

# Effects of Calcium $\beta$ -Hydroxy- $\beta$ -methylbutyrate (HMB) Supplementation During Resistance-Training on Markers of Catabolism, Body Composition and Strength

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Calcium  $\beta$ -hydroxy- $\beta$ -methylbutyrate (HMB) supplementation has been reported to reduce muscle catabolism and promote gains in fat-free mass and strength in subjects initiating training. However, whether HMB supplementation promotes these adaptations in trained athletes is less clear. This study examined the effects of HMB (as the calcium salt) supplementation during resistance training ( $6.9 \pm 0.7 \text{ hr} \times \text{wk}^{-1}$ ) on markers of catabolism, body composition and strength in experienced resistance-trained males. In a double-blind and randomized manner, 40 experienced resistance-trained athletes were matched and assigned to supplement their diet for 28 d with a fortified carbohydrate/protein powder containing either 0, 3 or  $6 \text{ g} \times \text{d}^{-1}$  of calcium HMB. Fasting venous blood and urine samples, dual energy X-ray absorptiometer-determined body composition, and isotonic bench press and leg press one repetition maximums (1 RM) were determined prior to and following 28 d of supplementation. HMB supplementation resulted in significant increases in serum and urinary HMB concentrations. However, no statistically significant differences were observed in general markers of whole body anabolic/catabolic status, muscle and liver enzyme efflux, fat/bone-free mass, fat mass, percent body fat, or 1 RM strength. Results indicate that 28 d of HMB supplementation ( $3$  to  $6 \text{ g} \times \text{d}^{-1}$ ) during resistance-training does not reduce catabolism or affect training-induced changes in body composition and strength in experienced resistance-trained males.

■ Key words: Exercise, sport nutrition, ergogenic aids.

## Introduction

The leucine metabolite  $\beta$ -hydroxy- $\beta$ -methylbutyrate (HMB) has recently become a popular dietary supplement purported to promote gains in fat-free mass (FFM) and strength during resistance-training. The rationale for this is related to observations that leucine and its metabolite  $\beta$ -ketoisocaproate (KIC) have been reported to inhibit protein degradation [11,12]. The anti-proteolytic effects of leucine and KIC have been suggested to be regulated by the leucine metabolite HMB [12]. Animal studies indicate that HMB is synthesized from KIC primarily as a byproduct of leucine metabolism and that approximately 5% of oxidized leucine is converted to HMB [16]. The addition of HMB to dietary feed improved colostral milk fat and sow growth performance [13], tended to improve the carcass quality of steers [15], and decreased markers of catabolism during training in horses [10]. Based on these findings, it has been hypothesized that supplementing the diet with leucine and/or HMB in humans may inhibit protein degradation during periods associated with increased proteolysis such as resistance-training.

Although much of the available literature on HMB supplementation in humans is preliminary in nature, there are several recently published articles and abstracts that support this hypothesis. In this regard, leucine infusion has been reported to decrease protein degradation in humans, suggesting leucine may serve as a regulator of protein metabolism [11]. Moreover, Nissen and colleagues reported significantly greater gains of FFM and strength in untrained men [12] and women [14] initiating resistance-training when administered  $1.5$  to  $3 \text{ g} \times \text{d}^{-1}$  of HMB (as the calcium salt) for 3 to 4 wks. These gains were associated with significantly less muscle enzyme efflux and urinary 3-methylhistidine excretion, suggesting that subjects ingesting HMB experienced less muscle catabolism during training [12]. Vukovich and coworkers [18] reported that 8 wks of HMB supplementation ( $3 \text{ g} \times \text{d}^{-1}$  of HMB as the calcium salt), compared to controls ingesting a placebo, significantly increased FFM ( $-0.58$  vs.  $1.5\%$ ), reduced fat mass ( $0.27$  vs.  $2.2\%$ ), and promoted greater gains in upper and lower extremity 1 RM strength in a group of elderly men and women initiating training. These preliminary findings suggest that dietary supplementation of  $1.5$  to  $3 \text{ g} \times \text{d}^{-1}$  of HMB may enhance training-induced changes in FFM and strength in untrained subjects initiating training [12,14,18].

However, it is less clear whether HMB supplementation reduces markers of catabolism and/or promotes greater gains in FFM and strength during resistance-training in well-trained athletes. Nissen and colleagues [12] reported that HMB supplementation ( $3 \text{ g} \times \text{d}^{-1}$  of HMB as the calcium salt) ingested with a vitamin/mineral fortified carbohydrate/protein meal replacement powder significantly increased FFM ( $\sim 2.7 \text{ kg}$ ) during the first 3 to 4 wks of a 7-wk off-season college football resistance-training in comparison to subjects ingesting an isoenergetic amount of orange juice without supplemental protein. However, there was no significant difference in FFM between the groups after 7 wks of training. In a previous study from this lab, we reported that 28 d of calcium HMB supplementation ( $3 \text{ g} \times \text{d}^{-1}$  of HMB as the calcium salt) during intense off-season college football resistance/agility training ( $\sim 8 \text{ hr/wk}$ ) did significantly affect FFM, isotonic lifting volume, or work performed during repetitive sprint performance [1,8]. We also found that HMB supplementation did not significantly affect muscle enzyme efflux or general markers of whole body catabolism.

