The Interactive effects of HMB and Glutamine supplementation on immune response of female students after a maximal exercise session

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ABSTRACT

Background: This study aimed to investigate the interactive effects of HMB and Glutamine supplementation on the amounts of IL-6 and IL-8 of non-athletic female students after a heavy resistance exercise session. For this purpose, 40 non-athletic female students with an average height of 165.00 ± 5.55 cm and mean weight of 71.40 ± 4.32 kg were selected and divided into four groups of 10 subjects (3 supplementation groups and one placebo group). Objective: write the main objective for your pap In the pre-test, subjects performed a session of weight training with 70 to 75% of one maximal repetition. The assessment of IL-6 and IL-8 in the previous steps was carried out after cool down and 2 hours after exercise. Results: The supplementation period was six days. During this period, each of the three experimental groups used HMB, glutamine, and a mixture of these two substances in the recommended amount. In this period, the control group used the placebo. Conclusion: After the supplementation period, post-test data were recorded in a manner similar to the pre-test. Levine's homogeneity of variance test, descriptive statistics, analysis of variance with repeated measures, and Tukey test at a significance level of 0.05 were used for analyzing the data. The results of ANOVA with repeated measures showed that IL-6 and IL-8 in glutamine, HMB, and combined groups revealed significant differences with the control group prior to the activity, after cool-down, and 2 hours after exercise (P<0.05). Based on the findings of this study, the improving impact of interleukin and butyrate beta hydroxysteroid supplements on immune system was confirmed. Consequently, because of possible side effects of combining these two supplements, despite the positive effects, it was not recommended.

INTRODUCTION

The immune system of athletes and its adaptability change during the years of exercise. During the exercise, some adaptations are created in the immune system. Hence, identifying the relationship between exercise, nutrition, and immune system function may result in appropriate exercise programs development and the health of athletes will improve [3].

In the research literature on the relationship between exercise and immune function, the nutritional supplements such as carbohydrates, amino acids, pharmaceuticals, minerals, and vitamins have been recommended as an important factor in the modulation of exercise-induced changes in immune function [4]. Many foods are involved in protein synthesis and energy production and may impact directly the immune cells and indirectly their performance [1]. Therefore, athletes’ diet is important.

The beta hydroxyl steroid beta-methyl butyrate (HMB) is a metabolite derived from leucine and its effects on immune system function and exercise performance has received less attention. Only have a few studies been conducted on the effects of this supplement in animal health-related indices. The findings of the conducted research have proved the safety of this supplement in animals [5,6,7]. Little studies have investigated the safety of HMB complementary in humans. Previous research has shown that using 1.5 to 4 grams per day for seven weeks does not imply any side effects [7,8]. Also, some studies have investigated the effects of HMB supplementation on body metabolism. The findings have indicated a positive effect of HMB supplementation on...
Based on previous research findings on the possible effects of HMB supplementation, it can be predicted that using this supplements along with regular exercise, especially resistance training, reduces exercise-induced muscle damage [9,10], and body fat [11,12]. Increases protein synthesis [10], improves recovery [13], and strengthens the immune system of athletes.

The glutamine is another supplement presumed to have a positive effect on the immune system of the human body. As a fuel and mediator of tri-carboxylic acid cycle (TCA), glutamine is involved in metabolism, and is used as an important fuel in the intestines and digestive system, and it is one of the most important energy substrates in the immune system [1].

Based on previous research findings, it is claimed that glutamine controls the negative biochemical effects of toxic substances which is generated after heavy exercise. For example, one research reported that the glutamine supplement creates ammonium cation by producing ammonia and linking it with hydrogen ions. Therefore, this material can be disposed along with chloride anion and body will be protected against toxic effects of ammonia [1].

Some researchers believe that glutamine may partially reduce the muscle burning which occurs followed by depletion of amino acid pools during heavy exercise. This is especially true for athletes who are over trained. Therefore, it is suggested that glutamine supplementation be conducted during recovery (2).

Given the importance of the immune response to exercise, contradictory research findings regarding the compatibility of the immune system with exercise, and prevalence of supplementation among the athletes in recent years, this study aims to investigate the interactive effect of HMB and Glutamine supplementation on IL6 and IL8.

Methodology:
A) Method:
This was an applied study. Because of using human samples, it was conducted through quasi-experimental method with one pre-test and two post-tests in three experimental groups and one control group.

B) Subjects and sampling method:
Table 1 shows the characteristics of subjects in this study. The study population consisted of 18-24 year-old students of Physical Education in Islamic Azad University of Karaj who entered to university in 2010-2013. After performing various steps, a total of 40 subjects were selected and randomly assigned into four GLU, HMB, GLU + HMB, and carbohydrate groups. All participants were healthy, had no history of alcohol use and smoking, and none of them was menstruating during the study. Table 2 is a summary of preliminary stages of selecting subjects. It also shows the implementation of main protocol. In 2 separate days with an interval of 6 days, subjects performed a session of weight training with 70 to 75% of one maximal repetition. Despite the fact that subjects followed the same diet in the dormitory, the subjects were asked to avoid any intense physical activity and using medications and nutritional supplements in the 24 hours before the exercise session.

