β-Hydroxy-β-methylbutyrate attenuates cytokine response during sustained military training

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ABSTRACT

This study tested the hypothesis that 23 days of β-hydroxy-β-methylbutyrate (HMB) supplementation can maintain muscle mass and attenuate the immune and inflammatory response in combat soldiers during highly intense military training. Soldiers were randomly assigned to either a HMB (n = 6) or placebo (PL; n = 7) group and provided with 3 g · day⁻¹ of either HMB or PL. During the final week of supplementation soldiers participated in extreme physical training, which included night navigation of 6–8 hours across difficult terrain carrying heavy loads combined with sleep deprivation (3.8 ± 3.0 h per night). Blood draws were performed prior to and following the supplementation period. Magnetic resonance imaging, which included diffusion tensor imaging sequence, was used for muscle fiber tracking analysis. Data was analyzed using a two-way mixed factorial analysis of variance. Magnitude-based inferences were used to provide inferences on the true effects that HMB may have had on the dependent variables compared to PL, calculated from 90% confidence intervals. Changes in tumor necrosis factor-α for HMB (−3.9 ± 8.2 pg · mL⁻¹) were significantly lower (P = .043) compared to the change in PL (+4.0 ± 3.7 pg · mL⁻¹). HMB ingestion was also very likely (92%-95% Likelihood) to lower granulocyte colony-stimulating factor and interleukin 10 compared to PL. During the final week of supplementation soldiers participated in extreme physical training, which included night navigation of 6–8 hours across difficult terrain carrying heavy loads combined with sleep deprivation (3.8 ± 3.0 h per night). Blood draws were performed prior to and following the supplementation period. Magnetic resonance imaging, which included diffusion tensor imaging sequence, was used for muscle fiber tracking analysis. Data was analyzed using a two-way mixed factorial analysis of variance. Magnitude-based inferences were used to provide inferences on the true effects that HMB may have had on the dependent variables compared to PL, calculated from 90% confidence intervals. Changes in tumor necrosis factor-α for HMB (−3.9 ± 8.2 pg · mL⁻¹) were significantly lower (P = .043) compared to the change in PL (+4.0 ± 3.7 pg · mL⁻¹). HMB ingestion was also very likely (92%-95% Likelihood) to lower granulocyte colony-stimulating factor and interleukin 10 compared to PL. In addition, HMB supplementation was likely (78%-87% likelihood) to reduce interferon-γ, interleukin 8, CX3CL1, and increase muscle volume for the adductor magnus (77% likelihood) compared to PL. In summary, the results of this study provide evidence that HMB supplementation may attenuate the inflammatory response to high intense military training, and maintain muscle quality.

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1. Introduction

Previous studies examining the physiological stress of sustained military operations have indicated that soldiers experience significant decrements in body mass, strength and power [1,2]. The physiological stress associated with these operations also result in significant elevations in inflammatory cytokine markers such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). These inflammatory cytokines have been shown to be significantly elevated following short (6 weeks) standardized military training [3], or longer-duration (4-months) military deployments to combat zones [4]. The cumulative effect of fatigue as the result of a 4-month deployment may result in an elevated cytokine response for up to 6-months following return [4]. Elevated inflammatory and immune responses are also reported following short-duration (7 days) intense military training under extreme environmental conditions [5]. In addition, sleep deprivation or sleep restriction, often associated with sustained military operations, also elevates pro-inflammatory markers [6,7]. The combination of sleep restriction or deprivation, in conjunction with extreme physical challenges, during intense military training appears to stimulate pro-inflammatory cytokines likely contributing to the anthropometric and performance changes associated with these stressors.

β-Hydroxy-β-methylbutyrate (HMB) is a derivative of the branched chain amino acid leucine. HMB has been demonstrated to enhance recovery and attenuate muscle damage resulting from high intensity exercise [8–12]. These studies often report a blunted creatine kinase response in both trained and untrained individuals to a physical stress [10,12], suggesting HMB supplementation may provide either a resiliency to muscle damage, or an enhanced recovery process. Although the precise mechanism of action is not clear, several investigations have suggested that HMB may inhibit the ubiquitin-proteasome-mediated proteolytic pathway leading to muscle degradation through several mechanisms. These mechanisms include activating PI3K/Akt-dependent mTOR and FoxO1/FoxO3a signaling pathways [13,14], attenuation of factors inhibiting the elongation phase of translation during protein synthesis [15], attenuation of proteolysis inducing factor [16], or inhibition of caspase activity [17]. It appears that decreases in the TNF-α and interferon-γ (IFN-γ) response by HMB administration is a common step in many of these proposed mechanisms [15,17]. Previous studies have shown that HMB supplementation (3 g · d⁻¹) can attenuate circulating TNF-α concentrations and TNF receptor expression on monocytes in experienced, trained men [11], as well as reduce C-reactive protein [9], and complement receptor type 3 (CR3) [8] responses to an acute muscle damaging resistance exercise protocol. Others have demonstrated that HMB supplementation is able to maintain lean body mass in muscle wasting diseases such as cancer [18] or autoimmune deficiency [19]. These observations suggest that HMB administration may be able to attenuate the initial immune response to intense exercise and reduce muscle and body mass loss during stressful physiological conditions.