There are several possible reasons for the discrepancy in results observed among studies. First, it is possible that untrained subjects initiating training may obtain greater benefit from HMB supplementation than experienced resistance-trained athletes. Second, it is possible that athletes undergoing intense training may need to supplement their diet with larger doses of HMB in order to obtain ergogenic benefit. Finally, since the athletes in the Nissen and colleagues study [12] ingested HMB with a vitamin/mineral fortified carbohydrate/protein meal replacement powder, it is possible that the results observed were due to differences in macronutrient intake, micronutrient intake, and/or that HMB acted synergistically with the ingredients contained in supplemental powder.

The purpose of this study was to determine whether ingesting 3 or  $6 \text{ g} \times \text{d}^{-1}$  of HMB with a vitamin/mineral fortified carbohydrate/protein meal replacement powder affects markers of catabolism, promotes lean tissue accretion, and/or enhances gains in strength during resistance-training in well-trained athletes in comparison to ingesting the vitamin/mineral fortified carbohydrate/protein meal replacement powder without HMB.

## Methods and Materials

### Subjects

Forty experienced resistance-trained males volunteered to participate in this study. Subjects were informed as to the experimental procedures and signed informed consent statements in adherence with the human subjects guidelines of The University of Memphis and the American College of Sports Medicine. Subject characteristics are presented in Table 1.

### Experimental design

In order to participate in the study, subjects had to 1) sign statements indicating they had no current or past history of anabolic steroid use; 2) be an experienced resistance-trained athlete ( $> 1 \text{ yr}$ ) who was currently training at least 3 hrs/wk with a program that included bench press and leg press/squat exercises; 3) submit a detailed description of their current training program; 4) not have ingested creatine, HMB, or beta-agonists for an 8-wk period prior to the start of supple-

**Table 1** Subject characteristics

| Variable   | Mean $\pm$ SEM |
|--|----------------|
| Age (yrs)  | 25.1 $\pm$ 1   |
| Weight (kg)  | 82.4 $\pm$ 2   |
| Height (cm)  | 178 $\pm$ 2    |
| Body Fat (%)   | 15.2 $\pm$ 1   |
| Resistance Training Experience (yrs)                                     | 5.5 $\pm$ 0.6  |
| Current Training ( $\text{hr} \times \text{wk}^{-1}$ )                   | 6.9 $\pm$ 0.5  |
| Bench Press 1 RM ( $\text{kg} \times \text{kg}^{-1}$ body weight)        | 1.4 $\pm$ 0.04 |
| Hip Sled/Leg Press 1 RM ( $\text{kg} \times \text{kg}^{-1}$ body weight) | 3.0 $\pm$ 0.8  |

mentation; and 5) agree not to ingest any other nutritional supplements, proposed ergogenic aids, or non-prescription drugs during the course of the study.

Subjects participated in two familiarization sessions. In the first familiarization session, the procedures of the study were explained, the subjects were weighed, training and medical history forms were completed, and the subjects were familiarized to the strength testing equipment and procedures. The subjects were instructed by a registered dietitian on how to report nutritional intake on nutritional log sheets. A certified strength and conditioning specialist instructed the subjects how to record training data (i.e. lifts performed, repetitions, amount of weight lifted, etc.) on training log forms. In the second session, subjects practiced using the bench press and hip sled/leg press strength testing equipment and were scheduled for pre-supplementation assessments. The investigators also clarified any questions the subjects had regarding methods of the study.

Pre-supplementation assessments included: 1) a 4-d nutritional intake assessment (including one weekend day); 2) donation of an 8 h fasting venous blood sample and a urine sample; 3) measurement of total body mass, total body water, and body composition; and 4) performance of 1 RM strength tests on the isotonic bench press and hip sled/leg press. Following these assessments, subjects were matched according to total body mass, FFM, years of training, hours per week of resistance-training, and training program type/volume. In a double-blind and randomized manner, subjects were then assigned to supplement their normal diet for 28 d with a vitamin/mineral fortified carbohydrate/protein powder (Neo-Lean™, Experimental & Applied Sciences, Golden, CO) containing  $81 \text{ g} \times \text{d}^{-1}$  of carbohydrate,  $75 \text{ g} \times \text{d}^{-1}$  of protein, and  $3 \text{ g} \times \text{d}^{-1}$  of fat with either 0 ( $n = 15$ ), 3 ( $n = 13$ ), or  $6 \text{ g} \times \text{d}^{-1}$  ( $n = 12$ ) of HMB (as the calcium salt) added to the formulation. Table 2 describes the ingredients contained in the carbohydrate/protein supplement.

