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ORIGINAL ARTICLE

Effects of β-hydroxy-β-methylbutyrate free acid and cold water immersion on post-exercise markers of muscle damage

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Abstract The aim of the current study was to examine the effects of cold water immersion (CWI) with and without the free acid form of β-hydroxy-β-methylbutyrate (HMB-FA) on markers of muscle damage following acute lower body resistance exercise. Forty recreationally resistance-trained men (22.3 ± 2.4 years) were randomly divided into one of the four groups: (1) Placebo (PL); (2) HMB-FA; (3) HMB-FA-CWI; (4) PL-CWI. HMB-FA groups ingested 3 g day^{-1} and CWI groups submersed their lower body into 10–12 °C water for 10-min post-exercise. No differences between groups were observed for CK; however, PL-CWI had significantly greater elevations in myoglobin 30-min post-exercise compared to HMB-FA ($p = 0.009$) and PL ($p = 0.005$), and HMB-FA-CWI was significantly greater than HMB-FA ($p = 0.046$) and PL ($p = 0.028$). No differences between groups were observed for IL-6 and IL-10, although CRP was significantly greater 24-h post-exercise for PL-CWI compared to HMB-FA-CWI ($p = 0.02$) and HMB-FA ($p = 0.046$). Only HMB-FA-CWI showed significantly ($p = 0.02$) greater improvements in average power per repetition. CWI appeared to elevate myoglobin compared to other groups, while HMB-FA may have attenuated the increase in CRP when combined with CWI. Nevertheless, HMB-FA or CWI treatments did not appear to provide benefit over PL for recovery. Instead, the combination of CWI and HMB-FA improved performance recovery compared to other groups.

Keywords Cytokines · C-reactive protein · Myoglobin · Recovery · HMB · Cryotherapy

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Introduction

Recovery from high-intensity exercise is key for athletes to overcome fatigue, regenerate skeletal muscle, and maintain athletic performance (Kenttä et al. 2002). Nutritional interventions and traditional athletic training therapies, such as cold water immersion (CWI), are commonly used strategies to enhance recovery from exercise. However, whether one mode of recovery is more advantageous than another has not been well established or validated. In addition, the role of combination therapies (using both nutritional and traditional treatment protocols) has not been examined. CWI is one of the most popular recovery modalities used to assist athletes in recovering from high-intensity exercise, but its efficacy remains unclear, especially as it relates to recovery from resistance exercise (Leeder et al. 2012). CWI is speculated to alter blood flow and reduce post-exercise inflammation in skeletal muscles in an attempt to attenuate the delayed-onset of muscle soreness (DOMS) and enhance muscle recovery (Swenson et al. 1996). Further, the results from a recent meta-analysis concluded that following strenuous exercise, CWI can alleviate symptoms of DOMS, reduce post-exercise elevation in creatine kinase (CK), and improve muscle power

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recovery, but may be limited in its ability to enhance recovery of muscle strength (Leeder et al. 2012).

There have been several studies that have demonstrated enhanced recovery by amino acid administration (Hoffman et al. 2010; Ratamess et al. 2003). Recent research has suggested that oral administration of the leucine metabolite β-hydroxy-β-methylbutyrate (HMB) may speed up the regenerative capacity of skeletal muscle following high-intensity exercise and attenuate markers of skeletal muscle damage (Wilson et al. 2013a). Wilson et al. (2013b) reported that short-term supplementation (2 days; 3 g day^{-1}) decreased indices of muscle damage (CK and 3-methylhistidine) following a high-volume intense resistance training session. It has been suggested that HMB stimulates muscle protein synthesis through an up-regulation of the mammalian target of rapamycin (mTOR), a kinase responsible for translation initiation of muscle protein synthesis (Wilkinson et al. 2013; Eley et al. 2007), and inhibits the ubiquitin–proteasome pathway, a system that degrades intracellular proteins (Smith et al. 2005).

The majority of research investigating the efficacy of HMB has utilized supplementation of calcium HMB (HMB-Ca) and has shown to be associated with greater increases in lean body mass, strength, and power in conjunction with a resistance training program of 1–12 weeks (Wilson et al. 2013a). However, some research has shown no effect from HMB-Ca supplementation in conjunction with a training program (Hoffman et al. 2004; Ransone et al. 2003). In a study investigating the timing of acute HMB-Ca ingestion, HMB-Ca was reported to attenuate the rise of lactate dehydrogenase, a serum indicator of muscle damage, to a greater degree when consumed before exercise compared to its ingestion following exercise or a placebo; however, the HMB-Ca supplementation did not alter maximal voluntary contraction or CK concentrations (Wilson et al. 2009). HMB-Ca has generally been shown to have a slow rate of appearance and takes approximately 60–120 min to reach peak plasma concentrations (Fuller et al. 2011). In contrast, when HMB is provided in its free acid form (HMB-FA), its absorption rate has been shown to be accelerated with greater peak plasma concentrations appearing approximately 30 min following ingestion (Fuller et al. 2011). The greater absorption rate and concentration are also suggested to result in a greater intramuscular HMB bioavailability and potentially be more effective at enhancing recovery from high-intensity exercise (Fuller et al. 2011; Wilkinson et al. 2013). Considering the greater absorption rate of HMB-FA, it may provide a greater benefit to exercise recovery by itself or in combination with more traditional therapies. Thus, the purpose of this study was to investigate the acute effects of HMB-FA supplementation, CWI, and the combination of the two modalities on post-exercise markers of muscle damage, inflammation, performance, and subjective measures of recovery.

