The effect of HMB ingestion on the IGF-I and IGF binding protein response to high intensity military training

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Objective: Insulin-like growth factor-I (IGF-I) is a metabolic and anabolic biomarker that has been proposed to reflect physiological adaptations resulting from multi-stressor environments. The bioactivity of IGF-I is regulated by seven different insulin-like growth factor binding proteins (IGFBPs) which act not only as carriers of IGF-I, but also function as a modulator of IGF-I availability and activity. Supplementing with β-hydroxy-β-methylbutyrate (HMB) has been shown to enhance physiological outcomes associated with intense training, and has been reported to augment the IGF-I response. The purpose of this study was to examine the effect of 23 days of HMB supplementation on circulating levels of IGF-I and IGFBPs in combat soldiers during highly intense military training.

Methods: Thirteen male soldiers from an elite infantry unit volunteered to participate in this double-blind, parallel design study. Soldiers were provided 3 g·day−1 of either HMB (n = 6) or placebo (PL; n = 7). During the study soldiers performed advanced military training with periods of restricted sleep and severe environmental stressors. Blood samples were obtained prior to (PRE) and approximately 18 h following the final supplement consumption (POST).

Results: No significant differences were observed for circulating IGF-1 concentrations between HMB and PL (p = 0.568). In addition, no differences were seen between the groups for IGFBP-1 (p = 1.000), IGFBP-2 (p = 0.855), IGFBP-3 (p = 0.520), IGFBP-4 (p = 0.103), IGFBP-5 (p = 0.886), or IGFBP-6 (p = 0.775). A significant difference was noted between HMB (169.9 ± 23.0 ng·ml−1) and PL (207.2 ± 28.0 ng·ml−1) for IGFBP-7 at POST (p = 0.042).

Conclusions: Although the results of this study do not support the influence of HMB supplementation on circulating concentrations of IGF-1 or IGFBPs1−6 during high intensity military training, it does present initial evidence that it may lower circulating IGFBP-7 concentrations. This may provide some indication of a reduced stress response, but further investigation on the physiological role of IGFBP-7 and military training is needed.

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1. Introduction

Insulin-like growth factor-I (IGF-I) is a metabolic and anabolic biomarker that has been examined in relation to physiological changes resulting from multi-stressor environments such as inadequate rest, high volume training, and poor nutrition [1–3]. Physiologically, IGF-I is known for having both mitogenic, and myogenic properties, and has been studied for its role in muscle remodeling and hypertrophy following training [2,4,5]. The bioactivity of IGF-I is regulated by seven different insulin-like growth factor binding proteins (IGFBPs) which act not only as carriers of IGF-I, which prolong the half-life of IGF-I, but also function as a modulator of IGF-I availability and activity [6]. Circulating IGF-I concentrations have been reported to be associated with lean body
mass and level of fitness [7]. Studies have also revealed a positive relationship between energy balance and IGF-I concentrations [2,8,9].

β-Hydroxy-β-methylbutyrate (HMB) is a metabolite of the branched-chain amino acid leucine and is produced endogenously in small amounts [10]. Supplementing with HMB has been shown to attenuate muscle loss and promote gains in strength and lean body mass by inhibiting protein degradation and stimulating protein synthesis [10–12]. HMB-induced elevation in net skeletal muscle protein balance, may be driven in part by increases in hepatic, and skeletal muscle IGF-1 [13,14]. A recent study reported that acute HMB supplementation can augment the IGF-1 response to resistance training [15], while others have reported no change in resting IGF-1 concentrations following 7-weeks of HMB supplementation [16].

