Clinical Nutrition 32 (2013) 704–712

Contents lists available at SciVerse ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Randomized control trials

Effect of β-hydroxy-β-methylbutyrate (HMB) on lean body mass during 10 days of bed rest in older adults

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ARTICLE INFO

Article history:
Received 12 October 2012
Accepted 22 February 2013

Keywords:
Muscle
Body composition
Protein synthesis
HMB
Beta-hydroxy-beta-methylbutyrate

SUMMARY

Background: Loss of muscle mass due to prolonged bed rest decreases functional capacity and increases hospital morbidity and mortality in older adults.
Objective: To determine if HMB, a leucine metabolite, is capable of attenuating muscle decline in healthy older adults during complete bed rest.
Design: A randomized, controlled, double-blinded, parallel-group design study was carried out in 24 healthy (SPPB $\geq$ 9) older adult subjects (20 women, 4 men), confined to complete bed rest for ten days, followed by resistance training rehabilitation for eight weeks. Subjects in the experimental group were treated with HMB (calcium salt, 1.5 g twice daily – total 3 g/day). Control subjects were treated with an inactive placebo powder. Treatments were provided starting 5 days prior to bed rest till the end rehabilitation phase. DXA was used to measure body composition.
Results: Nineteen eligible older adults (BMI: 21–33; age: 60–76 year) were evaluable at the end of the bed rest period (Control $n = 8$; Ca-HMB $n = 11$). Bed rest caused a significant decrease in total lean body mass (LBM) (2.05 ± 0.66 kg; $p = 0.02$, paired t-test) in the Control group. With the exclusion of one subject, treatment with HMB prevented the decline in LBM over bed rest −0.17 ± 0.19 kg; $p = 0.23$, paired t-test). There was a statistically significant difference between treatment groups for change in LBM over bed rest ($p = 0.02$, ANOVA). Sub-analysis on female subjects (Control = 7, HMB = 8) also revealed a significant difference in change in LBM over bed rest between treatment groups ($p = 0.04$, ANOVA). However, differences in function parameters could not be observed, probably due to the sample size of the study.
Conclusions: In healthy older adults, HMB supplementation preserves muscle mass during 10 days of bed rest. These results need to be confirmed in a larger trial.
This trial is registered at http://ClinicalTrials.gov under NCT00945581.

1. Introduction

Muscle inactivity due a prolonged period of immobility or bed rest induces rapid muscle atrophy and loss of force and power.[1–4] This is an undesirable consequence of hospitalization after illness or injury. A majority of hospitalized patients are 65 years or older, and their hospital lengths of stay tend to be longer.[5] About 65% of older patients experience a decrease in ambulatory function with hospitalization, and between 30 and 55% report a decline in activities of daily living.[6,7]

Healthy older adults have been reported to lose ~1 kg (about 6%) of lean tissue from the lower extremities after 10 days of bed rest, with an associated ~16% decline in isokinetic knee extensor strength.[8] In addition, they displayed a significant decline in maximal aerobic capacity.[9] This dramatic loss of muscle mass and strength is greater than is observed in young adults after 14 or 28 days of bed rest.[4,10] Muscle atrophy during bed rest is attributed to a marked decline in the rates of skeletal muscle protein synthesis,[4,11] although the role of accelerated muscle protein degradation in relation to the rate of synthesis cannot be ruled out. Resistance exercise has been

Abbreviations: SPPB, Short Physical Performance Battery score; LBM, Lean body mass.

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0261-5614/$ – see front matter
http://dx.doi.org/10.1016/j.clnu.2013.02.011

shown to provide a potent anabolic stimulus during bed rest.[12,13] However, exercise may not always be a feasible option, especially in situations involving physical impairments, surgery or severe injury. Thus dietary interventions have been considered and are currently being explored for their efficacy in ameliorating the debilitating impact of muscle atrophy due to bed rest.

Strategies for nutritional intervention have included increasing dietary protein intake from 0.5 g protein/kg/day to more than 1 g protein/kg/day in malnourished hospitalized patients. Increasing protein intake resulted in improved nitrogen balance and greater rates of whole body protein synthesis.[14] Another study showed that supplementation of the diet with essential amino acids (EAA) stimulated muscle protein synthesis and improved some functional outcomes in healthy older adults, confined to 10 days of bed rest, with no effect on muscle mass.[15] In addition, daily ingestion of EAA plus carbohydrate ameliorated the loss of lean body mass and muscle strength during 28 days of bed rest in young subjects.[10] Some of the beneficial effects of EAA have been attributed to the anabolic stimulus provided by branched chain amino acids (BCAA), leucine, isoleucine and valine.[16,17] However to date, no intervention has been identified that prevents the progression of muscle mass decline in older adults on bed rest.

Beta-hydroxy-beta-methylbutyrate (HMB) is a metabolite of leucine that, when ingested in combination with the amino acids glutamine and arginine, has been shown to preserve lean body mass in chronic disease conditions such as cancer and AIDS.[18,19] HMB has been shown to have an anabolic effect on muscle when ingested in conjunction with exercise (reviewed in[20]). In healthy exercising older adult subjects, HMB (3 g/d) when consumed for 8 weeks tended to increase fat-free mass gain ($p = 0.08$) and significantly increased the percentage of body fat loss ($p = 0.05$) compared with the placebo group.[21] In addition, a recent study has demonstrated that daily supplementation of non-exercising older women with HMB (plus arginine and lysine) for 12 weeks significantly improved their functionality and strength, with a trend toward improvement of whole body protein synthesis ($p = 0.08$).[22]Thus it seems plausible that supplementation of the diet with HMB would ameliorate the decrease in muscle protein synthesis and attenuate loss of muscle in older adults confined to bed rest. In addition, a recent preclinical study has demonstrated the positive effects of HMB on long term immobilized muscles and recovery from immobilization.[23]

In the current study we have tested the efficacy of HMB supplementation on the declines in muscle mass, strength and function that occur over 10 days of bed rest in older adults.

2. Subjects and methods

2.1. Subjects

A total of 24 subjects was randomized to treatment of which 6 subjects did not complete the study for the following reasons: 3 subjects had a positive D-dimer test so were considered a risk for bed rest and not allowed to go on bed rest, 1 subject (HMB group) had knee pain which prevented her from participating due to requirements for subject to be able to do lower extremity strength testing, 1 subject (Control group) had an adverse event (AE) (vomiting) resulting in an early exit and 1 subject (Control group) had a serious adverse event (SAE) (pulmonary embolism) during the recovery portion of the study (this subject was considered evaluable for the bed rest portion of the study). There were 8 Control subjects who were evaluable for bed rest (Phase I) and 7 Control subjects evaluable for entire study. For the HMB group, there were 11 subjects who were evaluable for the entire study (See CONSORT Diagram; Supplemental figure).

No major changes were made to eligibility criteria after trial commencement with the exception of allowing subjects with sporadic fish oil supplementation (<7 consecutive days) to be eligible, since we anticipated that short term supplementation would not impact muscle metabolism in the present trial. The number of subjects used was selected based on power analysis of our previous bed rest study in older adults that demonstrated significant declines in muscle mass and strength over bed rest. No interim analysis was planned or carried out.

All subjects provided written informed consent and signed authorizations for Health Insurance Portability and Accountability Act before enrolling in the study. The following inclusion criteria were verified at screening: male or female ≥60 to ≤79 years of age; body mass index (BMI) ≥ 20 but ≤35; ambulatory with a Short Performance Physical Battery (SPPB) score of ≥9 (fully functional with no mobility limitations); compliance with prescribed activity level. Exclusion criteria ruled out subjects who had undergone recent major surgery, had active malignancy (exception basal or squamous cell skin carcinoma or carcinoma in situ of the uterine cervix); history of Deep Vein Thrombosis (DVT) or other hypercoagulation disorders; refractory anemia; history of diabetes or fasting blood glucose value > 126 mg/dL; presence of partial or full artificial limb; kidney disease or serum creatinine > 1.4 mg/dL; evidence of cardiovascular disease assessed during resting or exercise electrocardiography (EKG); untreated hypothyroidism; liver disease; chronic or acute gastrointestinal (GI) disease; uncontrolled severe diarrhea, nausea or vomiting; were actively pursuing weight loss; were enrolled in other clinical trials; could not refrain from smoking over the bed rest study period or could not discontinue anticoagulant therapy over bed rest period. Potential subjects were also excluded if they were taking any medications known to affect protein metabolism (e.g. progestational agents, steroids, growth hormone, dronabinol, marijuana, HMB, free amino acid supplements, dietary supplements to aid weight loss).