Materials and methods

Participants

Forty recreationally resistance trained males with an average squat of 1.8 ± 0.3 times their body weight volunteered to participate in this randomized, double-blind, placebo-controlled study. None of the participants were competitive athletes, and all were recreationally lifting at study enrollment. Strict recruitment criteria were implemented to increase homogeneity of the sample, including between the ages of 18 and 35 years, a minimum of 1 year of resistance training experience, particularly with the squat, and the ability to squat a weight equivalent to their body weight. Following an explanation of all procedures, risks, and benefits, each participant gave his informed consent prior to participation in this study. The Institutional Review Board of the University approved the research protocol. Participants were not permitted to use any additional nutritional supplements or medications while enrolled in the study. Screening for nutritional supplements and performance enhancing drug use were accomplished via a health history questionnaire completed during participant recruitment. Participants were instructed not to partake in any personal recovery strategies while enrolled in the study including saunas, stretching routines, foam rollers, massages, additional hot/cold water therapy, etc.

Participants were randomly assigned to one of the four treatment groups as enrolled in the study: β-hydroxy-β-methylbutyrate free acid only (HMB-FA), placebo only (PL), HMB-FA and cold water immersion (HMB-FA-CWI), and PL and CWI (PL-CWI). Experimental group characteristics are presented in Table 1. Groups did not differ in age, body mass, height, body fat percentage, resistance training experience, or 1-RM strength.

Study protocol

Participants reported to the Human Performance Laboratory (HPL) on four separate occasions (T1–T4). During the first visit (T1), participants were tested for maximal strength (1-RM) on the barbell back squat, dead lift, and barbell split squat exercises. Prior to the second visit (T2), participants were instructed to refrain from all forms of exercise for a minimum of 72 h. Participants were also instructed to report to the HPL during T2–T4 in a 10-h fasted state. During T2, participants performed a lower body resistance exercise session which consisted of four sets of the squat, dead lift, and barbell split squat exercises.

Table 1 Experimental group characteristics

	PL	HMB-FA	PL-CWI	HMB-FA-CWI
Age (years)	23.8 ± 3.0	21.7 ± 2.0	22.5 ± 3.0	21.2 ± 1.7
Body mass (kg)	85.7 ± 5.4	81.1 ± 12.7	77.1 ± 7.7	87.6 ± 9.9
Height (m)	1.78 ± 0.06	1.77 ± 0.08	1.71 ± 0.07	1.71 ± 0.22
Body fat (%)	13.0 ± 3.4	13.1 ± 4.8	10.9 ± 4.1	11.6 ± 6.1
RTE (years)	7.6 ± 4.2	6.0 ± 2.5	5.7 ± 3.4	5.3 ± 2.0
1-RM Squat (kg)	148.0 ± 30.9	135.9 ± 34.3	148.7 ± 31.9	152.5 ± 17.4

Treatment groups: *PL* Placebo, *HMB-FA* β-hydroxy-β-methylbutyrate free acid only, *PL-CWI* cold water immersion only, *HMB-FA-CWI* β-hydroxy-β-methylbutyrate free acid and cold water immersion, *RM* repetition maximum, *RTE* resistance training experience

Data presented as mean ± SD

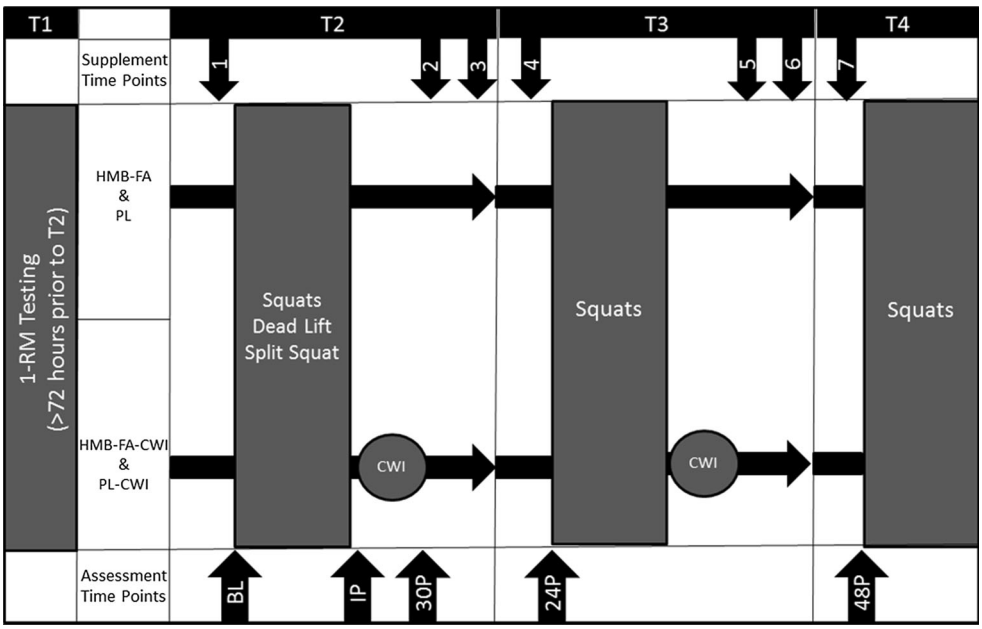

Fig. 1 Study protocol. Supplement time points: *1*, *4* and *7* 30 min prior to exercise; *2* and *5* 2-h post-exercise; *3* and *6* 6-h post-exercise. Assessment time points: *BL* baseline, *IP* immediately post-exercise, *30P* 30-min post-exercise, *24P* 24-h post-T2 exercise, *48P* 48-h post-T2 exercise