Physiological stresses associated with military training or deployment (e.g., environment, nutrition and sleep deprivation) have been well documented [17–20]. Nindl et al. [18] observed a 12.6% decrement in body mass, a 6.1% decrement in fat free mass, a 20% decrement in repetitive dynamic lifts, a 16% decrease in vertical jump and a 21% decrease in peak power following 8-weeks of intense Ranger training. In addition, they reported a significantly lower muscle cross-sectional area in both upper and lower limbs. During military deployment, periods of high intensity operations are also interspersed with lower intensity military activity. A 9-month deployment has been reported to result in significant decreases in body mass, aerobic capacity and upper body muscular power performance (~1.9%, −4.9% and −6.6%, respectively) [20]. These stresses have also been reported to pose significant challenges on the health and performance of soldiers [19]. It has been previously suggested that IGF-1, and its binding proteins, may serve as important biomarkers for a variety of health related outcomes; both positive (i.e., muscle hypertrophy, bone growth, and body composition) and negative (i.e., greater cancer risk and decreased longevity) [5]. Friedl et al. [3] reported a significant decline (approximately 50% decrease) in IGF-I concentrations during an 8-week United States Army Ranger training course. Although Nindl et al. [1] reported no change in IGF-I and IGFBP concentrations in male soldiers participating in a gender integrated 9-week basic combat training course, they did report that IGF-I concentrations were significantly elevated and IGFBP-2 was significantly reduced in the women soldiers. Others have reported significant decreases in IGF-I concentrations during 8-days of sustained, high intensity military training [2,21]. Significant elevations in IGFBPs1–3 have also been reported during these high intensity training days [21].

Investigations examining HMB supplementation and the IGF-I response have primarily used recreational or young, competitive athletes [15,16]. The physiological and psychological stresses associated with military training generally exceed that which is common among competitive athletes [17]. Thus, the potential for HMB supplementation to maintain the IGF-1 system (i.e., IGF-I and IGF binding proteins) during highly intense military training may be enhanced. Therefore, the purpose of this study was to examine the effect of HMB supplementation on circulating concentrations of IGF-I and IGF binding proteins in combat soldiers during highly intense military training.

2. Methods

2.1. Participants

Twenty-seven male soldiers from an elite combat unit of the Israel Defense Forces (IDF) 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 IDF Medical Corp approved this research study (ClinicalTrials.gov identifier: NCT02503007). Participants were not permitted to use any additional dietary supplementation and did not consume any androgens or any 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 two groups. The randomization procedure involved alternating group assignment of volunteers into either a group supplementing 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

The protocol used in this study has been previously described [22]. Briefly, all soldiers performed the same daily protocol during this 23-day study. During the first 10 days of the study soldiers were garrisoned on base and participated in 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 (day 18), soldiers participated in 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 with approximately 35 kg of equipment on their back (equating to approximately 40% of their body mass). The duration of the navigational exercise lasted between 6 and 8 h 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 accompanied with a sand storm) resulted in the cancellation of the navigational training. Soldiers remained in their camouflaged shelters until the afternoon of day 21. They were then returned to base and were subjected to excessive physical training that included 90 min of intense hand-to-hand combat (krav-maga training), 60 min of endurance training and an additional 60 min of resistance training. This intensive physical training continued 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 h per night) including two evenings of no sleep (days 18 and 22). All blood draws were conducted in a single day prior to (PRE) and approximately 18 h following the final supplement consumption (on day 24) (POST).

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 g per serving) per day (at meal time). The HMB supplement (marketed as BetaTor®, MTI, Ames, IA) consisted of β-hydroxy-β-methylbutyrate free acid, reverse osmosis water, debittering agent, stevia extract, 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. Both HMB and PL were obtained from Metabolic Technologies Inc. (Ames, IA). 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-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 following an overnight fast. All blood samples were collected into two Vacutainer® tubes, containing no anti-clotting agent and the second containing K2EDTA. The blood in the first tube was allowed to clot at room temperature for 30 min and subsequently centrifuged at 3000 × 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.