All subjects signed an informed consent after being informed of the procedures involved and of all possible risks. The study was performed at the University of Arkansas for Medical Sciences (UAMS) Clinical Research Center, Little Rock and approved by the Institutional Review Board of UAMS.

2.2. Study design

The study design was a prospective, randomized, double-blinded, placebo-controlled trial conducted at a single site (Table 1). At every screening visit, the subject eligibility status was verified, medication history collected and adverse events (AEs) recorded. The duration of the study was 13 months including recruitment, starting in late January 2010 and ending in late February 2011 when the last subject exited.

Eligible subjects were randomized to one of two groups. Sealed envelopes containing the subject treatment group assignment were prepared from randomization schedules generated by Abbott. Randomization schedules were computer-generated using a pseudo-random permuted blocks algorithm. The randomization was stratified by gender and age (60–69 years, 70–79 years). As eligible subjects were enrolled in the study by the UAMS site, they were sequentially assigned a subject number in ascending numerical order starting with the first envelope of the appropriate randomization stratum. Inside the envelope was a form indicating which study product should be given to the subject during the study period. Forms containing subject number assignments were prepared by Abbott, completed by the site and sent back to Abbott when a subject was enrolled. The groups were Control or Ca-HMB and each subject received 2 sachets of product per day.

Table 1
Study timeline and procedures.

Visits	Procedures
Screening visit 1	Eligibility criteria, informed consent, medical history, medication history
Screening visit 2	Physical examination, SPPB, fasting blood tests, resting EKG, distribute activity monitor and questionnaire (7-day collection)
Screening visit 3	VO_2 peak stress test, collect activity monitor and questionnaire
Pre-bed rest (Days 1–5)	Diet stabilization, treatment with HMB or placebo, 24 h urine collection, pre-bed rest strength and functional testing (day 3), pre-bed rest DXA (day 2), D-dimer test (day 4), AEs
10 days of bed rest (Day 6–15) – daily assessments	Diet stabilization, treatment with HMB or placebo, 24 h urine collection, 24 h nursing assessment, vital signs, fasted body weight, passive range of motion exercise, TED hose & SCD to prevent DVT, AEs
Day 6 (first day of bed rest)	Muscle protein synthesis study (8 h infusion), RMR, fasting blood draw
Day 13 (bed rest)	D-dimer test and ultrasound if positive D-dimer
Day 15 (final day of bed rest)	Muscle protein synthesis study (8-hr infusion), RMR, fasting blood draw
Day 16 (post bed rest)	Vital signs, fasted body weight, strength and functional testing, DXA, distribute product and product consumption records, discharge from CRC
Day 17 (post bed rest)	Return to CRC, VO2 peak test, distribute activity monitor and questionnaire (7-day collection), Initiate rehabilitation session
8 weeks (post bed rest)	Rehabilitation program (resistance training 3×/week), product consumption records, DXA (week 4 and week 8), strength measurement (week 2, 4, 6, 8), functionality measurements (week 4 and 8), AEs (weekly)

Abbreviations: AEs – adverse events, CRC – Clinical Research Center, DXA – dual X-ray absorptiometry, DVT – deep vein thrombosis, EKG – electrocardiogram, RMR – resting metabolic rate, SPPB – Short Physical Performance Battery, TED hose – Thrombo-Embolism Deterrent hose, SCD – Sequential Compression Device.

Each HMB sachet contained 1.5 g Ca-HMB (TSI, Salt Lake city, Utah), 4 g maltodextrin and 200 mg calcium with additional sweetener and flavoring agents. The composition of the Control sachet was identical to the HMB sachet with the exclusion of Ca-HMB. Both supplements were packaged indistinguishably by a third party manufacturer, except for the study code that was a 5-digit number that were identical for the two products except for the last digit. This study was a double-blinded study. Neither the investigators, their staff, Abbott scientists and staff involved in the study, or subjects were informed of the identity of any of the study products over the entire study period. The blind was maintained through the entire course of the study including the bed rest phase, rehabilitation phase and analysis of the data. The study center personnel were instructed not to analyze the contents of the study products or in any way seek to learn the identity of the study products. Subjects were instructed to consume twice daily (morning–evening) by mixing each sachet into a non-caloric, non-caffeinated, non-carbonated, non-milk-based beverage of their choice around or with their meal. Generally water was used to dissolve the product, Treatment with HMB or Control was initiated 5 days prior to bed rest and was continued until the end of the rehabilitation period.

For diet stabilization over the pre-bed rest and bed rest period, subjects were fed a metabolically controlled diet providing the RDA for protein intake (0.8 g protein/kg body weight per day). Total calorie needs were estimated using the Harris-Benedict equation for resting energy expenditure according to the following equation: For women = [655 + (9.56 × body weight in kg) + (1.85 × height in cm)–(4.68 × age in years)] × AF, or, For men = [66 + (13.7 × body weight in kg) + (5 × height in cm)–(608 × age in years] × AF, where AF = activity factor of 1.6 for the ambulatory and 1.35 for the bed rest periods. Given the total calorie and protein intakes, the remainder of the diet was manipulated to keep the non-protein calories at about 60% from carbohydrates and 40% from fat. Water was provided *ad libitum*.

Subjects were exited from study if they permanently discontinued product during the pre-bed rest period (Day 1 to Day 5), or if they discontinued product during the bed rest period and had completed less than 8 days of bed rest. Subjects with a positive D-dimer test or ultrasound for deep vein thrombosis (DVT) diagnosis were also exited from the study.

A subject's outcome data were classified as not evaluable for the analysis if one or more of the following events occurred: A. Subject received wrong product, contrary to the randomization scheme, B. Subject received excluded concomitant treatment defined as medications or dietary supplements that affect weight or metabolism (e.g. progestational agents, steroids, growth hormone, dronabinol, marijuana, HMB, free amino acid supplements, dietary supplements to aid weight loss, and fish oil supplements), C. subject had <67% of total study product consumption at final visit/exit as determined by product consumption records.

2.3. Bed rest and rehabilitation

After a diet stabilization of 5 days (ambulatory period), subjects remained in bed continuously for 10 days. While confined to bed rest, subjects were allowed to use the bedside commode for urination or were taken in a wheelchair for toileting. Subjects were given the option of taking a sponge bath or showering in a wheelchair. Prophylactic measures were taken to detect and prevent deep vein thrombosis including a blood D-dimer test followed by an ultrasound examination if D-dimer test was positive, passive range of motion exercise during bed rest, the use of Thrombo-Embolism Deterrent hose (TED) hose and Sequential Compression Device (SCD) over the bed rest period. Subjects were offered medication to help mitigate reflux problems associated with being supine. Subjects were constantly monitored by nursing staff and received a daily physical examination by the study physician.

During the 8 weeks following the bed rest period, subjects underwent resistance exercise training rehabilitation. Strength training consisted of circuit training for combined hip and knee extensors and flexors, and light upper body exercises. Subjects participated in strength training for 1 h, 3 days per week (24 total sessions). Three sets of each exercise were performed with a resistance that will allow 8–10 repetitions (approximately 80% of 1 RM) with appropriate rest periods between sets. Subjects were required to walk at their usual pace before and after strength training to allow for a warm-up and cool-down. Speed of contraction for the concentric component was approximately 2 s for full extension and the eccentric component was 4–6 s in duration. The 1 RM was determined weekly to ensure that each subject was exercising at the appropriate pace. The purpose of the rehabilitation phase of the experiment was to ensure that subjects regained their functional status after bed rest and also to determine if HMB had additional benefits in context of exercise.