<table>
<thead>
<tr>
<th>Group</th>
<th>Height</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine supplementation</td>
<td>165/70 ± 3/26</td>
<td>58/10 ± 5/32</td>
</tr>
<tr>
<td>HMB supplementation</td>
<td>165/00 ± 3/55</td>
<td>71/40 ± 4/32</td>
</tr>
<tr>
<td>Combined Supplementation</td>
<td>162/10 ± 2/80</td>
<td>58/30 ± 5/59</td>
</tr>
<tr>
<td>Control</td>
<td>165/70 ± 3/12</td>
<td>61/50 ± 5/19</td>
</tr>
</tbody>
</table>

C) Supplementation of subjects:
Every day, subjects used the solution of glutamine and HMB in the morning and evening. In the glutamine group, 0.1 g of glutamine per one kg of body weight [18] was used-i-e- half of it in each turns (morning and evening) was dissolved in a cup of water. In the HMB group, 3 g of HMB was used-i-e- half of it in each turns was dissolved in a cup of water. In the glutamine+ HMB group, 1.5 g of HMB and 7 g of glutamine was used-i-e- half of it in each turns (morning and evening) was dissolved in a cup of water. However, half of glutamine and half of HMB were separately dissolved in two glasses of water and were drunk in the morning and evening. Also, the control group received carbohydrates solved in a glass of water (0.1 gram per kilogram of body weight).

Procedure:
In the day of first test, after about 12 hours of fasting, subjects attended in 8 AM in the gym. The first blood sample was collected at 8:30. To reduce the effect of starvation on catabolism, subjects ate a standard breakfast including bread, butter, jam (approximately 300 kcal), and tea and relaxed for some moment. After 10 minutes of warm up, then, subjects conducted weights exercises with 70 to 75% of one maximum repetition intensity. The movements included seven moves including leg press, front thigh with machine, back of the thigh with machine, bench press (flat), the back shoulder press, cable presses behind the neck, and pressing two ends of
arms with an emphasis on eccentric phase of the movements. They were performed in three sets of 8 to 10 repetitions [14]. The rest interval between sets was one minute and between each movement was two minutes. After doing all the movements, subjects performed a five-minute cool-down. The blood samples were then taken after cooling and two hours after the activities [15]. The second phase of the work was done after six days of supplementation phase repeated in the same order. During the supplementation period, subjects continued their daily activity and feeding [16,17].

D) Method of data collection and laboratory analysis:
At each stage, approximately 10 ml of blood was taken from the brachial vein by the tubes. The samples were centrifuged at a speed of 5,000 rpm for 5 min. Their serum was separated and the concentrations of the studied variables were measured with special kits. The used kits were IL-8 Kit (Mediagnost Company of Germany) and IL-6 Kit (R & D Company of America).

E) Statistical methods:
The Kolmogorov-Smirnov test was used to assess the normality of data distribution. To test the hypotheses, the difference between pre-test and post-test at each step were calculated. Then, using analysis of variance with repeated measures on α=0.05, the hypotheses were examined. When the interaction between time and group was significant, further review was conducted in each group using analysis of variance with repeated measures and one-way analysis of variance (ANOVA). The statistical tests were performed in SPSS version 16.

Table 2: Summary of preliminary stages and main protocol of data collection

<table>
<thead>
<tr>
<th>7 days before the first test run</th>
<th>Selecting subjects by questionnaire</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 days before the first test run</td>
<td>Measurement of height, weight, determining one maximal repetition, familiarity with exercise protocol, and receiving informed consent</td>
</tr>
<tr>
<td>On the first test run</td>
<td>Fasting blood sampling</td>
</tr>
<tr>
<td></td>
<td>Eating breakfast</td>
</tr>
<tr>
<td></td>
<td>Conducting maximal exercise activity</td>
</tr>
<tr>
<td></td>
<td>Blood sampling immediately after cooling</td>
</tr>
<tr>
<td></td>
<td>Blood sampling 2 hours after maximal exercise</td>
</tr>
</tbody>
</table>

The results for IL-6 showed that the Mauchly’s sphericity hypothesis exists (P>0.05). The results of ANOVA with repeated measures showed a significant interaction between time and groups (P=0.000, F_{6,72}^*=8.980). Also, the main effect of time (P=0.000, F_{2,72}^*=48.688) and the main effect of group (P=0.000, F_{1,375}^*=10.775) was significant. Further review was conducted in each group using analysis of variance with repeated measures, one-way analysis of variance (ANOVA), and Tukey test. The post hoc test results can be seen in Table 3.