2.5. Biochemical analysis

Serum concentrations of IGF-I were analyzed using a commercially available Human IGF-I Quantikine ELISA assay (R & D Systems, Minneapolis, MN, USA), per manufacturer’s instructions, without modification. Listed sensitivities for IGF-I were 0.007–0.056 ng·mL−1. The intra-assay coefficient of variation for IGF-I assay was less than 5%. All samples were thawed once and analyzed in duplicate by the same technician using a BioTek Eon spectrophotometer for IGF-I. Serum concentrations of IGFBPs included IGFBP-1, -2, -3, -4, -5, -6, and -7 were measured using Luminex xMAP technology, Millipore MILLIPLEX MAP Human IGFBP panel kit-53 K (Millipore, Billerica, MA, USA) Sensitivities were 0.01, 0.63, 0.12, 0.69, 7.00, 0.07, and 0.04 ng·mL−1 for IGFBP-1, -2, -3, -4, -5, -6, and -7 respectively. The intra-assay CVs for the IGFBPs were less than 12.99%. 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 [23].

2.6. Statistical analysis

For data analysis, participants needed to consume 80% of the total possible supplement servings. Due to injuries (n = 2) and lack of compliance (n = 12), only 13 of the 27 participants were included in the final analysis (HMB = 6 and PL = 7). In consideration of the unequal sample size and low participant number, comparisons between groups were performed using the non-parametric Kruskal-Wallis test. Data were analyzed using SPSS software (version 23, SPSS Inc., Chicago, IL). All data are reported as mean ± SD.

3. Results

Participants included in the final analysis consumed 89.3 ± 6.8% of the possible servings. Among participants that began the study, no differences were found between HMB and PL in reported side effects. Gastrointestinal discomfort (cramps, bloating or and/or diarrhea) was reported in 14 of the original 27 participants. However, these complaints were reported evenly between the groups; seven of the complaints were made by participants in HMB and seven reported by PL. No other side effects were reported.

No significant differences (p = 0.094) were observed in the Δ changes (post − pre) in body mass between participants in HMB (−0.83 ± 1.29 kg) compared to PL (0.50 ± 1.13 kg). Changes in plasma HMB concentrations have been reported elsewhere [22]. Supplementation resulted in significantly greater (p = 0.025) plasma HMB concentrations at POST for HMB (33.9 ± 32.9 nmol·L−1) compared to PL (3.0 ± 1.9 nmol·L−1). Measures of IGF-1 and IGFBP binding proteins (means ± SD) are depicted in Table 1.

IGF-I concentrations were not significantly different (p = 0.568) between the groups following the supplementation period. Furthermore, no significant differences between HMB and PL were noted in IGFBP-1 (p = 1.000), IGFBP-2 (p = 0.855), IGFBP-3 (p = 0.520), IGFBP-4 (p = 0.103), IGFBP-5 (p = 0.886), and IGFBP-6 (p = 0.775). However, IGFBP-7 concentrations for HMB were significantly lower (p = 0.042) than PL at POST.

4. Discussion

The main findings of this study indicated that 23 days of HMB supplementation appeared to have no significant effect on circulating concentrations of IGF-I as well as IGFBPs 1–6 in combat soldiers during highly intense military training. However, HMB supplement intervention appeared to decrease resting IGFBP-7 concentrations compared to PL. The examination of IGF-I has been previously recommended as a biomarker reflecting physiological stress in military personnel [4,5]. In general, elevations in circulating concentrations of IGF-I are thought to be representative of a greater anabolic response [24], perhaps indicating adequate recovery from the strain of the training mission. However, others have suggested that IGF-I may have a bi-phasic response to chronic intense exercise [25], in which extreme increases in energy expenditure cause an initial decrease in circulating IGF-I concentrations, followed by a ‘rebound’ effect that results in an elevation from its baseline concentration. In consideration of the regulatory role that IGFBPs have in modulating IGF-I bioavailability and their biological activity in peripheral tissue [26], it would be expected that their response pattern would be similar to that of circulating IGF-I. The results of IGFBPs 1–6 observed in this study appear to be consistent with that notion.