In consideration that sustained combat operations or highly intense military training may result in both energy and sleep deficits, the use of a nutrient intervention to preserve lean mass and physical performance may be desirable. Therefore, the purpose of this study was to examine the hypothesis that HMB supplementation can attenuate muscle loss and the inflammatory response during highly intense, sustained military training. To the best of our knowledge, there are no studies known that have examined HMB supplementation in soldiers during intense military operations. In this study we examined the effect of 23 days of HMB supplementation on the immune and inflammatory response, and changes in muscle mass in combat soldiers during highly intense military training.

2. Methods and materials

2.1. Participants

Twenty-seven male soldiers from an elite combat unit of the Israel Defense Forces volunteered to participate in this double-blind, parallel-design study. Following an explanation of all procedures, risks and benefits, each participant provided his informed consent to participate in the study. The institutional review board of the Israel Defense Forces Medical Corp approved this research study. Participants were not permitted to use any additional dietary supplementation and did not consume any androgens or other performance enhancing drugs. Screening for performance enhancing drug use and additional supplementation was accomplished via a health questionnaire completed during participant recruitment. Soldiers were from the same unit and were randomly assigned to one of 2 groups. The randomization procedure involved alternating group assignment of volunteers into either a group supplemented with HMB (n = 14; age 20.1 ± 0.6 years; height: 1.75 ± 0.10 m; body mass: 70.2 ± 8.5 kg) or placebo (PL; n = 13; age 20.0 ± 0.9 years; height: 1.77 ± 0.04 m; body mass: 71.6 ± 5.4 kg).

2.2. Study protocol

During the 23-day study period all soldiers performed the same daily protocol. On days 1–10 soldiers were garrisoned on base and participated in the same advanced military training tasks that included combat skill development and conditioning. During days 11–17 soldiers were released for a week of rest and recovery. Upon reporting back for duty, soldiers were then subjected to a week (days 18–23) of extreme training with minimal recovery. On days 18 through 20, soldiers navigated 23.3 km per evening in difficult terrain carrying approximately 35 kg of equipment on their back (equating to approximately 40% of participant’s body mass). The duration of the navigational exercise lasted between 6 and 8 hours per evening. During daylight hours soldiers remained in camouflaged positions and maintained communication discipline. On the evening of day 20 a severe environmental stress (37°C ambient temperature during the evening accompanied with a sand storm) resulted in the cancellation of the navigational training. Soldiers remained in camouflaged shelters in the rough terrain until the afternoon of day 21. They were then returned to base and were subjected to excessive physical training that included 90 minutes of intense hand-to-hand combat (krav-maga training), 60 minutes of endurance training and an additional 60 minutes of...
resistance training. This intensive physical training was repeated on days 22 and 23. During the 6 days of intense training the soldiers slept a total of 22.5 h (3.8 ± 3.0 hours per night) including two evenings of no sleep (days 18 and 22). Blood draws and magnetic resonance imaging (MRI) measures were conducted in a single day prior to (PRE) and approximately 18 hours following the final supplement consumption (on day 24) (POST). All blood draws and MRI measures were performed at Soroka Medical Center.

2.3. HMB supplementation

Each serving of HMB (consumed in its free-acid form) and PL was provided in identical packets containing similarly flavored gel. Participants were required to consume three servings (1 gram per serving) per day (at meal time). The HMB supplement (marketed as BetaTor, MTI, Ames, IA, USA) consisted of Litesse polydextrose, HMB free acid, reverse osmosis water, orange flavor, stevia extract, potassium carbonate, and potassium sorbate. Each serving of PL consisted of a similar amount of Litesse polydextrose, reverse osmosis water, corn syrup, debittering agent, orange flavoring, stevia extract, citric acid, potassium sorbate, and xanthine gum powder. Both HMB and PL were obtained from Metabolic Technologies Inc (Ames, IA, USA). Participants were provided with weekly supplies of HMB and PL. Due to logistics associated with the training witnessing daily consumption was not possible. Participants were required to return all used and unused packets at the end of each week.

2.4. Blood measurements

Resting blood samples were obtained prior to each testing session. All blood samples were obtained following a 15-minutes 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, USA). Each participant’s blood samples were obtained at the same time of day during each session following an overnight fast. All blood samples were collected into two Vacutainer tubes, one containing no anticoagulant and the second containing K2EDTA. The blood in the first tube was allowed to clot at room temperature for 30 minutes and subsequently centrifuged at 3000 × g for 15 minutes 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.

2.5. Biochemical analysis

Serum concentrations of creatine kinase (CK) and lactate dehydrogenase (LDH) were analyzed using a commercially available kinetic assay (Sekisui Diagnostics, Charlottetown, PE, Canada; Sigma-Aldrich, St. Louis, MO, USA) per manufacturer’s instructions. Plasma concentrations of cytokines and chemokines included granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), monocyte chemoattractant protein-1 (MCP-1), fractalkine (CX3CL1), IFN-γ, interleukin-1β (IL-1β), interleukin-1 receptor antagonist (IL-1ra), IL-6, interleukin-8 (IL-8), interleukin-10 (IL-10), and tumor necrosis factor-alpha (TNF-α) were analyzed via multiplex assay, using the human cytokine/chemokine panel one (EMD Millipore, Billerica, MA, USA). In addition, plasma HMB concentrations were analyzed by gas chromatography–mass spectrometry and performed by Metabolic Technologies, Inc, using methods previously described to assess compliance and validate HMB in supplement packets [20]. All samples were thawed once and analyzed in duplicate by the same technician using a BioTek Eon spectro-photometer for CK and LDH concentrations (BioTek, Winooski, VT, USA), and MagPix for cytokine and chemokine concentrations (EMD Millipore). Mean intra-assay variability for all assays was below 10%.

