

Effect of leucine metabolite β -hydroxy- β -methylbutyrate on muscle metabolism during resistance-exercise training

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Nissen, S., R. Sharp, M. Ray, J. A. Rathmacher, D. Rice, J. C. Fuller, Jr., A. S. Connelly, and N. Abumrad. Effect of leucine metabolite β -hydroxy- β -methylbutyrate on muscle metabolism during resistance-exercise training. *J. Appl. Physiol.* 81(5): 2095–2104, 1996.—The effects of dietary supplementation with the leucine metabolite β -hydroxy- β -methylbutyrate (HMB) were studied in two experiments. In *study 1*, subjects ($n = 41$) were randomized among three levels of HMB supplementation (0, 1.5 or 3.0 g HMB/day) and two protein levels (normal, 117 g/day, or high, 175 g/day) and weight lifted for 1.5 h 3 days/wk for 3 wk. In *study 2*, subjects ($n = 28$) were fed either 0 or 3.0 g HMB/day and weight lifted for 2–3 h 6 days/wk for 7 wk. In *study 1*, HMB significantly decreased the exercise-induced rise in muscle proteolysis as measured by urine 3-methylhistidine during the first 2 wk of exercise (linear decrease, $P < 0.04$). Plasma creatine phosphokinase was also decreased with HMB supplementation (*week 3*, linear decrease, $P < 0.05$). Weight lifted was increased by HMB supplementation when compared with the unsupplemented subjects during each week of the study (linear increase, $P < 0.02$). In *study 2*, fat-free mass was significantly increased in HMB-supplemented subjects compared with the unsupplemented group at 2 and 4–6 wk of the study ($P < 0.05$). In conclusion, supplementation with either 1.5 or 3 g HMB/day can partly prevent exercise-induced proteolysis and/or muscle damage and result in larger gains in muscle function associated with resistance training.

resistance training

THE ANTICATABOLIC ACTIONS of leucine and certain metabolites of leucine such as α -ketoisocaproate (KIC) have been known for 35 years (7). Both leucine and KIC are proposed to decrease nitrogen and protein loss by inhibiting protein breakdown; however, extensions of this in vitro work to animals and humans have not clearly shown an anabolic effect except in situations of either severe stress or trauma in which proteolysis is greatly elevated (3, 6, 16, 18). This suggests either that KIC and leucine are active only during periods of excessive catabolism or that a further metabolic product of leucine (and KIC) may be variably produced depending on the prevailing metabolic milieu and may be responsible for the anticatabolic effects of these compounds. Based on several animal studies, we hypothesized that the leucine metabolite β -hydroxy- β -methylbutyrate (HMB), produced in the body from leucine via KIC, is responsible for the inhibitory effect on protein breakdown.

HMB is produced from KIC by the enzyme KIC-dioxygenase and, at least in the pig, is produced exclusively from leucine (23). Plasma concentrations of HMB range from 1 to 4 μ M but can increase 5- to

10-fold after leucine is fed (25). The cytosolic dioxygenase enzyme differs from the mitochondrial KIC-dehydrogenase enzyme in several aspects. The dioxygenase produces free HMB in the cytosol, whereas the dehydrogenase enzyme produces the CoA derivative of isovaleric acid in the mitochondria. The cytosolic dioxygenase enzyme requires iron and molecular O_2 for action, which are not required by the mitochondrial dehydrogenase enzyme (15). The cytosolic dioxygenase enzyme is present in large amounts in the liver compared with other tissues, including muscle, and it has a 20-fold higher substrate concentration for half-maximal enzyme velocity (K_m) than does the mitochondrial dehydrogenase. The high substrate concentration required by the dioxygenase enzyme compared with the liver concentration of KIC ($< 5 \mu$ M) suggests that HMB production in the body may be a first-order reaction controlled by enzyme and KIC concentrations. It has been calculated that, under normal conditions, $\sim 5\%$ of leucine oxidation proceeds via this pathway (23). If humans are assumed to have enzyme actions similar to those seen in pigs, a 70-kg human would produce from 0.2 to 0.4 g HMB/day depending on the level of dietary leucine. At leucine intakes of 20–50 g/day (which are used therapeutically), the concentrations of leucine and KIC in the liver increase and could result in HMB production reaching gram quantities per day.

The objective of the first study presented was to determine whether the administration of Ca-HMB to humans undergoing a regimen of stressful resistance exercise would result in the slowing of exercise-induced proteolysis. Second, because the popular literature suggests that the intake of high-protein concentrates enhances gains in muscle function achieved with resistance training, two levels of protein intake were used in the present study. Normal- (117 g/day) and high-protein (175 g/day) intakes were compared to determine whether very high protein intakes would increase muscle mass and/or muscle strength during resistance training. A second longer study was also conducted to determine whether the changes in body composition and strength seen during the first study were manifest over a longer period of time.

MATERIALS AND METHODS

Human Subjects

In both *studies 1* and *2*, potential subjects were excluded from the study if they had evidence or history of any of the following: diabetes mellitus; cardiac, liver, renal, or pulmonary diseases; recent joint or bone injury; or obesity. For *study 1*, subjects were also excluded if they had participated in a resistance-exercise program in the last 3 mo. Subjects were

week. The subjects were instructed not to begin any new exercise during the basal period. The test diets began on the Saturday of the basal period. Table 1 lists the sampling, training, dietary, and supplement schedules during *study 1*. Diets were meat free Monday evening through Friday morning of each week for the entire study. Urine was collected from 7:00 A.M. Wednesday to 7:00 A.M. Thursday and from 7:00 A.M. Thursday to 7:00 A.M. Friday each week during the study. On Thursday and Friday mornings of the baseline period and each week during the study, blood was collected from all subjects from a superficial forearm vein. Body composition was measured by total body electrical conductivity (TOBEC) on either Thursday or Friday of the baseline period and again on either Thursday or Friday at the end of the study.