The rest interval between each set and between all exercises was 90 s. The squat exercise was performed at 80 % of the participant's previously measured 1-RM, while the dead lift and barbell split squat exercises were performed at 70 % of the participant's previously measured 1-RM. Participants were verbally encouraged to perform as many repetitions as possible, but not to exceed ten repetitions in any set. This protocol was utilized to simulate a typical high-intensity lower body training routine during a hypertrophy phase of training (Hoffman et al. 2010). Participants then reported back to the HPL 24 (T3) and 48 h (T4) post-exercise. During T3 and T4, participants only performed four sets of the squat exercise using the same loading pattern and rest interval length as T2. The squat exercise performed on T3 and T4 was incorporated to assess performance recovery, mimicking an athlete who requires daily high-intensity training. One serving of HMB-FA or PL was consumed 30 min prior to each exercise session (T2–T4). In addition, servings were also provided at 2- and 6-h following the exercise sessions at T2 and T3. Therefore, participants in the HMB-FA groups received a total of 3 g HMB-FA on T2 and T3, while receiving 1 g HMB-FA on T4 (prior to resistance training only). This dosing strategy has also been implemented in previous studies investigating the effects of HMB (Wilson et al. 2013a). Participants were also asked to maintain a dietary log during the study. Blood samples and subjective measures of soreness, pain, and recovery were obtained at five time points: baseline (BL), immediately post-exercise (IP), and 30-min post-exercise (30P) during T2, and 24—(prior to exercise on T3), and 48 h (prior to exercise on T4) post-T2 (24P and 48P, respectively). The study's protocol is depicted in Fig. 1.

HMB-FA supplementation

Each serving of HMB-FA and PL was provided in identical packets containing similarly flavored gel. The HMB-FA

supplement consisted of 1 g of β-hydroxy-β-methylbutyrate free acid, reverse osmosis water, debittering agent, orange flavor, stevia extract, and potassium carbonate. Each serving of PL consisted of an equivalent amount of litesse polydextrose, citric acid, corn syrup, 10 % stevia powder, debittering agent, and orange flavoring. The HMB-FA and PL were obtained from Metabolic Technologies Inc. (Ames, IA). All HMB-FA and PL ingestion took place in the HPL and were witnessed by a study investigator to ensure 100 % compliance. In addition, blood plasma HMB concentrations were analyzed by gas chromatography–mass spectrometry and performed by Metabolic Technologies Inc. using methods previously described (Nissen et al. 1990) to assess compliance, and HMB content in supplement packets was validated by high-pressure liquid chromatography.

Cold water immersion (CWI)

Participants in the HMB-FA-CWI and PL-CWI groups were required to fully immerse their lower body into a metal tub (58.4 × 121.9 cm) filled 30 cm high with ice water at 10–12 °C following exercise. Participants immersed in the water up to their umbilicus for 10-min. Participants who were not in the CWI groups were required to remain in the HPL for the 10-min following exercise to ensure a similar post-exercise nutritional intervention opportunity among participants.

Performance measures

Prior to each exercise session, participants performed a standardized warm-up consisting of 5 min on a cycle ergometer, ten body weight squats, ten body weight walking lunges, ten dynamic walking hamstring stretches, and ten dynamic walking quadriceps stretches. The 1-RM tests were performed using methods previously described (Hoffman 2006). Each participant performed two warm-up sets using a resistance that was approximately 40–60 and 60–80 % of the participant's perceived maximum, respectively. Then, 3–4 subsequent trials were performed to determine the 1-RM. A 3–5 min rest period was provided between each trial. Trials not meeting the range of motion criteria for each exercise were discarded. The squat exercise required the participant to place an Olympic bar across the trapezius muscle at a self-selected location. Each participant descended to the parallel position which was attained when the greater trochanter of the femur reached the same level as the knee. The participant then ascended until full knee extension. The dead lift exercise required the participant to grasp an Olympic bar slightly wider than shoulder width with the arms in a fully extended position. A closed, open, or alternating hand grip was allowed and kept consistent for each participant. From a flexed position, the participant extended his hips and knees until the body assumed an erect standing position. The barbell split squat 1-RM was assessed only with the dominant leg forward using a prediction formula based on the number of repetitions performed to fatigue using a given weight (Brzycki 1993). The barbell split squat required the participant to place an Olympic bar across the trapezius muscle at a self-selected location. The participant assumed an alternating leg stance with the dominant leg forward. For each repetition, the participant flexed the dominant knee until it was over the dominant foot. The trailing knee was lowered to the floor without making contact, while the torso remained erect. The participant pushed off with both legs to return back to the starting position.

Lower body power during the squat exercise protocol was measured for each repetition with a Tendo^{TM} Power Output Unit (Tendo Sports Machines, Trencin, Slovak Republic). The Tendo^{TM} unit consists of a transducer that was attached to the end of the barbell which measured linear displacement and time. Subsequently, bar velocity was calculated and power was determined. Both peak and mean power output were recorded for each repetition and used for subsequent analysis. Average power per repetition was calculated as the average of the mean power outputs for each repetition performed divided by the number of repetitions performed. Test–retest reliability for the Tendo^{TM} unit in our laboratory has consistently shown $R > 0.90$.

Subjective measures of soreness, pain, and recovery

Participants were instructed to assess their subjective feelings of leg soreness and leg pain using a 15-cm visual analog scale (VAS). The scale was anchored by the words "lowest" and "highest" to represent extreme ratings where the greater measured value represented the greater feeling. Questions were structured as "my level of leg soreness is" and "my level of leg pain is". The validity and reliability of VAS in assessing fatigue and energy have been previously established (Lee et al. 1991).

Participants were instructed to assess their perceived recovery status at 24P and 48P prior to exercise and HMB-FA or PL ingestion. The scale followed a 0–10-point rating scale: 0 = very poorly recovered/extremely tired; 2 = not well recovered/somewhat tired; 4 = somewhat recovered; 5 = adequately recovered; 6 = moderately recovered; 8 = well recovered/somewhat energetic; 10 = very well recovered/highly energetic.

