Experimental Gerontology 48 (2013) 1303–1310

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Effect of calcium β-hydroxy-β-methylbutyrate (CaHMB) with and without resistance training in men and women 65+ yrs: A randomized, double-blind pilot trial

Jeffrey R. Stout [a,*], Abbie E. Smith-Ryan [b], David H. Fukuda [a], Kristina L. Kendall [c], Jordan R. Moon [d], Jay R. Hoffman [a], Jacob M. Wilson [e], Jeffery S. Oliver [f], Vikkie A. Mustad [f]

[a] Institute for Exercise Physiology and Wellness Research, University of Central Florida, Orlando, FL, USA
[b] Department of Exercise and Sport Science, University of North Carolina, Chapel Hill, NC, USA
[c] Department of Health and Kinesiology, Georgia Southern University, Statesboro, GA, USA
[d] United States Sports Academy, Daphne, AL, USA
[e] Department of Exercise and Sport Science, University of Tampa, Tampa, FL, USA
[f] Abbott Nutrition, Columbus, OH, USA

ARTICLE INFO

Article history:
Received 5 June 2013
Received in revised form 1 July 2013
Accepted 14 August 2013
Available online 24 August 2013

Section Editor: C. Leeuwenburgh

Keywords:
Exercise
Fat mass
Sarcopenia
Muscle quality
β-Hydroxy-β-methylbutyrate

ABSTRACT

Background: Evidence suggests CaHMB may impact muscle mass and/or strength in older adults, yet no long-term studies have compared its effectiveness in sedentary and resistance training conditions. The purpose of this study was to evaluate the effects of 24 weeks of CaHMB supplementation and resistance training (3 d wk^{-1}) or CaHMB supplementation only in ≥65 yr old adults.
Methods: This double-blinded, placebo-controlled, trial occurred in two phases under ad libitum conditions. Phase I consisted of two non-exercise groups: (a) placebo and (b) 3 g CaHMB consumed twice daily. Phase II consisted of two resistance exercise groups: (a) placebo and resistance exercise and (b) 3 g CaHMB consumed twice daily and resistance exercise (RE). Strength and functionality were assessed in both phases with isokinetic leg extension and flexion at $60°\cdot s^{-1}$ and $180°\cdot s^{-1}$ (LE60, LF60, LE180, LF180), hand grip strength (HG) and get-up-and-go (GUG). Dual X-Ray Absorptiometry (DXA) was used to measure arm, leg, and total body lean mass (LM) as well as total fat mass (FM). Muscle Quality was measured for arm (MQ_{HG} = HG/arm LM) and Leg (MQ_{60} = LE60/leg LM) (MQ_{180} = LE180/leg LM).
Results: At 24 weeks of Phase I, change in LE60 (+8.8%) and MQ_{180} (+20.8%) for CaHMB was significantly ($p < 0.05$) greater than that for placebo group. Additionally, only CaHMB showed significant ($p < 0.05$) improvements in total LM (2.2%), leg LM (2.1%), and LE_{180} (+17.3%), though no treatment effect was observed. Phase II demonstrated that RE significantly improved total LM (4.3%), LE60 (22.8%), LE180 (21.4%), HG (9.8%), and GUG (10.2%) with no difference between treatment groups. At week 24, only CaHMB group significantly improved FM (−3.8%) and MQ_{HG} (7.3%); however there was no treatment main effect for these variables.
Conclusion: CaHMB improved strength and MQ without RE. Further, RE is an effective intervention for improving all measures of body composition and functionality.

1. Introduction

It has been reported that one in three elderly over the 65 years of age suffers a fall each year (Doherty, 2003; Pijnappels et al., 2008). Age-related muscle loss has been associated with significant reductions in strength and power, yielding an increase in fall rates and thus accidental deaths (Doherty, 2003; Marcus, 1995). With a previously estimated $18.5 billion in annual health care costs in the United States (Janssen

Abbreviations: MQ, muscle quality; RE, resistance exercise; HG, hand grip; GUG, get-up-and-go; REPLA, placebo and resistance exercise; REHMB, CaHMB and resistance exercise; RBC, red blood cells; WBC, white blood cells; BUN, blood urea nitrogen; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; LDH, lactate dehydrogenase; DXA, dual-energy X-ray absorptiometry; PT, peak torque; DCER, Dynamic constant external resistance; AMPK, adenosine monophosphate kinase.

* Corresponding author at: Institute of Exercise Physiology and Wellness, University of Central Florida, 4000 Central Florida Blvd, Orlando, USA, FL 32816. Tel.: +1 407 823 0211.
E-mail addresses: jeffrey.stout@ucf.edu (J.R. Stout), abbsmith@email.unc.edu (A.E. Smith-Ryan), david.fukuda@yahoo.com (D.H. Fukuda), kkendall@georgiasouthern.edu (K.L. Kendall), Jordan@musclepharm.com (J.R. Moon), Jay.Hoffman@ucf.edu (J.R. Hoffman), jmwilson@ut.edu (J.M. Wilson), Jeffery.oliver@abbott.com (J.S. Oliver), vikkie.mustad@abbott.com (V.A. Mustad).

0531-5565
http://dx.doi.org/10.1016/j.exger.2013.08.007

et al., 2004), these are among the largest healthcare issues in aging populations and must be addressed through preventive intervention (Marcell, 2003).

Research has suggested that nutrition and/or physical activity may attenuate age-associated muscle loss by directly influencing myogenic processes and protein turnover (Evans, 1995). Nutrition and exercise strategies to increase muscle mass in elderly individuals include intakes of protein (1.2–1.6 g/kg bodyweight daily) above the current recommended dietary allowance (RDA) levels (Campbell et al., 1994), and resistance training (Bamman et al., 1998). Specifically, leucine has been reported to be the crucial amino acid within protein to combat loss of muscle and/or strength (Katsanos et al., 2005, 2006). One of the primary mechanisms by which leucine prevents muscle wasting is by its conversion to β-hydroxy-β-methylbutyrate (HMB) (Wilson et al., 2008). HMB has been suggested to mitigate muscle loss with aging, disease, or exercise stress by attenuating protein degradation (Eley et al., 2007; Wilkinson et al., 2013), and up-regulating protein synthesis (Wilkinson et al., 2013). Evidence of HMB's role in building muscle comes from human intervention trials among mostly young, exercising adults; although an emerging number of studies are available with HMB in the elderly with mixed results. Flakoll et al. (2004) reported that supplementing the diets of older (~76.7 yrs), sedentary women with 2 g CaHMB in combination with 5 g arginine, and 1.5 g lysine daily for 12 weeks significantly improved their functionality and fat free mass, while a year-long supplementation period in both men and women has been demonstrated to improve in lean mass but not strength or functionality (Baier et al., 2009). Vukovich et al. (2001) data suggested that 3 g HMB can have a positive effect on body composition and strength measures in older adults (70 ± 1 yr) that are initiating a moderate exercise program. In their study, the participants consumed 3 g of CaHMB per day and demonstrated a significant decrease in % fat but a non-significant increase in FFM using skinfold analysis when compared to the placebo group (Vukovich et al., 2001). In addition, they reported improvements in one-repetition maximum for the upper body pull-down exercise in the HMB group that was significantly greater than changes seen in the placebo group. However, no significant improvements were observed for lower body strength. It is unknown whether a longer period of HMB supplementation and training may have resulted in greater changes in FFM and strength. Clearly, additional studies are needed to understand the role of HMB alone, with and without resistance training, on muscle, strength and functionality in older men and women.