2.4. Body composition

Body weight was measured at baseline, after bed rest, and weekly during the 8 weeks of rehabilitation to the nearest 0.1 kg on

an Ohaus scale (Ohaus Corporation, model 15S, Florham Park, NJ). Nude body weight was calculated as total body weight minus hospital robe weight. Body height was measured to the nearest 0.1 cm without shoes using a stadiometer. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Measurements of body composition were conducted prior to and at the end of the 10-day bed rest period, and at the end of the rehabilitation period (week 8). DXA (Hologic Delphi W running QDR System Software Version 11.2) was used to estimate total and lower extremity lean body mass using a standard protocol.[8,15,24]

2.5. Strength testing

Strength measurements included: isokinetic knee extensor and flexor force (60° and 180°), leg press, standing plantar flexor force, and stair ascent and descent power. Dynamic concentric strength of the muscles involved with unilateral (dominant, unless contraindicated by pain or history of joint replacement) leg press, knee extension, and knee flexion were determined as the maximal load the subject could lift through the full range of motion one repetition only (1 RM) using Keiser pneumatic training equipment (Keiser Sports Health Equipment, Fresno, CA). One repetition maximum measurements were taken at baseline, after bed rest, and weekly throughout the rehabilitation period. Two baseline strength measurements were obtained, to account for learning effects. Standing plantar flexor strength measurements were determined as described before.[15]

2.6. Functionality

Lower extremity performance was measured using SPPB,[25] timed Get-Up-&-Go[26] and by the 5-item physical performance battery.[27] These tests were conducted prior to bed rest, after 10 days of bed rest, and at the end of the 8-week rehabilitation period.

2.7. Mixed skeletal muscle protein fractional synthesis rate (FSR)

Muscle FSR is an estimate of the capacity of the muscle to synthesize protein over time. Muscle FSR of mixed muscle protein was measured by the rate of incorporation of a stable isotope amino acid tracer (L-[$^{13}C_6$]phenylalanine) in muscle protein from the *vastus lateralis* over a fixed period of time. The tracer study encompassed an 8-h period in order to discern the overall effects of feeding and fasting on muscle protein synthesis. In order to maintain a relatively steady state during the 8 h of tracer infusion, in contrast to bolus feeding approaches,[24] subjects were given a small amount (30 ml) of a standardized liquid meal replacement (Ensure, Abbott Nutrition, Columbus, OH) every 30 min. The two sachets of HMB (3 g total) or placebo were dispensed to subjects in 3-dose intervals at approximately 2 h, 4 h and 6 h from the start of the study. This design was thought to be the most practical method of both minimizing subject discomfort due to fasting while assessing the overall effects of nutritional intervention on muscle protein metabolism. We performed muscle FSR measurements at day 6 and 15 (Table 1).

To initiate the isotope infusion study, an 18-gage polyethylene catheter was inserted into a vein on each forearm, one for blood sampling and the other for infusion of the stable isotope tracers. After a fasted blood sample was obtained for background amino acid enrichment, a priming dose (4.2 μmol/kg) of L-[ring-$^{13}C_6$] phenylalanine (Cambridge Isotope Labs, Andover, MA) was given. This was immediately followed by a continuous (0.07 μmol/kg/min) infusion of $^{13}C_6$-phenylalanine that was maintained throughout the experiment. Isotopic plateau of the infused tracer was achieved at 2 h, and a biopsy sample (~50 mg) was taken from the lateral portion of the *vastus lateralis* approximately 10–15 cm above the knee under local anesthesia (lidocaine HCl 1%) with a 5-mm Bergstrom needle with suction. The muscle was then cleansed of excess blood, connective tissue, and fat and immediately frozen in liquid nitrogen (−190 °C) until analysis. The two sachets of HMB or placebo were dispensed to subjects in 3-dose intervals at approximately 2 h, 4 h and 6 h from the start of the study. Subjects consumed the first dose of HMB or placebo immediately following the first muscle biopsy, and the final dose of HMB or placebo approximately 120 min before the second biopsy. The second biopsy was collected at the end of the 8-h infusion period. Subjects were fed several small meals during the 8-h infusion period to achieve steady state kinetics. Meals were provided in small doses at approximately 30 min intervals after the start of the procedure, and consisted of ~16 oz (total volume) of a standardized (HMB-free) meal replacement beverage (Ensure, Abbott Laboratories). Blood was sampled periodically for venous amino acid concentrations. From the two biopsy samples the synthesis rate of muscle protein was determined over 6 h (see calculations below).

Blood and muscle samples were processed and derivatized and used for the isotope enrichment measurements as described previously.[24] Mixed muscle fractional synthesis rate (FSR) for intracellular phenylalanine enrichment (intracellular enrichment) was calculated as the rate of L-[ring-$^{13}C_6$]phenylalanine tracer incorporation into muscle protein with the use of the following equation: FSR (%/h) = $\{(Et_1 - Et_0)/[Ep \times (t_1 - t_0)]\} \times 100$, where Et_0 is the enrichment of the protein-bound L-[ring-$^{13}C_6$]phenylalanine tracer from the first biopsy, Et_1 is the enrichment in the protein-bound L-[ring-$^{13}C_6$]phenylalanine tracer from the second biopsy, $(t_1 - t_0)$ is the L-[ring-$^{13}C_6$]phenylalanine tracer incorporation time (i.e. the time between biopsies), and Ep is the mean L-[ring-$^{13}C_6$]phenylalanine enrichment in the precursor pool. FSR was calculated using both the muscle intracellular free phenylalanine pool and the plasma phenylalanine enrichment as the precursor.[3,34]

2.8. Urinary HMB

Urinary HMB was conducted by Metabolic Technologies (Ames, IA) and was measured at the start of-bed rest and end of the bed rest period (Phase I) by a modified method of Nissen et al.[28] to verify compliance of product consumption over bed rest (See supplemental on-line table).

2.9. Statistical analysis

Analyzes were performed on the actual values (single time point or repeated measures, as appropriate), change from baseline to end of bed rest, and change from end of bed rest to end of study. Analysis of variance with treatment group in the model is the basis for the majority of the statistical analyzes that were performed. For variables with anticipated gender differences, gender and treatment by gender interaction were added to the model. Analysis of covariance was used for models that include baseline values. If the residuals from the analysis of variance were non-normal, Wilcoxon rank sum test was used to compare treatment groups. Paired *t*-tests by treatment group were used to test for differences from baseline to end of bed rest. Categorical variables were analyzed using Cochran–Mantel–Haenszel test. All main effects were tested using 2-sided, 0.05 level tests. Tests of interactions were 2-sided, 0.10 level tests. No adjustments were made to the significance levels for multiple variables. If a treatment group interaction with another factor in the model was significant, then treatment groups were compared at each level of the other factor using the stepdown Bonferroni (Holm) procedure to control for the number of comparisons made. All values are presented as means ± standard error of mean (SEM).

3. Results

There was no significant difference between groups at baseline for any of the characteristics indicated (Table 2). Additionally, at the end of bed rest, there was no significant difference in SPPB score (Wilcoxon rank sum test), body weight, total body fat, bone mineral density, fasted glucose, serum albumin, CRP or total cholesterol (ANCOVA). There were no statistically significant differences between the two study groups for the number of subjects reporting adverse events (AEs) in any system organ class (SOC) or for any specific preferred term (PT). Most of the AEs reported were common complaints associated with extended bed rest (back pain, constipation, headache) reported in previous bed rest studies. There were no serious adverse events (SAEs) associated with back pain or headaches. Two SAEs were reported during the study, one event in each group, both unrelated to study product. The SAE in the Control group was related to a pulmonary embolism and hypertension. The SAE in the HMB group was related to hyponatremia. Both subjects were discharged from the hospital in stable condition. Tables with more detailed information about the results are provided as supplemental material.

