.8	0.83	0.66
GGT, *IU/L*					
Control	19.9	−1.39	−7.0		
HMB	23.1	−1.88	−8.1	0.57	0.04
Iron, *mmol/L*					
Control	0.0197	−0.002	−9.7		
HMB	0.0201	−0.003	−16.3	0.16	0.008

[1] Results presented are least-square means from the combined analysis of the nine studies (n = 134 placebo and n = 128 HMB). Screen values are the initial values of the participating subjects and delta values are the change over the study.

[2] P-values for HMB effect and Experiment by HMB interaction as determined by ANOVA.

[3] Abbreviations used: SGOT, aspartate aminotransferase; SGPT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase.

blood pressure. Subjects given HMB had a decrease of ~3% in systolic blood pressure. Diastolic blood pressure decreased similarly to the decrease seen in the placebo-supplemented group. This change in blood pressure may be attributed to the additional calcium intake of ~400 mg/d with the Ca-HMB supplement. There is a variety of experimental evidence showing that calcium supplementation can affect blood pressure (Birkett 1998, Kok et al. 1986, McCarron and Reusser 1999, Sempos et al. 1986). Meta-analysis of the data in these experiments indicates that calcium lowers systolic blood pressure at the rate of ~0.39 mm Hg/(100 mg Ca·d). On the basis of this equation, the 400 mg of Ca given in the current study should have lowered systolic blood pressure ~1.56 mm Hg. The measured effect in subjects with systolic blood pressure >130 was much greater, i.e., 9.3 mm Hg total or 4.9 mm Hg net, suggesting that either the CaHMB is a more biologically available source of calcium (Sousa et al. 1996) or that HMB is having a more direct effect on cardiovascular function.

Blood potassium decreased < 2% in the HMB-supplemented subjects. The reason for this small decrease is not known but is likely due to the slightly different mineral intakes between the placebo- and the HMB-supplemented groups. The placebo supplement was not balanced for the calcium, phosphorus or potassium, which were contained in the HMB treatments. Thus, the daily dose of ~400 mg of calcium, ~135 mg of phosphorus and ~170 mg of potassium could have resulted in minor changes in ionic balance (although this represents only ~40, 14 and 5% of the estimated daily intake of Ca, P and K, respectively) (NRC 1989).

The only other significant difference in blood chemistry was a significantly greater albumin/globulin ratio in HMB-supplemented subjects. This was primarily the result of a relative increase in albumin and a relative decrease in globulin.

TABLE 6

Effect of 3 g/d supplemental β-hydroxy-β-methylbutyrate (HMB) on blood chemistry profiles in humans in a summary of 9 clinical studies[1]

	Screen	Delta	% Change	HMB effect[2]	Experiment by HMB[2]
Creatine kinase, *U/L*					
Control	157	84	54		
HMB	141	59	42	0.43	0.13
Glucose, *mmol/L*					
Control	4.88	0.089	1.8		
HMB	4.70	0.222	4.7	0.12	0.15
Uric acid, *mmol/L*					
Control	0.263	0.0077	2.9		
HMB	0.259	0.0131	5.1	0.39	0.37
Blood urea nitrogen, *mmol/L*					
Control	2.46	0.023	0.9		
HMB	2.50	−0.002	−0.1	0.75	0.02
Creatinine, *μmol/L*					
Control	91.1	−0.80	−0.9		
HMB	91.1	−2.48	−2.7	0.15	0.26
Blood urea nitrogen/creatinine, *wt/wt*					
Control	14.3	0.27	1.9		
HMB	14.4	0.27	1.9	0.99	0.006
Sodium, *mmol/L*					
Control	142	−0.478	−0.3		
HMB	142	−0.783	−0.6	0.16	0.20
Potassium, *mmol/L*					
Control	4.27	0.036	0.8		
HMB	4.30	−0.082	−1.9	0.003	0.07
Chloride, *mmol/L*					
Control	105	0.20	0.2		
HMB	105	0.51	0.5	0.62	0.92
Calcium, *mmol/L*					
Control	2.40	−0.032	−1.3		
HMB	2.42	−0.045	−1.9	0.30	0.21
Phosphorous, *mmol/L*					
Control	1.22	0.006	0.5		
HMB	1.26	−0.013	−1.0	0.45	0.16
Protein, *g/L*					
Control	72.6	−1.6	−2.2		
HMB	72.3	−1.7	−2.4	0.92	0.04
Albumin, *g/L*					
Control	43.9	−0.6	−1.4		
HMB	43.6	−0.2	−0.5	0.20	0.82
Globulin, *g/L*					
Control	28.7	−1.1	−3.8		
HMB	28.7	−1.5	−5.2	0.20	0.005
Albumin/Globulin					
Control	1.52	0.028	1.8		
HMB	1.50	0.075	5.0	0.03	0.02