2.6. Magnetic resonance imaging

Changes in muscle volume and integrity were assessed using MRI. Muscle integrity was determined through diffusion tensor imaging (DTI). DTI is a sensitive MRI technique to assess subclinical signs of muscle injury [21]. DTI assessment is predicated on cell membranes and other structures constraining water diffusion. Water movement can be evaluated by determining the 3 orthogonal directions of water diffusion, called eigenvectors, and their intensities, called eigenvalues [22]. From the 3 eigenvalues (λ1, 2, and λ3), parameters such as fractional anisotropy (FA) and apparent diffusion coefficients (ADC) can be calculated to evaluate the character of water diffusion in a voxel. These measures have been demonstrated to provide information about the integrity of skeletal muscle [23].

The MRI data were obtained using a 3.0-T whole body imager (Ingenia; Philips Medical Systems, Best, The Netherlands). During each measure, participants were placed supine in the scanner and imaged using phased-array surface coils. A position 20 cm above the patella was chosen as the image center and marked using an oil capsule. All scans were planned axially and consisted of 40 slices of 4-mm width for a foot-head coverage of 160 mm, and a field of view of 290 × 280 mm (RL × AP). Three image acquisitions were performed. A T1w Dixon was used for anatomical reference, a T2w Turbo spin-echo to assess any structural damage to the muscle, and a DTI sequence for muscle fiber tracking. The sequence parameters that were used are shown in Table 1.

Fat suppression (SPAIR – spectrally selective adiabatic inversion recovery) was used for the T2-T2 Spin Echo (TSE) and DTI scans. The DTI sequence was a 2D-EPID sequence imaged in two packages. The b-value was 400 s/mm² and imaged in 15 unique directions. Four muscles were analyzed: rectus femoris (RF), vastus lateralis (VL), adductor magnus (AM), and semitendinosus (ST). Muscle fiber tracking analysis was calculated by using the Philips FiberTrak software. A region of interest was hand-drawn for each of the 4 muscles on slices 15 and 25. The software then was allowed to delineate the muscle fibers using an algorithm that eliminated tracks if the fractional anisotropy was less than 0.1, if the change in angle was greater than 27° or if the fiber length was less than 10 mm. The same investigator performed all assessments.

2.7. Statistical analyses

Results are presented as means ± SD unless otherwise stated. Two-way mixed factorial analyses of variance [2 × 2; time
(pre-supplementation vs. post-supplementation) × treatment (PL vs HMB) were used to evaluate all blood and MRI data. If there was a significant main effect for time, then post-hoc analysis for each group was assessed using 95% confidence intervals (CIs) as described previously [11]. Percent change scores were calculated for each participant from pre- to post-testing. These percent change scores were averaged separately for the HMB and PL groups and 95% CIs were constructed around the mean percent change scores (Figs. 2–3). When the 95% CI includes zero, the mean percent change score is no different from zero, which can be interpreted as no statistical change (P > .05). However, if the 95% CI does not include zero, the mean percent change for that variable can be considered statistically significant (P < .05). The 95% CIs and graphs were calculated and created in Microsoft Excel (Version 2013; Microsoft Corp, Richmond, WA, USA), and all 2-way analyses of variance were performed with SPSS (version 23; SPSS Inc, Chicago, IL, USA).

Furthermore, to complement our null hypothesis testing, a more contemporary analytical approach was used by calculating the probability of practical significance [24]. Inferences on true effects HMB may have on blood and MRI variables compared to PL were measured using magnitude of differences, calculated from 90% CIs, as described previously by Batterham and Hopkins [25]. This approach uses the smallest worthwhile changes to establish the likelihood (in percentage terms) of the experimental condition having a positive, trivial, or negative effect. The change scores were analyzed using a published Excel spreadsheet [26], with the smallest, nontrivial change set at 20% of the grand standard deviation [25]. All data in Tables 1 to 3 are expressed as a mean effect ± 90% CI, with percent chances of a beneficial, trivial, or negative outcome. When clear interpretation was able to be made, a qualitative descriptor was assigned to the following quantitative chance of change: 0% to 25% unclear; 25% to 75% possible; 75% to 95%, likely; 95% to 99%, very likely; >99%, almost certain [27].

3. Results

To be considered compliant each participant needed to consume 80% of the total possible supplement servings. Due to injuries and compliance issues, only 13 of the 27 participants were included in the final analysis (HMB = 6 and PL = 7). These participants consumed 89.3% ± 6.8% of the possible servings. Of the 14 participants that were not included in the final analysis, 2 were injured during training (sprained ankle and chest contusion), and 12 participants did not meet the minimum compliance requirements of the study. No major side effects were reported, but 14 of the 27 participants did complain of gastrointestinal discomfort (cramps, bloating, or and/or diarrhea). Seven of these participants consumed the placebo, while the other 7 consumed HMB.

No significant interactions were observed between HMB and PL for body mass (F = 3.36, P = .094) from pre (72.6 ± 7.1 kg and 70.7 ± 6.6 kg, respectively) to post (71.7 ± 6.4 kg and 71.2 ± 6.9 kg, respectively).