3.1. Body composition

There was no significant change in total body weight over the bed rest period (Table 2). Fig. 1 also shows the change in total lean mass for individual subjects within each group over the bed rest period. Total lean mass measured by DXA significantly declined in the Control group at the end of the 10 d bed rest period (-2.05 ± 0.66 kg; $p = 0.02$, paired t-test) (Figs. 1 and 2). The HMB group preserved lean mass with an average loss of -0.60 ± 0.47 kg ($p = 0.23$, paired t-test) (Fig. 1). On a percent basis, the Control group lost an average of -4.6 ± 1.4% total lean mass over 10 days of bed rest whereas the HMB group lost an average of -1.2 ± 0.9%. Comparison of the change value in total lean mass over the bed rest period between treatments was not statistically significant ($p = 0.16$, ANOVA).

Within the HMB group ($n = 11$ subjects), all but one subject (indicated by X) showed preservation of total lean mass over bed rest (Fig. 1). This one subject showed a disproportional loss of total lean mass (>2 standard deviations above group average) over bed rest, and did not show a gain/recovery of muscle mass even after the exercise rehabilitation (data not shown). Thus this suggests an error in his baseline DXA measurement. This subject was considered an outlier and removed from subsequent DXA analysis. With the omission of the outlier, the HMB group lost an average of -0.17 ± 0.19 kg total lean mass ($p = 0.42$, paired t-test) versus Control (-2.05 ± 0.66 kg, $p = 0.02$, paired t-test) (Figs. 1 and 2A). Comparison of the change value in total lean mass over the bed rest period between treatment groups was statistically significant ($p = 0.02$, ANOVA). Similar results were obtained for leg lean mass (Fig. 2B) with the HMB group showing an average loss of -0.08 ± 0.17 kg ($p = 0.65$, paired t-test) versus the Control (-1.01 ± 0.35 kg, $p = 0.02$, paired t-test). There was a statistically significant difference between treatment groups for the change in leg lean mass over the bed rest period ($p = 0.02$, ANOVA).

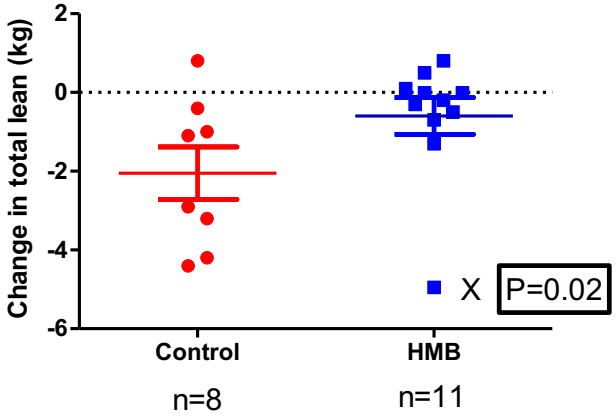

Fig. 1. Change in total lean mass in individual subjects over 10-day bed rest in Control (●) and HMB (■). Line with error bars represent mean ± SEM for each group. Values from all subjects (Control $n = 8$; HMB $n = 11$). X indicates potential outlier from HMB group. Difference between treatment groups was non-significant ($p = 0.16$, ANOVA). When data are analyzed from all Control subjects ($n = 8$) and HMB subjects excluding potential outlier, thus a total of 10 subjects, the difference between treatment groups is significant ($p = 0.02$, ANOVA).

We carried out a sub-analysis on female subjects, since there was a greater number and more equal distribution of females in the study (Control $n = 7$; HMB $n = 8$). This sub-analysis revealed that compared to the Control group, HMB significantly attenuated muscle loss (total lean and leg lean mass) over bed rest. For total lean mass, HMB group lost -0.2 ± 0.24 kg and Control group lost -1.89 ± 0.75 kg ($p = 0.04$, ANOVA).

At the end of the exercise rehabilitation period, there was no significant difference from baseline in the change value for total lean mass from within each group: Control: -0.43 ± 0.67 ($p = 0.55$, paired t-test) and HMB: 0.07 ± 0.26 ($p = 0.80$, paired t-test). However, when comparing the overall change value for leg lean mass from baseline to end of rehabilitation, the HMB group showed a tendency of a gain of 0.71 ± 0.33 kg ($p = 0.06$, paired t-test), whereas the change value for the control group was -0.06 ± 0.22 kg ($p = 0.78$, paired t-test; Fig. 2B).

Additional DXA data analysis revealed that bed rest caused a significant loss of lean mass in arms of the Control group but not the HMB group (Table 3). In addition there was a significant

Table 2
Subject characteristics at baseline and post bed rest.

Female/Male	Control ($n = 7/1$)		HMB ($n = 8/3$)	
Age (yrs)	67.1 ± 1.7		67.4 ± 1.4	
BMI (kg/m^2)	26.5 ± 1.2		24.9 ± 1.0	
25-OH-vitamin D (ng/ml)	25.26 ± 3.37 ($n = 7$)		28.63 ± 4.03 ($n = 11$)	
	Pre-bed rest	Post bed rest	Pre bed rest	Post bed rest
Body weight (kg)	71.36 ± 5.55	69.99 ± 5.37	67.24 ± 2.98	66.16 ± 2.99
Total body fat (kg)	26.15 ± 2.38	26.45 ± 2.66	22.69 ± 1.91	22.64 ± 1.82
Bone mineral density (g/cm^2)	1.14 ± 0.04	1.14 ± 0.04	1.11 ± 0.03	1.11 ± 0.03
Fasted glucose (mg/dL)	90.88 ± 4.79	82.13 ± 4.35	89.55 ± 3.25	84.18 ± 4.00
Total cholesterol (mg/dL)	228.88 ± 20.28	201.88 ± 12.07	205.73 ± 8.60	185.82 ± 7.35
Serum albumin (g/dL)	4.33 ± 0.12	3.83 ± 0.1	4.42 ± 0.06	4.00 ± 0.07
CRP (mg/L)	2.71 ± 0.63	7.99 ± 5.95	1.99 ± 0.39	2.06 ± 0.51
SPPB score	11.63 ± 0.26	11.50 ± 0.27	11.55 ± 0.25	11.45 ± 0.28

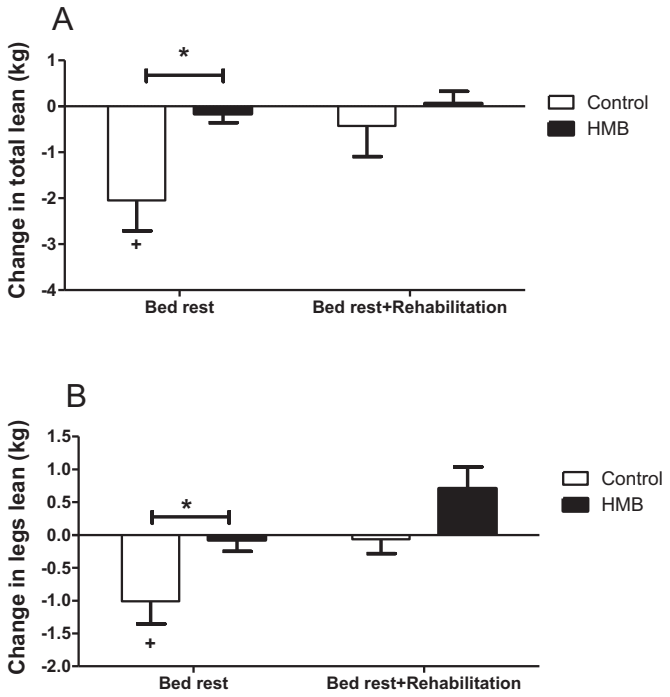

Fig. 2. Body composition (DXA) changes over bed rest. Top panel (A): change in total lean mass over 10-day bed rest (bed rest) and change from baseline to end of exercise rehabilitation (bed rest + rehab). Values are presented as mean ± SEM for Control ($n = 8$) and HMB ($n = 10$, excluding potential outlier). (+) Difference from pre-bed rest value ($p = 0.02$, paired t-test); (*) difference between treatment groups ($p = 0.02$, ANOVA). Bottom panel (B): change in leg lean mass over bed rest and rehabilitation. (+) Difference from pre-bed rest value ($p = 0.02$, paired t-test); (*) difference between treatment groups ($p = 0.02$, ANOVA); trend toward increase from baseline to end of rehabilitation for HMB group ($p = 0.06$, paired t-test) and non-significant for Control group.

increase in fat mass of the arms in Control but not the HMB group. The HMB group also had a significant loss of total body fat mass (−0.52 ± 0.22, $p = 0.04$, paired t-test) over bed rest which was not observed in the Control group (Table 3). Additional data are available in a supplemental on-line table.