[1] Results presented are least-square means from the combined analysis of the nine studies (n = 134 placebo and n = 128 HMB). Screen values are the initial values of the participating subjects and delta values are the change over the study.

[2] P-values for HMB effect and Experiment by HMB interaction as determined by ANOVA.

TABLE 7

Effect of 3 g/d supplemental β-hydroxy-β-methylbutyrate (HMB) on blood hematology in humans in a summary of 9 clinical studies[1]

	Screen	Delta	% Change	HMB effect[2]	Experiment by HMB[2]
WBC,[3] $\times 10^9$/L					
Control	6.56	−0.25	−3.8		
HMB	6.55	−0.54	−8.2	0.11	0.73
RBC, $\times 10^{12}$/L					
Control	4.76	−0.08	−1.7		
HMB	4.78	−0.14	−2.9	0.08	0.13
Hemoglobin, g/L					
Control	146	−2.5	−1.7		
HMB	147	−3.8	−2.6	0.12	0.41
Hematocrit, L/L					
Control	0.432	−0.0039	−0.9		
HMB	0.434	−0.0087	−2.0	0.05	0.10
MCV, μm^3					
Control	91	0.7	0.8		
HMB	91	0.4	0.4	0.12	0.30
MCH, pg					
Control	30.8	−0.14	−0.5		
HMB	30.8	−0.10	−0.3	0.87	0.52
MCHC, g/L					
Control	344	−2.6	−0.8		
HMB	339	−2.1	−0.6	0.62	0.02
Platelets, $\times 10^9$/L					
Control	237	−2.0	−0.8		
HMB	236	−5.2	−2.2	0.47	0.60
Polycytes, %					
Control	54.0	−0.38	−0.7		
HMB	53.2	−0.85	−1.6	0.64	0.57
Lymphocytes, %					
Control	35.6	−0.53	−1.5		
HMB	36.3	−0.01	0	0.53	0.47
Monocytes, %					
Control	6.29	0.88	14.0		
HMB	6.12	1.19	19.4	0.24	0.41
Eosinophils, %					
Control	3.15	0.08	2.5		
HMB	3.39	−0.21	−6.2	0.24	0.28
Basophils, %					
Control	0.82	0.09	11.0		
HMB	0.85	0.01	1.2	0.41	0.43
Polycytes, $\times 10^9$/L					
Control	3.59	−0.12	−3.3		
HMB	3.55	−0.36	−10.1	0.15	0.87
Lymphocytes, $\times 10^9$/L					
Control	2.30	−0.14	−6.1		
HMB	2.33	−0.17	−7.3	0.61	0.14
Monocytes, $\times 10^9$/L					
Control	0.41	0.04	9.8		
HMB	0.40	0.04	10.0	0.79	0.41
Eosinophils, $\times 10^9$/L					
Control	0.21	−0.007	−3.3		
HMB	0.22	−0.028	−12.7	0.19	0.52
Basophils, $\times 10^9$/L					
Control	0.065	0.006	9.2		
HMB	0.062	0.007	11.3	0.95	0.92

[1] Results presented are least-square means from the combined analysis of the nine studies (n = 133 placebo and n = 129 HMB). Screen values are the initial values of the participating subjects and delta values are the change over the study.

[2] P-values for HMB effect and Experiment by HMB interaction as determined by ANOVA.

[3] Abbreviations used: WBC, white blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC mean corpuscular hemoglobin concentration.