3.1 Blood data

3.1.1 HMB

Changes in plasma HMB concentrations can be observed in Fig. 1. A significant interaction (F = 5.46, P = .038) was observed between HMB and PL. Plasma HMB concentrations at POST were significantly different (P = .029) between the groups.

All inflammatory cytokine data and muscle damage markers (means ± SD) are depicted in Table 2. In addition, magnitude-based inference comparisons for these markers are also reported in this table.

3.1.2 G-CSF

No significant interaction (F = 3.6, P = .095) was observed for plasma G-CSF concentrations, indicating that the pre to post change for HMB (−34.8 ± 39.5 pg · mL⁻¹) was not significantly different from the change in PL (14.0 ± 41.9 pg · mL⁻¹). Magnitude-based inferences indicated that HMB supplementation likely attenuated (92% likelihood effect) the G-CSF response compared with the effect of PL (a difference ± 90% CI of −49 ± 46.2 pg · mL⁻¹ between the Δ HMB − Δ PL).

3.1.3 GM-CSF

No significant interaction (F = 0.45, P = .52) was observed for plasma GM-CSF concentrations, indicating that the pre to post change for HMB (1.0 ± 28.2 pg · mL⁻¹) was not significantly different from the change in PL (9.1 ± 13.2 pg · mL⁻¹). Magnitude-based inferences indicated that HMB supplementation possibly attenuated (63% likelihood effect) the GM-CSF response compared with the effect of PL (a difference ± 90% CI of −8.1 ± 20.0 pg · mL⁻¹ between the Δ HMB − Δ PL).

3.1.4 CX3CL1

No significant interaction (F = 2.1, P = .18) was observed for plasma CX3CL1 concentrations, indicating that the pre to post change for HMB (−10.8 ± 34.8 pg · mL⁻¹) was not significantly different from the change in PL (18.0 ± 49.0 pg · mL⁻¹). Magnitude-based inferences indicated that HMB supplementation likely attenuated (78% likelihood effect) the CX3CL1 response compared with the effect of PL (a difference ± 90% CI of −38 ± 43.7 pg · mL⁻¹ between the Δ HMB − Δ PL).

3.1.5 IFN-γ

No significant interaction (F = 2.4, P = .16) was noted for plasma IFN-γ concentrations, indicating that the pre to post change for HMB (−13.2 ± 40.8 pg · mL⁻¹) was not significantly different from the change in PL (28 ± 46.8 pg · mL⁻¹). Table 2

<table>
<thead>
<tr>
<th>Sequence parameter</th>
<th>3D T1w mDIXON</th>
<th>T2-TSE</th>
<th>DTI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repetition time (ms)</td>
<td>14</td>
<td>5380</td>
<td>2917</td>
</tr>
<tr>
<td>Echo time (ms)</td>
<td>1.56, 4.0</td>
<td>70</td>
<td>56</td>
</tr>
<tr>
<td>In-plane voxel size (mm)</td>
<td>1.1 × 1.1</td>
<td>1.0 × 1.0</td>
<td>3.0 × 3.0</td>
</tr>
<tr>
<td>Turbo factor</td>
<td>—</td>
<td>18</td>
<td>—</td>
</tr>
<tr>
<td>EPI train length</td>
<td>—</td>
<td>—</td>
<td>47 (single-shot)</td>
</tr>
<tr>
<td>SENSE factor (AP)</td>
<td>1.5</td>
<td>1.3</td>
<td>2.0</td>
</tr>
<tr>
<td>No. of averages</td>
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<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Scan time (min)</td>
<td>1:32</td>
<td>2:09</td>
<td>9:26</td>
</tr>
</tbody>
</table>

Table 1 – Magnetic resonance acquisition parameters
indicates that HMB ingestion likely (87.2% likelihood effect) attenuated the IFN-γ response compared with the effect of the placebo (a difference ± 90% CI of –42 ± 47.3 pg · mL⁻¹ between the Δ HMB – Δ PL).

3.1.6. IL-1ra
No significant interaction (F = 0.99, P = .35) was noted for plasma IL-1ra concentrations, indicating that the pre to post change for HMB (–3.0 ± 20.7 pg · mL⁻¹) was not significantly different from the change in PL (8.5 ± 17.4 pg · mL⁻¹). Magnitude-based inferences indicated that HMB ingestion possibly attenuated (74% likelihood effect) the IL-1ra response compared to PL (a difference ± 90% CI of –11 ± 18.9 pg · mL⁻¹ between the Δ HMB – Δ PL).

3.1.7. IL-6
No significant interaction (F = 0.79, P = .40) was noted for plasma IL-6 concentrations, indicating that the pre to post change for HMB (–1.08 ± 3.46 pg · mL⁻¹) was not significantly different from the change in PL (7.6 ± 14.2 pg · mL⁻¹). Table 2 indicates that the effect of HMB supplementation possibly (74.5% likelihood effect) attenuated the IL-6 response compared to PL (a difference ± 90% CI of –6.6 ± 11.2 pg · mL⁻¹ between the Δ HMB – Δ PL).

