Effects of $\beta$-hydroxy-$\beta$-methylbutyrate free acid and cold water immersion on expression of CR3 and MIP-1$\beta$ following resistance exercise


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Gonzalez AM, Fragala MS, Jajtner AR, Townsend JR, Wells AJ, Beyer KS, Boone CH, Pruna GJ, Mangine GT, Bohner JD, Fukuda DH, Stout JR, Hoffman JR. Effects of $\beta$-hydroxy-$\beta$-methylbutyrate free acid and cold water immersion on expression of CR3 and MIP-1$\beta$ following resistance exercise. Am J Physiol Regul Integr Comp Physiol 306: R483–R489, 2014. First published February 5, 2014; doi:10.1152/ajpregu.00542.2013.—The inflammatory response to muscle-damaging exercise requires monocyte mobilization and adhesion. Complement receptor type 3 (CR3) and macrophage inflammatory protein (MIP)-1$\beta$ enables monocyte recruitment, adhesion, and subsequent infiltration into damaged muscle tissue. The purpose of this study was to examine the effects of cold water immersion (CWI) and/or $\beta$-hydroxy-$\beta$-methylbutyrate free acid (HMB-FA) on CR3 expression and MIP-1$\beta$ concentration after four sets of up to 10 repetitions of squat, dead lift, and split squat exercises at 70–80% 1-repetition maximum. Thirty-nine resistance-trained men (22.2 ± 2.5 yr) were randomly divided into four groups: 1) placebo (PL), 2) HMB-FA, 3) HMB-FA-CWI, and 4) PL-CWI. The HMB-FA groups ingested 3 g/day, and CWI groups were submersed into 10–12°C water for 10 min after exercise. Blood was sampled at baseline (PRE), immediately post- (IP), 30 min post- (30P), 24 h post- (24P), and 48 h post- (48P)-exercise. Circulating MIP-1$\beta$ was assayed and CR3 expression on CD14+$\beta$-methylene was measured by flow cytometry. Without treatment, CR3 expression significantly elevated at 30P compared with other time points ($P = 0.030–0.047$); HMB-FA significantly elevated the percentage of monocytes expressing CR3 between IP and 24P ($P = 0.046$) and between IP and 48P ($P = 0.046$). No time effect was observed for MIP-1$\beta$ concentration. The recovery modalities showed to attenuate the rise in CR3 following exercise. Additionally, supplementation with HMB-FA significantly elevated the percentage of monocytes expressing CR3 during recovery. Although the time course that inflammatory responses are most beneficial remains to be determined, recovery modalities may alter immune cell mobilization and adhesion mechanisms during tissue recovery.

THE MECHANICAL STRESS of resistance exercise causes significant skeletal muscle damage, which initiates a subsequent local inflammatory response (24, 25). This response results in a mobilization and infiltration of leukocytes to the damaged tissue (5, 33). Macrophage inflammatory protein (MIP)-1$\beta$ and complement receptor type 3 (CR3) are part of the acute immune response that enables monocyte recruitment, adhesion, and subsequent infiltration into the damaged muscle tissue. CR3 is a $\beta_2$-integrin that mediates leukocyte adhesion, migration, and phagocytosis in response to inflammatory stimuli (3, 29). CR3 contains a combination of pro-inflammatory cell surface receptors (CD11b and CD18) that facilitate the binding of monocytes to cell adhesion molecules on endothelial cells, allowing monocyte infiltration of the damaged muscle tissue (11, 40). Macrophage inflammatory protein (MIP)-1$\beta$ is a critical pro-inflammatory chemokine for inducing inflammation and regulating tissue homeostasis (17, 31). MIP-1$\beta$ exerts its effects by binding to cell surface receptors (CCR5) initiating a cascade of intracellular events that lead to numerous cell functions including chemotaxis, degranulation, phagocytosis, and mediator synthesis (17).