No significant differences in IGF-I concentrations were noted in this study in response to intense military training. This was consistent with other studies examining male soldiers during basic training [1], but differs from other investigations that have reported a significant decline in IGF-I concentrations in response to high intensity military training [23, 21,27]. Differences between these studies and the present investigation could be partly attributed to differences in the duration of sustained training exercise and nutrient intake. Although the supplementation period was 23-days, the duration of high intensity activity was only six days. Previous studies have reported significant reductions in IGF-I concentrations during intense military training ranging from 8– [22] to 21 days [3,27]. These studies were also associated with sleep restriction (3–4 h a day) and nutrient depletion. Interestingly, similar to the present study Nindl et al. [1] observed no change in IGF-I concentrations during a 4-month basic training course in male soldiers. No changes in body mass were also reported, suggesting that changes in IGF-I concentrations may be more sensitive to nutrient depletion than sleep deprivation or intense activity. Although the soldiers in this present investigation performed intense military training and experienced extreme sleep restriction, they were provided regular meal rations. Previous studies have found a positive relationship between nutrient balance and IGF-I concentrations [2,8,9]. It is likely that maintaining normal nutrient feedings and avoiding a nutrient deficit can minimize disruption to the IGF-I axis. This is supported by a previous investigation reporting that IGF-I concentrations return to resting concentrations during refeeding periods, but continue to decrease when soldiers return to a food restricted state [3].

The response pattern of IGF binding proteins appear to vary dependent on the physiological stresses imposed [26,28]. This is likely related

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Pre</th>
<th>Post</th>
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</thead>
<tbody>
<tr>
<td>IGF-I</td>
<td>PL</td>
<td>104.6 ± 36.9</td>
<td>107.0 ± 27.4</td>
</tr>
<tr>
<td></td>
<td>HMB</td>
<td>119.8 ± 27.1</td>
<td>109.0 ± 10.7</td>
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<tr>
<td>IGFBP-1</td>
<td>PL</td>
<td>45.2 ± 14.5</td>
<td>36.1 ± 10.1</td>
</tr>
<tr>
<td></td>
<td>HMB</td>
<td>68.2 ± 20.0</td>
<td>33.0 ± 21.0</td>
</tr>
<tr>
<td>IGFBP-2</td>
<td>PL</td>
<td>3614 ± 1606</td>
<td>2933 ± 1223</td>
</tr>
<tr>
<td></td>
<td>HMB</td>
<td>3943 ± 1288</td>
<td>2905 ± 1900</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>PL</td>
<td>2164 ± 1993</td>
<td>1993 ± 306</td>
</tr>
<tr>
<td></td>
<td>HMB</td>
<td>2233 ± 758</td>
<td>2151 ± 520</td>
</tr>
<tr>
<td>IGFBP-4</td>
<td>PL</td>
<td>5272 ± 908</td>
<td>5878 ± 745</td>
</tr>
<tr>
<td></td>
<td>HMB</td>
<td>5012 ± 1167</td>
<td>5086 ± 963</td>
</tr>
<tr>
<td>IGFBP-5</td>
<td>PL</td>
<td>34.4 ± 7.0</td>
<td>36.4 ± 9.8</td>
</tr>
<tr>
<td></td>
<td>HMB</td>
<td>38.3 ± 17.0</td>
<td>41.8 ± 14.9</td>
</tr>
<tr>
<td>IGFBP-6</td>
<td>PL</td>
<td>20.5 ± 9.4</td>
<td>18.4 ± 9.3</td>
</tr>
<tr>
<td></td>
<td>HMB</td>
<td>17.9 ± 3.8</td>
<td>17.8 ± 6.2</td>
</tr>
<tr>
<td>IGFBP-7</td>
<td>PL</td>
<td>196.5 ± 43.0</td>
<td>207.2 ± 28.0</td>
</tr>
<tr>
<td></td>
<td>HMB</td>
<td>179.4 ± 31.0</td>
<td>169.9 ± 23.0</td>
</tr>
</tbody>
</table>