The only significant difference in blood hematology was an ~0.5% lower hematocrit in the HMB-supplemented group. This difference was also reflected nonsignificantly in ~1% lower red cell numbers and hemoglobin. An explanation for these differences is not apparent, but in all cases, the ending values were not significantly different between the treatments. Other experiments in animals indicate that HMB can increase hematocrit, decrease granulocytes and increase lymphocytes (Sandberg et al. 1997, Nissen et al. 1994c, Siwicki et al. 1998b).

Previous research has shown that feeding HMB to a wide variety of animals has not had any adverse effects on health or growth (Nissen et al. 1994a and 1994b, Peterson et al. 1999a and 1999b, Van Koevering et al. 1993 and 1994). In fact, the most consistent effect of HMB in animals has been a positive effect on health. In sheep, pigs, cattle, chickens and fish, there have been positive effects noted on both immune function and improved resistance to infectious agents (Nissen et al. 1990a, 1994b and 1994c, Ostaszewski et al. 1998, Peterson et al. 1999a and 1999b, Siwicki et al. 1998a and 1998b).

The Circumplex Model of emotion has been used in humans to assess emotional balance. In this model, emotion is described as a continuum (circle) of positive (pleasant) and negative (unpleasant) emotions that are either activated or unactivated. By scoring how the subject feels about a word (an emotional feeling), a profile of emotional balance is estimated. In the current study, the only significant change in emotion was a decrease in "Unactivated Unpleasant Affect" when HMB was supplemented. This emotional category is described by the words dull, tired, drowsy, sluggish, bored and droopy. Subjects taking HMB appeared to have less of this negative emotion. This study did not attempt to assess the cause of this effect, but most of the studies did involve some sort of exercise component; it may be that HMB enhanced the effects of this exercise, which could translate to less of an Unactivated Unpleasant Affect.

Incidences of all adverse symptoms were quite low except for coughing and nasal congestion, which indicates many subjects had upper respiratory infections during the study. Although the responses to questions did not differ between the placebo and the HMB groups, there were some trends for differences. The HMB-supplemented subjects tended to have less diarrhea and less loss of appetite. There was also a trend for a greater number of stiff joints reported in the HMB-supplemented group. However, the HMB-supplemented group started out with a much lower incidence of stiff joints and the ending value was actually numerically lower than the placebo-supplemented group, suggesting this was simply a chance occurrence.

In summary, the only definitive effects of HMB were positive in nature, especially relating to lowering plasma cholesterol and blood pressure. These data suggest that the popular use of supplemental HMB at 3 g/d as an ergogenic aid for exercise is well tolerated and safe in humans.

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LITERATURE CITED

Agresti, A. (1990) Categorical Data Analysis. John Wiley and Sons, New York, NY.
Bastiaanse, E. M., Hold, K. M. & Van der Laarse, A. (1997) The effect of