3.2. Muscle strength and functional changes

A number of different strength measurements were performed. These included knee extensor strength (Isokinetic − 60° and 180°; Isotonic − 1RM), knee flexor strength (Isokinetic − 60° and 180°) and standing plantar flexor. In general, there was a greater numerical loss of strength in the Control group compared to the HMB group over the 10-day bed rest period, although the change was not statistically significant within each group. In addition, the difference between treatment groups did not reach statistical significance at the end of bed rest.

Figure 3 represents changes in isokinetic knee extensor (60° and 180°) strength over the bed rest and recovery period. Over the bed rest period, isokinetic knee extensor (60°) strength declined in the Control group (−12.54 ± 7.84 Nm/s, $p = 0.15$, paired t-test) whereas it was maintained in the HMB group (0.67 ± 6.91 Nm/s, $p = 0.93$, paired t-test). This represented a percent decline of −8.6 ± 4.5% in Control compared to HMB (0.0 ± 5.2%). There was no statistical difference between the change value for the treatment groups at the end of bed rest ($p = 0.84$, ANOVA) (Fig. 3A). Both groups showed significant improvement in knee extensor (60°) strength from the end of bed rest value to the end of the recovery period: Control (22.24 ± 8.16 Nm/s, $p = 0.03$, paired t-test); HMB (23.64 ± 9.61 Nm/s, $p = 0.03$, paired t-test). There was no statistical difference between the treatment groups at the end of recovery. The HMB group had a significant increase in strength from baseline to the end of recovery ($p = 0.0041$, paired t-test) whereas the Control group did not ($p = 0.24$, paired t-test) (Fig. 3A).

Similarly for isokinetic knee extensor (180°) (Fig. 3B), there was a much larger decline in strength over bed rest in the Control group (−11.00 ± 8.31 Nm/s) than in the HMB group (−0.18 ± 7.07 Nm/s) but the change was not statistically different from baseline within each group. There was no statistical difference for the change value over bed rest between the treatment groups ($p = 0.10$, ANOVA). Although numerically higher, there was no significant improvement in knee extensor (180°) strength from end of bed rest to end of recovery: Control (15.90 ± 8.06 Nm/s, $p = 0.10$, paired t-test); HMB (11.15 ± 8.49 Nm/s, $p = 0.22$, paired t-test). The HMB group showed significant strength gains above baseline value at the end of recovery ($p = 0.03$, paired t-test) whereas the Control group did not ($p = 0.36$, paired t-test).

There was no significant decline in functionality as evaluated using SPPB, Get-Up-&-Go or 5-item physical performance tests in either group over the bed rest period. The mean SPPB score remained >11 in both groups at the end of bed rest (Table 2). The mean Get-Up-&-Go time remained <10 s in both groups pre and post bed rest. There was also no significant difference between groups for the cumulative 5-item physical performance score at the end of bed rest. There was no significant difference between treatment groups at the end of bed rest for any of the above described functionality measures. At the end of the recovery period compared to baseline value, there was a significant improvement (decrease) in time to Get-Up-&-Go within each group compared to baseline (Control: −1.35 ± 0.35; HMB: −0.74 ± 0.30, $p < 0.05$, paired t-test). Additional data are available in supplemental on-line tables.

Table 3
Body composition by DXA over bed rest period.

	Control ($n = 8$)				HMB ($n = 10$)			
	Pre-bed rest	Post bed rest	Change over bed rest	p-value[a]	Pre-bed rest	Post bed rest	Change over bed rest	p-value[a]
Total lean Mass (Kg)	42.22 ± 3.60	40.18 ± 3.26	−2.05 ± 0.66	**0.0178**	39.67 ± 2.03	39.50 ± 2.06	−0.17 ± 0.19	0.4177
Appendicular Lean Mass (ALM) (arms + legs) (Kg)	16.99 ± 1.73	15.66 ± 1.52	−1.33 ± 0.41	**0.0137**	15.44 ± 0.85	15.33 ± 0.91	−0.10 ± 0.17	0.5604
Leg Lean (Kg)	12.80 ± 1.28	11.79 ± 1.09	−1.01 ± 0.35	**0.0223**	11.37 ± 0.54	11.29 ± 0.62	−0.08 ± 0.17	0.6541
Arm Lean (Kg)	4.19 ± 0.47	3.87 ± 0.44	−0.32 ± 0.09	**0.0084**	4.07 ± 0.31	4.04 ± 0.30	0.02 ± 0.06	0.6715
Trunk Lean (Kg)	21.91 ± 1.79	21.36 ± 1.65	−0.55 ± 0.26	0.0711	21.15 ± 1.11	21.15 ± 1.10	−0.01 ± 0.24	0.9806
Total body fat mass (Kg)	26.15 ± 2.38	26.45 ± 2.66	0.30 ± 0.46	0.5297	23.10 ± 2.06	22.58 ± 2.01	−0.52 ± 0.22	**0.0401**
Leg fat mass (Kg)	9.13 ± 0.79	9.22 ± 0.93	0.10 ± 0.22	0.6799	8.05 ± 0.63	7.87 ± 0.65	−0.19 ± 0.11	0.1179
Arm fat mass (Kg)	2.91 ± 0.29	3.14 ± 0.32	0.22 ± 0.07	**0.0139**	2.40 ± 0.26	2.44 ± 0.29	0.04 ± 0.04	0.4414
Trunk fat mass (Kg)	13.22 ± 1.52	13.25 ± 1.66	0.03 ± 0.23	0.8898	11.84 ± 1.29	11.48 ± 1.16	−0.36 ± 0.25	0.1819

Data represents mean ± SEM. Bold values represent $p < 0.05$.
[a] Change within group by t-test.

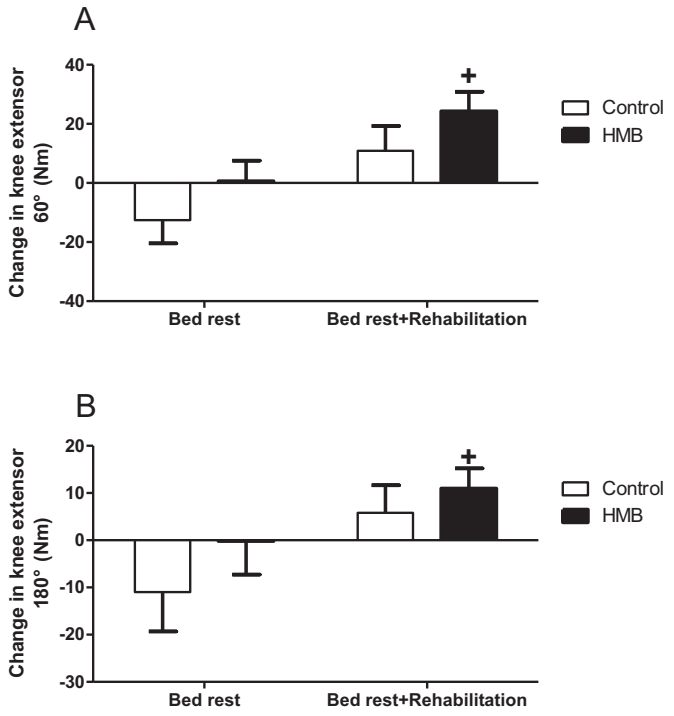

Fig. 3. Top panel (A): change in isokinetic knee extensor (60°) strength over 10-day bed rest and 8-week exercise rehabilitation. Values are presented as mean ± SEM. (+) Difference from pre-bed rest value ($p = 0.004$, paired t-test). Lower panel (B): change in isokinetic knee extensor (180°) strength over 10-day bed rest and 8-week exercise rehabilitation. (+) Difference from pre-bed rest value ($p = 0.03$, paired t-test).