EXPERIMENTAL PERIOD/TRAINING-SUPPLEMENTS. Weight training began on Friday evening of the last baseline day and continued three times per week throughout the study. Dietary supplements were also begun at this time and continued throughout the study. On Thursday and Friday mornings of each week, blood was collected and vital signs, including body weight, were measured.

WEIGHT-TRAINING REGIMEN. The strength-training program consisted of concentric and eccentric isotonic lifting exercises that worked each muscle group once or twice weekly with either free weights (FW) or weight machines (WM). Sessions alternated between upper and lower body exercises. The subjects lifted three times per week with at least 1 day of rest between sessions. During the 3-wk period, each subject exercised 10 times (5 upper body and 5 lower body). The initial weight lifted was calculated from the 1 RM by multiplying the 1 RM by 90%. It was estimated that this would allow for three to five repetitions before failure. Each exercise included 2 sets of 10 repetitions at 30 and 60% of the subject's 1 RM as a warmup, followed by 3 sets of 3–5 repetitions at 90% of 1 RM. When necessary, weights were adjusted to assure failure on the third to fifth repetitions. The exercises for the upper body day were bench press (FW), latissimus pulldown (WM), seated rows (WM), Cybex-Pec-Fly (WM), seated preacher curls (FW), inclined dumbbell curls (FW), and triceps pushdown (WM). The lower body exercises were seated leg press (WM), standing calf raises (WM), leg curls (WM), leg extension (WM), 45° inclined sit-up, inclined leg lift, and inverted sit-ups (back extension).

After each session, the average weight lifted on the last two sets was incremented by 2% (and rounded to the closest increment of weight for each exercise machine), and these values were used as the target weight to be lifted at the next session. Each session was monitored by trained supervisors who recorded weights and judged whether changes in weight were necessary to produce failure after three to five repetitions. The sit-ups, inclined leg lifts, and back extensions were conducted to exhaustion. No weight was added during these exercises except for four subjects who held additional weight to the chest during the inverted sit-ups to force exhaustion in <100 repetitions.

CALCULATION OF MUSCLE WORK. Muscle strength was assessed by first calculating the average weight lifted during the last three working sets of each exercise (failure at four to six repetitions). The average weight was then multiplied by the number of repetitions the weight was lifted to yield a work index. Upper body strength (composite scores) was calculated by adding the work indexes for each exercise in the upper body group, whereas the lower body strength was calculated by adding the work indexes for all the lower body exercises except for the standing calf raises. The standing calf raises were not used in the calculation because some subjects could lift the entire weight stack, whereas some other subjects

could not tolerate the weight on their shoulders. The individual upper and lower body weights lifted and abdominal exercise efforts as well as the total weight lifted for combined upper and lower body are presented in RESULTS.

DIETARY CONTROL. Meals were supplied to the subjects as frozen entrees and packed lunches. The frozen entrees were selected from commercially available foods (Weight Watchers, Tyson Healthy Portions, Healthy Choice). Diets were meat free from Monday evening through Friday morning to allow for measurement of endogenous 3-methylhistidine (3-MH) from Wednesday morning through Friday morning (10).

Washout of the dietary 3-MH was tested by comparing the two consecutive urine collections. During the basal period, the overall averages of urinary 3-MH excretion for the first and second collection periods were 237 ± 14 and 281 ± 16 $\mu\text{mol/day}$, respectively. Paired *t*-test analysis showed a trend for an increase in urine 3-MH from *collection 1* to *collection 2* ($P = 0.10$). There were no significant differences among the treatments during either collection period ($P > 0.82$). These findings suggest that the timing of urine collection and 3-MH analyses were appropriate and that either washout of dietary 3-MH was complete or dietary 3-MH was a negligible part of the total 3-MH excretion.

Samples of all meals were saved, freeze ground, and analyzed for nitrogen. The major difference between the control and high-protein groups was the substitution of three protein shakes per day for some solid food in the high-protein group. To keep the caloric intake of all groups as equal as possible, the subjects selected for the high-protein group did not receive a sack lunch but instead consumed one of the three protein shakes with juice for lunch. Total nitrogen intakes averaged 18.7 g/day (117 g protein/day) in the control groups and 28.0 g/day (175 g protein/day) in the high-protein groups.

The subjects were instructed to mix the powder with either the orange juice provided or water. The volume of each serving was 0.94 liter (16 oz). Special instructions were given to the protein-supplemented subjects to ensure that they completely consumed the entire mixed drink. This included washing the mixing container with water and consuming the wash water.