Subjects maintained their usual individualized training program and recorded all training on training log sheets during the supplementation period. Following the 28-d supplementation period, subjects underwent post-supplementation assessments in a similar manner as the pre-supplementation tests. Specifically, diet was recorded for 4 d; subjects donated an 8-h fasting venous blood and urine sample; body mass, body water, and body composition were determined; and, subjects per-

**Table 2** Ingredient list for the NeoLean™ supplement used in the H0, H3, and H6 groups (calculated from total daily servings)

| Ingredient             | NeoLean™ |
|------------------------|----------|
| <i>Macronutrients</i>  |          |
| Carbohydrate (g)       | 81       |
| Protein (g)            | 75       |
| Fat (g)                | 3        |
| <i>Vitamins</i>        |          |
| Vitamin A (mg)         | 1.5      |
| Vitamin D (mcg)        | 1.5      |
| Vitamin C (mg)         | 180      |
| Vitamin E (mg)         | 30       |
| Thiamin (mg)           | 2.25     |
| Riboflavin (mg)        | 2.55     |
| Niacin (mg)            | 28.5     |
| Vitamin B-6 (mg)       | 3.0      |
| Vitamin B-12 (mcg)     | 3        |
| Pantothenic Acid (mg)  | 8.25     |
| Folic Acid (mcg)       | 300      |
| Biotin (mcg)           | 45       |
| <i>Minerals</i>        |          |
| Sodium (mg)            | 870      |
| Calcium (mg)           | 1800     |
| Magnesium (mg)         | 525      |
| Potassium (mg)         | 1770     |
| Zinc (mg)              | 22.5     |
| Manganese (mg)         | 6        |
| Copper (mg)            | 3.4      |
| Iron (mg)              | 15       |
| Phosphorus (mg)        | 1800     |
| Iodine (mcg)           | 225      |
| Selenium (mcg)         | 75       |
| Chromium (mcg)         | 600      |
| Molybdenum (mcg)       | 150      |
| <i>Other Nutrients</i> |          |
| L-Carnitine (mg)       | 300      |
| Choline (mg)           | 300      |
| Boron (mg)             | 3        |
| Garcinia Cambogia (mg) | 750      |

Values are calculated based on Reference Daily Intake (RDI) values for food label percent translations.

formed 1 RM strength tests on the isotonic bench press and hip sled/leg press.

### Procedures

Supplements were prepared in powder form with identical texture, taste and appearance and independently packaged/labeled in single-serving foil packets for double-blind administration by a food science lab. Subjects mixed the supplement powder into water, milk, or juice and ingested the solution following morning, mid-day and evening meals. Consequently, individual supplement packets contained a single-serving of the carbohydrate/protein supplement with either 0, 1, or 2 g/serving of HMB. Subject compliance in taking the supplements was verified by collecting empty supplement packets at the conclusion of the study, assessing blood and urine samples for HMB concentrations, and post-study questionnaires. Subjects had to turn in all empty packets and complete all aspects of the study in order to receive payment and/or incentives for participating in the study (i.e. \$ 100 for four cans of Phosphagen® [Experimental & Applied Sciences, Golden CO]).

Subjects maintained their normal diet throughout the supplementation period. Nutritional intake was monitored for 4 d prior to the initiation of supplementation and during the final week of supplementation. Nutritional records were evaluated and analyzed by a registered dietitian using the Food Processor III nutritional analysis software (Nutritional Systems, Salem, OR).

Prior to donating blood and urine samples, subjects: 1) observed a meat-free diet for 3 d in order to normalize the effects of diet on 3-methylhistidine and HMB concentrations as previously described [12]; 2) did not exercise for 48 h; and 3) observed an overnight 8-h fast. Venous blood samples were obtained via venipuncture from an antecubital vein in the forearm using standard phlebotomy procedures from 6.00 to 8.00 am. Venous blood was collected into two 10 mL serum separation tubes (SST) and a 5 mL anticoagulant tube containing K<sub>3</sub> (EDTA). The SST's were centrifuged at 5,000 rev × min<sup>-1</sup> for 10 min using a Biofuge 17 R centrifuge (Heraeus Inc., Germany). Serum from one SST was transferred into microcentrifuge tubes and frozen at -80 °C for subsequent analysis. Serum from the remaining SST was transferred into a 10 ml plain sterile tube. The plain and EDTA tubes were refrigerated and shipped overnight in cold containers to Ciba Corning Diagnostic Laboratories (St. Louis, MO) for clinical analysis. A complete clinical chemistry panel (31 items) was run on serum samples using the Technicon DAX model 96-0147 automated chemistry analyzer (Technicon Inc., Terry Town, NY) following standard clinical procedures. Cell blood counts with percent differentials were run on whole blood samples using a Coulter STKS automated analyzer using standard procedures (Coulter Inc., Hialeah, FL). Urine samples were collected in sterile urine collection containers and frozen at -80 °C. Frozen serum and urine samples were shipped overnight to the Department of Animal Science at Iowa State University for blind determination of HMB concentrations in serum and urine, using procedures previously described [12].