3.3. Muscle protein synthesis

Six hour FSR was determined in 8 Control subjects and 10 HMB subjects (one HMB subject was unable to participate in the procedure) at the start of bed rest and on the last day of bed rest. In the Control subjects, FSR decreased by 21% (from 0.1028 ± 0.0042%/h- pre-bed rest to 0.0800 ± 0.0124%/h- post bed rest). However this change did not reach statistical significance ($p = 0.14$, paired t-test). For the HMB group, FSR was maintained (from 0.0978 ± 0.0062%/h- pre-bed rest to 0.0962 ± 0.0129%/h- post bed rest, $p = 0.91$, paired t-test). There was no significant treatment difference for the change values over bed rest ($p = 0.30$, ANOVA).

4. Discussion

Our study shows that nutritional intervention with HMB (3 g/day) preserves muscle mass in older adult subjects during extended bed rest. Consistent with previous findings, bed rest caused a substantial loss of muscle in older adults,[8,9] and HMB was able to ameliorate this muscle loss.

Subjects were maintained at a protein intake of 0.8 g/kg bw/day which was chosen to be consistent with the RDA for protein supplementation in adults and mimics the scenario that patients encounter when hospitalized. Due to the stringent enrollment criteria for older adult subjects, we predominantly enrolled female subjects (Control $n = 7$, HMB $n = 8$). We therefore performed a sub-analysis on females alone which revealed the same trends as seen in the whole group. We started the HMB supplementation 5 days prior to the initiation of bed rest to ensure high endogenous levels of HMB at start of bed rest, since the exact kinetics of HMB in humans is not well established. We instituted an 8 week exercise rehabilitation program to help the subjects regain the muscle and strength lost over 10 days of bed rest. Interestingly, many subjects who received the HMB treatment had gains in leg muscle mass and strength above the baseline value at the end of the rehabilitation period. This could be attributed to either a synergistic effect of HMB and exercise or because the HMB group had higher lean mass/strength values at the end of bed rest than the Control group.

Bed rest caused significant loss of FFM at the whole body level (about 5% loss) as well as from the extremities (about 7% loss from legs) and HMB preserved muscle mass over bed rest. This effect attained statistical significance in women and was close to significance in the fully evaluable sample. There was one exception in the HMB group who showed significant loss of muscle over the bed rest period (>2 SD greater than the average for the group) along with further muscle loss over the 8 week exercise/rehabilitation period. We explored the possibility that this subject was losing muscle mass due to infection but he did not have elevated CRP concentrations or significant changes in body weight over the study. This subject did not have abnormal loss of muscle strength over the bed rest period either. We confirmed that this subject had received HMB supplementation by urine analysis for HMB. Thus we postulate that there was an error in this subject's baseline DXA measurement leading to errors in interpreting subsequent change values from baseline. This subject was considered an outlier and was thus excluded from all the body composition analysis. When he was excluded from analysis, the results were comparable to the sub-analysis in women, in that HMB significantly preserved muscle mass over bed rest. We therefore conclude that provision of HMB starting before bed rest and during the bed rest period attenuates muscle mass losses in older adults.

Since the subjects were relatively healthy and had good mobility (SPPB ≥ 9) to begin with, we did not see a decline in the SPPB, Get-Up-&-Go, or 5-item physical performance test score post bed rest in either group. This is consistent with what was reported earlier.[9] These tests may be more sensitive in evaluating people with lower functionality (i.e. hospitalized older adult patients) rather than healthy community dwelling older adults. Interestingly, there was a significant improvement in Get-Up-&-Go time from baseline time in both groups at the end of recovery/rehabilitation that points to the positive effects of exercise rehabilitation on functionality.

The results of all the strength and functional tests show that the variability is too high in this older adult population to be able to observe any significant difference between groups in studies using small numbers of subjects. Although we had powered the current study based on our previous bed rest study results,[15] there appears to be considerable higher variability in the baseline strength of the healthy older adult subjects of the present study. A power analysis using the current strength data revealed that 50–75 subjects per group would be required to see a statistically significant difference between groups. We have no logical explanation for the higher variability in the present study. We therefore think that although we did our best to perform all the different muscle function tests in the present study, the variability within each treatment groups are too high to make a conclusion regarding the effect of HMB on muscle strength/function over bed rest.

A recent finding indicated that improved strength gains were only observed when vitamin D concentrations were higher than 30 ng/ml, while improved mass was found to be independent of the vitamin D status.[29] We therefore performed a post-hoc analysis to see whether the data from the present study would show a similar relation. Although we found that a number of our subjects had vitamin D levels <30 ng/ml, we did not observe a relation between improved strength only when vitamin D levels were high. This could possibly be due to the small sample size of the present study, the relative short duration of supplementation in our study together with the variability in our strength data.

We studied muscle metabolism using a stable isotope infusion protocol, combined with muscle biopsies that enable the measurement of the fractional synthesis rate of muscle protein (FSR). The protocol involved the provision of sip feeding throughout the 8 h protocol to prevent the effects of prolonged starvation and a total of 3 g of HMB was provided over 3 doses over the period in the HMB group. The results showed a numerical advantage to the HMB group, but were not statistically significant. This result seems to be inconsistent with the observed preservation of lean body mass in the HMB group. We have previously found that bed rest causes a decrease in muscle protein synthesis[4] and experimental interventions that reduce loss of muscle mass in bed rest do so by ameliorating the normal decline in FSR with bed rest in young subjects.[15] However, these studies measured FSR on a 24 h basis that includes both fasted and fed periods, while in the present study we only measured during the fed state. Also, over the course of the bed rest period, HMB was delivered as a bolus, whereas in the FSR protocol HMB was given in smaller doses. It could be that a peak concentration of HMB is needed to elicit a stimulation of FSR but was not achieved in the tracer protocol. Although our measured values of muscle FSR were not significantly different between treatment groups, it is nonetheless possible that preservation of muscle during bed rest by HMB could have been due to the stimulation of muscle protein synthesis. We therefore believe that the experimental design possibly minimized the likelihood of observing a significant treatment difference. A positive effect of HMB on muscle FSR would be expected since HMB has been shown to stimulate muscle protein synthesis *via* activation of mammalian target of rapamycin (mTOR) in preclinical models.[30,31]

Another potential mechanism of action of HMB on preservation of muscle mass could *via* down regulation of muscle protein degradation, as has been demonstrated in catabolic states such as cancer cachexia and sepsis.[18,30,32] In those states, HMB has been shown to down regulate upstream regulators such as NFκB and Foxo involved in muscle atrophy.[33] It may be possible that an increase in muscle protein degradation plays a bigger role in bed rest induced muscle atrophy than previously believed since changes in LBM over time reflect an altered relationship between protein synthesis and breakdown. It could be that the HMB effect is predominantly on breakdown, and we missed that response by measuring only FSR. Lastly, HMB has been shown to down regulate myonuclear apoptosis that increases over immobilization in muscle leading to muscle atrophy.[23] This provides another mechanism by which HMB could potentially preserve muscle during bed rest.

We and others have done several interventional studies in young[10] and older adults[15] that were confined to bed rest and provided nutritional supplements or other treatment such as hormonal treatment,[34] supplements with branched chain[16,17] or essential amino acids[10,13,15,35,36] and resistance exercise.[12,37] EAA were found to attenuate muscle mass loss during bed rest in young subjects in one study[10] but not in another.[36] The only intervention study carried out to date in healthy older adults on bed rest involved the use of EAA supplements for treatment.[15] In that study, EAA treatment resulted in preservation of some functional measures and stimulated muscle protein synthesis but did not prevent loss of lean mass.[15] This suggests multiple mechanisms may contribute to preservation of muscle mass and function over bed rest and a combination of interventions may be more effective than just one alone.

Our current study shows that HMB is an effective nutritional intervention for preservation of muscle mass in healthy older adults confined to bed rest. It may be that a greater availability of EAAs was required than we provided, by using the RDA as a guideline for protein intake, for a stimulatory effect of HMB on muscle FSR to reach statistical significance. In that light, an effective therapeutic regimen for preventing muscle decline over extended hospitalization may involve a combination approach of using HMB plus EAA (or a high quality protein source that provides EAAs) and possibly vitamin D to effectively preserve muscle mass, strength and function. In addition, providing calories and micronutrients may also help address the malnutrition problem that has been reported in large number of hospitalized patients.