$\beta$-Hydroxy-$\beta$-methylbutyrate (HMB), a metabolite of the amino acid leucine, has shown to improve muscular recovery and reduce indices of muscle damage when supplemented in conjunction with resistance exercise (22, 45–47). Recently, HMB in free acid form (HMB-FA) has been shown to attenuate circulating tumor necrosis factor-α (TNF-α), a pro-inflammatory cytokine responsible for signaling the migration of neutrophils and macrophages to the site of muscle damage, following muscle-damaging resistance exercise (42). Additionally, HMB appears to stimulate muscle protein synthesis through an upregulation of the mammalian target of rapamycin (mTOR) (4, 20) and attenuate muscle protein degradation by inhibiting the ubiquitin-proteasome pathway (37, 38). However, the mechanisms underlying the inflammatory contributions to tissue remodeling in vivo are yet to be determined. Studies investigating the role of HMB on monocyte recruitment via CR3 and MIP-1$\beta$ may better elucidate its role in altering the inflammatory cascade to muscle damage.

Cold water immersion (CWI) is a common modality used to enhance recovery from high-intensity exercise. A recent meta-analysis investigating the efficacy of CWI showed benefits in alleviating symptoms of delayed-onset of muscle soreness (DOMS), reducing postexercise elevations in creatine kinase (CK), and improving muscle power recovery (15). Some evidence also suggests that CWI may suppress cellular components of the immune response such as decreasing lymphocyte proliferation and downregulating the immune cascade (35). Additionally, CWI has been shown to alter inflammation by blunting the rise in total number of leukocytes and CK following exhaustive intermittent exercise (30). However, to date, the effects of CWI on immune recruitment and adhesion are not fully understood.

The effects of CWI and HMB-FA on the modulation of CR3 and the pro-inflammatory chemokine MIP-1$\beta$ have not previously been investigated. However, it conceivable that CWI and HMB-FA may alter immune cell mobilization and when combined may be incorporated into a feasible strategy to enhance recovery. Thus the purpose of the present study was to inves-
tigate the effects of acute HMB-FA supplementation alone and in conjunction with CWI on circulating MIP-1β and monocyte expression of CR3 following muscle-damaging resistance exercise. Specifically, we sought to examine the effect of CWI and HMB-FA on CD14+ monocyte CR3 expression and circulating MIP-1β concentration following an acute bout of high-intensity resistance exercise. Examination of these markers may provide further insight into the immune cell mobilization and adhesion mechanisms of these recovery modalities, as well as provide a mechanism to facilitate tissue recovery from intense exercise and other injuries or conditions.

MATERIALS AND METHODS

Participants. Thirty-nine resistance-trained men (22.2 ± 2.5 yr; 82.8 ± 10.0 kg; 1.7 ± 0.1 m; 11.9 ± 4.5% body fat) with an average squat of 1.8 ± 0.3 times their body weight, and 6.0 ± 3.0 yr of resistance training experience 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 yr, a minimum of 1 yr of resistance training experience, particularly with the squat, and the ability to squat a weight equivalent to their body weight. After an explanation of all procedures, risks, and benefits, each participant gave his informed consent before 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 was accomplished via a health history questionnaire completed during participant recruitment. Participants were instructed not topartake in any additional 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 four treatment groups as enrolled in the study: β-hydroxy-β-methylbutyrate free acid and cold-water immersion (HMB-FA-CWI), and PL and CWI (PL-CWI). Experimental group characteristics are displayed in Table 1. Groups did not differ in age, body mass, height, body fat percentage, resistance training experience, or 1-repetition maximum (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) and 35 yr, a minimum of 1 yr of resistance training experience, or 1-repetition maximum (1-RM) strength. Randomization of treatment groups was performed by a study investigator to ensure 100% compliance. In addition, participants also were administered the supplement (or placebo) at 2 and 6 h after exercise. 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 (before resistance training only). 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 (PRE), immediately postexercise (IP), and 30 min postexercise (30P) during T2, and 24- and 48 h postexercise (24P and 48P, respectively). The study 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 was obtained from Metabolic Technologies (Ames, IA). All HMB-FA and PL ingestion took place in the HPL and was 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 using methods previously described to assess compliance and validate HMB in supplement packets (19).