A potential, but unexplored benefit of HMB is its effect on fat tissue. Recently, Wilson et al. (2012) found that HMB lowered body fat from old to very old age in rats. Studies evaluating elderly individuals have demonstrated that strength training improves muscle quality (MQ) (Tracy et al., 1999) and decreases intramuscular fat (Newman et al., 2003). Muscle quality is a measure of strength relative to muscle mass and is considered a predictor of health status, mortality and a better indicator of muscle function than strength alone (Tracy et al., 1999). In theory, if HMB decreases fat while at the same time increasing muscle mass, then this may be reflected in an increase in MQ measurements.

There is a need, however, to examine the potential benefit of HMB with and without resistance training, in older human populations. Therefore the purpose of this study was to evaluate the effects of 12 and 24 weeks of HMB supplementation on muscle mass, fat mass, MQ, strength, and function with and without a progressive resistance training program in healthy older men and women following an adequate protein diet.

2. Methods

2.1. Participants

Participants were screened for the following inclusion criteria: male or female ≥65 years; Geriatric Nutritional Risk Index ≥ 92 (21); BMI > 20.0 kg m^{-2}, but <30.0 kg m^{-2} and ambulatory (Bouillanne et al., 2005). The exclusion criteria were major surgery within four weeks of enrollment; active malignant disease; immunodeficiency disorder; history of diabetes; partial or full artificial limb; significant cardiovascular, metabolic or endocrine disease; antibiotic use within one week of enrollment; history of allergies to product ingredients; major disease of the gastrointestinal tract, significant neurological or psychological disorder; actively pursuing weight loss; and currently taking an excluded concomitant treatment including weight loss or gain aids (e.g., steroids, meal replacements, protein and/or amino acids).

2.2. Study design

This randomized, double-blinded, placebo-controlled, mixed factorial clinical trial was conducted in two phases (Fig. 1): Phase I consisted of two non-exercise (NE) groups: (a) ad libitum diet plus placebo (NE_{PLA}) and (b) ad libitum diet plus CaHMB (NE_{HMB}). Phase II consisted of two resistance exercise (RE) groups: (a) ad libitum diet plus placebo and resistance exercise (RE_{PLA}) and (b) ad libitum diet plus CaHMB and resistance exercise (RE_{HMB}). After recruitment and enrollment for Phase I was completed, recruitment and enrollment for Phase II was initiated. No subject was allowed to participate in both Phase I and Phase II. Phase II included all the same outcome measures in Phase I, with the addition of specific resistance exercise outcome measures (i.e., 5RM strength for the bench press, leg press, and leg extension exercises). Study volunteers signed informed consent forms that were approved by the university's institutional review board.

After eligibility was determined, participants were randomly assigned to treatment groups by a computer-generated, pseudo-random permuted block algorithm for Phases I and II. The randomization was further stratified by sex to balance randomization for each gender. Upon enrollment participants were sequentially assigned a subject number and given a corresponding randomization stratum. In a double-blind fashion, participants were given a placebo (PLA, 200 mg calcium + 4 g carbohydrate as packets of powder) or calcium beta-hydroxy-beta-methylbutyrate (HMB, 1.5 g CaHMB + 4 g carbohydrate as packets of powder). Instructions were given to mix their assigned study product in a non-carbonated, non-alcoholic beverage of their choice (e.g., milk, water, juice) and drink ad libitum. Two packets were mixed and consumed every day during the study period.

The study evaluation period for each participant in Phases I and II was 24 weeks. Testing was performed at week 0 (pre-test), 12 weeks (mid-test), and 24 weeks (post-test), and consisted of body composition, muscle strength, functional movement, three-day dietary recall, blood markers, and urinalysis for HMB consumption. All testing took place on the same day such that blood draws, urinalysis, and body composition were tested in the morning after a 12-hour fast. All tests were performed in a metabolic and body composition laboratory at the university.

2.3. Compliance

Product intake was recorded on individual intake logs, which were returned to the laboratory and monitored. Urinary HMB levels were used as markers to indicate test treatment compliance. The first morning urine void was collected at pre-, mid-, and post-testing using a 'clean catch' method previously described (Nissen et al., 1996). Urine samples (100 μl) were placed in 2 mL tubes, frozen at −70° C, and sent on dry ice to Metabolic Technologies (Ames, IA) for analysis. HMB levels increased from pre- to mid-testing (1010 ± 1408 nmol ml^{-1}) and from pre- to post-testing (972 ± 1182 nmol ml^{-1}) for the NE_{HMB} and RE_{HMB} groups compared to minimal changes of 11.7 ± 25.7 nmol ml^{-1} and 25.5 ± 43.3 nmol ml^{-1}, respectively, for the NE_{PLA} and RE_{PLA} groups.

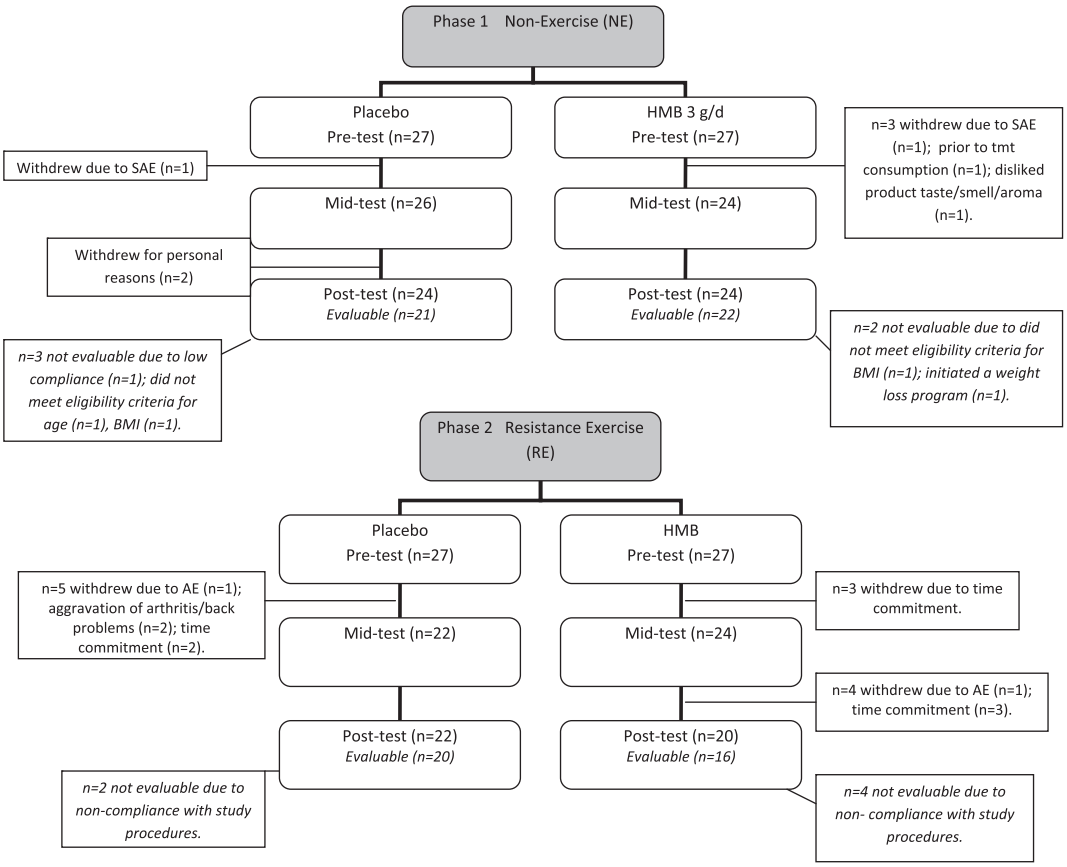

Fig. 1. Subject randomization and evaluability flowchart for non-exercising group (A) and exercising group (B).