All data are reported as means ± SD. * Significant difference between PL and HMB.
to the different physiological roles and responsibilities that the individual binding proteins have [26,28]. IGF binding proteins can either potentiate or inhibit the IGF response [26]. IGFBP-1 and IGFBP-2 in the circulation are primarily un saturated and are major modulators of IGF concentrations, and IGFBP-1 appears to be inversely related to insulin concentrations [28]. IGFBP-2 and IGFBP-4 appear to have inhibitory effects on IGF-I induced DNA synthesis, while IGFBP-5 potentiates the mitogenic effects of IGF-1 [29]. IGFBP-5 is also thought to be the major IGF binding protein secreted by skeletal muscle [26], while IGFBP-3 is the primary carrier of IGF-I and provides for an adequate reservoir of IGF-I in the circulation [28]. IGFBP-6 has been suggested to have antiapoptotic activity enhancing cell survival [30]. IGFBP-7 is also referred to as IGF binding protein-related protein 1 (IGFBP-rP1), elevations in IGFBP-7 are suggested to be reflective of a greater risk of cancer [31,32]. However, others have reported that elevations in IGFBP-7 are related to tumor suppression and increased cell apoptosis, while inhibiting IGF and insulin signaling [33]. Increased expression of IGFBP-7 has also been suggested to be associated with insulin resistance and metabolic syndrome [34]. The lack of any change in IGFBPs 1–6 is consistent with the response noted in IGF-I, and also similar to that reported by Nindl et al. [1]. Similar to IGF-I, IGF binding proteins have been postulated to serve as reliable indicators of nutritional state [21]. Considering that nutrient intake in this study was adequate, the maintenance of IGFBP concentrations appears to be consistent in the ability of these soldiers to maintain physiological homeostasis during the 6 days of intense training.

This investigation, to the best of our knowledge, is the first to report on changes in IGFBP-7 concentrations during military training, or in healthy individuals performing intense exercise. The physiological relevance associated with the significant decrease observed in IGFBP-7 concentrations in HMB is therefore difficult to explain. However, a decrease in IGFBP-7 has been associated with a reduced risk for both metabolic syndrome [34] and cancer [31,32]. Although speculative, a reduction of IGFBP-7 in HMB could be considered to be a potentially positive change. Still, the physiological significance of changes in IGFBP-7 concentrations in active, healthy individuals requires further examination.

A major limitation of this study was compliance among the soldiers that volunteered. The similar occurrences of gastrointestinal discomfort from participants in both HMB and PL suggest that issues of compliance were unrelated to side effects associated with the supplement. The low compliance was believed to be related more to the method of supplement delivery. The gel like substance was likely not the ideal method of supplement delivery in a field study occurring in hot and difficult conditions. It also needs to be acknowledged that circulating IGF-I and IGFBP concentrations may not provide a clear picture of the IGF-I response. The response of IGF-I is quite complex in that it is released of supplement delivery and in a field study occurring in hot and difficult conditions. It also needs to be acknowledged that circulating IGF-I and IGFBP concentrations may not provide a clear picture of the IGF-I response. The response of IGF-I is quite complex in that it is released from the liver into the circulation, but it is also released in a paracrine/autocrine fashion to exert its biological effect [5]. In this present study, we were unable to provide any measure of change in the intramuscular IGF-I response, thus limiting our interpretation to circulating concentrations only. In the present study, HMB supplementation did not appear to have any influence on the changes in the circulating concentrations of IGF-I or IGF binding proteins 1–6. Considering that energy intake or expenditure was not measured, we can only speculate that the lack change in IGF-I and IGF binding proteins 1–6 may have been related to the soldiers consuming an adequate energy intake. During intense military training, sleep deficit/restriction and intense activity is an accepted part of the training. However, in actual combat or controlled training environments the ability to maintain appropriate energy intake is critical. The results of this study suggest that circulating concentrations of IGF-I and IGFBPs appeared to be maintained, regardless of supplement usage. HMB supplementation did appear to reduce IGFBP-7, however additional research is warranted to provide further insight on the physiological role that changes in IGFBP-7 have during high intensity training with restricted sleep.

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