membrane cholesterol content on ion transport processes in plasma membranes. Cardiovasc. Res. 33: 272–283.
Birkett, N. J. (1998) Comments on a meta-analysis of the relation between dietary calcium intake and blood pressure. Am. J. Epidemiol. 148: 223–228.
Bloch, K. (1944) Some aspects of the metabolism of leucine and valine. J. Biol. Chem. 115: 255–263.
Cheng, W., Phillips, B. & Abumrad, N. (1998) Effect of HMB on fuel utilization, membrane stability and creatine kinase content of cultured muscle cells. FASEB J. 12: A950 (abs.).
Clark, R. H., Feleke, M., Din, M., Yasmin, T., Singh, G. Khan, F. & Rathmacher, J. A. (2000) Nutritional treatment for acquired immunodeficiency virus-associated wasting using β-hydroxy-β-methylbutyrate, glutamine and arginine: a randomized, double-blind, placebo-controlled study. JPEN 24: 133–139.
Friedewald, W. T., Levy, R. I. & Fredrickson, D. S. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. 18: 499–502.
Halle, M., Berg, A., Baumstark, M. W. & Keul, J. (1999) Association of physical fitness with LDL and HDL subfractions in young healthy men. Int. J. Sports Med. 20: 464–469.
Janskiewiez, J., Popov, K. M. & Harris, R. A. (1996) α-Ketoisocaproate dioxygenase, a dual-specificity enzyme corresponds to alloantigen F. FASEB J. 9: A318 (abs.).
Kok, F. J., Vandenbroucke, J. P., Van Der Heide-Wessel, C. & Van Der Heide, R. M. (1986) Dietary sodium, calcium, and potassium, and blood pressure. Am. J. Epidemiol. 123: 1043–1048.
McCarron, D. A. & Reusser, M. E. (1999) Finding consensus in the dietary calcium-blood pressure debate. J. Am. Coll. Nutr. 18: 398S–405S.
Mutoh, T., Kumano, T., Nakagawa, H. & Kuriyama, M. (1999) Role of tyrosine phosphorylation of phospholipase C gamma1 in the signaling pathway of HMG-CoA reductase inhibitor-induced cell death of L6 myoblasts. FEBS Lett. 446: 91–94.
National Research Council (1989) Recommended Dietary Allowances, 10th ed. National Academy Press, Washington, DC.
Nissen, S. L. & Abumrad, N. N. (1997) Nutritional role of the leucine metabolite β-hydroxy-β-methylbutyrate (HMB). J. Nutr. Biochem. 8: 300–311.
Nissen, S., Faidley, T. D., Zimmerman, D. R., Izard, R. & Fisher, C. T. (1994a) Colostral milk fat percentage and pig performance are enhanced by feeding the leucine metabolite β-hydroxy β-methylbutyrate to sows. J. Anim. Sci. 72: 2332–2337.
Nissen, S., Fuller, J. C., Jr., Sell, J., Ferket, P. R. & Rives, D. V. (1994b) The effect of β-hydroxy-β-methylbutyrate on growth, mortality and carcass qualities of broiler chickens. Poult. Sci. 73: 137–155.
Nissen, S., Kuhlman, G. & Roth, J. A. (1990a) Modulation of ovine lymphocyte function by leucine and leucine metabolites. In: Amino Acids: Chemistry, Biology and Medicine (Lubec, G., ed.), pp. 817–820. ESCOM, Leiden, The Netherlands.
Nissen, S., Morrical, D. & Fuller, J. C., Jr. (1994c) The effects of the leucine catabolite β-hydroxy-β-methylbutyrate (HMB) on the growth and health of growing lambs. J. Anim. Sci. 77 (suppl. 1): 243.
Nissen, S., Panton, L., Fuller, J., Jr., Rice, D. & Sharp, R. (1997) Effect of feeding β-hydroxy-β-methylbutyrate (HMB) on body composition and strength of women. FASEB J. 11: A150 (abs.).
Nissen, S., Panton, L., Wilhelm, R. & Fuller, J. C., Jr. (1996a) Effect of β-hydroxy-β-methylbutyrate (HMB) supplementation on strength and body composition of trained and untrained males undergoing intense resistance training. FASEB J. 10: A287 (abs.).
Nissen, S., Sharp, R., Ray, M., Rathmacher, J. A., Rice, J., Fuller, J. C., Jr., Connelly, A. S. & Abumrad, N. N. (1996b) The effect of the leucine metabolite β-hydroxy β-methylbutyrate on muscle metabolism during resistance-exercise training. J. Appl. Physiol. 81: 2095–2104.
Nissen, S., Van Koevering, M. & Webb, D. (1990b) Analysis of β-hydroxy-β-methyl butyrate in plasma by gas chromatography and mass spectrometry. Anal. Biochem. 188: 17–19.