It remains to be established whether combining these approaches are advantageous. However, one can hypothesize that bed rest/immobilization would induce acute loss of muscle mass in hospitalized older adults with other co-morbidities such as cancer, COPD, chronic lung, liver or heart failure or around surgical procedures. Here a therapeutic combination approach for reducing muscle loss becomes absolutely necessary to address malnutrition, inflammation and other confounding factors that contribute to muscle loss in those situations.

Currently the correlation between muscle mass and strength is still unresolved. Part of the controversy stems from analysis of data from longitudinal aging studies that indicates a disproportionate loss of muscle strength preceding loss of muscle mass.[38] Still other studies do show a correlation between muscle mass and strength.[39,40] Lower extremity muscle mass has also been shown to predict functionality performance in mobility-limited older adults.[41] In addition, intervention studies in cancer cachexia patients who experience acute loss of muscle have demonstrated that preservation of muscle mass leads to improvements in functionality and quality of life outcomes.[42] Since bed rest causes an acute loss of muscle over a short period of time with concurrent loss of strength, this could be analogous to the chronic wasting disease states that show a strong correlation between muscle mass and strength. Nevertheless, there is need for more studies in hospitalized older adults to better understand the relationship between the acute loss of muscle mass that occurs during hospitalization and loss of strength/functionality.

In conclusion, HMB was able to prevent the acute decline in muscle mass in older adults over 10 days of bed rest and this will most likely translate into maintenance of muscle strength/function during extended immobilization (i.e. hospitalization).

Conflict of interest

R. Wolfe is a member of the Abbott Research Advisory Board and received compensation. S. Pereira, J. Oliver and N. Edens are employed by Abbott Nutrition. N. Deutz, N. Hays and C. Evans have no conflict of interest to declare.

Acknowledgments

The authors wish to thank Lulu Xu, Rick Williams and John Thaden for analysis of the samples.

The research was supported by a grant from Abbott Nutrition, Abbott Laboratories, Columbus, OH. Study design, planning and statistical analysis were done in conjunction with Abbott Nutrition. All authors had full access to study data. The corresponding author and principal investigator R. Wolfe has final responsibility for the decision to submit the publication.

N. Deutz, R. Wolfe and S. Pereira were involved in data analysis and writing of the manuscript. R. Wolfe, N. Hays, S. Pereira and N. Edens were involved in the study design and data collection, and C. Evans was involved in data collection and collation. J. Oliver was responsible for data analysis and statistical analysis. All authors have reviewed and approved the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.clnu.2013.02.011.