On the same day that blood and urine samples were collected, subjects performed 1 RM strength tests. A warm-up on the bench press was followed by 3 to 5 progressive 1 RM attempts. Hand position on the bar was recorded and the weight plates were standardized between trials. Subjects were required to maintain good lifting form (i.e., feet maintaining contact with the floor, no arching of the back off of the bench, no bouncing of the weight off of the chest). Once 1 RM was determined on the bench press, subjects rested for 10 min and began warming up for the hip sled/leg press test. The leg press 1 RM test was performed on an AMF hip sled (AMF, Jefferson, IA). Subjects were positioned on their back in an adjustable back/shoulder support. The adjustable back/shoulder support was moved to allow each subject to be positioned so that their knees were bent passed 90 degrees with their thighs approximately one inch from their chest and their feet were comfortably positioned. Back/shoulder support position, foot placement position, athletic shoes worn, and the weight plates used were standardized between trials. Subjects were required to maintain standardized lifting form. Subjects typically made 4 to 6 lifts before achieving their 1 RM on the leg press. Strength tests were performed in a competitive environment with financial incentives awarded to the subjects based upon their performance in order to motivate the subjects to perform to the best of their ability. All 1 RM tests were performed under the supervi-

sion of certified strength and conditioning specialists using standardized lifting criteria [2, 7, 19].

Subjects were instructed to not exercise and to fast for 4 hr prior to body composition assessments. Total body mass was measured on a calibrated digital scale with a precision of  $\pm 0.02$  kg (Sterling Scale, Co., Southfield, MI). Total body water was estimated [17] using a Valhalla 1990b Bioelectrical Impedance Analyzer (Valhalla Scientific, San Diego, CA). Whole body (excluding cranium) body composition measurements were determined using a Hologic QDR-2000 dual energy X-ray absorptiometer (DEXA) with the Hologic version V7, REV F software (Waltham, MA) using procedures previously described [6, 8]. DEXA measures the amount of bone, fat, and fat-free/soft tissue mass that falls within standardized density ranges. The DEXA scans regions of the body (right arm, left arm, trunk, right leg, left leg) to determine the amount of bone mass, fat mass, and fat-free/soft tissue mass within each region. The scanned bone, fat, and fat-free/soft tissue mass for each region was then subtotaled to determine whole body (excluding cranium) values. Percent body fat is calculated by dividing the amount of measured fat mass by total scanned mass (sum of bone mass, fat mass, and fat-free/soft tissue mass). DEXA has been shown to be a highly reliable ( $r = 0.99$ ) and precise method (coefficient of variation of 0.5–1%) for determining individual body composition segments [3–5, 9].

Subjects were positioned according to standardized criteria during the initial scan. DEXAs were performed under the supervision of a certified radiology technician. Quality control (QC) calibration procedures were performed on a spine phantom (Hologic X-CALIBER Model DPA/QDR-1 anthropometric spine phantom) prior to each testing session according to procedures previously described [6, 8]. Mean coefficients of variation in BMC and BMD measurements obtained in the lateral and array modes ranged between 0.41 to 0.55% throughout the life of the unit. Test-retest reliability studies performed on male athletes with this DEXA machine yielded mean deviation for total BMC and total fat-free/soft tissue mass of 0.31% with a mean intraclass correlation of 0.985 [6].

### Statistical analysis

Data were analyzed by a  $2 \times 2$  repeated measures analysis of variance (ANOVA) using SPSS for Windows Version 7.5 software (SPSS, Inc., Chicago, IL). Delta scores (post-prevalues) were calculated on selected variables and analyzed by one way ANOVA. In addition, a general linear model statistical analysis was performed on body composition data in order to determine whether there was a dose related linear change in body composition data as previously described [12]. Data are presented as means  $\pm$  standard error of means. Data were considered significantly different when the probability of error was 0.05 or less.

## Results

### Side effects

Analysis of post-study questionnaires revealed that subjects tolerated the supplementation protocol well with no reports of medical problems or symptoms.