3.1.8. IL-8
A significant interaction (F = 19.7, P = .058) was observed for plasma IL-8 concentrations, indicating that the pre to post change for HMB (–0.28 ± 6.4 pg · mL⁻¹) was different from the change in PL (6.5 ± 11.6 pg · mL⁻¹). In addition, a significant main effect (F = 11.0, P = .008) for time was noted. HMB supplementation likely decreased (80.5% likelihood effect) the IL-8 response compared to PL (a difference ± 90% CI of –6.7 ± 10.0 pg · mL⁻¹ between the Δ HMB – Δ PL).

3.1.9. IL-10
No significant interaction (F = 4.6, P = .06) was observed for plasma IL-10 concentrations, indicating that the pre to post change for HMB (–2.2 ± 5.5 pg · mL⁻¹) was not significantly different from the change in PL (4.1 ± 4.0 pg · mL⁻¹). Magnitude-based inferences indicated that HMB supplementation very likely attenuated (95% likelihood effect) the IL-10 response compared with the effect of PL (a difference ± 90% CI of –6.3 ± 5.7 pg · mL⁻¹ between the Δ HMB – Δ PL).

3.1.10. TNF-α
A significant interaction (F = 5.2, P = .043) was observed for plasma TNF-α concentrations, indicating that the pre to post change for HMB (–3.9 ± 8.2 pg · mL⁻¹) was different from the change in PL (4.0 ± 3.7 pg · mL⁻¹). However, HMB supplementation very likely (92% likelihood effect) attenuated the TNF-α response compared to PL (a difference ± 90% CI of –10.8 ± 7.2 pg · mL⁻¹ between the Δ HMB – Δ PL).

The remaining cytokines demonstrated no significant pre and post changes between HMB and PL for IL-1b (F = 0.04, P = .84), and MCP-1 (F = 0.58, P = .46). Furthermore, the magnitude-based inference analyses indicated that comparisons between HMB and PL on these inflammatory markers were unclear.

### Table 2 – Circulating cytokine concentrations (pg · mL⁻¹) and muscle damage markers in HMB and PL in response to intense military training

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Pre</th>
<th>Post</th>
<th>%Increase</th>
<th>%Trivial</th>
<th>%Decrease</th>
<th>Qualitative inference of HMB vs PL</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-CSF</td>
<td>PL</td>
<td>75.7 ± 48.4</td>
<td>89.7 ± 34.9</td>
<td>2.7</td>
<td>5.4</td>
<td>91.8</td>
<td>Very likely attenuated</td>
</tr>
<tr>
<td></td>
<td>HMB</td>
<td>130.3 ± 63.0</td>
<td>95.6 ± 24.3</td>
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<tr>
<td>GM-CSF</td>
<td>PL</td>
<td>33.1 ± 27.7</td>
<td>42.2 ± 27.9</td>
<td>16.6</td>
<td>20.3</td>
<td>63.2</td>
<td>Possibly attenuated</td>
</tr>
<tr>
<td></td>
<td>HMB</td>
<td>76.7 ± 46.8</td>
<td>77.8 ± 44.3</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CX3CL1</td>
<td>PL</td>
<td>97.6 ± 62.0</td>
<td>115.6 ± 27.1</td>
<td>8.6</td>
<td>13.8</td>
<td>77.6</td>
<td>Likely attenuated</td>
</tr>
<tr>
<td></td>
<td>HMB</td>
<td>137.8 ± 55.6</td>
<td>127.1 ± 22.4</td>
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<tr>
<td>IFN-γ</td>
<td>PL</td>
<td>48.8 ± 29.2</td>
<td>77.2 ± 51.3</td>
<td>4.3</td>
<td>8.5</td>
<td>87.2</td>
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<tr>
<td></td>
<td>HMB</td>
<td>176.2 ± 52.5</td>
<td>163.0 ± 53.7</td>
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<tr>
<td>IL-10</td>
<td>PL</td>
<td>9.3 ± 8.2</td>
<td>13.4 ± 9.1</td>
<td>1.6</td>
<td>3.9</td>
<td>94.5</td>
<td>Very likely attenuated</td>
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<tr>
<td></td>
<td>HMB</td>
<td>12.9 ± 10.0</td>
<td>10.7 ± 8.3</td>
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<tr>
<td>IL-1ra</td>
<td>PL</td>
<td>36.2 ± 16.0</td>
<td>44.7 ± 10.9</td>
<td>10.9</td>
<td>15.3</td>
<td>73.8</td>
<td>Possible attenuated</td>
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<tr>
<td></td>
<td>HMB</td>
<td>74.6 ± 47.5</td>
<td>71.6 ± 34.1</td>
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<td>IL-6</td>
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<td>15.9</td>
<td>74.5</td>
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<td></td>
<td>HMB</td>
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<td>IL-8</td>
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<td>20.1 ± 17.9</td>
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<td>6.4</td>
<td>13.2</td>
<td>80.5</td>
<td>Likely attenuated</td>
</tr>
<tr>
<td></td>
<td>HMB</td>
<td>31.5 ± 11.3</td>
<td>31.2 ± 10.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1</td>
<td>PL</td>
<td>279.5 ± 50.9</td>
<td>286.6 ± 77.1</td>
<td>65.5</td>
<td>20.2</td>
<td>14.3</td>
<td>Unclear</td>
</tr>
<tr>
<td></td>
<td>HMB</td>
<td>221.4 ± 64.8</td>
<td>247.6 ± 80.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>PL</td>
<td>18.1 ± 7.7</td>
<td>22.1 ± 9.6</td>
<td>2.2</td>
<td>5.9</td>
<td>92.0</td>
<td>Likely attenuated</td>
</tr>
<tr>
<td></td>
<td>HMB</td>
<td>29.8 ± 15.2</td>
<td>25.9 ± 9.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK (IU · L⁻¹)</td>
<td>PL</td>
<td>126.0 ± 55.2</td>
<td>83.9 ± 53.2</td>
<td>93.8</td>
<td>4.6</td>
<td>1.5</td>
<td>Likely increased</td>
</tr>
<tr>
<td></td>
<td>HMB</td>
<td>79.3 ± 29.1</td>
<td>106.7 ± 65.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH (IU · L⁻¹)</td>
<td>PL</td>
<td>354.4 ± 70.9</td>
<td>358.2 ± 46.4</td>
<td>83.8</td>
<td>11.1</td>
<td>5.1</td>
<td>Likely increased</td>
</tr>
<tr>
<td></td>
<td>HMB</td>
<td>293.1 ± 59.5</td>
<td>349.7 ± 46.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All data are reported as means ± SD.
3.1.11. Muscle damage blood markers

