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## Effects of BCAA Plus Glucose Supplement Timing on Inflammatory Response Indicators after a Resistant Exercise

Guoqiang Geng,Junqiang Qiu,Longyan Yi,Yangmengrou Pan,Meiyao Li Beijing Sport University

**Objective** A single bout of high-intensity exercise (such as resistant exercise) may result in oxidative stress and impaired immunity, for example, excessive-inflammation and compensatory immunosuppression during the recovery period. BCAA supplement has been reported that it can reduce immunosuppression and excessive inflammation after high-intensity resistant exercise. The purpose of this study was conducted to compare the effects of one-time BCAA plus glucose supplement at two different points of time, two hours before exercise and after it immediately, then figure out a better timing for improving human's impaired immune function after resistant exercise.

**Methods** The study was a randomized, controlled, one-blind crossover trial. The entire test lasted two weeks. It recruited 16 non-trained healthy male college students. They were divided into two groups (each group contained 8 people), pre-exercise supplement group (group A) and post-exercise supplement group (group B). All subjects have not had other high-intensity exercises at least 1week before this study. Also, they did not have any exercise-induced injury or physical discomfort.

All subjects needed to ingest BCAA plus glucose and placebo supplement in 2 weeks respectively and they consumed them at 2 different timing (group A or B). Because of digestion time of the capsule, subjects of group A consumed supplements 0.5h before exercise, group B need to consume them after exercise immediately. At the test day, subjects were asked not to eat anything 3 hours before their resistant exercise. Besides, subjects' diets were recorded for 2 weeks experience, to make sure if they consumed other excessive essential amino-acid which may influence this study. And the study compared each subject's 2 different results and figured out whether one-time BCAA plus glucose supplement could improve human's impaired immune function after resistant exercise and which timing was better.

This exercise was designed to be a circular centrifugal movement which contained 3 different training subjects, including 5 sets of 95%10RM seated leg curl machine training, 3 sets of the maximum Dropjumps and 2 sets of the maximum strength continuous 20m frog jump. The whole resistant exercise lasted about 1 hour. All subjects went through the same exercise and rest 7 days, then they repeated it in next week.

All subjects were taken 8 blood samples in vein in 2 weeks. In other words, there were 4 timing of taking subjects' blood samples in each test. Samples would be placed on a shaker for 1h at-20°C, before being centrifuged for 10 mins at 3,000 g.

The test indicators of this study included serum interleukin-6 (IL-6), serum C-reactive protein (CRP), serum immunoglobulin a (Iga) and DOMS soreness. Among them, the level serum IL-6 was tested using a human interleukin 6 ELISA kit in a double antibody one-step sandwich enzyme-linked immunosorbent assay. The serum C-reactive protein index was tested by a human C-reactive protein (CRP) ELISA kit, which is an experimental method for enzyme-linked immunosorbent assay. Serum Iga was analyzed by collecting peripheral anticoagulation and then using an automated biochemical analyzer. The degree of muscle soreness was evaluated using a visual simulation evaluation method. Participants will be asked to perform and hold a squat (90°knee angle) whilst they rated their perceived muscle soreness on a 200 mm visual analog scale The scale consisted of a line from 0 mm (no pain) to 200mm.

Supplement protocol: The pure BCAA supplement contained a ratio of 2:1:1 (leucine, isoleucine, and valine, respectively), a dose of 40 mg per kilogram of body weight and glucose supplement was designed to a dose of 24 mg per kilogram of body weight. The form of BCAA was the capsule, and the glucose supplement was in the powder form; each serving was mixed with 300 ml of water. The placebo was the same dose of oligosaccharides in capsule, and artificial sweetener in 300ml of water to match the taste of glucose supplement.

Data analysis was performed using the PC version of SPSS 19.0 software. All indicators were statistically analyzed using repeated measures analysis. If the interaction of supplement x timing was significant, the independent sample t test was performed between the groups, and the paired sample t test or one-way ANOVA was performed in the group.