Cold-water immersion. After exercise, participants in the HMB-FA-CWI and PL-CWI groups were required to fully immerse their lower body into a metal tub (58.4 cm × 121.9 cm) filled 30 cm high with ice water at 10–12°C. Participants immersed in the water up to their umbilicus for 10 min. Reductions in femoral artery blood flow and muscle temperature have previously been reported after 10 min of CWI in both 8°C and 22°C water (7). Participants who were not in the CWI groups were required to remain in the HPL for the 10 min after exercise.

Table 1. Experimental group characteristics

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>HMB-FA</th>
<th>PL-CWI</th>
<th>HMB-FA-CWI</th>
</tr>
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<tr>
<td>n</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age, yr</td>
<td>23.8 ± 3.0</td>
<td>21.2 ± 1.3</td>
<td>22.5 ± 3.0</td>
<td>21.2 ± 1.7</td>
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<tr>
<td>Body mass, kg</td>
<td>85.7 ± 5.4</td>
<td>80.4 ± 13.3</td>
<td>77.1 ± 7.7</td>
<td>87.6 ± 9.9</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.78 ± 0.06</td>
<td>1.77 ± 0.08</td>
<td>1.71 ± 0.07</td>
<td>1.71 ± 0.22</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>13.0 ± 3.4</td>
<td>12.3 ± 4.2</td>
<td>10.9 ± 4.1</td>
<td>11.6 ± 6.1</td>
</tr>
<tr>
<td>Resistance training experience, yr</td>
<td>7.6 ± 4.2</td>
<td>5.3 ± 1.3</td>
<td>5.7 ± 3.4</td>
<td>5.3 ± 2.0</td>
</tr>
<tr>
<td>1-RM squat, kg</td>
<td>148.0 ± 30.9</td>
<td>136.1 ± 36.4</td>
<td>148.7 ± 31.9</td>
<td>152.5 ± 17.4</td>
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<tr>
<td>1-RM squat/body mass</td>
<td>1.72 ± 0.34</td>
<td>1.68 ± 0.29</td>
<td>1.94 ± 0.42</td>
<td>1.75 ± 0.20</td>
</tr>
<tr>
<td>1-RM deadlift, kg</td>
<td>151.4 ± 26.7</td>
<td>146.0 ± 39.0</td>
<td>157.3 ± 20.3</td>
<td>166.1 ± 20.0</td>
</tr>
<tr>
<td>1-RM split squat, kg</td>
<td>101.4 ± 22.7</td>
<td>97.0 ± 16.8</td>
<td>108.2 ± 22.2</td>
<td>114.8 ± 16.9</td>
</tr>
</tbody>
</table>

Data are means ± SD; n = number of subjects. 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.
exercise to ensure a similar postexercise nutritional intervention opportunity among participants.

**Performance measures.** Before each exercise session, participants performed a standardized warm-up consisting of 5 min on a cycle ergometer, 10 body weight squats, 10 body weight walking lunges, 10 dynamic walking hamstring stretches, and 10 dynamic walking quadriceps stretches. The 1-RM tests were performed using methods previously described by Hoffman (8). Each participant performed two warm-up sets using a resistance that was ∼40–60% and 60–80% of his perceived maximum, respectively. After this, 3–4 subsequent trials were performed to determine the 1-RM. A 3- to 5-min rest period was provided between each trial. A certified strength and conditioning specialist observed each repetition to ensure proper range of motion, and verbal signals were given when the subject reached the appropriate final descent position. 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 position. Each participant descended to the parallel position that 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 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. For each repetition, the participant flexed his perceived maximum, respectively. After this, 3–4 subsequent trials were performed to determine the 1-RM. A 3- to 5-min rest period was provided between each trial. A certified strength and conditioning specialist observed each repetition to ensure proper range of motion, and verbal signals were given when the subject reached the appropriate final descent position. 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 that 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. To minimize the risk for injury, 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 (2). 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.

**Blood measurements.** During T2, PRE blood samples were obtained after 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. To standardize each participant following the resistance exercise protocol, participants remained in the supine position for the full 30-min recovery phase before 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 preexercise blood samples were drawn (24P and 48P, respectively) following a 1-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$_2$EDTA. 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 was placed into separate 1.8-ml microcentrifuge tubes and frozen at −80°C for later analysis.