### Training and diet

No differences were observed among groups in total lifting volume during the training/supplementation period. Table 3 presents dietary intake data among groups ingesting the carbohydrate/protein powder containing  $0 \text{ g} \times \text{d}^{-1}$  of HMB (H0),  $3 \text{ g} \times \text{d}^{-1}$  of HMB (H3), or  $6 \text{ g} \times \text{d}^{-1}$  of HMB (H6). Dietary supplementation of the carbohydrate/protein powder significantly increased mean energy intake ( $4.1 \pm 1.5 \text{ kcal} \times \text{kg}^{-1} \times \text{d}^{-1}$ ,  $p = 0.01$ ), mean carbohydrate intake ( $0.5 \pm 0.2 \text{ g} \times \text{kg}^{-1} \times \text{d}^{-1}$ ,  $p = 0.04$ ), and mean protein intake ( $0.84 \pm 0.1 \text{ g} \times \text{kg}^{-1} \times \text{d}^{-1}$ ,  $p = 0.001$ ) in all groups combined. However, no significant interactions were observed among groups in pre- and post-supplementation energy intake, carbohydrate, protein, or fat intake.

### Chemistry profiles

Table 4 presents selected markers for catabolism and muscle/liver enzymes for the H0, H3, and H6 groups. All blood variables evaluated remained within normal limits for individuals engaged in heavy exercise training. In comparison to the H0 group, serum (H0  $0.7 \pm 0.4$ ; H3  $22.3 \pm 8$ ; H6  $37.4 \pm 15 \mu\text{M}$ ,  $p = 0.03$ ) and urinary (H0  $30 \pm 15$ ; H3  $783 \pm 292$ ; H6  $1.301 \pm 437 \mu\text{M}$ ,  $p = 0.009$ ) HMB concentrations were significantly greater in the H3 and H6 groups. There was some evidence that the change in total creatine kinase (CK) levels in the H6 group tended to be lower than the change observed in the H0 group (H0  $96 \pm 62$ ; H3  $-11 \pm 30$ ; H6  $-114 \pm 92 \text{ IU}$ ). However, this difference was not significantly different ( $p = 0.09$ ). No significant group  $\times$  time interactions were observed among groups in creatinine, urea nitrogen, the ratio of urea nitrogen to creatinine, uric acid, lactate dehydrogenase (LDH), alanine

**Table 3** Dietary intake data for the H0, H3, and H6 supplemented groups

| Variable  | Group       | Day 0 | Day 28 |                     | p     |
|---|-------------|-------|--------|---------------------|-------|
| Energy Intake<br>(kcal $\times$ kg $^{-1}$ $\times$ d $^{-1}$ )       | H0 $\times$ | 36.4  | 42.4   | Group               | 0.6   |
|   | $\pm$       | 3.2   | 2.7    | Time                | 0.01  |
|   | H3 $\times$ | 34.1  | 37.6   | Group $\times$ Time | 0.71  |
|   | $\pm$       | 3.2   | 2.7    |                     |       |
|   | H6 $\times$ | 35.8  | 38.7   |                     |       |
|   | $\pm$       | 3.4   | 2.8    |                     |       |
| Carbohydrate<br>Intake<br>(g $\times$ kg $^{-1}$ $\times$ d $^{-1}$ ) | H0 $\times$ | 5.1   | 6.0    | Group               | 0.33  |
|   | $\pm$       | 0.5   | 0.4    | Time                | 0.04  |
|   | H3 $\times$ | 4.6   | 4.9    | Group $\times$ Time | 0.51  |
|   | $\pm$       | 0.5   | 0.4    |                     |       |
|   | H6 $\times$ | 4.7   | 5.0    |                     |       |
|   | $\pm$       | 0.5   | 0.4    |                     |       |
| Protein Intake<br>(g $\times$ kg $^{-1}$ $\times$ d $^{-1}$ )         | H0 $\times$ | 1.5   | 2.4    | Group               | 0.57  |
|   | $\pm$       | 0.1   | 0.1    | Time                | 0.001 |
|   | H3 $\times$ | 1.5   | 2.3    | Group $\times$ Time | 0.90  |
|   | $\pm$       | 0.1   | 0.1    |                     |       |
|   | H6 $\times$ | 1.4   | 2.2    |                     |       |
|   | $\pm$       | 0.2   | 0.2    |                     |       |
| Fat Intake<br>(g $\times$ kg $^{-1}$ $\times$ d $^{-1}$ )             | H0 $\times$ | 1.1   | 0.9    | Group               | 0.53  |
|   | $\pm$       | 0.1   | 0.1    | Time                | 0.06  |
|   | H3 $\times$ | 1.1   | 1.0    | Group $\times$ Time | 0.96  |
|   | $\pm$       | 0.1   | 0.1    |                     |       |
|   | H6 $\times$ | 1.3   | 1.1    |                     |       |
|   | $\pm$       | 0.2   | 0.1    |                     |       |

$\times$  Represents group mean.  $\pm$  Represents standard error of mean.