Blood measurements

During T2, BL blood samples were obtained following a 15-min equilibration period. Additional blood samples were also drawn IP and 30P. All blood samples were obtained using a 20-gauge Teflon cannula placed in a superficial forearm vein using a three-way stopcock with a male luer

lock adapter. The cannula was maintained patent using an isotonic saline solution (Becton–Dickinson, Franklin Lakes, NJ). IP blood samples were taken within 1 min of exercise cessation. Following the resistance exercise protocol, participants remained in the supine position for the full 30-min recovery phase prior to the 30P blood sample being drawn, except for the participants in the CWI groups, who spent the first 10 min of the 30 min in the ice bath. All T2 blood samples were drawn with a plastic syringe while the participant was in a supine position. During T3 and T4, only pre-exercise blood samples were drawn (24P and 48P, respectively) following a 15-min equilibration period. These blood samples were obtained from an antecubital arm vein using a 20-gauge disposable needle equipped with a Vacutainer® tube holder (Becton–Dickinson, Franklin Lakes, NJ). Each participant's blood samples were obtained at the same time of day during each session.

All blood samples were collected into two Vacutainer® tubes, one containing no anti-clotting agent and the second containing K_2EDTA. A small aliquot of whole blood was removed from the second tube and used for determination of hematocrit and hemoglobin. The blood in the first tube was allowed to clot at room temperature for 30 min and subsequently centrifuged at 3,000×*g* for 15 min along with the remaining whole blood from the second tube. The resulting plasma and serum were placed into separate 1.8-mL microcentrifuge tubes and frozen at −80 °C for later analysis.

Biochemical analysis

Creatine kinase (CK) concentrations were analyzed with the use of a spectrophotometer and a commercially available enzymatic kit (Sekisui Diagnostics, Charlottetown, PE, Canada). Myoglobin concentrations were determined using enzyme-linked immunosorbent assays (ELISA) (Calbiotech, Spring Valley, CA). Determination of serum immunoreactivity values was determined using a BioTek Eon spectrophotometer (BioTek, Winooski, VT). To eliminate inter-assay variance, all samples for a particular assay were thawed once and analyzed in the same assay run by a single technician. All samples were run in duplicate with a mean intra-assay variance of 2.60 % for CK and 5.73 % for myoglobin.

Hemoglobin was analyzed in triplicate from whole blood using an automatic analyzer (Hemocue®, Cypress, CA). Hematocrit was analyzed in triplicate from whole blood via microcentrifugation (Statspin® Critspin, Westwood, MA) and microcapillary technique. Coefficient of variation for each assay was 3.73 % for hemoglobin and 0.65 % for hematocrit. Plasma volume shifts following the workout were calculated using a previously established formula (Dill and Costill 1974).

Circulating levels of C-reactive protein (CRP), Interluken-6 (IL-6), and Interluken-10 (IL-10) were assessed by Magpix (EMD Millipore, Billerica, MA). CRP was assayed by the human CVD panel three (EMD Millipore, Billerica, MA), while the remaining variables were assayed by the human cytokine/chemokine panel one (EMD Millipore, Billerica, MA, USA) according to manufacturer's guidelines. Samples were analyzed in duplicate, with an average coefficient of variation of 7.22 % for CRP, 11.47 % for IL-6, and 10.94 % for IL-10.

Dietary logs

Participants were instructed to maintain their normal dietary intake leading up to the experimental trial. Participants were then instructed to self-report dietary intake as accurately as possible during T2 and T3 by recording each food item and estimated amount consumed. Dietary data were analyzed to identify differences between the groups. Participants were instructed not to eat or drink (except water) within 10 h of reporting to the HPL for subsequent visits. FoodWorks® dietary analysis software (McGraw Hill, New York, NY) was used to analyze the dietary logs for total kilocalorie intake (kcal) and macronutrient distributions.

Statistical analysis

All data were analyzed using repeated measures analysis of covariance (ANCOVA) with IP or T2 as the covariate. One way analysis of variance (ANOVA) was employed for significant interactions using delta values followed by LSD post hoc for pairwise comparisons. To assess changes from PRE to 48P, a repeated measure ANOVA was used to detect changes within each individual experimental group across time points. Prior to analysis, all data were assessed to ensure normal distribution, homogeneity of variance and sphericity. Changes in dietary composition were analyzed using one-way ANOVA. Results were considered significant at an alpha level of $p \leq 0.05$. All data are reported as mean ± SD. Using the equation: effect size (ES) = (control mean − experimental mean)/[the pooled standard deviation of peak serum CK levels (μL)], the study by Wilson et al. (2013b) had an ES of 1.55 = [(604 − 322)/182.5 μL]. Power was set at 80 with an α level of 0.05 using an ES of 1.55. We estimated a minimum of 28 subjects or 7 in each group were needed for the study.

Results

Performance measures

Squat repetitions

There were no significant differences between the groups for changes in squat repetitions performed on T2–T4 (Fig. 2). All groups significantly decreased squat repetitions on T3 as compared to T2 ($p = 0.002$–0.038).