**Cell staining.** Blood samples were obtained from fresh, anticoagulated (K$_2$EDTA), whole blood, and analyzed in duplicate. Erythrocytes were first lysed from 350 μl of whole blood with BD Pharm Lyse solution (BD Biosciences, Franklin Lakes, NJ) within 30 min of collection. Samples were then washed three times in staining buffer containing 1× phosphate-buffered saline containing fetal bovine serum (BD Pharmingen; BD Biosciences) by centrifugation and aspiration. Leukocytes were then resuspended in 100 μl BD Pharmingen. Direct staining methods were used to label CR3 and CD14. Allophycocyanin (APC)-conjugated anti-CD11b (D12; BD Pharmingen), and PerCP Cy5.5-conjugated anti-CD14 (M5E2; BD Pharmingen) were used in the receptor labeling process. Surface staining was performed by adding 20 μl of directly conjugated APC-anti-CD11b and 5 μl of directly conjugated PerCP Cy5.5-anti-CD14 to the cell suspension and incubating in the dark for 30 min at 20°C. Cells were resuspended in 1.0 ml of stain buffer for flow cytometry analysis.

**Flow cytometry.** Flow cytometry analysis of stained cells was run on a BD C6 Accuri Flow Cytometer (BD Biosciences, San Jose, CA) equipped with BD Accuri analysis software (BD Biosciences). Forward and side scatter along with four fluorescent channels of data
were collected using two lasers providing excitation at 488 and 640 nm. Monocytes were determined by initial gating based on forward and side scatter, followed by gating for CD14+ cells as also described by Tallone and colleagues (39). A minimum of 10,000 events, defined as CD14+ monocytes, were obtained with each sample (Fig. 2).

Analysis of monocyte subpopulations was completed by quadrant analyses, in which CD14 was compared with CR3. Mean fluorescence of CR3 on CD14+ cells was recorded, representing the expression of CR3 per cell (6). Compensation for fluorescence spillover was set based on manufacturer recommendations (BD Biosciences).

Biochemical analysis. Circulating levels macrophage inhibiting protein (MIP-1β) was assessed by Magpix (EMD Millipore, Billerica, MA) via the human cytokine/chemokine panel one (EMD Millipore). Samples were analyzed according to manufacturer’s guidelines with an average coefficient of variation 6.25%.

CK was analyzed with the use of a spectrophotometer and a commercially available enzymatic kit (Sekisui Diagnostics, Charlotte-town, PE, Canada) per manufacturer’s instructions. Determination of serum immunoreactivity values was determined using a BioTek Eon spectrophotometer (BioTek, Winooski, VT). To eliminate interassay 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 2.99%.

Dietary logs. Participants were instructed to maintain their normal dietary intake leading up to the experimental trial. Participants were then instructed to record dietary intake as accurately as possible during T2 and T3. Dietary data were analyzed to identify differences between 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 recalls for total kilocalorie intake (kcal) and macronutrient distributions.

Statistical analysis. The Shapiro-Wilk tests were used to verify the normal distribution of data, which were not normally distributed. The nonparametric Kruskal-Wallis one-way ANOVA test was performed to compare experimental groups at each time point. Subsequently, each experimental group was analyzed individually across time points using Friedman’s ANOVA as a nonparametric alternative to repeated measures ANOVA. Changes in dietary composition were analyzed using repeated measures ANOVA. Results were considered significant at an α level of P ≤ 0.05. All data are reported as means ± SD.