HMB was provided by Metabolic Technologies (Ames, IA) and was synthesized and purified as previously described (5). The subjects received the supplement as the calcium salt (monohydrate) of HMB (Ca-HMB), which was >99% HMB as assessed by high-performance liquid chromatography. HMB mixed in 0.94-liter (16-oz) bottles of prepared orange juice (Minute Maid, Coca-Cola, Atlanta, GA) was administered to the subjects. The servings of juice and HMB were prepared weekly during the study. Two stock solutions of HMB and orange juice were made: one was 0.15 g HMB/ml orange juice and the other was 0.3 g HMB/ml orange juice. Five milliliters of a stock solution were added to each 0.94-liter (16-oz) bottle of orange juice after an equal volume of juice from the bottle was removed to yield either 0.75 or 1.5 g HMB/bottle. Each bottle of orange juice contained one-half of the daily dosage of HMB. The subjects were instructed to keep the juice refrigerated and to take one bottle of juice in the morning and one bottle of juice in the evening. Independent tests showed no degradation of HMB for at least 2 wk in the orange juice even when it was stored at room temperature.

BLOOD COLLECTION/ANALYSIS. Blood samples were collected from a superficial forearm vein into Vacutainers (Vacutainer Systems, Becton-Dickinson, Rutherford, NJ) after an overnight fast by the subjects. Plasma from EDTA-treated blood was collected and frozen for 3-MH, plasma amino acid, and HMB analyses. The blood samples were processed on the day

of collection and analyzed for electrolytes, red and white blood cell numbers, muscle creatine phosphokinase (CK), lactate dehydrogenase (LDH), plasma creatinine, alkaline phosphatase, γ -glutamyl transpeptidase, serum glutamic-oxaloacetic transaminase (SGOT), and serum glutamic-pyruvic transaminase (SGPT) by Roche Biochemical Laboratories (St. Louis, MO). Plasma was analyzed for HMB by gas chromatography-mass spectrometry (12). Plasma-free amino acids were measured by high-performance liquid chromatography (Waters Pico Tag System).

URINE COLLECTIONS. Two 24-h urine collections were made each week. After the total volume of urine collected was measured, a sample was frozen at -20°C for later analysis of nitrogen (macro-Kjeldahl), creatinine, HMB, and 3-MH. Urine 3-MH and HMB were quantified by previously described gas chromatography-mass spectrometry techniques (12, 13).

BODY COMPOSITION. Estimates of whole body lean and fat masses were measured by using TOBEC (model HA-2, EM Scan, Springfield, IL). The subjects were scanned, and fat tissue was predicted by using equations supplied by the manufacturer and derived from data generated by the University of Illinois (Urbana-Champaign) (9). Scanning of all subjects in the morning before breakfast was not logistically possible. Therefore, the subjects were scanned between 9 A.M. and 4 P.M. Because body composition measures are influenced by the state of hydration and food intake, an attempt was made to more truly estimate lean body mass in the fasted state. Fat mass measured by TOBEC was subtracted from the fasting body weight to yield a fasting lean mass for all subjects. This assumes that only fat-free mass (FFM) will be affected by food and/or water consumption and that fat mass will remain the same regardless of fluid and/or food intake. We hypothesized that this calculation gave a more realistic estimate of lean tissue mass, which would have been influenced by a fluctuation in fluid and food intake throughout the day. This assumption was verified in a separate experiment where 12 male subjects were scanned in the morning after an overnight fast and then scanned again in the afternoon after two meals. In the morning, the fat mass and FFM were 24.2 ± 2.6 and 68.1 ± 2.5 kg, respectively, and in the afternoon, fat mass and FFM were 23.8 ± 2.5 and 69.7 ± 2.4 kg, respectively. The FFM was significantly higher in the afternoon ($P < 0.001$, paired t -test), but the fat mass was not significantly different between the two times. By using the fasting weight minus the fat mass, the corrected FFM was 68.5 ± 3.0 kg, which was not significantly different from the morning value of 68.1 kg. Thus the assumption that food intake will not affect fat mass was valid, and a correction to calculate fasting FFM was appropriate.

FFM was therefore calculated by subtracting the fat mass from the fasting body weight to measure true FFM. These calculated values vs. the actual "fed" TOBEC values did not change the statistical interpretation of the data (HMB effect, $P < 0.09$), but corrected values showed a decrease in the FFM gained over the 3 wk compared with uncorrected values, with the corrected values being more realistic values.

Study 2

Thirty-two male volunteers 19–22 yr of age were selected for the study. Body weight averaged 99.3 ± 3.4 kg with a range of 72–136 kg and height was 185 ± 1.5 cm with a range of 170–198 cm. Almost all subjects were engaged in some form of exercise program before the study.

Experimental Design

Body composition was measured with TOBEC as in *study 1* except that all subjects were measured after an overnight

fast. Measurements were made on the Friday morning before the start of the experiment and each Friday thereafter. Strength measurements were made the week before the study and consisted of three standard strength measurements: the bench press, the squat, and the hang clean. Treatments and exercise regimens were started on Monday. The exercise regimen consisted of weight training 6 days/wk, which included work on all major muscle groups, and lasted from 2 to 3 h/day. Aerobic training was also included in the exercise workout at least three times per week.

No dietary control was imposed in *study 2*, and the subjects were instructed to eat normally. Most meals were obtained at the Iowa State University athletic-training table. In addition, a nutrient shake was available during each training session. This was available to all subjects regardless of treatment and was served in the weight-training area.

The subjects were randomly assigned to one of two supplements, one of which contained HMB, so they did not know whether they were receiving HMB. Because we found no effect of added nutrient supplementation on HMB effects in *study 1*, HMB was delivered in the nutrient shake identical to that used in *study 1* (MET-Rx). The placebo was an orange drink mix that contained calories equal to the nutrient shake but no added protein. Thus the two groups likely had different protein intakes, although we roughly estimated the total protein intake of the placebo group to be ~ 180 g/day and the HMB group to be ~ 200 g/day.