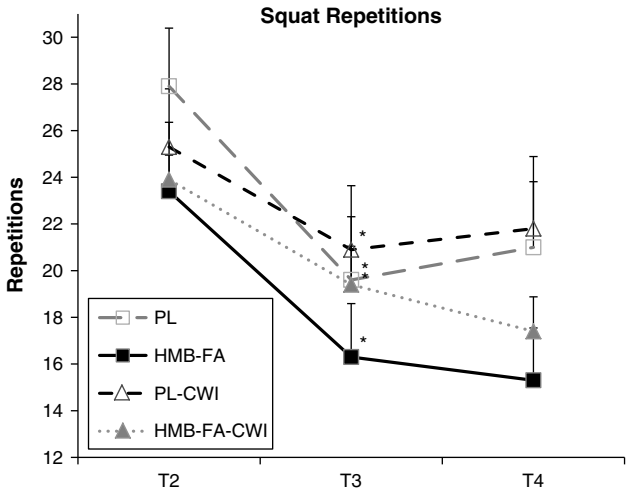

Fig. 2 Squat repetitions performed at 80 % 1-repetition maximum over four sets with 90-s rest intervals. Treatment groups: *PL* Placebo; *HMB-FA* β-hydroxy-β-methylbutyrate free acid only; *PL-CWI* cold water immersion only; *HMB-FA-CWI* β-hydroxy-β-methylbutyrate free acid and cold water immersion. Time points: *T2* lower body resistance exercise session; *T3* 24 h following T2; *T4* 48 h following T2. *Mean value was significantly different from previous time point

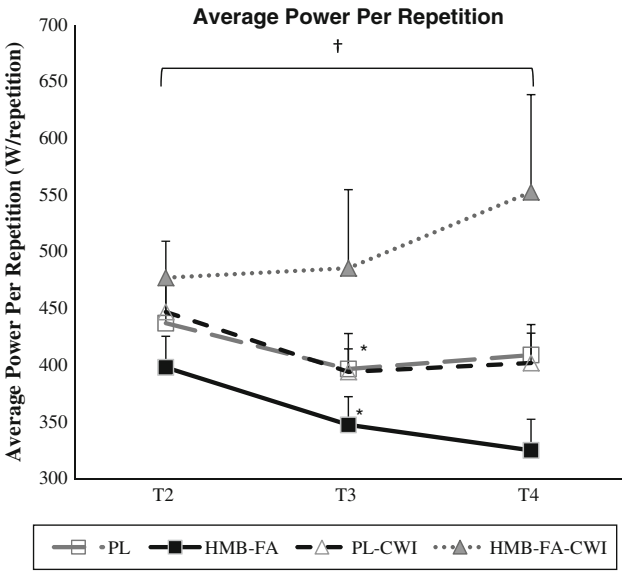

Fig. 3 Average power per repetition performed at 80 % 1-repetition maximum over four sets with 90-s rest intervals. Treatment groups: *PL* Placebo; *HMB-FA* β-hydroxy-β-methylbutyrate free acid only; *PL-CWI* cold water immersion only; *HMB-FA-CWI* β-hydroxy-β-methylbutyrate free acid and cold water immersion. Time points: *T2* lower body resistance exercise session; *T3* 24 h following T2; *T4* 48 h following T2. *Mean value was significantly different from previous time point. †Mean difference between T2 and T4 was significantly greater for HMB-FA-CWI than all other treatment groups ($p = 0.02$)

Average power per repetition

The change in average power per repetition from T2 to T4 was significantly greater ($p = 0.02$) for HMB-FA-CWI (96.8 ± 190.9 W/repetition) than PL (−46.7 ± 91.3 W/repetition), HMB-FA (−73.1 ± 80.2 W/repetition), and PL-CWI (−49.1 ± 57.9 W/repetition).

Average power per repetition between T2 and T3 was significantly reduced ($p = 0.03$) with HMB-FA and PL-CWI (Fig. 3).

Subjective measures of soreness, pain, and recovery

Leg soreness

There were no significant differences between the groups for changes in feelings of leg soreness across time points. All groups significantly increased feelings of leg soreness between BL and IP ($p = 0.0001$). Feelings of leg soreness between IP and 30P significantly decreased ($p = 0.005$–0.011) with PL and PL-CWI. Feelings of leg soreness between 30P and 24P significantly increased ($p = 0.001$–0.02) with PL and HMB-FA. Feelings of leg soreness between 24P and 48P significantly increased ($p = 0.003$–0.029) with HMB-FA and PL-CWI.

Leg pain

There were no significant differences between the groups for changes in feelings of leg pain across time points. All groups significantly increased feelings of leg pain between BL and IP ($p = 0.0001$–0.002). All groups significantly decreased feelings of leg pain between IP and 30P ($p = 0.001$–0.022). Feelings of leg pain between 24P and 48P significantly increased ($p = 0.008$–0.036) with HMB-FA, PL-CWI, and HMB-FA-CWI.

Recovery

There were no significant differences between the groups for changes in perceived recovery status between 24P and 48P. Perceived recovery status between 24P and 48P significantly decreased ($p = 0.012$–0.02) with HMB-FA and HMB-FA-CWI.

Biochemical analysis

Plasma HMB concentration

Plasma HMB concentrations were significantly greater than placebo for HMB-FA and HMB-FA-CWI at all time points ($p \leq 0.01$). HMB-FA-CWI significantly elevated plasma HMB concentrations from baseline at IP and 30P. HMB-FA significantly elevated plasma HMB concentration from baseline at IP, 30P, and 48P (Fig. 4).