A trend \((F = 4.30, P = .062)\) towards an interaction was noted in plasma CK concentrations, indicating that the pre to post change for HMB \((27.4 \pm 72.0 \text{ IU} \cdot \text{L}^{-1})\) trended to be different from the change in PL \((-39.1 \pm 42.3 \text{ IU} \cdot \text{L}^{-1})\). Magnitude-based inferences indicated that HMB experienced a likely increase \((94\% \text{ likelihood})\) in the CK response compared to PL \((\text{a difference} \pm 90\% \text{ CI of} \ 67 \pm 5.8 \text{ IU} \cdot \text{L}^{-1} \text{ between the } \Delta \text{HMB} - \Delta \text{PL})\). No significant interaction \((F = 2.60, P = .19)\) was noted in changes in plasma LDH concentrations between HMB and PL, indicating that the pre to post change for HMB \((56.6 \pm 73.2)\) was not different from the change in PL \((3.8 \pm 62.1)\). Magnitude-based inferences indicated that HMB experienced a likely increase \((84\% \text{ likelihood})\) in the LDH response compared to PL \((\text{a difference} \pm 90\% \text{ CI of} \ 53 \pm 5.8 \text{ IU} \cdot \text{L}^{-1} \text{ between the } \Delta \text{HMB} - \Delta \text{PL})\) (see Table 2).

3.2. MRI and DTI data

3.2.1. Muscle volume

No significant interactions were observed between HMB and PL in changes in muscle volume for RF \((F = 0.97, P = .35)\), VL \((F = 0.05, P = .83)\), ST \((F = 0.03, P = .87)\) or AM \((F = 1.82, P = .21)\). However, a main effect for time was noted in AM \((F = 9.48, P = .01)\). Further, the mean percent change scores revealed a significant increase in AM muscle volume for HMB only (Fig. 2). In addition, Table 3 shows the effect of HMB possibly \((77\% \text{ likelihood effect})\) increased AM muscle volume compared to PL \((\text{a difference} \pm 90\% \text{ CI of} \ 41 \pm 134 \text{ cm}^3 \text{ between the } \Delta \text{HMB} - \Delta \text{PL})\).

3.2.2. FA and ADC

No significant interactions were observed between HMB and PL for changes in FA in RF \((F = 0.98, P = .34)\), VL \((F = 0.49, P = .50)\), ST \((F = 0.2, P = .67)\), or AM \((F = 0.70, P = .80)\). While there was only a trend for significant main effect \((F = 2.0 \text{ to } 3.9, P = .18- .07)\), the mean percent change scores revealed significant decreases for the RF and ST muscle groups in the placebo group only (see Fig. 3). Magnitude-based inferences indicated a possible increase in FA \((75\% \text{ likelihood})\) in ST for HMB in comparison to PL (a difference \pm 90\% CI of 0.05 ± 0.09 between

![Fig. 1 – Plasma HMB concentrations. *Significantly different from HMB PRE; #significantly different from PL. All data are reported as means ± SD.](image)
the Δ HMB – Δ PL). All other comparison of muscle groups were unclear (see Table 3). No significant interactions were noted between HMB and PL for changes in ADC in RF (F = 0.65, P = .44), VL (F = 2.02, P = .18), ST (F = 0.03, P = .90), or AM (F = 0.20, P = .66). HMB supplementation likely (86% likelihood effect) increased ADC in the VL compared to PL (a difference ± 95% CI of 41 ± 134 cm³ between the Δ HMB – Δ PL). All other comparisons were unclear (see Table 3).

4. Discussion

The results of this study indicate that 23 days of HMB supplementation can attenuate inflammatory cytokine markers and maintain muscle quality during highly intense military training with sleep deprivation. Although this is the first study to examine the potential benefits of HMB ingestion in soldiers, it does appear to support previous investigations indicating that HMB ingestion can attenuate the cytokine response to a muscle damaging protocol in both short (4 days) [9,11] and long (12 weeks) [28] duration supplement protocols. The attenuation of the cytokine response to high intensity stress has been suggested to inhibit the ubiquitin-proteasome mediated proteolytic pathway and reduce the proteolytic effects of various muscle degrading stimuli [15,17]. Decreases in both TNF-α and IFN-γ concentrations observed with HMB are consistent with other studies indicating the role that attenuating these cytokine responses have on mediating the catabolic effects associated with prolonged physical stress [15,17,29]. In addition, HMB ingestion was also shown to attenuate IL-6, IL-8, IL-10, IL-1ra, CX3CL1, and G-CSF response to combined high intense training and sleep restriction.