Statistics

The general linear models procedure of the Statistical Analysis System (17) was used to statistically analyze the data. Because the major objective of the experiment was to determine the dose-responsive effect of HMB over the 3-wk period, an analysis of variance model was used that included the main effects of protein and dose-responsive linear and quadratic effects of HMB supplementation and protein by HMB interaction. Only the main effects of HMB supplementation and the main effect of protein intake are presented because no significant protein intake \times HMB supplementation interactions were found. A pooled SE for HMB is given for each measurement. The SE for HMB is most indicative of the variation among HMB groups. In *study 2*, differences between the two treatment groups were determined with a t -test. Differences were considered significant if $P < 0.05$. Trends were determined for $0.05 < P < 0.11$, and differences were considered not significant for $P > 0.11$.

RESULTS

General

In *study 1*, two subjects withdrew during the first week of exercise because of incompatibility of the study requirements with their schedules. Compliance was $>95\%$ concerning food consumption and exercise training. One subject (3 g HMB/day and high protein) admitted to major violations of the dietary protocol on two of the sample-collection days; therefore, his blood and urine data were not included in the analysis on these days. Other minor violations in dietary protocol were 2–4 days removed from the data collections and were not considered serious enough to exclude the data collected from these subjects. One subject did not exercise for two sessions because one leg was injured.

Table 2. Protein, fat, and energy intake of subjects during basal week and 3 wk of exercise and supplementation

	Ca-HMB, g/day			Protein Intake, g/day		SE
	0	1.5	3.0	117 (Normal)	175 (High)	
Protein intake, g/day (measured)						
Basal	109	110	116	112	111	± 4.19
Week 1	155	148	131	117	172	± 4.31
Week 2	156	151	128	118	172	± 4.88
Week 3	155	150	137	117	178	± 4.31
Fat intake, g/day (calculated)						
Basal	62	63	63	63	63	± 0.23
Week 1	44	44	44	57	31	± 0.16
Week 2	45	45	45	58	31	± 0.23
Week 3	45	44	45	58	32	± 0.36
Caloric intake, MJ/day (calculated)						
Basal	9.627	9.807	9.753	9.732	9.728	± 0.06
Week 1	9.899	10.092	10.025	9.724	10.284	± 0.07
Week 2	9.937	10.201	9.966	9.740	10.330	± 0.08
Week 3	9.920	9.979	9.904	9.652	10.217	± 0.06

Values are means; SE, pooled SE of means for HMB; $n = 41$ subjects in combined treatment groups. Subjects were assigned at random to 1 of 3 HMB dosages and 2 protein levels. Protein intake was calculated from nitrogen analysis of each foodstuff times 6.25. Fat intake and caloric intake were calculated from manufacturers' food labels.

Another subject could not complete the pectoral exercise because of a previous shoulder injury but was not dropped from the study because all other exercises were done. Four subjects dropped out of the second study for personal reasons. No adverse effects were noted in either study.

Table 3. Body weight and composition of subjects supplemented with Ca-HMB during basal period and after 3 wk of exercise

	Ca-HMB, g/day			Protein Intake, g/day		Significance			SE
	0	1.5	3.0	117	175	HMB Linear	HMB Quadratic	Protein	
Body weight, kg									
Basal	84.0	81.3	81.5	82.2	82.3	0.54	0.96	0.78	± 2.89
Week 3	82.6	81.0	81.1	81.2	81.9	0.71	0.88	0.97	± 2.86
Body weight change from basal, kg									
Week 3	-1.41	-0.26	-0.41	-1.00	-0.39	0.03	0.11	0.14	± 0.32
Body fat, kg									
Basal	15.0	14.1	14.6	14.2	15.0	0.89	0.80	0.69	± 1.58
Week 3	13.2	13.1	13.0	12.6	13.6	0.94	0.95	0.57	± 1.43
Body fat change from basal, kg									
Week 3	-1.82	-1.07	-1.62	-1.62	-1.38	0.74	0.19	0.60	± 0.37
Body lean, kg									
Basal	69.0	67.2	66.9	68.0	67.4	0.50	0.80	0.81	± 2.17
Week 3	69.4	68.0	68.1	68.6	68.4	0.69	0.82	0.92	± 2.20
Body lean change from basal, kg									
Week 3	0.40	0.80	1.21	0.62	0.99	0.11	0.71	0.30	± 0.36

Values are means; SE, pooled SE of means for HMB; $n = 41$ subjects in combined treatment group. Subjects were assigned at random to 1 of 3 HMB dosages and 2 protein levels. Body weight was measured after an overnight fast. Body fat was measured by TOBEC. Body lean was estimated by subtracting body fat weight from fasting body weight. Significance, probability of a significant effect of protein supplementation or linear effect of HMB supplementation.

Study 1

Dietary intake. The calculated intakes of protein, fat, and calories are listed in Table 2. The high-protein group had 50% greater protein intake than the normal-protein subjects. Total energy intake averaged ~ 9.707 MJ (2,320 kcal)/day in normal-protein subjects and ~ 10.293 MJ (2,460 kcal)/day in high-protein subjects. This resulted in a total intake of ~ 10.878 additional MJ (2,600 kcal) over the 3-wk study by the high-protein subjects.