2.4. Blood markers

Fasting blood samples were drawn from the antecubital vein at pre- and post-testing only. Samples were sent to a commercial laboratory for analysis (Quest Diagnostics, Norman, OK). Markers analyzed included total protein, albumin, prealbumin, hemoglobin, hematocrit, red blood cells (RBC), white blood cells (WBC), differentials (percent and absolute values for lymphocytes, monocytes, neutrophils, eosinophils, and basophils), platelet count, glucose, blood urea nitrogen (BUN), creatinine, sodium, potassium, chloride, calcium, magnesium, phosphorus, uric acid, total cholesterol, triglycerides, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), lactate dehydrogenase (LDH), and total bilirubin.

2.5. Dietary assessment

Participants completed a three-day dietary recall at pre-, mid-, and post-testing. Instructions for participants were to write down everything consumed during two weekdays and one weekend day. These data were entered into a software program (Food Processor, Version 8.6.0, ESHA Research, Salem, Oregon) that provided calculations of absolute daily protein intake (g), relative daily protein intake (g kg^{-1}), and daily caloric intake (kcal). Average values for protein and caloric intake across each three-day period were recorded.

2.6. Body composition

Whole-body scans were performed at pre-, mid-, and post-testing following a 12-hour fast using a dual-energy X-ray absorptiometry (DXA) scanner (Lunar Prodigy Advanced, Madison, WI, Software version 10.50.086). Images were analyzed with manufacturer-provided software (LUNAR Radiation body composition program). Total fat mass (FM), total lean mass (LM), regional leg lean mass (left and right), and regional arm lean mass (left and right) were measured. Previously determined intraclass correlation coefficients (ICC) and standard errors of measurement (SEM) for test–retest reliability using the DXA scanner in this laboratory to measure 11 men and women 24–48 hours apart for total FM, LM, regional leg LM, and regional arm LM were 0.97 and 0.93 kg, 0.99 and 0.61 kg, 0.99 and 0.03 kg, 0.99 and 0.02 kg, respectively.

2.7. Strength and function assessment

2.7.1. Handgrip strength

Handgrip (HG) strength was measured on the participant's dominant hand (hand used to write with) using a standard hand-held dynamometer (DHS-176, Detecto, Webb City, MO). The dynamometer handle was adjusted so that the middle phalange of the third digit was comfortably perpendicular to the long axis of the handle. In an upright standing position, arms adducted, dominant forearm flexed to 90°, and the wrist in a neutral position, participants were asked to squeeze the dynamometer handle as forcefully as possible for 3 to 5 s. Three trials were performed with about 30 second rest between trials. Strong verbal encouragement was provided for each trial. Force output in kg was recorded for each trial, and the average of the three trials was analyzed as the representative HG strength value.

2.7.2. Isokinetic leg strength

Peak torque (PT) during maximal voluntary concentric isokinetic leg extension and flexion muscle actions was measured at pre-, mid-, and post-testing using a calibrated isokinetic dynamometer (LIDO Multi-Joint II, Loredan Biomedical, West Sacramento, CA) at randomly ordered angular velocities of $60°\cdot s^{-1}$ and $180°\cdot s^{-1}$. All isokinetic testing was performed on the dominant leg (assessed by kicking preference) unless

the participant had a pre-existing condition that precluded testing that leg, in which case the non-dominant leg was used for all testing. The leg used at pre-testing was reassessed at mid- and post-testing. The center of the rotation of the knee joint was visually aligned with the dynamometer's axis of rotation, and restraining straps were positioned over the hips and dominant thigh to prevent extraneous movements. The lever arm was strapped to the distal leg just proximal to the malleoli. At each angular velocity, participants performed four movement-specific warm-up (practice) repetitions, which consisted of leg extension and flexion muscle actions at 25%, 50%, 75%, and 100% of their maximal perceived effort. Immediately following the warm-up repetitions, each participant performed three maximal leg extension and leg flexion muscle actions in a 'push-pull' fashion as hard and fast as they could for a total of six muscle actions (three extensions and three flexions). Three minutes of rest was allowed between velocities. The average PT across the three repetitions was used for analysis.

2.7.3. Bench press, leg press, and leg extension strength

Dynamic constant external resistance (DCER) strength testing was performed only for Phase II (RE_{PLA} and RE_{HMB} groups) at pre-, mid-, and post-testing. Participants completed a five repetition maximum (5RM) test for the following exercises in order: bilateral leg extension, bench press, and bilateral leg press. Prior to the 5RM testing, a five-minute warm-up was completed on a stationary cycle ergometer (Monark 828E, Vansbro, Sweden) at a self-selected intensity. Participants then completed two warm-up sets of 10 repetitions at 55% and 65% of their perceived maximum load. A maximum of five 5RM attempts were allowed with three to five minute rest between attempts. Each successive load was increased by 2–4 kg for the bench press or 4–8 kg for the leg extension and leg press. When the subject was unable to complete five repetitions during an attempt, the load (kg) from the previous attempt was recorded as the 5RM. When the subject completed five repetitions during five successive attempts despite load increases, the final load (kg) used was recorded as the 5RM.

2.7.4. Get-up-and-go test

The get-up-and-go (GUG) test, which has been previously described elsewhere (Podsiadlo and Richardson, 1991), was performed at pre-, mid-, and post-testing as a measure of functionality. GUG was measured as the time it took for a participant seated in a chair to stand up (without assistance from their hands), walk forward briskly along a straight line on the floor measured out to 3 m, turn around 180°, walk back toward the chair briskly along the same three-meter line, turn around 180° again, and sit back down in the chair (without assistance from their hands). A standard digital stopwatch, press-back wooden chair without padding, and red tape to mark the three-meter course on a level, tiled concrete surface were used for the GUG test. The stopwatch was started upon the initiation of movement by the subject to stand up, stopped when the subject was seated again, and recorded to the nearest 0.01 s. Three attempts were performed, and the average of the three was used as the representative GUG score.

2.7.5. Muscle quality

Muscle quality (MQ) was calculated as muscle strength relative to muscle mass. MQ has been used and described previously as an indicator of muscle function (Lynch et al., 1999; Tracy et al., 1999). MQ was calculated for three separate tests: HG (kg) ÷ arm lean mass (kg) = MQ_{HG} (kg kg^{-1}); leg extension PT (Nm) at $60°\cdot s^{-1}$ ÷ leg lean mass (kg) = MQ_{60} (Nm kg^{-1}); and leg extension PT (Nm) at $180°\cdot s^{-1}$ ÷ leg lean mass (kg) = MQ_{180} (Nm kg^{-1}).