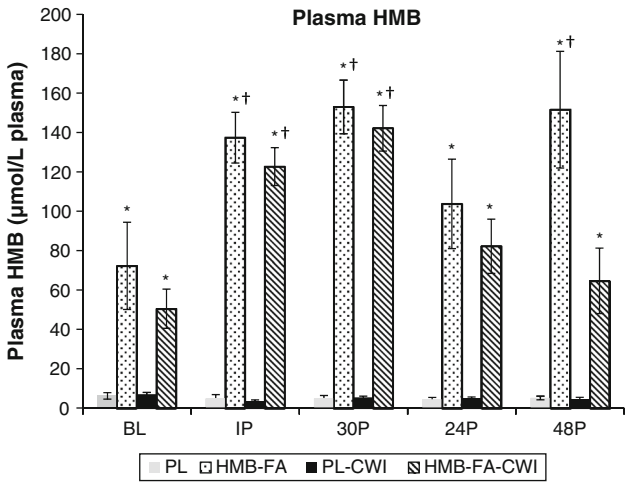

Fig. 4 Plasma β-hydroxy-β-methylbutyrate concentration. Treatment groups: *PL* Placebo; *HMB-FA* β-hydroxy-β-methylbutyrate free acid only; *PL-CWI* cold water immersion only; *HMB-FA-CWI* β-hydroxy-β-methylbutyrate free acid and cold water immersion. Time points: *BL* Baseline; *IP* immediately post-exercise; *30P* 30-min post-exercise; *24P* 24-h post-exercise; *48P* 48-h post-exercise. *Mean value was significantly greater than PL. †Mean value was significantly greater than BL

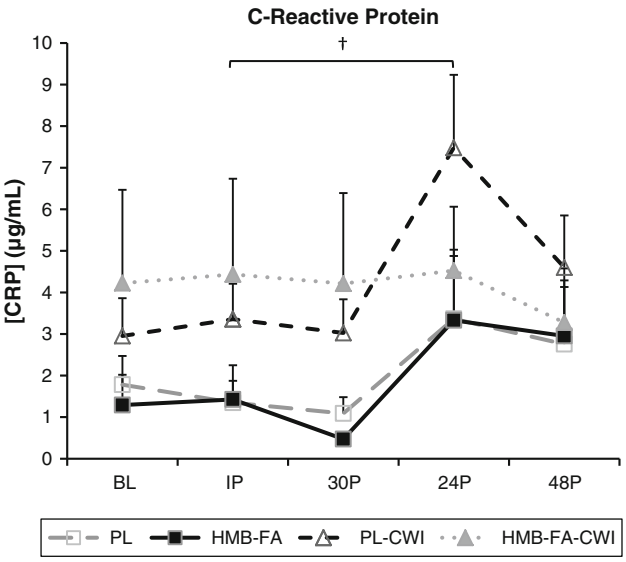

Fig. 5 Acute effects of β-hydroxy-β-methylbutyrate free acid and/or cold water immersion on C-reactive protein. Treatment groups: *PL* Placebo; *HMB-FA* β-hydroxy-β-methylbutyrate free acid only; *PL-CWI* cold water immersion only; *HMB-FA-CWI* β-hydroxy-β-methylbutyrate free acid and cold water immersion. Time points: *BL* baseline; *IP* immediately post-exercise; *30P* 30-min post-exercise; *24P* 24-h post-exercise; *48P* 48-h post-exercise. †Mean increase was significantly greater for PL-CWI than HMB-FA-CWI ($p = 0.02$) and HMB-FA ($p = 0.046$)

Creatine kinase

There were no significant differences between the groups for changes in CK concentrations across time points. All groups significantly increased CK concentrations between BL and IP (50.2 % increase) ($p = 0.0001$–0.005) and between IP and 24P (163.4 % increase) ($p = 0.002$–0.009). No significant differences were observed between 24P and 48P for any group.

C-reactive protein

The change in CRP concentration from IP to 24P was significantly greater ($p = 0.02$ and $p = 0.046$, respectively) in PL-CWI (4.1 ± 5.2 ng mL^{-1}) than HMB-FA-CWI (0.09 ± 3.1 ng mL^{-1}) and HMB-FA (0.38 ± 1.5 ng mL^{-1}) (Fig. 5).

Myoglobin

The change in myoglobin concentrations from IP to 30P was significantly greater ($p = 0.005$ and $p = 0.009$, respectively) in PL-CWI (75.1 ± 52.4 ng mL^{-1}) compared to PL (25.5 ± 18.6 ng mL^{-1}) and HMB-FA (28.5 ± 43.0 ng mL^{-1}). Additionally, the change in myoglobin concentrations from IP to 30P was significantly greater ($p = 0.028$ and $p = 0.047$, respectively) in HMB-FA-CWI (63.2 ± 22.7 ng mL^{-1}) compared to PL and HMB-FA.

PL, HMB-FA, and HMB-FA-CWI significantly increased myoglobin concentration between BL and IP ($p = 0.0001$–0.023). PL, PL-CWI, and HMB-FA-CWI significantly increased myoglobin concentration between IP and 30P ($p = 0.0001$–0.002) (Fig. 6).

Interleukin-6

There were no significant differences between the groups for changes in IL-6 concentrations across time points. Only PL-CWI significantly increased IL-6 concentration between BL and IP ($p = 0.033$).

Interleukin-10

There were no significant differences between the groups for changes in IL-10 concentrations across time points. Between BL and IP, PL-CWI significantly increased ($p = 0.044$) IL-10 concentrations. Between IP and 30P, HMB-FA-CWI significantly increased ($p = 0.012$) IL-10 concentrations. Between 30P and 24P, PL-CWI and HMB-FA-CWI significantly decreased ($p = 0.005$ and $p = 0.026$, respectively) IL-10 concentrations.