Body composition. Body composition and weight changes are listed in Table 3. The 0-, 1.5-, and 3-g HMB groups lost 1.41, 0.26, and 0.41 kg, respectively, over the 3 wk of study ($P < 0.03$, negative linear effect of HMB). There was no significant effect of protein level on body weight change. The body-composition analysis showed 1–1.8 kg of fat lost over the 3-wk period. The loss of fat was not significantly different among the groups. Lean tissue gain tended to increase in a dose-responsive manner with HMB supplementation (0.4, 0.8, and 1.2 kg lean gain for 0-, 1.5-, and 3.0-g HMB groups, respectively; $P < 0.11$, linear).

Muscle strength. The changes in muscle work and strength are listed in Table 4 and Fig. 1. The total work (number of repetitions \times weight) at the beginning and end of the study is listed in Table 4. All subjects increased the amount of weight lifted in each exercise and the total number of abdominal efforts during the 3-wk training period (net change). No differences in the amount of weight lifted were seen between the normal- and high-protein groups for any of the exercises. HMB increased the number of abdominal exercises and the lower body weight lifted more markedly than it increased the upper body weight lifted. The increase in lower body weight lifted during the study was greater in the HMB-supplemented groups than in the unsupplemented group ($P < 0.01$). The unsupplemented subjects increased the number of abdominal efforts by 14%

Table 4. Muscle strength before and after 3 wk of resistance exercise in subjects supplemented with Ca-HMB

	Ca-HMB, g/day			Protein Intake, g/day		HMB Linear	SE
	0	1.5	3.0	117	175		
Total upper body lift, kg							
Week 1	2,020	2,075	1,896	1,988	2,005	0.39	± 102.9
Week 3	2,213	2,215	2,115	2,169	2,194	0.49	± 100.8
Net	193.6	140.5	219.5	180.7	188.4	0.69	± 47.7
Total lower body lift, kg							
Week 1	2,206	2,001	1,941	2,043	2,055	0.07	± 99.2
Week 3	2,350	2,390	2,428	2,407	2,372	0.63	± 113.1
Net	144.2	389.0	487.6	363.4	317.0	0.009	± 86.7
Abdomen, total efforts							
Week 1	53.3	47.1	49.8	47.4	52.8	0.57	± 4.0
Week 3	60.8	69.5	75.7	67.3	70.1	0.17	± 7.5
Net	7.5	22.5	25.9	20.0	17.3	0.05	± 6.3
Total strength, kg							
Week 1	4,226	4,075	3,837	4,031	4,060	0.12	± 172
Week 3	4,563	4,605	4,544	4,575	4,566	0.94	± 194
Net	337.8	529.4	707.1	544.1	505.4	0.02	± 105

Values are means; SE, pooled SE of means for HMB; $n = 41$ subjects in combined treatment groups. Subjects were assigned at random to 1 of 3 HMB dosages and 2 protein levels. Protein intake did not significantly affect any strength measurements. Total upper body and lower body lifts were assessed by first calculating average weight lifted during last 3 working sets of each exercise and then multiplying this by average repetitions in each set. Failure was usually at 4–6 repetitions. Total strength is combined upper and lower total weight lifted. HMB linear, probability of a significant linear effect of HMB supplementation.

during the 3 wk, whereas both HMB-supplemented groups increased the number of abdominal efforts ~50% over the 3-wk study ($P < 0.05$). The total strength (combined upper and lower body weight totals) increased by 8% in the unsupplemented subjects during the 3-wk period, whereas in the 1.5- and 3.0-g HMB-supplemented groups, total strength increased by 13 and 18.4%, respectively ($P < 0.02$). Figure 1 graphically depicts the increase in total strength gains over the 3-wk period. Expressing the data as maximum weight lifted vs. total weight lifted (work) resulted in a similar HMB effect.

Muscle protein breakdown. Plasma CK and LDH levels are presented in Table 5. Plasma CK levels in subjects not supplemented with HMB increased to 15,868 U/ml after 1 wk of exercise. HMB-supplemented subjects had lower levels of CK, but because of extreme variation in the concentrations among subjects, it was not significant. By week 3, HMB supplementation had

decreased plasma CK levels in a dose-responsive manner ($P < 0.05$). Protein intake did not affect plasma CK levels. Plasma LDH levels followed the same pattern as CK levels except for a less dramatic increase during the first week. HMB supplementation also tended to decrease plasma LDH in a dose-responsive manner in weeks 2 and 3 ($P < 0.08$ and $P < 0.07$, respectively).

The rate of 3-MH loss in urine is also presented in Table 5. The percent change in urinary 3-MH from the basal level is depicted in Fig. 2. One week after exercise started, urinary 3-MH increased 94% in subjects not supplemented with HMB, 85% in subjects supplemented with 1.5 g HMB/day, and 50% in subjects supplemented with 3 g HMB/day. In week 2 of the experiment, urinary 3-MH in control subjects was still 27% above the basal level. However, urinary 3-MH was 4 and 15% below the basal level for the 1.5 and 3.0 g HMB/day supplemented subjects, respectively ($P < 0.001$, linear effect of HMB). During the third week of the study, HMB did not have a significant effect on urinary 3-MH, although the trends continued. Expressed as a percentage of muscle broken down per day, the unsupplemented group increased from 3% of the total muscle breakdown per day to 6%/day, whereas the 1.5 and 3 g HMB/day groups increased from 3 to 5.5 and 3 to 4.5%/day, respectively. High protein increased urine volume ($P < 0.05$), which resulted in a lower creatinine concentration in the urine ($P < 0.05$). There was no effect of HMB on either urine volume or creatinine concentration. The end result was that there were no significant effects of either HMB or protein on total urine creatinine output per day (data not shown).