2.8. Resistance exercise

For Phase II, the progressive resistance exercise (RE) program consisted of three sessions per week for 21 weeks (excluding three weeks of testing). The volume of RE (number of sets per exercise per week) was as follows: week 1 was pre-testing only, weeks 2 and 3 included one set per exercise, week 4 was two sets, weeks 5–10 were three sets, week 11 was one or two sets, week 12 was mid-testing only, weeks 13 and 14 were one set, week 15 was two sets, weeks 16–22 were three sets, week 23 was one or two sets, and week 24 was post-testing only. All RE sessions were completed in the laboratory using the same equipment used for testing and were supervised by certified personal trainers. During each training session, participants completed one to three sets of 8–12 repetitions for each exercise. Exercises included the bench press, lat pulldown, bilateral leg press, hack squat, and bilateral leg extension. For the bench press, bilateral leg press, and bilateral leg extension exercises, 80% of the one repetition maximum (1RM) determined at pre-testing was used as the load and was progressively increased throughout the study. For the lat pulldown and hack squat exercises, a self-selected load was used to achieve 8–12 repetitions. Each exercise set was separated by 2–5 min of rest. The loads were progressively increased by 2–4 kg for the bench press, lat pulldown, and leg extension or 4–8 kg for the leg press and hack squat when participants were comfortably able to complete 12 repetitions during the last two sets of any exercise for two consecutive sessions.

2.9. Statistical analyses

As this was a pilot study, a relatively small sample size (n of approximately 20 evaluable subjects for each treatment group/exercise group combination) was chosen in order to gather data that may be used in the design of future studies. Because of the exploratory nature of this study, the primary analysis was the evaluable analysis: a participant's outcome data were classified as evaluable until one or more of the following events occurred: participant took an excluded concomitant treatment (e.g., steroids, anabolic agents, amino acid/protein supplements); assigned to the control group, but exhibited a urinary HMB >0.5 μmol ml^{-1} at mid- or post-testing; did not have body composition measurement pre- or post-testing; reported an average total protein intake <0.8 g kg^{-1} body mass; consumed <67% of study product; and in the RE_{PLA} and RE_{HMB} groups completed <60% of RE sessions.

Values are reported as mean raw change scores and the corresponding standard errors. Analysis of variance (ANOVA) and/or paired t-tests were used to analyze the change from baseline to 12 weeks and baseline to 24 weeks for body composition, muscle strength, functionality, muscle quality, dietary intake, blood markers, and HMB urinalysis data. If the residuals from ANOVA did not follow a normal distribution (not attributable to outliers), a Wilcoxon rank-sum test was used to compare groups. The factors used in the model were: treatment × sex, treatment, and sex. If there was a significant interaction ($p < 0.10$), then treatment differences were investigated separately for each sex using the Stepdown Bonferroni (Holm) procedure. Adjusted p-values from this procedure are presented in the footnotes of the tables for significant ($p < 0.05$) treatment effect values for sex. Adverse events and serious adverse events were analyzed by appropriate categorical techniques.

SAS® version 9.1.3 and 9.2 (SAS, Inc., Cary, NC) were used for all statistical analyses. All main effects were tested with two-sided, 0.05 alpha level tests, while interaction effects were assessed with two-sided, 0.10 alpha level tests.

3. Results

3.1. Phase I—no resistance exercise

During the course of the 24-week study, 43 subjects completed Phase I and were considered protocol evaluable [$n = 21$ in NE_{PLA}, $n = 22$ in NE_{HMB}]. Table 1 contains the demographic and baseline characteristics.

Table 1
Characteristics of study participants.

	Study Phase I		Study Phase II	
	NE_{PLA}	NE_{HMB}	RE_{PLA}	RE_{HMB}
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Males/females	14/11	13/12	11/13	11/13
Age, yrs	72 ± 1	73 ± 1	73 ± 1	73 ± 1
Weight, kg	76 ± 4	73 ± 2	68 ± 3	74 ± 3
BMI kg m^{-2}	25 ± 1	26 ± 05	25 ± 1	26 ± 1
Protein intake, g/kg bwt	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
Body composition				
Total lean mass, kg	24 ± 2	22 ± 1	21 ± 1	23 ± 1
Leg lean mass, kg	16 ± 1	14 ± 1	14 ± 1	15 ± 1
Total fat mass, kg	25 ± 2	24 ± 1	22 ± 1	24 ± 1
Isokinetic peak torque (Nm)				
Extensor 60°·s^{-1}	97 ± 9	80 ± 8	75 ± 8	84 ± 9
Flexor 60°·s^{-1}	56 ± 5	49 ± 4	40 ± 3	46 ± 4
Extensor 180°·s^{-1}	61 ± 5	49 ± 5	51 ± 6	56 ± 6
Flexor 180°·s^{-1}	42 ± 4	35 ± 2	32 ± 2	34 ± 2
Hand grip strength, kg	31 ± 3	32 ± 2	26 ± 3	29 ± 2
Muscle quality (MQ)				
MQ 60° s Nm kg^{-1}	5.9 ± 0.3	5.4 ± 0.4	5.2 ± 0.4	5.3 ± 0.3
MQ 180° s Nm kg^{-1}	3.8 ± 0.2	3.2 ± 0.2	3.5 ± 0.3	3.5 ± 0.2
MQ hand grip	5.8 ± 0.2	6.4 ± 0.2	5.4 ± 0.2	5.5 ± 0.2
Get up and go, s	5.2 ± 0.2	5.4 ± 0.4	5.6 ± 0.3	6.0 ± 0.6
Activities of daily living	6.3 ± 0.3	6.4 ± 0.3	6.5 ± 0.3	6.6 ± 0.3

3.1.1. Body weight and composition

With the exception of an increase in arm lean mass at post-testing, no significant changes in mass or body composition were observed in the NE_{PLA} group (Table 2). However, the NE_{HMB} group experienced significant increases in total weight, total LM and leg LM at both mid- and post- testing compared to baseline pre-testing (Table 2). Both arm lean mass and total FM also had significant increased at post- but not mid-test (Table 2). No significant differences were observed between groups.

3.1.2. Strength and functionality

Leg extensor PT did not change for either 60°·s^{-1} or 180°·s^{-1} throughout the study within the NE_{PLA} group (Table 2). For the NE_{HMB} group, leg extensor PT 60°·s^{-1} significantly increased at post-testing, and for 180°·s^{-1} increased at both mid- and post-testing. Differences between groups (NE_{PLA} vs NE_{HMB}) were significant at post-testing for 60°·s^{-1}. Isokinetic leg flexor PT did not change at mid- or post-testing except for the following: leg flexor PT transiently decreased for 60°·s^{-1} at mid-testing in the NE_{HMB} group but was not different from baseline at post-testing; there was a non-significant ($p = 0.052$) increase for 180°·s^{-1} at post-test in both treatment groups.

Handgrip strength did not change from baseline for either the NE_{PLA} or NE_{HMB} groups (Table 2). Get-up-and-go time improved in the NE_{HMB} group at mid-testing (−0.2 ± 0.1 s, $p = 0.04$) and in both groups at post-testing (−0.6 ± 0.2 s, $p < 0.01$, −0.5 ± 0.1 s, $p < 0.01$, NE_{PLA} and NE_{HMB}, respectively), with no differences between groups.

3.1.3. Muscle quality

MQ_{60} was unaffected at mid- and post-testing for the NE_{PLA} and NE_{HMB} groups, respectively (Table 3). MQ_{180} remained unchanged at mid- and post-testing for the NE_{PLA} group, but increased at mid- and post- for the NE_{HMB} group (Table 3). MQ_{HG} did not change at mid- or post-testing for either the NE_{PLA} or NE_{HMB} group, respectively.

3.2. Phase II—resistance exercise

During the course of the 24-week study, 36 subjects completed Phase II and were considered protocol evaluable [n = 20 in RE_{PLA}, n = 16 in RE_{HMB}]. Table 1 contains the demographic and baseline characteristics for these participants.