**Table 4** Selected markers of catabolism and muscle/liver enzyme efflux for the H0, H3, and H6 supplemented groups

| Variable  | Group | Day 0 | Day 28 | p            |       |
|---|-------|-------|--------|--------------|-------|
| Creatinine<br>$\mu\text{mol} \times \text{L}^{-1}$  | H0 ×  | 110   | 107    | Group        | 0.03  |
|   | ±     | 3     | 3      |              |       |
|   | H3 ×  | 101   | 96     | Group × Time | 0.72  |
|   | ±     | 3     | 3      |              |       |
| H6 ×  | 107   | 99    |        |              |       |
| ±   | 4     | 3     |        |              |       |
| Urea Nitrogen<br>$\text{mmol} \times \text{L}^{-1}$ | H0 ×  | 4.8   | 5.4    | Group        | 0.06  |
|   | ±     | 0.2   | 0.3    |              |       |
|   | H3 ×  | 5.6   | 6.3    | Group × Time | 0.56  |
|   | ±     | 0.3   | 0.3    |              |       |
| H6 ×  | 5.7   | 5.9   |        |              |       |
| ±   | 0.4   | 0.3   |        |              |       |
| Urea Nitrogen/<br>Creatinine Ratio                  | H0 ×  | 10.9  | 12.6   | Group        | 0.005 |
|   | ±     | 0.6   | 0.9    |              |       |
|   | H3 ×  | 13.6  | 16.4   | Group × Time | 0.60  |
|   | ±     | 0.7   | 0.8    |              |       |
| H6 ×  | 13.4  | 15.0  |        |              |       |
| ±   | 1.0   | 1.1   |        |              |       |
| Uric Acid<br>$\mu\text{mol} \times \text{L}^{-1}$   | H0 ×  | 476   | 416    | Group        | 0.52  |
|   | ±     | 34    | 42     |              |       |
|   | H3 ×  | 444   | 369    | Group × Time | 0.58  |
|   | ±     | 37    | 37     |              |       |
| H6 ×  | 409   | 377   |        |              |       |
| ±   | 32    | 37    |        |              |       |
| CK $\text{IU} \times \text{L}^{-1}$                 | H0 ×  | 332   | 429    | Group        | 0.29  |
|   | ±     | 88    | 93     |              |       |
|   | H3 ×  | 244   | 233    | Group × Time | 0.09  |
|   | ±     | 30    | 39     |              |       |
| H6 ×  | 425   | 312   |        |              |       |
| ±   | 100   | 68    |        |              |       |
| LDH $\text{IU} \times \text{L}^{-1}$                | H0 ×  | 152   | 212    | Group        | 0.53  |
|   | ±     | 6     | 37     |              |       |
|   | H3 ×  | 153   | 234    | Group × Time | 0.59  |
|   | ±     | 7     | 37     |              |       |
| H6 ×  | 149   | 178   |        |              |       |
| ±   | 9     | 25    |        |              |       |
| ALT $\text{IU} \times \text{L}^{-1}$                | H0 ×  | 28.2  | 26.5   | Group        | 0.81  |
|   | ±     | 3.3   | 2.6    |              |       |
|   | H3 ×  | 29.8  | 29.5   | Group × Time | 0.64  |
|   | ±     | 3.2   | 2.4    |              |       |
| H6 ×  | 29.3  | 30.3  |        |              |       |
| ±   | 3.8   | 4.4   |        |              |       |
| AST $\text{IU} \times \text{L}^{-1}$                | H0 ×  | 24.5  | 24.8   | Group        | 0.68  |
|   | ±     | 2.5   | 1.8    |              |       |
|   | H3 ×  | 24.5  | 25.6   | Group × Time | 0.94  |
|   | ±     | 1.2   | 1.6    |              |       |
| H6 ×  | 26.8  | 26.9  |        |              |       |
| ±   | 2.7   | 2.6   |        |              |       |

× Represents group mean. ± Represents standard error of mean.

aminotransferase (ALT), or aspartate aminotransferase (AST). Additionally, no significant interactions were observed among groups in gamma-glutamyl transferase, total protein, albumin, globulin, glucose, electrolytes, lipid profiles, total bilirubin, hemoglobin, hematocrit, red blood cells, white blood cells, or types of lymphocytes.