Plasma amino acids. Plasma amino acids were measured during the basal period and at the end of the study. The plasma concentration of most amino acids

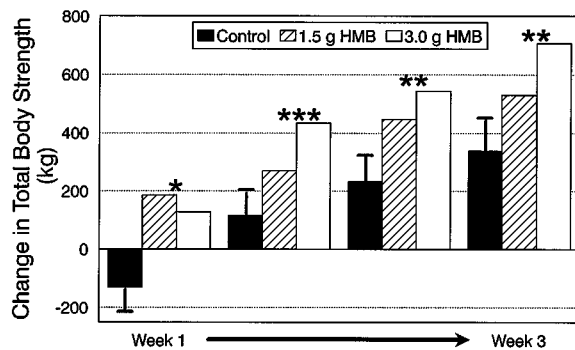


Fig. 1. Change in muscle strength (total of upper and lower body exercises) from week 1 to week 3 in subjects supplemented with Ca- β -hydroxy- β -methylbutyrate (HMB). Each set of bars represents one complete set of upper and lower body workouts. *** $P < 0.01$; ** $P < 0.02$; * $P < 0.03$ (significant linear effect of HMB supplementation).

Table 5. Plasma, creatine phosphokinase, and lactate dehydrogenase as indicators of muscle damage and urine 3-MH as an indicator of proteolysis in subjects undergoing resistance exercise and supplemented with Ca-HMB

	Ca-HMB, g/day			Protein Intake, g/day		Significance		SE
	0	1.5	3.0	117	175	HMB Linear	Protein	
Plasma creatine phosphokinase, U/ml								
Basal	245	302	219	257	253	0.74	0.95	61.8
Week 1	15,868	15,355	7,859	9,251	16,804	0.17	0.08	3,255.8
Week 2	1,408	643	724	1,051	798	0.21	0.63	308.7
Week 3	666	388	304	471	434	0.05	0.92	104.7
Plasma lactate dehydrogenase activity, U/ml								
Basal	172	176	162	163	177	0.30	0.05	5.4
Week 1	581	505	354	400	560	0.12	0.13	79.8
Week 2	203	184	179	188	190	0.08	0.73	7.8
Week 3	187	171	169	169	182	0.07	0.08	5.3
Urine 3-MH, μ mol/day								
Basal	250	253	279	268	254	0.33	0.55	19.75
Week 1	484	467	419	491	423	0.13	0.06	30.11
Week 2	318	242	239	284	248	0.001	0.04	14.41
Week 3	347	335	334	335	342	0.69	0.80	24.07
Urine 3-MH change from basal, μ mol/day								
Week 1	234	214	140	223	169	0.04	0.15	31.00
Week 2	68	-11	-41	16	-5	0.001	0.38	20.63
Week 3	97	82	54	67	88	0.40	0.58	32.44

Values are means; SE, pooled SE of means for HMB; $n = 41$ subjects in combined treatment groups. Subjects were assigned at random to 1 of 3 HMB dosages and 2 protein levels. Significance, probability of a significant linear effect of HMB supplementation or effect of protein intake.

was not significantly changed by either protein intake or HMB supplementation. However, the sum of all essential amino acids in plasma increased 32% in subjects not receiving HMB but decreased 9 and 18% in the 1.5 and 3 g HMB/day supplemented subjects, respectively (linear effect of HMB, $P < 0.04$). In addition, subjects consuming the high-protein diet had 26% lower plasma glycine ($P < 0.05$) and 25% lower plasma serine concentrations ($P < 0.01$). Plasma proline in the high-protein group increased by 17% during the study ($P < 0.05$), whereas plasma proline in the control subjects only increased 4%.

Plasma and urine HMB. Plasma HMB was measured after an overnight fast (~ 12 h after the last HMB consumption). Plasma HMB concentrations remained constant in subjects receiving no HMB. However, plasma HMB increased in a dose-responsive manner in the 1.5

and 3.0 g HMB/day groups, with levels increasing from basal levels of 2.8 μ M to levels of 10.7 and 20.3 μ M, respectively ($P < 0.0001$). Urine HMB was measured in the 2-day quantitative urine collections. Urine HMB varied from 10 to 30 mg of free acid equivalents per day during the basal period. Supplementation with 1.5 g HMB/day resulted in an increase to ~ 450 – 500 mg of free acid equivalent lost in urine per day over the 3-wk period ($P < 0.0001$). This amounted to $\sim 43\%$ of the HMB fed after correction for endogenous production. Supplementation with 3.0 g HMB/day increased the loss of HMB in urine to ~ 950 – 1200 mg (free acid equivalents), which again is less than one-half of that fed ($P < 0.0001$).