Dietary logs

Analysis of dietary intake revealed no significant differences between the groups for total kilocalorie intake, macronutrient distributions, or protein intake relative to body weight

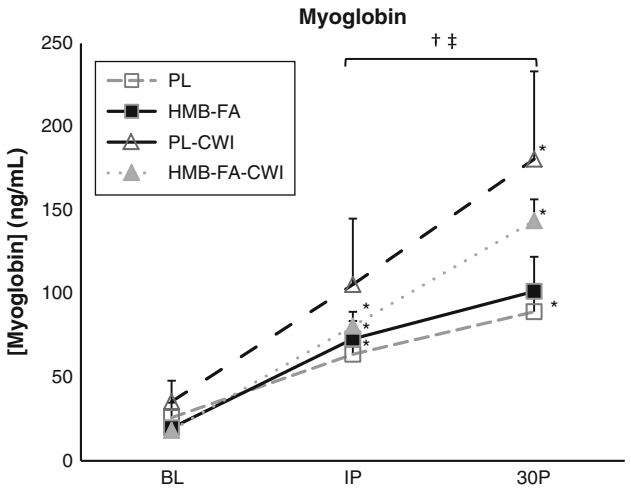

Fig. 6 Acute effects of β-hydroxy-β-methylbutyrate free acid and/or cold water immersion on myoglobin. Treatment groups: *PL* Placebo; *HMB-FA* β-hydroxy-β-methylbutyrate free acid only; *PL-CWI* cold water immersion only; *HMB-FA-CWI* β-hydroxy-β-methylbutyrate free acid and cold water immersion. Time points: *BL* baseline; *IP* immediately post-exercise; *30P* 30-min post-exercise. *Mean value was significantly different from previous time point. †Mean increase was significantly greater for PL-CWI than PL ($p = 0.005$) and HMB-FA ($p = 0.009$). ‡Mean increase was significantly greater for HMB-FA-CWI than PL ($p = 0.028$) and HMB-FA ($p = 0.047$)

over the course of the study protocol. The average caloric intake per day on T2 and T3 was PL: 2,384.7 ± 901.9 kcal; HMB-FA: 2,410.9 ± 687.0 kcal; PL-CWI: 2,998.3 ± 858.6 kcal; and HMB-FA-CWI: 2,822.2 ± 974.9 kcal. The average protein intake relative to body weight on T2 and T3 was PL: 1.6 ± 0.8 g kg^{-1} day^{-1}; HMB-FA: 1.4 ± 0.6 g kg^{-1} day^{-1}; PL-CWI: 2.1 ± 1.0 g kg^{-1} day; and HMB-FA-CWI: 2.0 ± 1.0 g kg^{-1} day^{-1}.

Plasma volume shifts

Relative to BL, plasma volume decreased at IP, −14.4 ± 4.9 %; decreased at 30P, −1.3 ± 4.7 %; increased at 24P, 1.3 ± 5.5 %; and increased at 48P, 1.5 ± 6.6 %. However, the difference between the groups was not significant. Blood variables were not corrected for plasma volume shifts due to the importance of molar exposure at the tissue receptor level.

Discussion

The aim of the present study was to investigate the acute effects of HMB-FA, CWI, and the combination of treatments on post-exercise markers of muscle damage, inflammation, and performance in recreationally resistance trained men. Although the recovery modalities did not have a profound effect on muscle recovery, the main findings suggest that CWI treatment may elevate an acute index of muscle damage (myoglobin) when compared to other treatment groups, while HMB-FA treatment may have attenuated the increase in CRP when compared to CWI treatment. However, it appears that HMB-FA or CWI treatments by themselves did not provide any benefit over PL on all measures of muscle recovery. Rather, the combination of CWI and HMB-FA appears to improve recovery of average power per repetition when compared to all other treatment groups.

The physiological response to acute muscle damage is inflammation, which involves the production of cytokines (Calle and Fernandez 2010). IL-6 acts as both a pro-inflammatory and anti-inflammatory cytokine in response to resistance training (Calle and Fernandez 2010) and may play a role in the remodeling process of damaged muscle tissue (Pedersen et al. 2001). In the current study, no treatment effect was observed in IL-6 concentration in response to the resistance training protocol. Additionally, no time effect for IL-6 was observed for any group post-exercise. Acute bouts of resistance training have previously shown to elicit elevations in IL-6 concentrations, particularly after eccentric resistance exercise (Phillips et al. 2010; MacIntyre et al. 2001; Croisier et al. 1999). However, similar to our findings, a number of studies have reported no changes in post-exercise IL-6 concentration, albeit in untrained individuals (Uchida et al. 2009; Buford et al. 2009). Furthermore, the peak concentration time for IL-6 has differed among studies ranging from 3 (Peake et al. 2006) to 24 h (Smith et al. 2000; MacIntyre et al. 2001) post-exercise. Given the time points for blood collection in this study, it is possible that an unmeasured peak in IL-6 may have occurred between 30P and 24P. In addition, the exercise protocol may not have elicited enough damage in all of the subjects as indicated in the large standard deviation in the cytokine response.

IL-10 is a cytokine that mediates the anti-inflammatory effects of exercise; therefore, acute elevations appear to be beneficial for recovery (Petersen and Pedersen 2005). In the present study, IL-10 was seen to be significantly elevated between IP and 30P for the HMB-FA-CWI group and significantly decreased between 30P and 24P for the PL-CWI and HMB-FA-CWI groups; however, there were no significant differences between treatment groups. Few studies have reported increases in IL-10 concentration following resistance training (Hirose et al. 2004; Smith et al. 2000), while conflicting results on the ability of cold exposure to increase IL-10 concentrations exist (Banfi et al. 2010; Tseng et al. 2012). Similar to our findings, topical cooling following eccentric exercise has been reported to not alter IL-10 concentrations post-exercise (Tseng et al. 2012). Although speculative, the significant elevation of IL-10 observed at 30 min post-exercise for the HMB-FA-

CWI group may have contributed to maintaining average power output over the course of the study by suppressing the action of macrophages on the exercised muscle (Calle and Fernandez 2010).