**Results** In this study, all the data from the two weeks were divided into four groups according to the timing of supplementation and the supplementary category. They were the pre-exercise, placeco group(called A- group), the post-exercise, placeco group (called B- group), the pre-exercise, BCAA plus glucose group(A+ group), the post-exercise, BCAA plus glucose group(B+ group). The data of A- group and A+ group were compared to determine the effectiveness of BCAA plus glucose supplement on anti-inflammatory response. Also, it's the same goal to compare the data of B- group and B+ group. Then, through comparison between the the pre-exercise, BCAA plus glucose group and the post-exercise, BCAA plus glucose group, it is determined which supplemental timing IS better for lower resistant exercise-induced inflammatory response.

The results showed that serum IL-6 levels were  $208.35\pm11.34$  pg/ml in the pre-exercise, BCAA plus glucose group and  $273.08\pm4.76$  pg /ml in the pre-exercise, placeco group at 24 h after exercise. There was significantly lower in A+ group(p<0.05); the serum IL-6 level in the post-exercise, BCAA plus glucose group was 169.99±10.85 pg/ml, while the serum IL-6 value of  $303.78\pm8.79$  pg/ml in the post-exercise, placeco group. There was a significant decrease in B+ group comparing with the level of B-group (p < 0.05). Besides, the serum IL-6 level in the post-exercise, placeco group was lower than that in the pre-exercise, BCAA plus glucose group, and there was a significant difference at 24 h after exercise (p<0.05).

Serum CRP at 24 h after exercise, the serum CRP value of the pre-exercise, BCAA plus glucose group was 4.26  $\pm$  0.29 mg /L, significantly lower than the data of CRP in pre-exercise, placeco group (4.64  $\pm$  0.35 mg/L,p < 0.05). Similarly, the supplement The serum CRP level of post-exercise, BCAA plus glucose group was 3.75 $\pm$ 0.44 mg/L, which was significantly different from it in post-exercise, placeco group (p<0.05). At timing of 48h after exercise, the serum CRP level of the B+ group was 3.92 $\pm$ 0.24mg/L, which was significantly lower than the serum CRP value of the B- group after 48h (4.4 $\pm$ 0.29 mg/L), and there was a significant difference (p<0.05). In addition, at the timing of 24 hour after exercise, the serum CRP level of the post-exercise, BCAA plus glucose group was significantly lower than it in pre-exercise, placeco group, and there was a significant difference (p<0.05). There was no significant difference in the data of exercise–induced immunoglobulin of each group after centrifugation. As a direct indicator of the systemic inflammatory response, serum IL-6 and CRP can significantly reflect the inflammatory response induced by BCAA combined with glucose supplementation at 24 and 48 hours after exercise. And instead of Supplement before exercise, it is better for lowering resistant exercise-induced inflammatory response to supply it after exercise immediately.

The degree of delayed onset muscle soreness is considered to be a clear indicator of the local inflammatory response. The Visual Analogue Scale(VAS) of delayed onset muscle soreness (DOMS) was  $2.63\pm1.55$  in A+ group at 24 h after exercise, was significantly lower than it ( $3.31\pm1.54$ ) in the A-group (p<0.05). And the level of VAS in the B+ group was  $1.27\pm0.72$ , which was significantly lower than it in the B- group at 24 h after exercise ( $2.86\pm1.77$ , p<0.05). At 48 h after exercise, the DOMS level in the post-exercise, BCAA plus glucose group( $1.36\pm0.85$ ) was significantly lower than the 48-hour DOMS level in the post-exercise, placeco group ( $4.4\pm0.29$  mg/L). In addition, at the timing of 24 hour after exercise, the VAS of DOMS in the post-exercise, BCAA plus glucose group was significantly lower than it in pre-exercise, placeco group(9.0.05).

**Conclusions** Acute and one-time BCAA plus glucose supplement can reduce the exercise-induced systemic inflammatory response (serum IL-6 and serum CRP) and local inflammatory response (DOMS) 24 h and/or 48 h after a resistant exercise.

Besides, compared with a supplementation before a resistant exercise, it's more effective to supply BCAA plus glucose after the exercise immediately for reducing body's exercise-induced inflammatory response damage.