Table 2
Body composition (kg) and isokinetic strength (Nm) and changes after 12 week (mid-test) and 24 week (post-test) supplementation with Placebo (PLA) or HMB in non-exercising (NE) study subjects.

	NE_{PLA}		NE_{HMB}		Treatment main effects[a]
	Mean ± SEM	Within group p value	Mean ± SEM	Within group p value	
Weight, kg					
Mid-test	0.2 ± 0. 3	–	0.8 ± 0.2	<0.01	–
Post-test	−0.3 ± 0. 5	–	0.8 ± 0.3	0.02	–
Total lean mass, kg					
Mid-test	0.2 ± 0.1	–	0.4 ± 0.1	<0.01	–
Post-test	0.2 ± 0.1	–	0.5 ± 0.1	<0.01	–
Leg lean mass, kg					
Mid-test	0.1 ± 0.1	–	0.3 ± 0.1	0.01	–
Post-test	0.1 ± 0.1	–	0.3 ± 0.1	<0.01	ns (0.09)
Arm lean mass, kg					
Mid-test	0.04 ± 0.03	–	0.05 ± 0.03	ns(0.06)	–
Post-test	0.11 ± 0.04	0.02	0.07 ± 0.03	0.03	–
Total fat mass, kg					
Mid-test	0.2 ± 0.3	–	0.3 ± 0.2	–	–
Post-test	0.3 ± 0.4	–	0.6 ± 0.3	0.03	ns (0.06)
Isokinetic peak torque (Nm)					
Extensor, 60°·s^{-1}					
Mid-test	2.7 ± 2.2	–	4.8 ± 3.2	–	–
Post-test	−1.2 ± 2.1	–	7.7 ± 3.5	0.04	0.04
Flexor, 60°·s^{-1}					
Mid-test	1.7 ± 3.3		−6.3 ± 2.6	0.03	ns (0.08)
Post-test	0.5 ± 2.5		1.4 ± 1.5	–	–
Extensor, 180°·s^{-1}					
Mid-test	5.0 ± 3.1	–	7.2 ± 1.7	<0.01	*[1]
Post-test	2.9 ± 3.2	–	8.5 ± 1.9	<0.01	–
Flexor, 180°·s^{-1}					
Mid-test	2.7 ± 3.0	–	2.9 ± 2.0	–	–
Post-test	5.9 ± 2.8	ns (0.052)	7.3 ± 3.6	ns (0.052)	–
Grip strength, kg					
Mid-test	−0.3 ± 0.8	–	−0.3 ± 1.1	–	–
Post-test	0.6 ± 0.8	–	0.02 ± 0.9	–	–
GUG, s					
Mid-test	−0.3 ± 0.2	–	−0.2 ± 0.1	0.04	–
Post-test	−0.6 ± 0.2	<0.01	−0.5 ± 0.1	<0.01	–

* indicates significant treatment group by gender interaction (treatment p-value indicated below for gender).
[a] p values reported for significant main effects unless there was a significant treatment by gender interaction, in which case results are indicated in footnote.
[1] Females: ns (0.07).

3.2.1. Body weight and composition

There were no significant changes in total body weight at mid- or post-testing for either the RE_{PLA} or RE_{HMB} groups, respectively; however, weight loss was significantly greater in RE_{HMB} males vs. RE_{PLA} at mid-test (Table 4). Total, leg, and arm lean mass increased in both groups with significant ($p < 0.05$) differences ($RE_{PLA} > RE_{HMB}$) for men in total and arm lean mass at both mid- and post-testing. Fat decreased in both groups at mid- and only in RE_{HMB} at post-testing (Table 4) with no differences between groups.

3.2.2. Strength and functional movement

Bench press, leg press, and leg extensor 5RM significantly increased at mid- and post-testing for both the RE_{PLA} and RE_{HMB} groups (Table 4) with no differences between groups. Isokinetic leg extensor PT at both 60°·s^{-1} and 180°·s^{-1} increased at mid- and post-testing with no differences between groups. Isokinetic leg flexor PT 60°·s^{-1} tended to increase at mid- but not post-testing, while leg flexor PT 180°·s^{-1} increased at post-testing in the RE_{PLA} group and at mid-testing in the RE_{HMB} group ($p = 0.052$), with no differences between groups. Handgrip strength increased from baseline at both mid- and post-test for

Table 3
Muscle quality changes after 12 week (mid-test) and 24 week (post-test) supplementation with Placebo (PLA) or HMB in non-exercising (NE) study subjects.

	NE_{PLA}		NE_{HMB}		Treatment main effects[a]
	Mean ± SEM	Within group	Mean ± SEM	Within group	
MQ 60°·s^{-1} Nm/kg					
Mid-test	0.1 ± 0.2	–	0.2 ± 0.2	–	–
Post-test	−0.1 ± 0.1	–	0.3 ± 0.2	–	–
MQ 180°·s^{-1} Nm/kg					
Mid-test	0.2 ± 0.2	–	0.5 ± 0.1	<0.01	*[1]
Post-test	0.1 ± 0.2	–	0.5 ± 0.1	<0.01	0.049
MQ HG					
Mid-test	−0.1 ± 0.2	–	−0.3 ± 0.3	–	–
Post-test	0.02 ± 0.1	–	−0.2 ± 0.2	–	–

* indicates significant treatment group by gender interaction (treatment p-value indicated below for gender).
[a] p values reported for significant main effects unless there was a significant treatment by gender interaction, in which case results are indicated in footnote.
[1] Females: 0.01.

both the RE_{PLA} and RE_{HMB} groups, respectively, with no differences between groups (Table 4). Likewise, GUG time significantly decreased from baseline to mid- and post-testing for both the RE_{PLA} and RE_{HMB} groups, respectively, with no differences between groups (Table 4).

3.2.3. Muscle quality

MQ_{60} increased with no differences between groups; mid-test values were not significant for RE_{HMB} (Table 5). MQ_{180} significantly increased at mid- and post-testing for the RE_{PLA} group, but did not significantly change from baseline to mid- or post-testing for the RE_{HMB} group, with no differences between groups (Table 5). MQ_{HG} did not significantly change from baseline to mid- or post-testing for the RE_{PLA} group, however, the RE_{HMB} group increased at mid- and post-testing, with no differences between groups (Table 5).

3.3. Blood chemistries—Phases I and II

Analysis of blood chemistries revealed no clinically-significant differences within or between PLA and HMB treatments. The mean values for total protein and SGOT were collectively higher for those who took the placebo than HMB ($p = 0.04$ and $p = 0.046$, respectively); while all values were within the expected and normal ranges. Uric acid was higher for those who took the HMB treatment than the placebo ($p < 0.01$), however, the mean uric acid values fell within the expected and normal range. Absolute lymphocytes were higher for NE_{HMB} than NE_{PLA} ($p < 0.01$), however, the percent lymphocytes were not different among groups. There were no differences among groups for any other blood markers.

3.4. Adverse events—Phases I and II

Overall, 25 adverse events (AEs; 24 mild; 1 moderate) and 2 serious AEs (SAEs) were reported during both Phases I and II. The AEs were found to be "probably none" or "not" related to the study protocol or product by the study physician. Four participants discontinued the study due to AEs and/or SAEs. One withdrew from the RE_{HMB} group after reporting a moderate AE due to pneumonia and another withdrew from the RE_{PLA} group after reporting a fracture due to fall. The two participants who reported SAEs exited from the study between mid- and post-testing. One participant in the NE_{HMB} group was diagnosed with a urinary tract infection, was hospitalized, and later died. Another man in the NE_{PLA} group was diagnosed with throat cancer and withdrew from the study. Neither AE/SAE was related to the study protocol or product as determined by the study physician. No statistical differences or clinically-relevant findings among the numbers of AEs were reported between the HMB and PLA groups.