Other measurements. Plasma sodium, potassium, chloride, calcium, and phosphorus and red and white blood cell counts were unaffected by either HMB supplementation or protein intake. Also, the levels of the plasma enzymes GGT and alkaline phosphatase were unaffected by either HMB supplementation or protein intake. Values for these parameters were all within normal ranges. The plasma enzymes SGOT and SGPT showed slight but not significant increases with exercise, and there was no significant effect of HMB on these increases. There was an effect of protein intake on SGOT and SGPT. Whereas SGOT was increased in both protein groups, SGOT in the high-protein group during week 1 was increased more than in the normal-protein group ($P < 0.05$). SGPT decreased to basal values in the normal-protein group over the 3 wk while still remaining higher in the high-protein subjects at the end of the 3 wk ($P < 0.01$). No adverse reactions or other symptoms were measured relative to either HMB supplementation or protein intake. In the high-protein

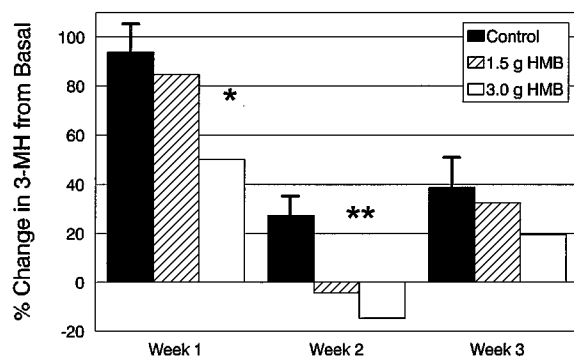


Fig. 2. Change in urinary 3-methylhistidine (3-MH) in subjects undergoing exercise-resistance training and supplemented with Ca-HMB. * $P < 0.04$; ** $P < 0.001$ (significant linear effect of HMB supplementation).

Table 6. Summary of body weight, fat mass, and strength measured in resistance-training men over a 7-wk study

	Control			HMB + Nutrient Supplement			<i>P</i>
	Week 0	Week 7	Change	Week 0	Week 7	Change	
Body weight, kg	100.1	101.2	1.1	99.2	101.8	2.6	<0.31
Fat mass, kg	16.5	16.8	0.31	15.7	15.9	0.25	<0.94
Bench press	315	321	5.4	299	314	15.0	<0.01
Squat	380	405	25.0	388	420	32.0	<0.28
Hang clean	221	250	29.0	222	252	30.0	<0.91

Values are means; $n = 34$ subjects in combined treatment groups. Control, group that received a carbohydrate-based drink; HMB + nutrient supplement, group that received 3 g Ca-HMB/day mixed in a nutrient powder. Body weight and fat mass were measured after an overnight fast. Fat mass was measured by TOBEC (see METHODS). Strength measures were expressed as maximal weight in pounds that could be lifted once (1 repetition maximum). *P* values were determined by conducting a *t*-test between the 2 test groups.

subjects, plasma creatinine levels decreased 12% after 3 wk ($P < 0.002$) when compared with the control subjects.

Study 2

The results of *study 2* are presented in Table 6 and Fig. 3. Over the period of exercise, all subjects tended to increase body weight and fat weight, although there was no significant effect of supplementation on these measures. The gain in FFM depicted in Fig. 3 indicates that the HMB-supplemented subjects showed significant increase in FFM at the earliest measurements. By *day 14* and through *day 39*, the FFM gained by the HMB-supplemented group was significantly more than in the unsupplemented group ($P < 0.05$). On the last day of the study, FFM was not significantly different between the groups.

Strength measurements are also presented in Table 6. These represent 1 RMs for the bench press and the squat lift. The bench press was significantly increased by almost threefold with HMB supplementation ($P < 0.01$). Although the squat lift increase was numerically higher with the HMB treatment, it was not significant.

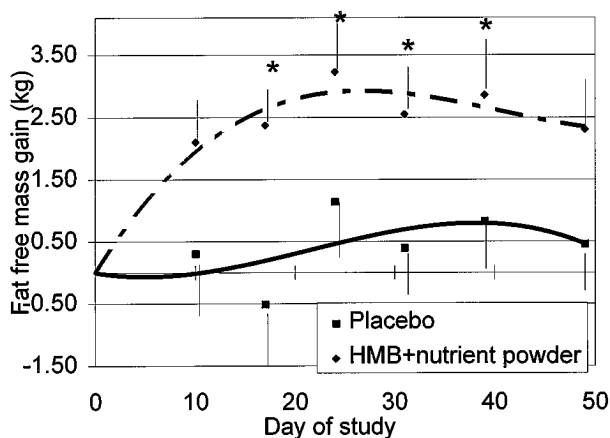


Fig. 3. Change in fat-free mass during *study 2* as measured by total body electrical conductivity. Values are means \pm SE for control group ($n = 13$) that received a carbohydrate drink (Placebo) and HMB group ($n = 15$) that received 3 g Ca-HMB/day mixed in a nutrient powder (HMB+nutrient powder). Lines, best fit of data to a cubic equation. *Significant difference between control and HMB groups at a given time, $P < 0.05$.

DISCUSSION

The major finding of this study is that HMB supplementation resulted in an enhancement of muscle function in humans undergoing resistance exercise. This effect was clearly shown by increases in muscle strength and is supported by increased lean tissue mass in both studies and decreased biochemical indicators of muscle damage. The most direct evidence of altered muscle metabolism by HMB was the 20% decrease in 3-MH loss in urine and a 20–60% decrease in the levels of enzymes, indicating muscle damage in the plasma. These changes suggest that HMB prevents or slows muscle damage as well as partially preventing the increase in proteolysis associated with intense muscular work. The decrease in muscle proteolysis is consistent with *in vitro* studies with leucine and KIC that suggest that leucine and metabolites act directly to decrease muscle proteolysis (22). This is, however, the first demonstration that the administration of either leucine or its catabolites can alter both muscle mass and strength in normal humans consuming adequate protein. The exact mechanism of the effect of HMB on muscle metabolism is not known, but at least two potential hypotheses can be put forth to explain these results.