Results of this study demonstrate that HMB supplementation can result in a possible decrease in circulating IL-6. Only a limited number of investigations have examined HMB ingestion and the IL-6 response. These previous studies have reported no change in the response of IL-6 concentrations following 7 weeks of supplementation in elite, adolescent athletes [30], or to a bout of resistance exercise following 14 weeks of training in previously untrained men [28]. Although IL-6 has been proposed to have many physiological functions, including a role as a pro-inflammatory cytokine [31], evidence also suggests that it can inhibit the production of TNF-α and IL-1β [32], or promote the formation of anti-inflammatory cytokines such as IL-10 [33]. In addition, IL-6 is thought to have both protective and restorative roles that improve muscle recovery [34].

HMB supplementation also appeared to attenuate the circulating IL-8 response to intense military training program with sleep restriction. IL-8 is a marker of inflammation [35] and has been reported to be elevated following consecutive days of intense exercise [36]. However, a previous study reported significantly lower IL-8 concentrations following 3 days of simulated firefighting and sleep restriction (4 hours per night) compared to the same physical stress in participants who slept 8 hours per evening [37]. The investigators suggested that the lower cytokine response during the restricted sleep trial was likely related to the lower physical activity performed by that group (performance of each task was self-paced). In the present study, soldiers in HMB and PL worked together as a group and were required to complete all tasks as a unit. Therefore, the volume and intensity of work was the same for all, thus the change in resting IL-8 concentrations seen in this study was likely the effect of the supplement.

Plasma G-CSF concentrations appeared to be very likely decreased as the result of HMB supplementation. This is the first study to our knowledge to indicate that HMB can attenuate the G-CSF response to intense physical stress. The physiological role of G-CSF is to stimulate the production and activation of neutrophils [38] and is often reported to be elevated following muscle damaging activity [38,39]. Attenuation in G-CSF as a result of HMB supplementation contrasts with Kraemer and colleagues [28] who reported no change in the G-CSF response to resistance exercise subsequent to 14-weeks of HMB supplementation in previously untrained individuals. This likely reflects the differences in the stress imposed by these two studies. Recent evidence indicates that regulation of G-CSF is dependent upon the type of inflammatory stimulus [39]. Resistance training, using a consistent volume and intensity, will result in a lower level of muscle damage as training progresses [40]. However, the combination of extreme physical stress and restricted sleep experienced by the participants of this present study was an extreme and novel experience even for soldiers participating in advanced

Fig. 2 – Mean percent change scores ± 95% CIs for MRI Muscle Volume measures for RF, VL, ST, and AM. Black circles indicate placebo group; white circles, HMB group. * indicates a significant difference when 0 is outside of the 95% CI.
infantry training. The reduced G-CSF observed in HMB likely reflected an adaptation from the supplement.

The response of IL-10 observed in this study is consistent with the changes in the IL-6 response. IL-10 is generally reported to be an anti-inflammatory marker and has been suggested to be stimulated by elevations in IL-6 [41]. Although the possible attenuation in IL-6 concentrations during HMB may have influenced the IL-10 response, the physiological relevance of increasing the anti-inflammatory cytokines during exercise has been questioned [42]. Generally, the pro-inflammatory response precedes and directly initiates the anti-inflammatory response [43], which may explain the blunting of both pro- and anti-inflammatory cytokines. Elevations in IL-10 are also suggested to result in a negative feedback response resulting in a decrease in IL-8, TNF-α, and IL-6 concentrations [41]. Although our results do not appear to support this, this may be a function in the timing of the blood measurements. It is possible that this feedback loop may have exerted itself subsequent to the return to base. Although the soldiers were still physically stressed following the navigational training – the removal from the stressful environment, and exercise occurring in cooler environments, may have allowed for some recovery. Still, participants were subjected to intense physical training and sleep restriction that is reflected in the pro-inflammatory response of PL.

Examination of changes in the circulating CX3CL1 response to physical stress or exercise is limited. The main role of CX3CL1 is promotion of leukocyte binding and adhesion, as well as activation of target cells [44]. Similarly, GM-CSF and IL-1ra are also involved in the inflammatory response during exercise or stress [45]. The attenuation of CX3CL1, GM-CSF, and IL-1ra in HMB is consistent with the attenuation of many of the pro-inflammatory markers seen to the nutrient intervention used in this study. However, the response of GM-CSF to HMB contrasted to that reported by others [28], who indicated no change in the GM-CSF response during 14 weeks of HMB supplementation and resistance exercise. Again, this is likely a function of the differences in stress imposed by the two studies. HMB, as a nutrient intervention, may provide greater resiliency during shorter periods of highly intense physical stress than a longer duration training routine.

Comparisons in changes of the other cytokine markers (MCP-1 and IL-1β) between HMB and PL were unclear. The response of IL-1β contrasted with a previous investigation examining the effect of HMB supplementation on the cytokine response to exercise [25], while the response of MCP-1 appeared to be consistent with that same investigation. Both IL-1β and MCP-1 are considered to be pro-inflammatory mediators [41]. However, it is possible that changes in the circulating concentration may not reflect the role of several of these cytokines within the muscle [42]. The elevation in some inflammatory markers but not others also reflects the complex workings of the inflammatory and immune response to physical stress, muscle damage and disease [35,42].