Table 4
Body composition (kg) and isokinetic strength (Nm) and changes after 12 week (mid-test) and 24 week (post-test) supplementation with placebo (PLA) or HMB in resistance-exercising (RE) study subjects.

	RE_{PLA}		RE_{HMB}		Treatment main effects[a]
	Mean ± SEM	Within group p value	Mean ± SEM	Within group p value	
Weight, kg					
Mid-test	0.3 ± 0.2	ns(0.08)	−0.6 ± 0.4	–	*[1]
Post-test	0.4 ± 0.3	–	−0.5 ± 0.6	–	ns (0.06)
Total lean mass, kg					
Mid-test	0.7 ± 0.1	<0.01	0.4 ± 0.2	0.03	*[2]
Post-test	0.9 ± 0.1	<0.01	0.7 ± 0.2	0.01	*[3]
Leg lean mass, kg					
Mid-test	0.4 ± 0.1	<0.01	0.2 ± 0.1	ns (0.08)	*[4]
Post-test	0.6 ± 0.1	<0.01	0.5 ± 0.2	0.01	–
Arm lean mass, kg					
Mid-test	0.21 ± 0.03	<0.01	0.11 ± 0.04	0.01	*[5]
Post-test	0.24 ± 0.04	<0.01	0.13 ± 0.06	ns (0.053)	*[6]
Total fat mass, kg					
Mid-test	−0.4 ± 0.2	0.02	−0.9 ± 0.3	0.01	–
Post-test	−0.5 ± 0.3	ns (0.096)	−0.9 ± 0.4	0.04	–
Bench press, 5RM					
Mid-test	16 ± 2	<0.01	19 ± 1	<0.01	*[7]
Post-test	31 ± 3	<0.01	32 ± 2	<0.01	–
Leg press, 5RM					
Mid-test	118 ± 14	<0.01	131 ± 18	<0.01	–
Post-test	259 ± 23	<0.01	275 ± 32	<0.01	–
Leg extensor, 5RM					
Mid-test	36 ± 4	<0.01	37 ± 4	<0.01	–
Post-test	78 ± 6	<0.01	74 ± 6	<0.01	–
60°·s^{-1} extensor peak torque, Nm					
Mid-test	10.8 ± 3.1	<0.01	5.3 ± 2	0.03	–
Post-test	17.1 ± 3.8	<0.01	9.1 ± 2	<0.01	ns(0.09)
180°·s^{-1} extensor peak torque, Nm					
Mid-test	5.0 ± ±2	0.01	3.4 ± 2.0	–	–
Post-test	11.0 ± 3	<0.01	5.7 ± 2.5	0.04	–ns(0.09)
60°·s^{-1} flexor peak torque, Nm					
Mid-test	4.7 ± 1.3	<0.01	3.3 ± 1.8	ns (0.09)	–
Post-test	7.0 ± 5.0	–	−2.1 ± 2.2	–	–
180°·s^{-1} flexor peak torque, Nm					
Mid-test	2.2 ± 1.4	–	3.0 ± 1.4	ns (0.052)	–
Post-test	3.8 ± 1.8	0.048	−0.1 ± 1.8	–	ns (0.08)
Grip strength, kg					
Mid-test	2.1 ± 0.5	<0.01	2.1 ± 0.6	<0.01	–
Post-test	2.6 ± 0.6	<0.01	2.8 ± 0.7	<0.01	–
GUG, s					
Mid-test	−0.4 ± 0.2	0.04	−0.5 ± 0.2	.0.01	–
Post-test	−0.6 ± 0.3	0.045	−0.7 ± 0.3	.0.03	–

* indicates significant treatment group by gender interaction (treatment p-value indicated below for gender).
[a] p values reported for significant main effects unless there was a significant treatment by gender interaction, in which case results are indicated in footnote.
[1] Males: 0.01.
[2] Males: 0.02.
[3] Males: 0.03.
[4] Males: ns (0.09).
[5] Males: 0.01.
[6] Males: 0.005.
[7] Males: ns (0.06).

Table 5
Muscle quality changes after 12 weeks (Mid-test) and 24 weeks (post-test) supplementation with placebo (PLA) or HMB in resistance-exercising (RE) study subjects.

	RE_{PLA}		RE_{HMB}		Treatment main effect[a]
	Mean ± SEM	Within group p value	Mean ± SEM	Within group p value	
MQ60°·s^{-1} Nm/kg					
Mid-test	0.6 ± 0.2	0.01	0.2 ± 0.1	ns (0.09)	–
Post-test	1.1 ± 0.3	<0.01	0.5 ± 0.2	0.01	–
MQ 180°·s^{-1} Nm/kg					
Mid-test	0.3 ± 0.1	0.03	0.2 ± 0.1	ns (0.098)	–
Post-test	0.8 ± 0.2	0.01	0.3 ± 0.2	ns (0.07)	–
MQ HG					
Mid-test	0.2 ± 0.1	ns (0.098)	0.3 ± 0.1	0.03	–
Post-test	0.2 ± 0.1	ns (0.08)	0.4 ± 0.1	0.02	–

[a] p values reported for significant main effects.

4. Discussion

Phase I of the current study demonstrated that prolonged supplementation with CaHMB improved total lean mass (LM), strength, function and MQ without resistance exercise. In addition, the progressive, high-intensity resistance training protocol used in Phase II resulted in increased LM, strength, and MQ, with or without CaHMB. Moreover, the CaHMB intervention in Phase II resulted in a significant decreased total fat mass along with the increased total lean mass and arm MQ from the training. Therefore, these data suggest that strength, MQ, body composition, and functionality in healthy older men and women can be improved through CaHMB supplementation, with and without resistance training.

Recent evidence suggests that CaHMB combined with arginine and lysine (HMB-Arg-Lys) may partially blunt or reverse the age-related changes in muscle mass (Baier et al., 2009; Flakoll et al., 2004). While the current study only supplemented with CaHMB, the changes in total lean body mass (0.40 kg NE, 0.39 kg RE) were similar to values (0.20 kg & 0.56 kg) reported by Flakoll et al. (2004) and Baier et al. (2009), respectively. Furthermore, our data show that CaHMB increased leg lean mass 2.1% at both 12 and 24 weeks, which was similar to Flakoll et al. (2004) who demonstrated an increase of 1.1% in thigh, arm and forearm limb circumference.

It is currently thought that the effects of CaHMB in aging muscle are mediated by altering protein balance. For example, Wilson et al. (2012) demonstrated that CaHMB prevented the decline in muscle fiber dimensions from young to old age using a rat model. These effects may have been mediated by blunting the genes that regulate protein breakdown. Moreover, Baier et al. (2009) suggested that CaHMB may improve whole body protein metabolism in elderly individuals over the age of 65.