Hypothesis 1: HMB Inhibition of Proteolytic Processes

The decrease in urine 3-MH is consistent with a decrease in muscle protein turnover. Numerous studies have shown that incubation of the muscle with either KIC or leucine inhibits proteolysis in muscle (8). This could explain the effect of HMB in the first week of exercise when unsupplemented subjects lost strength while HMB-supplemented subjects gained strength. Supporting a decrease in muscle proteolysis was the decrease in essential plasma amino acids in blood with HMB supplementation. Because these plasma amino acid values were obtained from overnight-fasted subjects, the major contributor to plasma amino acids would be endogenous muscle proteolysis. Last, there were also other indications of less muscle-specific damage in the HMB-supplemented subjects undergoing muscle-damaging heavy-resistance exercise, such as the decrease in plasma levels of CK and LDH. Lower plasma CK and LDH suggest that less inflammation

and/or damage to the muscle plasma membrane may have occurred.

Hypothesis 2: Participation of HMB in an Unknown Process

The metabolic function and fate of HMB are not fully understood. The data presented here suggest that over one-half of the HMB fed is metabolized in the body. Based on the known biochemistry, the most likely metabolic fate of HMB would be conversion to HMG-CoA (2). Alternatively, preliminary studies have shown that HMB may also be covalently linked in some form in the tissues. Hydrolysis with acid and base resulted in 10–100 μ M HMB concentrations in tissues (24). This suggests that HMB may be part of some structural component within tissues or membranes. Although it is not known what specific chemical combinations of HMB are produced in the tissues, there are at least two possibilities. The first is through esterification to CoA derivatives or phosphorylation, which can occur with hydroxy acids and hydroxy amino acids through the hydroxyl group. The other possibility is that HMB forms a polymer or copolymer in the tissues. The chemically similar compound β -hydroxybutyrate (BHB) has been found to polymerize in plants, bacteria, and animal tissues (19). In animals, it has been proposed that poly-BHB is a component of the calcium channel of the cell membrane (14). Because HMB and BHB are very similar chemically and HMB has been shown to be covalently bound in tissues, HMB could be present in the cell as a polymer or copolymer.

The loss of almost one-half of the supplemented HMB via the urine suggests that the kidney does not actively reabsorb HMB. This is similar to the metabolism of many water-soluble vitamins and BHB in that urinary losses are proportional to blood levels. Studies with 40-kg pigs showed that feeding 2 g of HMB resulted in 200 μ M plasma HMB concentrations that peaked 2 h after administration. Subsequently, plasma concentrations decreased, with a half-life between 2 and 3 h (24). Thus, in the present study, plasma concentrations of HMB could have reached 100–200 μ M in the period of 2–3 h after HMB was consumed. This could have resulted in large initial losses of HMB in urine that may have diminished as plasma HMB concentrations fell. This is supported by the observation that plasma concentrations of HMB are increased 5- to 10-fold, whereas urine losses of HMB were 10- to 20-fold higher in the HMB-supplemented subjects than in the un-supplemented subjects. These data suggest that feeding HMB twice per day may not have been ideal for maximum effectiveness.

The enhancement of muscle protein metabolism by HMB in this exercise/stress model could also have relevance in other stressful situations. However, an effect of HMB could be predicted based on the effectiveness of the HMB precursors leucine and KIC in slowing protein loss during starvation (4, 11), trauma (18), and burns (1, 18). The exercise/stress model used here caused an increase in proteolysis that also occurs with chronic wasting diseases or acute stress found with

burns and severe trauma. Further studies will be necessary to determine whether HMB could be useful in preventing or slowing the proteolytic process in disease.

General Effect and Effects of Protein Supplementation

The resistance-exercise regimen used in *study 1* resulted in marked anabolic response with weight training, although in the second study where the frequency and length of exercise were longer, the net effects of resistance exercise without HMB supplementation appeared to be minimal. The role of protein intake in moderating this anabolic response is less clear. The popular literature and the preponderance of commercial protein products designed for exercise training suggest that a greater intake of protein is needed. This is based on the notion that muscle anabolism must increase the requirement of dietary protein. However, controlled studies defining the protein and amino acid requirements of resistance-training humans have not been extensively reported (21). This controversy is neither answered in the present study nor will it be easily answered in future clinical studies because of the myriad of potential confounding experimental conditions. These variables include intensity of training, frequency of training, the timing of training, the genetic potential for muscle growth, and the interaction of other nutrients on muscle anabolism. It should be noted that protein intake of the control group was already twice the RDA of protein intake for maintaining nitrogen balance. Thus, although there was no significant effect of protein supplementation above twice the RDA, it is unclear whether protein supplementation between the RDA and twice the RDA can benefit the anabolic response to resistance training. No significant protein intake \times HMB interactions were noted relative to any of the reported measures, suggesting that the HMB effects on metabolism are additive with and independent of protein intakes.

In summary, dietary supplementation of 3 g of HMB/day to humans undergoing intense resistance-training exercise resulted in an increased deposition of FFM and an accompanying increase in strength. Muscle proteolysis was also decreased with HMB, which was accompanied by lower plasma levels of muscle damage-indicating enzymes and an \sim 50% decrease in the concentrations of plasma essential amino acids. The mechanism by which HMB impacts muscle proteolysis and function is not currently known.

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