RESULTS

The lower-body resistance training protocol utilized in this study resulted in significant muscle damage as reflected by a significant decrease in performance and a significant rise in CK concentrations. However, no significant differences were noted between experimental groups for squat repetitions performed or CK concentrations between trials. All groups significantly decreased squat repetitions on T3 compared with T2 (−23.7 ± 22.2%). All groups significantly increased CK concentrations between PRE (136.8 ± 100.8 IU/l) and IP (205.5 ± 130.0 IU/l) and between IP and 24P (543.4 ± 331.2 IU/l). Analysis of dietary intake revealed no significant differences between groups for total kilocalorie intake, macronutrient distributions, or protein intake relative to body weight during the study protocol. All groups consumed a daily average of 2675.9 ± 895.7 kcal, 299.9 ± 116.4 g carbohydrate, 149.6 ± 75.0 g protein, and 101.5 ± 48.1 g fat. The average protein intake relative to body weight was 1.8 ± 0.9 g/kg.

CR3 expression (reported as mean fluorescence) on CD14+ monocytes. Expression of CR3 on CD14+ monocytes is represented in Fig. 3. No significant time point differences (P =

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Fig. 2. Gating procedures for CD14+ cells. A: CD14+ cells selected from the specified region based on forward (FSC) and side scatter (SSC) properties. B: two-dimensional histogram displaying fluorescence characteristics of cells in selected region. Cells positive for CR3 and CD14 are displayed in the top right quadrant.

Fig. 3. Acute effects of HMB-FA and/or CWI on on expression (mean fluorescence) of complement receptor 3 (CR3) on CD14+ monocytes. Treatment groups were PL, HMB-FA, PL-CWI, and β-hydroxy-β-methylbutyrate free acid and cold water immersion (HMB-FA-CWI). Time points were PRE, IP, 30P, 24P, and 48P. *PL significantly elevated expression of CR3 at 30P compared with PRE (P = 0.030), 24P (P = 0.047), and 48P (P = 0.047).
Circulating MIP-1β did not appear to be strongly influenced by the resistance training protocol or the recovery treatments. Previous research has shown significant elevations in MIP-1β following a marathon run (21). However, similar to our results, previous studies have found no change in MIP-1β following 300 maximal eccentric contractions of the quadriceps (23) or 3 sets of 6-RM of the back squat, front squat, and leg extension exercises (32). As MIP-1β is a pro-inflammatory chemokine for regulating tissue repair (17, 31), we had expected to see elevations in circulating concentrations following the resistance training protocol that would be attenuated by recovery treatments. However, it is possible that the mode of exercise may dictate changes in circulating MIP-1β, where circulating changes are only observed with exhaustive aerobic type exercise. It is also possible that large observed inter-individual variations in circulating MIP-1β among participants may have masked any response to the exercise protocol or treatment effects. Large variations in circulating MIP-1β among participants is not uncommon (12) as MIP-1β is secreted by most immune cells and its production has shown to be induced by various other pro-inflammatory cytokines, including TNF-α and IFNγ, and downregulated by IL-4 and IL-10 (17).

When no treatment was employed, the PL group significantly increased the expression of CR3 at 30 min postexercise compared with other time points. Previous investigations on the expression of CR3 (CD11b/CD18) and its component, CD11b, following exercise have yielded conflicting results. Pizza and colleagues (27) showed CD11b expression was elevated above baseline levels at 24, 72, and 96 h after eccentric exercise of the forearm flexors of untrained subjects, whereas Saxton and colleagues (34) observed no changes in CD11b expression was up to 72 h after a bout of eccentric isokinetic contractions of the quadriceps or 40 min of high-intensity stepping exercise in recreationally active subjects. Additionally, others have reported no changes in CD11b expression following eccentric exercise in recreationally active subjects (32). As MIP-1β is secreted by most immune cells...
following submaximal cycle ergometry (14) and running (10). Conflicting findings are likely due to differences in training status of participants, as training status has been previously shown to impact the expression of CR3 on immune cells (10, 27). As CR3 mediates leukocyte adhesion, migration, and phagocytosis in response to inflammatory stimuli (3, 29), it is possible that the observed elevation at 30 min postexercise without treatment in the current study would result in enhanced monocyte activity following exercise. Follow-up studies are needed to examine actual monocyte infiltration after high-intensity resistance exercise in relation to the expression of CR3.