References

1. Brower P, Hicks D. Maintaining muscle function in patients on bed rest. *Am J Nurs* 1972 Jul;**72**(7):1250–3. PubMed PMID: 4482582. Epub 1972/07/01. eng.
2. Edwards H, Rose EA, King TC. Postoperative deterioration in muscular function. *Arch Surg* July 1, 1982;**117**(7):899–901.
3. Creditor MC. Hazards of hospitalization of the elderly. *Ann Intern Med* 1993 Feb 1;**118**(3):219–23. PubMed PMID: 8417639. Epub 1993/02/01. eng.
4. Ferrando AA, Lane HW, Stuart CA, Davis-Street J, Wolfe RR. Prolonged bed rest decreases skeletal muscle and whole body protein synthesis. *Am J Phys* 1996 Apr;**270**(4 Pt 1):E627–33. PubMed PMID: 8928769. Epub 1996/04/01. eng.
5. He W, Sengupta M, Velkoff VA, DeBarros KA. *Current population reports 65+ in the United States*. Washington DC: US Census Bureau US Government Printing Office; 2005. 23–209.
6. Hirsch CH, Sommers L, Olsen A, Mullen L, Winograd CH. The natural history of functional morbidity in hospitalized older patients. *J Am Geriatr Soc* 1990 Dec;**38**(12):1296–303. PubMed PMID: 2123911. Epub 1990/12/01. eng.
7. Sager MA, Franke T, Inouye SK, Landefeld CS, Morgan TM, Rudberg MA, et al. Functional outcomes of acute medical illness and hospitalization in older persons. *Arch Intern Med* 1996 Mar 25;**156**(6):645–52. PubMed PMID: 8629876. Epub 1996/03/25. eng.
8. Kortebein P, Ferrando A, Lombeida J, Wolfe R, Evans WJ. Effect of 10 days of bed rest on skeletal muscle in healthy older adults. *JAMA* 2007 Apr 25;**297**(16):1772–4. PubMed PMID: 17456818. Epub 2007/04/26. eng.
9. Kortebein P, Symons TB, Ferrando A, Paddon-Jones D, Ronsen O, Protas E, et al. Functional impact of 10 days of bed rest in healthy older adults. *J Gerontol* 2008 Oct;**63**(10):1076–81. PubMed PMID: 18948558. Epub 2008/10/25. eng.
10. Paddon-Jones D, Sheffield-Moore M, Urban RJ, Sanford AP, Aarsland A, Wolfe RR, et al. Essential amino acid and carbohydrate supplementation ameliorates muscle protein loss in humans during 28 days bedrest. *J Clin Endocrinol Metab* 2004 Sep;**89**(9):4351–8. PubMed PMID: 15356032. Epub 2004/09/10. eng.
11. Paddon-Jones D, Sheffield-Moore M, Cree MG, Hewlings SJ, Aarsland A, Wolfe RR, et al. Atrophy and impaired muscle protein synthesis during prolonged inactivity and stress. *J Clin Endocrinol Metab* 2006 Dec;**91**(12):4836–41. PubMed PMID: 16984982. Epub 2006/09/21. eng.
12. Ferrando AA, Tipton KD, Bamman MM, Wolfe RR. Resistance exercise maintains skeletal muscle protein synthesis during bed rest. *J Appl Physiol* 1997 Mar;**82**(3):807–10. PubMed PMID: 9074967. Epub 1997/03/01. eng.
13. Fitts RH, Romatowski JG, Peters JR, Paddon-Jones D, Wolfe RR, Ferrando AA. The deleterious effects of bed rest on human skeletal muscle fibers are exacerbated by hypercortisolemia and ameliorated by dietary supplementation. *Am J Physiol Cell Physiol* 2007 Jul;**293**(1):C313–20. PubMed PMID: 17409123. Epub 2007/04/06. eng.
14. Bos C, Benamouzig R, Bruhat A, Roux C, Valensi P, Ferriere F, et al. Nutritional status after short-term dietary supplementation in hospitalized malnourished geriatric patients. *Clin Nutr* 2001 Jun;**20**(3):225–33. PubMed PMID: 11407869. Epub 2001/06/16. eng.
15. Ferrando AA, Paddon-Jones D, Hays NP, Kortebein P, Ronsen O, Williams RH, et al. EAA supplementation to increase nitrogen intake improves muscle function during bed rest in the elderly. *Clin Nutr* 2010 Feb;**29**(1):18–23. PubMed PMID: 19419806. Epub 2009/05/08. eng.
16. Stein TP, Donaldson MR, Leskiw MJ, Schluter MD, Baggett DW, Boden G. Branched-chain amino acid supplementation during bed rest: effect on recovery. *J Appl Physiol* 2003 Apr;**94**(4):1345–52. PubMed PMID: 12471043. Epub 2002/12/10. eng.
17. Stein TP, Schluter MD, Leskiw MJ, Boden G. Attenuation of the protein wasting associated with bed rest by branched-chain amino acids. *Nutrition* 1999 Sep;**15**(9):656–60. PubMed PMID: 10467608. Epub 1999/09/01. eng.
18. May PE, Barber A, D'Olimpio JT, Hourihane A, Abumrad NN. Reversal of cancer-related wasting using oral supplementation with a combination of beta-hydroxy-beta-methylbutyrate, arginine, and glutamine. *Am J Surg* 2002 Apr;**183**(4):471–9. PubMed PMID: 11975938. Epub 2002/04/27. eng.
19. Clark RH, Feleke G, Din M, Yasmin T, Singh G, Khan FA, et al. Nutritional treatment for acquired immunodeficiency virus-associated wasting using beta-hydroxy beta-methylbutyrate, glutamine, and arginine: a randomized, double-blind, placebo-controlled study. *J Parenter Enteral Nutr* 2000 May-Jun;**24**(3):133–9. PubMed PMID: 10850936. Epub 2000/06/13. eng.
20. Wilson GJ, Wilson JM, Manninen AH. Effects of beta-hydroxy-beta-methylbutyrate (HMB) on exercise performance and body composition across varying levels of age, sex, and training experience: a review. *Nutr Metab (Lond)* 2008;**5**:1. PubMed PMID: 18173841. Pubmed Central PMCID: 2245953. Epub /01/05. eng.
21. Vukovich MD, Stubbs NB, Bohlken RM. Body composition in 70-year-old adults responds to dietary beta-hydroxy-beta-methylbutyrate similarly to that of young adults. *J Nutr* 2001 Jul;**131**(7):2049–52. PubMed PMID: 11435528. Epub 2001/07/04. eng.
22. Flakoll P, Sharp R, Baier S, Levenhagen D, Carr C, Nissen S. Effect of beta-hydroxy-beta-methylbutyrate, arginine, and lysine supplementation on strength, functionality, body composition, and protein metabolism in elderly women. *Nutrition* 2004 May;**20**(5):445–51. PubMed PMID: 15105032. Epub 2004/04/24. eng.
23. Hao Y, Jackson JR, Wang Y, Edens N, Pereira SL, Alway SE. {beta}-Hydroxy-{beta}-methylbutyrate reduces myonuclear apoptosis during recovery from hind limb suspension-induced muscle fiber atrophy in aged rats. *Am J Physiol Regul Integr Comp Physiol* 2011 Jun 22. PubMed PMID: 21697520. Epub 2011/06/24. Eng.
24. Deutz NE, Safar A, Schutzler S, Memelink R, Ferrando A, Spencer H, et al. Muscle protein synthesis in cancer patients can be stimulated with a specially formulated medical food. *Clin Nutr* 2011 Dec;**30**(6):759–68. PubMed PMID: 21683485. Epub 2011/06/21. eng.
25. Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, et al. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *J Gerontol* 1994 Mar;**49**(2):M85–94. PubMed PMID: 8126356. Epub 1994/03/01. eng.
26. Mathias S, Nayak US, Isaacs B. Balance in elderly patients: the "get-up and go" test. *Arch Phys Med Rehabil* 1986 Jun;**67**(6):387–9. PubMed PMID: 3487300. Epub 1986/06/01. eng.
27. Murphy MA, Olson SL, Protas EJ, Overby AR. Screening for falls in community-dwelling elderly. *J Aging Phys Activ* 2003;**11**:64–78.
28. Nissen S, Van Koevering M, Webb D. Analysis of beta-hydroxy-beta-methyl butyrate in plasma by gas chromatography and mass spectrometry. *Anal Biochem* 1990 Jul;**188**(1):17–9. PubMed PMID: 2221358. Epub 1990/07/01. eng.
29. Fuller Jr JC, Baier S, Flakoll P, Nissen SL, Abumrad NN, Rathmacher JA. Vitamin D status affects strength gains in older adults supplemented with a combination of beta-hydroxy-beta-methylbutyrate, arginine, and lysine: a cohort study. *J Parenter Enteral Nutr* 2011 Nov;**35**(6):757–62. PubMed PMID: 21807930. Epub 2011/08/03. eng.
30. Smith HJ, Mukerji P, Tisdale MJ. Attenuation of proteasome-induced proteolysis in skeletal muscle by {beta}-hydroxy-{beta}-methylbutyrate in cancer-induced muscle loss. *Cancer Res* 2005 Jan 1;**65**(1):277–83. PubMed PMID: 15665304. Epub 2005/01/25. eng.
31. Hill DS, Szewczyk N, Smith K, Brookfield R, Loughna P, Rathmacher J, et al. β-hydroxy-β-methyl butyrate (HMB) stimulates mammalian target of rapamycin complex 1 (mTORc1) signaling via a phosphatidylinositol-3-kinase (PI3K)-dependent pathway. *FASEB J* 2011;**25**:1059.10.
32. Kovarik M, Muthny T, Sispera L, Holecek M. Effects of beta-hydroxy-beta-methylbutyrate treatment in different types of skeletal muscle of intact and septic rats. *J Physiol Biochem* 2010 Dec;**66**(4):311–9. PubMed PMID: 20725872. Epub 2010/08/21. eng.
33. Eley HL, Russell ST, Tisdale MJ. Attenuation of depression of muscle protein synthesis induced by lipopolysaccharide, tumor necrosis factor, and angiotensin II by beta-hydroxy-beta-methylbutyrate. *Am J Physiol Endocrinol Metab* 2008 Dec;**295**(6):E1409–16. PubMed PMID: 18854427. Epub 2008/10/16. eng.
34. Zachwieja JJ, Smith SR, Bray GA, Lovejoy JC, Witt TL, DeLany JP, et al. Effect of the leucine metabolite ß-hydroxy-ß-methylbutyrate on muscle protein synthesis during prolonged bedrest. *FASEB J* 1999;**13**:A1025.
35. Paddon-Jones D, Sheffield-Moore M, Urban RJ, Aarsland A, Wolfe RR, Ferrando AA. The catabolic effects of prolonged inactivity and acute hypercortisolemia are offset by dietary supplementation. *J Clin Endocrinol Metab* 2005 Mar;**90**(3):1453–9. PubMed PMID: 15598679. Epub 2004/12/16. eng.
36. Brooks N, Cloutier GJ, Cadena SM, Layne JE, Nelsen CA, Freed AM, et al. Resistance training and timed essential amino acids protect against the loss of muscle mass and strength during 28 days of bed rest and energy deficit. *J Appl Phys* 2008 Jul;**105**(1):241–8. PubMed PMID: 18483167. Pubmed Central PMCID: 2494840. Epub 2008/05/17. eng.
37. Brooks NE, Cadena SM, Vannier E, Cloutier G, Carambula S, Myburgh KH, et al. Effects of resistance exercise combined with essential amino acid supplementation and energy deficit on markers of skeletal muscle atrophy and regeneration during bed rest and active recovery. *Muscle Nerve* 2010 Dec;**42**(6):927–35. PubMed PMID: 20928906. Pubmed Central PMCID: 2991371. Epub 2010/10/12. eng.
38. Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, et al. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. *Journals Gerontol* 2006 Oct;**61**(10):1059–64. PubMed PMID: 17077199. eng.
39. Newman AB, Haggerty CL, Goodpaster B, Harris T, Kritchevsky S, Nevitt M, et al. Strength and muscle quality in a well-functioning cohort of older adults: the Health, Aging and Body Composition Study. *J Am Geriatr Soc* 2003 Mar;**51**(3):323–30. PubMed PMID: 12588575. eng.
40. Ferrando AA, Sheffield-Moore M, Yeckel CW, Gilkison C, Jiang J, Achacosa A, et al. Testosterone administration to older men improves muscle function: molecular and physiological mechanisms. *Am J Physiol Endocrinol Metab* 2002 Mar;**282**(3):E601–7. PubMed PMID: 11832363. Epub 2002/02/08. eng.
41. Reid KF, Naumova EN, Carabello RJ, Phillips EM, Fielding RA. Lower extremity muscle mass predicts functional performance in mobility-limited elders. *J Nutr Health Aging* 2008 Aug-Sep;**12**(7):493–8. PubMed PMID: 18615232. Pubmed Central PMCID: 2544628. Epub 2008/07/11. eng.
42. Fearon KC, Von Meyenfeldt MF, Moses AG, Van Geenen R, Roy A, Gouma DJ, et al. Effect of a protein and energy dense N-3 fatty acid enriched oral supplement on loss of weight and lean tissue in cancer cachexia: a randomised double blind trial. *Gut* 2003 Oct;**52**(10):1479–86. PubMed PMID: 12970142. Pubmed Central PMCID: 1773823. eng.