The initiation of resistance exercise in aging populations has resulted in robust changes in lean body mass (Charette et al., 1991; Delecluse et al., 2004; Fielding et al., 2002; Hunter et al., 2004; Schwartz and Evans, 1995). We are aware of only one previous study that has examined the effects of CaHMB in conjunction with resistance exercise in the elderly (Vukovich et al., 2001). Interestingly, the present results partially disagreed with Vukovich et al. (2001) who found that CaHMB combined with resistance training increased anthropometrically-determined fat free mass more than resistance training alone. However, the decreases in fat mass observed in the CaHMB group (RE_{HMB}) in the present study were consistent with the decreases in percent fat in the CaHMB group reported by Vukovich et al. (2001). It should be noted that Vukovich et al. (2001) used lower intensities (70% vs. 80% 1RM) and frequencies (2 vs. 3 days) for resistance exercise than used in phase II of the present study. Moreover, the resistance training program used in the current study resulted in 26–41% attrition, which was higher than the 19–23% attrition observed during the 8-weeks of exercise used by Vukovich et al. (2001). Breen and Phillips (2011) recently suggested that low-intensity and high-repetition resistance exercise may be more effective for older adults. Future studies should consider examining the efficacy of lower-intensity resistance training, with and without protein and/or CaHMB supplementation, for improving exercise compliance in the elderly.

Research suggests that after the age of 74, 30% of men and 66% of women in the United States are incapable of lifting objects greater than 4.5 kg (Jette and Branch, 1981). However, the neuromuscular system is highly plastic and displays the capacity to respond to repeated loading via an increase in strength in elderly populations (Charette et al., 1991; Delecluse et al., 2004; Fielding et al., 2002; Hunter et al., 2004; Moss et al., 1997; Schwartz and Evans, 1995). The current study demonstrated that CaHMB alone (without training) elicited an increase in strength, as well as GUG performance. Furthermore, both resistance training alone, and when combined with CaHMB, increased upper and lower body strength, with no differences between conditions.

Muscle quality has been suggested to be a better indicator of muscle function than strength alone, especially when evaluating elderly adults (Newman et al., 2003). While CaHMB had no effect on overall strength compared to resistance training alone, it did increase MQ. Sarcopenia is purposefully defined to include the age-related loss of muscle mass and function due to the observation that muscle mass alone may not account for the rapid deterioration of strength losses with age (Jette and Branch, 1981). Therefore, it is possible that the reversal of this process may also be predicated on increases in strength, without a concomitant increase in muscle size in older adults. This hypothesis is consistent with early work by Sale (1988) who demonstrated an extended time course of neural versus hypertrophic adaptations to resistance exercise in the elderly. It is likely that CaHMB is enhancing strength through increasing the short-term energetic capacity of myofibers (Pinheiro et al., 2012). It was recently demonstrated that four weeks of CaHMB administration in male Wistar rats increased intramuscular ATP and glycogen content by up to 2- and 5-fold respectively (Pinheiro et al., 2012). It is therefore possible that such metabolic changes enhance strength per unit area of lean mass. However, further research is needed in human models to examine the possible change in energetic capacity and its effect on muscle quality.

One interesting finding regarding the CaHMB treatment in the present study was its effect on fat mass, which was consistent with Vukovich et al. (2001). CaHMB alone did not cause fat loss. However, resistance training with or without CaHMB resulted in a significant loss of fat mass at week 12, whereas only the CaHMB group was able to further the reduction in fat mass at week 24. Resistance exercise is not thought to be a potent stimulus for total body fat loss (Binder et al., 2005; Ismail et al., 2012). However, CaHMB is thought to improve metabolic capacity and fat utilization of myofibers (Bruckbauer et al., 2012). In fact, recent evidence suggested that CaHMB supplementation improved fatty acid oxidation, adenosine monophosphate kinase (AMPK), and Sirt1 and Sirt3 activity in adipocytes and in muscle cells (Bruckbauer et al., 2012). Collectively, these proteins act to improve mitochondrial biogenesis, fat oxidation, and energy metabolism. Thus, it is conceivable that the combination of elevated LM, coupled with greater MQ and metabolic capacity resulted in significant fat loss at 24 weeks in the RE_{HMB} group. Due to the elevated comorbidities associated with sarcopenic obesity (Chung et al., 2012), future studies are needed to further investigate the potential combination of resistance exercise and CaHMB supplementation for reducing fat mass.

5. Conclusion

In conclusion, the present data supports the consensus that 24 weeks of resistance training is an effective intervention for improving LM, strength, functional movement and MQ in elderly men and

women. However, there are three potential limitations: 1) low adherence to high intensity resistance exercise programs, 2) discontinuation of resistance exercise will result in rapid loss of benefits, and 3) in frail elderly, resistance exercise may not be adequate to reverse loss of muscle function (Bamman et al., 2007; Moss et al., 1997). Accordingly, non-exercise interventions (nutritional or pharmacological) that can improve body composition, MQ and functionality, are critically important. The findings of the present pilot study indicate that CaHMB without RE enhances strength and MQ in elderly men and women, thereby supporting its potential as a nutritional intervention to prevent sarcopenia and its associated functional decline in people as they age.

Competing interests

J.R. Stout is a science advisor for Abbott Nutrition and received compensation. V. Mustad and J. Oliver are employed by Abbott Nutrition. A. Smith-Ryan, D. Fukuda, J.R. Hoffman, K. Kendall, J. Moon, and J. Wilson have no conflict of interest to declare.

Acknowledgments

The research was supported by a grant from Abbott Nutrition, 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, J.R. Stout has the final responsibility for the decision to submit the publication.

References

Baier, S., Johannsen, D., Abumrad, N., Rathmacher, J.A., Nissen, S., Flakoll, P., 2009. Year-long changes in protein metabolism in elderly men and women supplemented with a nutrition cocktail of β-hydroxy-β-methylbutyrate (HMB), l-arginine, and l-lysine. J. Parenter. Enter. Nutr. 33 (1), 71–82.

Bamman, M.M., Clarke, M.S., Feeback, D.L., Talmadge, R.J., Stevens, B.R., Lieberman, S.A., Greenisen, M.C., 1998. Impact of resistance exercise during bed rest on skeletal muscle sarcopenia and myosin isoform distribution. J. Appl. Phys. 84 (1), 157–163.

Bamman, M.M., Petrella, J.K., Kim, J.S., Mayhew, D.L., Cross, J.M., 2007. Cluster analysis tests the importance of myogenic gene expression during myofiber hypertrophy in humans. J. Appl. Phys. 102 (6), 2232–2239.

Binder, E.F., Yarasheski, K.E., Steger-May, K., Sinacore, D.R., Brown, M., Schechtman, K.B., Holloszy, J.O., 2005. Effects of progressive resistance training on body composition in frail older adults: results of a randomized, controlled trial. J. Gerontol. A Biol. Sci. Med. Sci. 60 (11), 1425–1431.

Bouillanne, O., Morineau, G., Dupont, C., Coulombel, I., Vincent, J.P., Nicolis, I., Aussel, C., 2005. Geriatric Nutritional Risk Index: a new index for evaluating at-risk elderly medical patients. Am. J. Clin. Nutr. 82 (4), 777–783.

Breen, L., Phillips, S.M., 2011. Skeletal muscle protein metabolism in the elderly: interventions to counteract the 'anabolic resistance' of ageing. Nutr. Metab. (Lond.) 8 (68). http://dx.doi.org/10.1186/1743-7075-8-68.

Bruckbauer, A., Zemel, M.B., Thorpe, T., Akula, M.R., Stuckey, A.C., Osborne, D., Wall, J.S., 2012. Synergistic effects of leucine and resveratrol on insulin sensitivity and fat metabolism in adipocytes and mice. Nutr. Metab. (Lond.) 9 (1), 77.