Ostaszewski, P., Kozlowska, E., Siwicki, A. K., Krzyzanowski, J., Fuller, J. C., Jr. & Nissen, S. (1998) The immunomodulating activity of dietary 3-hydroxy-3-methylbutyrate (HMB) in weanling pigs. J. Anim. Sci. 76 (suppl. 1): 136.
Peterson, A. L., Qureshi, M. A., Ferket, P. R. & Fuller, J. C., Jr. (1999a) Enhancement of cellular and humoral immunity in young broilers by the dietary supplementation of β-hydroxy-β-methylbutyrate. Immunopharm. Immunotoxicol. 21: 307–330.
Peterson, A. L., Qureshi, M. A., Ferket, P. R. & Fuller, J. C., Jr. (1999b) In vitro exposure with β-hydroxy-β-methylbutyrate enhances chicken macrophage growth and function. Vet. Immunol. Immunopathol. 67: 67–78.
Pierno, S., De Luca, A., Tricarico, D., Roselli, A., Natuzzi, F., Ferrannini, E., Laico, M. & Camerino, D. C. (1995) Potential risk of myopathy by HMG-CoA reductase inhibitors: a comparison of pravastatin and simvastatin effects on membrane electrical properties of rat skeletal muscle fibers. J. Pharmacol. Exp. Ther. 275: 1490–1496.
Rabinowitz, J. L. (1955) The biosynthesis of radioactive β-hydroxyisovaleric acid in rat liver. J. Am. Chem. Soc. 77: 1295–1297.
Rudney, H. (1957) The biosynthesis of beta-hydroxy-beta-methylglutaric acid. J. Biol. Chem. 227: 363–377.
Russell, J. A. (1980) A circumplex model of affect. J. Pers. Soc. Psychol. 39: 1161–1178.
Sabourin, P. J. & Bieber, L. L. (1981) Subcellular distribution and partial characterization of an α-ketoisocaproate oxidase of rat liver: formation of β-hydroxyisovaleric acid. Arch. Biochem. Biophys. 206: 132–144.
Sabourin, P. J. & Bieber, L. L. (1983) Formation of β-hydroxyisovalerate by an α-ketoisocaproate oxygenase in human liver. Metabolism 32: 160–164.
Sandberg, L., Miller, P. & Fuller, J. C., Jr. (1997) Effect of β-hydroxy-β-methylbutyrate on the physiological response to exercise and conditioning in horses. J. Anim. Sci. 75 (suppl. 1): 198.
Sempos, C., Cooper, R., Kovar, M G., Johnson, C., Drizd, T. & Yetley, E. (1986) Dietary calcium and blood pressure in National Health and Nutrition Examination Surveys I and II. Hypertension 8: 1067–1074.
Siwicki, A. K., Morand, M., Fuller, J. C., Jr., Nissen, S., Kazua, K. I., Glabski, E. & Ostaszewski, P. (1998a) Influence of 3-hydroxy-3-methylbutyrate (HMB) on specific cellular immune response after *in vitro* and *in vivo* immunization with *Yersinia ruckeri* antigen. J. Anim. Sci. 76 (suppl. 1): 136.
Siwicki, A. K., Ostaszewski, P., Fuller, J. C., Jr., Nissen, S. & Studnicka, M. (1998b) *In vitro* effects of 3-hydroxy-3-methylbutyrate (HMB) on measures of immune function and immunocompetence in fish. J. Anim. Sci. 76 (suppl. 1): 136.
Sousa, M. F., Abumrad, N. N., Martins, C., Nissen, S. & Riella, M. C. (1996) Calcium β-hydroxy-β-methylbutyrate. Potential role as a phosphate binder in uremia: in vitro study. Nephron 72: 391–394.
Van Koevering, M. T., Gill, D. R., Smith, R. A., Owens, F. N., Nissen, S. & Ball, R. L. (1993) Effect of β-hydroxy-β-methyl butyrate on the health and performance of shipping-stressed calves. Oklahoma State Univ. Res. Rep., pp. 312–316.
Van Koevering, M. & Nissen, S. (1992) Oxidation of leucine and α-ketoisocaproate to β-hydroxy-β-methylbutyrate in vivo. Am. J. Physiol. 262: E27–E31.
Van Koevering, M. T., Dolezal, H. G., Gill, D. R., Owens, F. N., Strasia, C. A., Buchanan, D. S., Lake, R. & Nissen, S. (1994) Effects of β-Hydroxy-β-methylbutyrate on performance and carcass quality of feedlot steers. J. Anim. Sci. 72: 1927–1935.
Yeagle, P. L. (1991) Modulation of membrane function by cholesterol. Biochimie 73: 1303–1310.
Zabin, I. & Bloch, K. (1951) The utilization of butyric acid for the synthesis of cholesterol and fatty acids. J. Biol. Chem. 192: 261–266.