The HMB-FA, CWI, and combination of the two treatments all similarly attenuated the rise in CR3 expression observed in the PL group at 30 min postexercise. Additionally, the HMB-FA group significantly elevated the percentage of monocytes expressing CR3 for up to 48 h following resistance exercise. To our knowledge, no studies have investigated the effects of HMB or CWI on CR3 and MIP-1β expression in vivo. However, in vitro studies in chicken and fish models have shown increased macrophage cell proliferation and phagocytosis suggesting the potential for HMB to be used as a possible dietary immunomodulator (26, 36). Additionally, leucine appears to exert a greater effect on immune function compared with other branch chained amino acids (13), which has been partly explained by the differential ability of leucine to stimulate muscle protein synthesis through an upregulation of mTOR (16, 18). HMB, a metabolite of leucine, may play a major role in the modulation of immune function. Recent in vivo research by Townsend and colleagues (42) also showed that HMB-FA attenuated circulating TNF-α, another pro-inflammatory cytokine, immediately postexercise and attenuated TNF-α receptor expression at 30 min postexercise. It appears that HMB-FA may be effective in altering the immune response after resistance exercise. Contrary to our findings for CR3 expression, Townsend et al. (42) did not observe any differences in circulating TNF-α or its receptors with CWI treatment. An important consideration to the interpretation of our findings is the relatively high daily protein consumption (1.8 ± 0.9 g/kg) of participants. While groups did not differ in daily protein consumption, prior research has shown that protein supplementation may positively impact postexercise CK concentrations (9). Additionally, incremental exercise to exhaustion has shown to increase leukocyte concentration in conjunction with elevated levels of CD11b (44), whereas CWI has shown to blunt the rise in total number of leukocytes postexercise (30). It may be that this attenuation in leukocyte concentration may also attenuate the rise in CR3 accompanied with muscle damaging exercise.

Limited research has been conducted on CR3 expression on monocytes in response to resistance training, but the present results may help us understand the in vivo leukocyte infiltration processes necessary for tissue repair. Although the treatment strategies employed in this study attenuated the rise in CR3 expression after exercise and HMB-FA showed to significantly elevate the percentage of monocytes expressing CR3 for up to 48 h after resistance exercise, the effects of the subsequent inflammatory events are yet to be determined. An increased expression of CR3 likely facilitates the infiltration of neutrophils and monocytes into the exercise-damaged muscle as a necessary stage in skeletal muscle repair; however, neutrophils may also play a role in furthering muscle damage by releasing oxygen free radicals and proteases that potentially cause additional tissue injury (1, 41). Neutrophils are the first subpopulation of leukocytes to appear at the injury site (28), yet the time points in which pro-inflammatory and anti-inflammatory cytokines become most beneficial for recovery have yet to be determined (43). Thus results should be interpreted as a component of the complex multifactorial series of events in the entire inflammatory process of tissue repair. Future research is needed to better understand the timeline of CR3 and MIP-1β expression following resistance exercise relating to the magnitude of muscle repair.

**Perspectives and Significance**

This study is the first to investigate the effects of traditional recovery modalities on CR3 expression and MIP-1β concentrations after resistance exercise. The significant peak expression of CR3 at 30 min postexercise was attenuated by HMB-FA, CWI, and the combination of the two treatments. Treatments with HMB-FA and/or CWI showed no increases in CR3 expression after exercise or during recovery. However, supplementation of HMB-FA significantly elevated the percentage of monocytes expressing CR3 for up to 48 h following resistance exercise. No time or treatment effect was observed for any experimental group for MIP-1β concentrations. Research on recovery modalities has focused on altering or alleviating the inflammatory process following acute exercise-induced muscle damage. However, it is not yet known at what time point during the acute inflammatory response it is most sensible to interfere with the natural repair progress, or if it is advisable to alter it at all. An understanding of the β2 integrin CR3 and its response to resistance training provides a unique insight into the inflammatory response. The treatment modalities in this study showed to attenuate the rise in CR3 expression at 30 min postexercise and HMB-FA showed to elevate the percentage of monocytes expressing CR3 between IP and 24P and between IP and 48P; however, this alleviation did not contribute to a more rapid recovery or improve subsequent performance.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


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