Campbell, W.W., Crim, M.C., Dallal, G.E., Young, V.R., Evans, W.J., 1994. Increased protein requirements in elderly people: new data and retrospective reassessments. Am. J. Clin. Nutr. 60 (4), 501–509.

Charette, S., McEvoy, L., Pyka, G., Snow-Harter, C., Guido, D., Wiswell, R.A., Marcus, R., 1991. Muscle hypertrophy response to resistance training in older women. J. Appl. Physiol. 70 (5), 1912–1916.

Chung, J.Y., Kang, H.T., Lee, D.C., Lee, H.R., Lee, Y.J., 2012. Body composition and its association with cardiometabolic risk factors in the elderly: a focus on sarcopenic obesity. Arch. Gerontol. Geriatr. 56 (1), 270–278.

Delecluse, C., Colman, V., Roelants, M., Verschueren, S., Derave, W., Ceux, T., Stijnen, V., 2004. Exercise programs for older men: mode and intensity to induce the highest possible health-related benefits. Prev. Med. 39 (4), 823–833.

Doherty, T.J., 2003. Invited review: aging and sarcopenia. J. Appl. Physiol. 95 (4), 1717–1727.

Eley, H.L., Russell, S.T., Baxter, J.H., Mukerji, P., Tisdale, M.J., 2007. Signaling pathways initiated by beta-hydroxy-beta-methylbutyrate to attenuate the depression of protein synthesis in skeletal muscle in response to cachectic stimuli. Am. J. Physiol. Endocrinol. Metab. 293 (4), E923–E931.

Evans, W.J., 1995. What is sarcopenia? J. Gerontol. A Biol. Sci. Med. Sci. 50, 5–8 (Special Issue).

Fielding, R.A., LeBrasseur, N.K., Cuoco, A., Bean, J., Mizer, K., Singh, M.A.F., 2002. High-velocity resistance training increases skeletal muscle peak power in older women. J. Am. Geriatr. Soc. 50 (4), 655–662.

Flakoll, P., Sharp, R., Baier, S., Levenhagen, D., Carr, C., Nissen, S., 2004. Effect of β-hydroxy-β-methylbutyrate, arginine, and lysine supplementation on strength, functionality, body composition, and protein metabolism in elderly women. Nutrition 20 (5), 445–451.

Hunter, G.R., McCarthy, J.P., Bamman, M.M., 2004. Effects of resistance training on older adults. Sports Med. 34 (5), 329–348.

Ismail, I., Keating, S.E., Baker, M.K., Johnson, N.A., 2012. A systematic review and meta-analysis of the effect of aerobic vs. resistance exercise training on visceral fat. Obes. Rev. 13 (1), 68–91.

Janssen, I., Shepard, D.S., Katzmarzyk, P.T., Roubenoff, R., 2004. The healthcare costs of sarcopenia in the United States. J. Am. Geriatr. Soc. 52 (1), 80–85.

Jette, A.M., Branch, L.G., 1981. The Framingham disability study: II. Physical disability among the aging. Am. J. Public Health 71 (11), 1211–1216.

Katsanos, C.S., Kobayashi, H., Sheffield-Moore, M., Aarsland, A., Wolfe, R.R., 2005. Aging is associated with diminished accretion of muscle proteins after the ingestion of a small bolus of essential amino acids. Am. J. Clin. Nutr. 82 (5), 1065–1073.

Katsanos, C.S., Kobayashi, H., Sheffield-Moore, M., Aarsland, A., Wolfe, R.R., 2006. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. Am. J. Physiol. Endocrinol. Metab. 291 (2), E381–E387.

Lynch, N.A., Metter, E.J., Lindle, R.S., Fozard, J.L., Tobin, J.D., Roy, T.A., Hurley, B.F., 1999. Muscle quality. I. Age-associated differences between arm and leg muscle groups. J. Appl. Physiol. 86 (1), 188–194.

Marcell, T.J., 2003. Sarcopenia: causes, consequences, and preventions. J. Gerontol. 58 (10), M911–M916.

Marcus, R., 1995. Relationship of age-related decreases in muscle mass and strength to skeletal status. J. Gerontol. A Biol. Sci. Med. Sci. 50 (Spec No:86–7).

Moss, B.M., Refsnes, P.E., Abildgaard, A., Nicolaysen, K., Jensen, J., 1997. Effects of maximal effort strength training with different loads on dynamic strength, cross-sectional area, load-power and load-velocity relationships. Eur. J. Appl. Physiol. Occup. Physiol. 75 (3), 193–199.

Newman, A.B., Haggerty, C.L., Goodpaster, B., Harris, T., Kritchevsky, S., Nevitt, M., Visser, M., 2003. Strength and muscle quality in a well-functioning cohort of older adults: the health, aging and body composition study. J. Am. Geriatr. Soc. 51 (3), 323–330.

Nissen, S., Sharp, R., Ray, M., Rathmacher, J.A., Rice, D., Fuller, J.C., Abumrad, N., 1996. Effect of leucine metabolite β-hydroxy-β-methylbutyrate on muscle metabolism during resistance-exercise training. J. Appl. Physiol. 81 (5), 2095–2104.

Pijnappels, M., Reeves, N.D., van Dieën, J.H., 2008. Identification of elderly fallers by muscle strength measures. Eur. J. Appl. Physiol. 102 (5), 585–592.

Pinheiro, C.H., Gerlinger-Romero, F., Guimarães-Ferreira, L., de Souza-Jr, A.L., Vitzel, K.F., Nachbar, R.T., Curi, R., 2012. Metabolic and functional effects of beta-hydroxy-beta-methylbutyrate (HMB) supplementation in skeletal muscle. Eur. J. Appl. Physiol. Occup. Physiol. 112 (7), 2531–2537.

Podsiadlo, D., Richardson, S., 1991. The timed "up & go": a test of basic functional mobility for frail elderly persons. J. Am. Geriatr. Soc. 39 (2), 142.

Sale, D.G., 1988. Neural adaptation to resistance training. Med. Sci. Sports Exerc. 20 (5 Suppl.), S135–S145.

Schwartz, R.S., Evans, W.J., 1995. Effects of exercise on body composition and functional capacity of the elderly. J. Gerontol. A Biol. Sci. Med. Sci. 50, 147–150 (Special Issue).

Tracy, B.L., Ivey, F.M., Hurlbut, D., Martel, G.F., Lemmer, J.T., Siegel, E.L., Hurley, B.F., 1999. Muscle quality. II. Effects of strength training in 65-to 75-yr-old men and women. J. Appl. Physiol. 86 (1), 195–201.

Vukovich, M.D., Stubbs, N.B., Bohlken, R.M., 2001. Body composition in 70-year-old adults responds to dietary β-hydroxy-β-methylbutyrate similarly to that of young adults. J. Nutr. 131 (7), 2049–2052.

Wilkinson, D.J., Hossain, T., Hill, D.S., Phillips, B.E., Crossland, H., Williams, J., Atherton, P.J., 2013. Effects of leucine and its metabolite β-hydroxy-β-methylbutyrate on human skeletal muscle protein metabolism. J. Physiol. 591, 2911–2923.

Wilson, G.J., Wilson, J.M., Manninen, A.H., 2008. 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.) 5, 1.

Wilson, J.M., Grant, S.C., Lee, S.R., Masad, I.S., Park, Y.M., Henning, P.C., Kim, J.S., 2012. Beta-hydroxy-beta-methyl-butyrate blunts negative age-related changes in body composition, functionality and myofiber dimensions in rats. Int. J. Sport Nutr. 9, 18.