Previous research has reported that basic military training did not result in significant changes in the inflammatory response following 4-months of training [46]. However, during short-term (7 days), high stress military training significant elevations in the inflammatory cytokines TNF-α, IL-1β, and IL-6 have been reported [1,47]. In addition, elevations in IL-4, IL-6 and IL-8 have also been reported following long duration (~60 days) intense US Army Ranger training that required soldiers to march with loads of 30 to 40 kg, travel approximately 320 km, consume 2200 kcal per day and sleep 0 to 5 hours per night [48]. The inflammatory markers only returned to baseline values following 2 to 6 weeks following the course. The present study, using a similar relative physical stress and sleep restriction patterns, albeit for a week, was able to demonstrate that HMB supplementation may prevent the inflammatory response to intensity military training.

The cytokine response to muscle-damaging events is quite complex and difficult at times to ascertain [45]. Although HMB appeared to reduce the inflammatory response to exercise, both CK and LDH concentrations for HMB appeared to be likely increased in comparison to PL. This is consistent with other studies indicating that HMB may not have an effect on muscle damage markers [9,11], but contrasts with others suggesting that HMB ingestion can reduce muscle damage [10,12]. Recently, some investigators have suggested that the relationship between inflammatory markers and muscle damage markers are not clear, as significant elevations in CK and myoglobin were reported without any change in inflammatory cytokine markers [49].
Sustained short-duration military operations have been demonstrated to result in significant decreases in muscle mass [2]. The use of HMB as a nutrient intervention appeared to increase muscle volume for AM compared to PL. However, comparisons for the other muscles assessed were unclear. A previous investigation reported significant fatigue in knee extensors and plantar flexors during a 21-hour simulated military operation [50]. Although we reported no benefit from HMB in either the VL or RF, a greater increase in volume for the AM may provide for greater stability during navigation across a difficult and rocky terrain carrying a load.

In addition to examining muscle volume changes, DTI was conducted to provide a sensitive method of assessing subclinical signs of muscle injury [21,51]. The DTI technique assesses diffusion of water molecules and its direction in the 3-dimensional muscle microstructure [22]. In healthy tissue the integrity of the structure results in barriers to diffusion which is greater in some areas than others [51]. FA represents the increase in diffusivity into tissue following trauma. Thus, a decrease in FA values represents a disruption to the integrity of the muscle and cause greater diffusion [51]. In contrast, the ADC reflects the degree of diffusion in each direction of the muscle by the length of its axis. An increase in ADC indicates greater skeletal muscle disruption as a consequence of an increase in fluid diffusion into muscle [22]. Magnitude-based inferences indicated that FA for ST was possibly increased during HMB, and 95% CIs also indicated significant decreases in FA for both RF and ST muscle groups for PL only, suggesting that HMB provided some degree of protection in muscle integrity.

These results do appear to be consistent with the inflammatory cytokine response observed. Several investigators have suggested that HMB may inhibit the ubiquitin-proteasome-mediated proteolytic pathway by attenuating the inflammatory response to muscle damaging protocols that result in muscle degradation [13,15–17]. The results of this study appear to provide additional support for this hypothesis. In regards to ADC, a likely increase in change was noted in HMB when compared to PL for VL only, all other comparisons were unclear. The lack of consistency in the HMB-PL comparisons in the DTI measures is not well understood. Previous investigations employing DTI assessments in muscle damaging protocols are very limited, and each appear to have used a very specific and different damaging protocol: marathon run [49] and repeated eccentric contractions of 15 sets × 20 repetitions at 0.52 rad · s⁻¹ [51]. Froeling and colleagues [21] investigated DTI changes in experienced marathon runners and reported a broad response range among the six muscle groups measured two days following the marathon. In contrast, Germak et al., [51] specifically focused on the VL muscle and examined a novel damaging protocol for that specific muscle in recreationally active individuals. Results demonstrated clear damage confirmed by both invasive and DTI measures. The results of this present study are more consistent with that of Froeling et al [21] in which participants were experienced in the activity, and although the activity is known to be highly stressful, it was not designed to maximize muscle damage. Further, experienced participants such as soldiers involved in advanced infantry training likely have a degree of sensitization or resiliency to the repeated stress of training [52,53].

A major limitation of this study was the lack of compliance among many of the participants. The lack of compliance was not related to side effects associated with the supplement, as similar complaints for taste and gastrointestinal discomfort were noted for both PL and HMB. It is likely that a gel like substance may not have been an ideal method of supplement delivery in a field study occurring in the heat and in difficult conditions. In addition, the week of rest prior to the intense field training was also a potential factor for loss of compliance, but it also allowed some degree of recovery for soldiers prior to the intense training. Regardless, the results of this study does provide evidence that HMB supplementation may attenuate the inflammatory response to high intensity military training, which may have offered some benefit for maintaining muscle quality. As such the hypothesis of the study that HMB supplementation can attenuate muscle loss and the inflammatory response during highly intense, sustained military training is accepted. Additional research is needed to substantiate the potential benefits that HMB supplementation may have on reducing the inflammatory response during sustained intense military operations, and its potential implication for sustained military performance.

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REFERENCES