**Table 5** DEXA body composition data for the H0, H3, and H6 supplemented groups

| Variable                   | Group | Day 0 | Day 28 | p            |      |
|----------------------------|-------|-------|--------|--------------|------|
| Scanned<br>Mass<br>(kg)    | H0 ×  | 74.6  | 75.1   | Group        | 0.74 |
|                            | ±     | 4.3   | 4.3    |              |      |
|                            | H3 ×  | 76.3  | 77.3   | Group × Time | 0.34 |
|                            | ±     | 3.7   | 3.6    |              |      |
| H6 ×                       | 78.6  | 80.0  |        |              |      |
| ±                          | 3.7   | 3.7   |        |              |      |
| Fat/Bone Free<br>Mass (kg) | H0 ×  | 59.9  | 60.2   | Group        | 0.38 |
|                            | ±     | 2.6   | 2.5    |              |      |
|                            | H3 ×  | 61.3  | 62.0   | Group × Time | 0.46 |
|                            | ±     | 1.6   | 1.6    |              |      |
| H6 ×                       | 64.1  | 65.1  |        |              |      |
| ±                          | 2.5   | 2.5   |        |              |      |
| Fat Mass<br>(kg)           | H0 ×  | 12.3  | 12.4   | Group        | 0.99 |
|                            | ±     | 2.0   | 1.9    |              |      |
|                            | H3 ×  | 12.5  | 12.8   | Group × Time | 0.81 |
|                            | ±     | 2.8   | 2.6    |              |      |
| H6 ×                       | 11.9  | 12.3  |        |              |      |
| ±                          | 2.0   | 2.0   |        |              |      |
| Bone Mass<br>(g)           | H0 ×  | 2434  | 2434   | Group        | 0.65 |
|                            | ±     | 129   | 121    |              |      |
|                            | H3 ×  | 2517  | 2533   | Group × Time | 0.35 |
|                            | ±     | 95    | 90     |              |      |
| H6 ×                       | 2569  | 2591  |        |              |      |
| ±                          | 118   | 122   |        |              |      |
| Body Fat<br>(%)            | H0 ×  | 15.6  | 12.4   | Group        | 0.93 |
|                            | ±     | 1.4   | 1.9    |              |      |
|                            | H3 ×  | 15.2  | 15.5   | Group × Time | 0.87 |
|                            | ±     | 2.4   | 2.1    |              |      |
| H6 ×                       | 14.5  | 14.8  |        |              |      |
| ±                          | 2.1   | 2.1   |        |              |      |

× Represents group mean. ± Represents standard error of mean.

#### Total body mass and body water

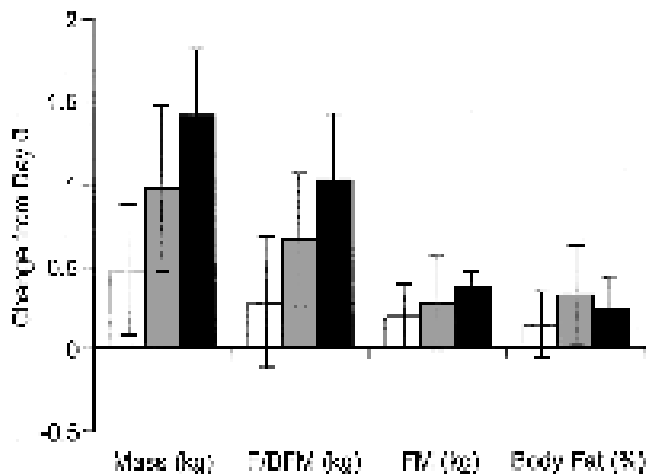
No significant differences were observed among H0, H3, and H6 groups, respectively, in changes in total body weight ( $0.43 \pm 0.5$ ;  $0.71 \pm 0.5$ ;  $0.82 \pm 0.3$  kg,  $p = 0.81$ ) or total body water expressed as a percentage of total body mass ( $-0.4 \pm 0.3$ ;  $0.2 \pm 0.3$ ;  $0.5 \pm 0.4\%$ ,  $p = 0.12$ ).

#### Body composition

Table 5 presents DEXA determined body composition data obtained prior to and following 28 d of supplementation while Fig. 1 presents mean changes in body composition values from Day 0. No significant interactions were observed in total scanned mass, fat/bone free mass (F/BFM), fat mass, bone mass or percent body fat. In addition, general linear model statistical analysis revealed non-significant linear trends in total scanned mass ( $p = 0.17$ ), fat/bone free mass ( $p = 0.22$ ), fat mass ( $p = 0.63$ ), bone mass ( $p = 0.25$ ), and percent body fat ( $p = 0.91$ ).

#### Strength

Table 6 presents pre- and post-supplementation 1 RM strength results for the H0, H3, and H6 supplemented groups. No significant interactions were observed among groups in bench press or leg press 1 RM values. Moreover, no significant differences were observed among H0, H3, and H6 groups, respectively, in



**Fig. 1** Changes in DEXA determined scanned body mass (Mass), fat/bone-free mass (F/BFM), fat mass (FM) and percent body fat observed for the H0 (open bars), H3 (shaded bars), and H6 (dark bars) supplemented groups. Data are means  $\pm$  standard error of means.