Table 3: Results of IL-6 rate before the activity, after cool-down, and 2 hours after exercise, the results of ANOVA with repeated measurements, and the results of within- groups and between- group tests.

<table>
<thead>
<tr>
<th>Group</th>
<th>Before the activity</th>
<th>After cooling down</th>
<th>Two hours after activity</th>
<th>Intergroup comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamine</td>
<td>t=0.17 ± 0.13</td>
<td>t=0.25 ± 0.12</td>
<td>t=0.56 ± 0.19</td>
<td>F = 23.968, P = 0.000</td>
</tr>
<tr>
<td>HMB</td>
<td>t=0.05 ± 0.10</td>
<td>t=0.06 ± 0.07</td>
<td>t=0.06 ± 0.07</td>
<td>F = 1/126, P = 0.358</td>
</tr>
<tr>
<td>Combined</td>
<td>t=0.08 ± 0.05</td>
<td>t=0.08 ± 0.08</td>
<td>t=0.31 ± 0.11</td>
<td>F = 70/182, P = 0.000</td>
</tr>
<tr>
<td>Control</td>
<td>-0.11 ± 0.14</td>
<td>-0.09 ± 0.18</td>
<td>-0.10 ± 0.11</td>
<td>F = 0/31, P = 0.029</td>
</tr>
<tr>
<td>Comparison</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>between groups</td>
<td>= 12/503 *P = 0.000</td>
<td>= 5/522 *P = 0.003</td>
<td>F = 16/792 *P = 0.000</td>
<td></td>
</tr>
</tbody>
</table>

The results for IL-8 showed that the Mauchly’s sphericity hypothesis exists (P>0.05). The results of ANOVA with repeated measures showed a significant interaction between time and groups (P=0.001, F_{6,72}^*=4.435). Also, the main effect of time (P=0.000, F_{2,72}^*=46.741) and the main effect of group (P=0.013, F_{1,3}=4.109) was significant. Further review was conducted in each group using analysis of variance with repeated measures, one-way analysis of variance (ANOVA), and Tukey test. The post hoc test results can be seen in Table 4.
Discussion:
This study aimed to investigate the supplementation effect of HMB in combined with Glutamine on immune response of female students after a maximal exercise session. The results of present study showed that one maximal activity increases IL-6 and IL-8. This increase immediately after cool-down was 4.31% for IL-6 and 12.57% for IL-8. This increase was consistent with the results of Revere and colleagues (1994), Wayne Stokes et al. (1997), Esther Vicky et al. (1999), Timothy et al. (1999), Moold Wano et al. (2000), Schmidt and colleagues (2000), Ney Mann et al. (2001), Margili et al. (2005), and Gooyayum and colleagues (2007).

By continuing the activities, especially maximal activity, and reducing carbohydrate, it was predicted that increased exercise stress increases considerably IL-6 and partially IL-8. This increase is justifiable by reduction of carbohydrate, reduction of muscle glycogen, and also somewhat through reduction of amino acid pool which creates major metabolism changes.

The results showed that after glutamine supplementation, the levels of IL-6 and IL-8 before the activity decreased 5.17% and 12.57%, respectively. However, the results showed that after supplementation with glutamine, the amount of IL-6 and IL-8 after cool-down revealed significant difference with before the activity and 2 hours after exercise.

After loading glutamine supplementation, it is possible that during the activity, considerable metabolic changes leading to increased secretion of six-eight IL do not occur. It can also be said that, the secretion of 6-8 IL decreased by changing the activity of immune system, since these two cytokines are proinflammatory cytokines and are affected by the activity of immune system.

The results showed that the levels of IL-6 and IL-8 before the activity decreased 1.43% and 7.69%, respectively after HMB supplementation. However, the results showed that after supplementation with HMB, the amount of IL-6 and IL-8 after cool-down and 2 hours after exercise did not reveal any significant difference with before the activity.

It seems that HMB supplementation does not lead to metabolic changes and considerable improvement in the activity of immune system. The reduced secretion of six and eight interleukin after the supplementation period is justifiable by increased fat metabolism, and decreased blood glucose depletion and muscle glycogen after the HMB supplementation.

The results showed that after combined supplementation, the levels of IL-6 and IL-8 before the activity decreased 2.60% and 10.89%, respectively. However, the results showed that after combined supplementation, the amount of IL-6 and IL-8 after 2 hours of exercise revealed significant difference with before the activity.

Overall, the results showed that IL-6 and IL-8 in glutamine, HMB, and combined groups had significant difference with the control group prior to the activity, after cooling, and 2 hours after exercise.

Conclusion:

Based on the findings of this study, the improving impact of interleukin and butyrate beta hydroxysteroid supplements on immune system is confirmed. To sum up, because of possible side effects of combining these two supplements, despite the positive effects, they are not recommended.

REFERENCES