Effects of CWI

CWI provided no advantage over the PL group on performance recovery for the barbell squat exercise. The benefit of post-exercise CWI remains unclear as a number of studies have reported equivocal results regarding exercise recovery (Versey et al. 2013). There have been only a limited number of studies that have investigated the effects of CWI following dynamic resistance exercise. A recent meta-analysis has indicated that although CWI may not be effective for improving recovery of muscle strength, it does appear to enhance recovery of muscle power (Leeder et al. 2012). This is consistent with our findings in that CWI did not improve the number of repetitions performed during T3 and T4, but we were not able to support the effect of CWI on recovery of muscle power. Additionally, Leeder et al. (2012) suggested that CWI was not effective for alleviating the subjective feeling of DOMS following eccentric-based exercise, which is in agreement with our data showing CWI treatment did not attenuate subjective feelings of leg soreness, pain, or recovery in comparison to the PL group.

While CWI did not attenuate elevations in CK, it appeared that CWI alone resulted in greater elevations in myoglobin 30 min post-exercise (30P). CWI has been reported to reduce peripheral blood perfusion, altering inflammation response within the muscle, and possibly delay the recovery process (Eston and Peters 1999). In support of our findings, a recent study by Tseng et al. (2012) demonstrated that topical cooling can actually delay recovery from exercise-induced muscle damage yielding greater elevations in CK and myoglobin during 48–72 h post-exercise. In contrast, Leeder et al. (2012) suggested small, yet significant, benefits of CWI attenuating CK levels in the blood 24–72 h following high intensity and eccentric-based exercise. Although a significant difference between treatment groups for CK response was not seen in the present study, the significant elevation of myoglobin at 30 min post-exercise in CWI may have been due to the reduced blood perfusion in the lower extremities, limiting the clearance rate of myoglobin from the exercised muscle. However, this does not explain why we failed to see significantly greater elevations of myoglobin 30 min post-exercise in the HMB-FA-CWI treatment group.

CWI also appeared to result in the highest elevation in CRP at 24P. CRP has been shown to induce anti-inflammatory cytokines in circulating monocytes and suppress the synthesis of pro-inflammatory cytokines in tissue macrophages (Pue et al. 1996). Repeated bouts of anaerobic exercise have previously shown to increase CRP at 24-h post-exercise by ~1,100 % (Meyer et al. 2001) and ~480 % (Ingram et al. 2009) from resting concentrations. In the current study, PL increased CRP by 89 % at 24P, while PL-CWI increased by 150 %. Interestingly, the concentration of CRP at 24P for PL-CWI was even greater than those previously reported. This is in contrast to Ingram et al. (2009) which showed that CWI following repeated bouts of anaerobic exercise did not significantly alter CRP at 24-h post-exercise. Since CRP has both pro-inflammatory and anti-inflammatory properties necessary for the process of muscle repair (Black et al. 2004), acute elevations of CRP are likely beneficial for recovery, although chronically elevated CRP has been associated with cardiovascular disease (Kones 2009). CWI appears to result in a significantly greater elevation in CRP at 24P compared to the other treatment groups; however, more research is needed to determine if significant elevations are beneficial for acute recovery.

Effects of HMB-FA

In the present study, administration of HMB-FA (3 g day^{-1}) did not improve subsequent performance or provide an attenuation of post-exercise markers of muscle damage and inflammation. Previous research utilizing a similar resistance training protocol showed improvements in squat performance recovery at T3 and T4 and a blunted rise in CK; however, that study used competitive strength/power athletes and participants were administered a substantially greater amount of amino acids pre-and post-exercise (84 g protein day^{-1}) (Hoffman et al. 2010). Similarly, Wilson et al. (2013b) examined recovery following a full-body resistance training bout in highly trained men and reported that 3 g day^{-1} HMB-FA, administered in a similar fashion as the current study, attenuated the rise in CK and perceived recovery status 48-h post-exercise, with no effect on CRP levels. The acute dose of HMB-FA in the present study may not have provided a sufficient stimulus to improve recovery or attenuate muscle damage in recreationally trained participants. Alternatively, other investigations have shown attenuations of the rise in post-exercise CK in untrained populations when a combination of 3 g day^{-1} HMB-Ca and 0.3 g day^{-1} α-ketoisocaproate was administered for 2 weeks prior to a single bout of eccentric resistance exercise (Van Someren et al. 2005), as well as when a 3 g serving of HMB-Ca was administered 1 h prior to exercise (Wilson et al. 2009). Therefore, future studies should examine the effect of a loading phase and/or ingestion of 3 g day^{-1} HMB-FA as a bolus prior to exercise in resistance trained men.

Combination of treatments

To the best of our knowledge, this is the first study to examine the combined effects of CWI and HMB-FA on recovery from a damaging bout of exercise. The combination of HMB-FA (3 g day^{-1}) and CWI allowed for a greater recovery of average power per repetition compared to the other treatment groups. Recovery of muscle power has been observed with CWI (Leeder et al. 2012), while research is lacking on acute recovery of muscle power using HMB. In the current study, only the combination group (HMB-FA-CWI) maintained average power production. Interestingly, HMB-FA also seemed to attenuate the increase in CRP when combined with CWI, while CWI alone resulted in significantly greater elevations in CRP at 24P. It appears that further research is needed to determine whether significant elevations of CRP are beneficial for acute recovery. Nevertheless, the combination of treatments did improve recovery of average power per repetition for the barbell squat exercise along with the attenuation of CRP.

Conclusion

Acute treatment of HMB-FA (3 g day^{-1}) or CWI does not appear to attenuate post-exercise markers of muscle damage, inflammation, or performance following acute lower body resistance exercise in the young men in the current study. However, the combination of treatments appears to enhanced recovery of average power per repetition and attenuate CRP response of CWI. The present study has limitations concerning participants, supplemental dosing, and blood collection time points; therefore, future research should examine the effects of 3 g HMB-FA ingestion as a bolus prior to resistance training and the longitudinal effects of HMB-FA in conjunction with resistance exercise.

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Conflict of interest The authors declare that they have no conflict of interests.

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