**Table 6** 1 RM strength test data for the H0, H3, and H6 supplemented groups

| Variable         | Group       | Day 0 | Day 28 |                     | p    |
|------------------|-------------|-------|--------|---------------------|------|
| Bench Press (kg) | H0 $\times$ | 113.5 | 115.3  | Group               | 0.96 |
|                  | $\pm$       | 7.8   | 7.4    | Time                |      |
|                  | H3 $\times$ | 116.5 | 117.7  | Group $\times$ Time |      |
|                  | $\pm$       | 7.8   | 7.4    |                     |      |
|                  | H6 $\times$ | 115.0 | 118.2  |                     |      |
|                  | $\pm$       | 6.9   | 6.5    |                     |      |
| Leg Press (kg)   | H0 $\times$ | 247.3 | 249.5  | Group               | 0.86 |
|                  | $\pm$       | 16.4  | 16.3   | Time                |      |
|                  | H3 $\times$ | 255.2 | 263.7  | Group $\times$ Time |      |
|                  | $\pm$       | 13.4  | 14.0   |                     |      |
|                  | H6 $\times$ | 249.7 | 256.5  |                     |      |
|                  | $\pm$       | 12.4  | 12.9   |                     |      |

$\times$  Represents group mean.  $\pm$  Represents standard error of mean.

overall gains in 1 RM strength when bench press and leg press values were combined ( $3.1 \pm 6.1$ ;  $9.0 \pm 3.5$ ;  $8.3 \pm 3.9$  kg,  $p = 0.63$ ).

## Discussion

Previous studies indicated that HMB supplementation ( $1.5$  or  $3$  g  $\times$  d $^{-1}$ ) during 3 to 8 wks of training promoted significantly greater changes in FFM, fat loss, and/or strength while decreasing markers of catabolism in untrained men [12,18] and women [14,18] initiating a resistance-training program. Moreover, HMB supplementation with a carbohydrate/protein meal replacement supplement during 7 wks of off-season college football resistance-training promoted greater gains in FFM during the first 3 to 4 wks of training in comparison to subjects ingesting an isoenergetic amount of orange juice. Collectively, these findings suggest that HMB supplementation may enhance training-induced adaptations.

However, in contrast to these findings, we previously reported that 28 g of HMB supplementation ( $3$  g  $\times$  d $^{-1}$ ) during off-season football resistance/agility training did not significantly affect FFM or strength gains in well-trained athletes [1,8]. In comparing these findings to previous studies, there appeared to be several differences between studies which may have accounted for the discrepancy in results observed. In the present study, we attempted to control for some of these factors by supplementing the subjects diet with a vitamin/mineral fortified carbohydrate/protein powder containing 0, 3, or 6 g  $\times$  d $^{-1}$  of HMB. This ensured: 1) that the only difference between placebo and HMB supplements was the HMB content of the supplements; 2) that by increasing mean protein intake from  $1.5 \pm 0.1$  to  $2.3 \pm 0.1$  g  $\times$  kg $^{-1}$   $\times$  d $^{-1}$  subjects would in all likelihood maintain a positive nitrogen/protein balance during training; and 3) that a dose response of HMB supplementation during training could be evaluated. Additionally, we performed identical statistical analysis procedures on the data.

Results of the present study indicate that supplementing the diet with a vitamin/mineral fortified carbohydrate/protein powder containing 3 and 6 g  $\times$  d $^{-1}$  of HMB did not significantly affect markers of anabolic/catabolic status, FFM, fat mass, or gains in upper and lower extremity 1 RM strength in resistance-trained athletes in comparison to ingesting the vitamin/mineral fortified carbohydrate/protein powder alone. However, it should be noted that, although not statistically significant, the mean changes in CK enzyme efflux, FFM, and overall 1 RM strength observed were similar to values previously reported [12,14,18]. Further, although not statistically significant, there appeared to be a dose-related response in mean changes in FFM and strength among groups receiving 0, 3 and 6 g  $\times$  d $^{-1}$  of HMB.

Whether supplementing the diets of these athletes for a longer period of time and/or incorporating additional training, dietary and/or experimental controls would have resulted in statistically significant results remains unclear. It is also unclear whether experienced resistance-trained athletes are less responsive to HMB supplementation than untrained counterparts initiating training or whether there is greater variability in responsiveness to HMB supplementation among experienced resistance-trained athletes. Nevertheless, results of the present study do not support contentions that HMB supplementation during training provides ergogenic value to experienced resistance-trained athletes. Additional research is necessary to determine whether HMB supplementation during resistance-training in male and female athletes affects markers of catabolism and proteolysis, promotes lean tissue accretion, and/or enhances gains in strength.

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