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The Effect of BCAA Supplementation on Serum C - Reactive Protein and Creatine Kinase after Acute Resistance Exercise in Soccer Players

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ABSTRACT

While regular exercise training is associated with numerous health benefits, a single bout of physical exercise has been shown to induce inflammatory process and cellular damage. However, natural supplementation may attenuate these in athletes. Hence, the present study was conducted to assess the effect of BCAA supplementation on serum C - reactive protein, creatine kinase (CK) after acute resistance exercise in soccer players. 20 male subjects (age: 21.5 ±1.7 years; height: 177.3 ± 1.1 cm; weight: 77.2 ±2.2 kg) completed 3 sets of 8-10 repetitions at their 8-10 repetition maximum to volitional fatigue. The exercise order consisted of the high pull, leg curl, standing overhead press, leg extension, lat pull-down, leg press, and bench press. In a double-blind, posttest-only control group design, subjects consumed 200 mg.kg^{-1} BW of either BCAA or placebo (carbohydrate (dextrin)) 30 minutes prior to exercise. To identify ck activity and HS-CRP, venous blood samples were obtained 30 min prior to and immediately following exercise and at 1 hr, 2 hrs, 24 hrs post exercise. Data were statistically analyzed using 2-way repeated measure ANOVA and Bonfferoni test. Baseline CK and Hs-CRP were determined 30 minutes before the exercise test. Baseline serum values for CK, Hs-CRP were not different between groups in the 30 minutes before the exercise test ($p>0/05$). However there were significant increases ($p<0/05$) between the pre exercise and post exercise values for CK, Hs-CRP from 24 hrs posttest, ($p< 0.05$). Importantly, the BCAA supplementation significantly reduced this change in CK from 24hrs post-test, and Hs-CRP 24hrs post-test ($p< 0.05$). Conclusions: These results indicate that supplementary BCAA decreased serum concentrations of the intramuscular enzyme CK and inflammation biomarker Hs-CRP following resistance exercise. This observation suggests that BCAA supplementation may reduce the muscle damage and inflammation biomarker associated with resistance exercise.

Key words: branch-chain amino acid, creatine kinase, C - reactive protein, resistance exercise.

INTRODUCTION

Exercise-induced muscle damage and its clinical corollary (delayed onset on muscular soreness, DOMS) often occur due to the predominance of eccentric exercise. The injury itself is a mechanical disturbance of the sarcomeres provoked by an inflammatory response [1-5]. PGE2 is the direct cause of the pain, whereas leukotrienes increase the vascular permeability and attract neutrophils to the site of the damage. Together, these two factors result in the feeling of inflammation felt by the sportsperson after a hard training session [6]. In contrast, the other type of muscle inflammation commonly found in high performance sports, which occurs 1-2 days after a heavy exercise session and/or competition [1,7]. Some previous studies declared that intense exercise such as extended periods of running, strength training or sprinting, can lead to disruption of the normal muscle ultrastructure and impairment of muscle function [8-11]. These changes are accompanied by an increase in muscle proteins in blood, which are useful markers of skeletal muscle injury. In attempting to quantify this damage, creatine kinase (CK) has been used as blood measurements of intramuscular enzymes [12, 13].

Moreover, acute physical exercise is accompanied by inflammatory responses which are also found in the plasma concentrations of various substances which affect the function of leukocytes, including inflammatory cytokines such as TNF- α, the inflammatory macrophages protein-1 and IL-1, as well as acute-phase proteins, including High-sensitivity C-reactive protein (Hs-CRP). Hs-CRP is an acute phase protein involved in systemic inflammation [7]. In fact exercise-induced muscle damage and inflammation lead to changes and imbalances in the immunological system along with an “acute phase response” [7]. Hs-CRP has the analytic and assay characteristics that are most conducive for clinical use and has shown a dose-response relationship to coronary heart disease that is independent of other major risk factors [14].

It is assumed that the suppression of muscle damage and/or inflammation can be attenuated the muscle soreness induced by exercise [14]. Furthermore, various supplemental protocols have been investigated as a method to attenuate levels of muscle damage, and subsequent reductions in functionality of the muscle, following a bout of resistance exercise [16-19]. Many of these investigations have utilized supplements containing amino acids, though the composition of specific amino acids has been highly variable. Of particular interest have been the branched-chain amino acids (BCAAs), due to their relative abundance in skeletal muscle. BCAA supplementation has also been shown to reduce the level of delayed-onset muscle soreness (DOMS) experienced 24 h post-exercise and attenuated the decrease in leg-flexion torque 48 h post-exercise [20]. For example, Greer et al (2007) reported that acute ingestion of 50g of BCAAs resulted in significantly lower CK levels at 4, 24, and 48 hours following a cycling protocol of 90 minutes at 55% of V02 peak [21]. Also recently Shimomura et al, administered 100 mg/kg body weight of BCAAs or a placebo to women (21–24 years of age) prior to resistance exercise and found that muscle soreness was lower after BCAA ingestion compared to a placebo [22]. But Zebblin et al has been shown that BCAA supplementation administration before mild resistance exercise had no effect on serum CK activity [23]. However, the actions and effects of BCAA supplementation on inflammation Following a Resistance Exercise Bout have not previously been reported.

Consequently according to there is not enough research about the acute effects of BCAA supplementation on levels of CRP and muscle damage after resistance exercise, Also no research was found about the effects of BCAA supplementation after a single bout of resistance exercise on blood levels of these risk factors (Hs-CRP) in healthy soccer players' men. Hence, this study

was conducted to investigate the acute effect of BCAA supplementation after one session resistance exercise on muscle damage and Hs-CRP.

MATERIALS AND METHODS

Subjects

20 young soccer players (age: 21.5 ±1.7 years; height: 177.3 ± 1.1 cm; weight: 77.2 ±2.2 kg, Mean ±SD) in a randomized and double-blind design were divided in two supplemental and placebo groups. They have three session training per week. In addition, they have no past history of kidney, heart and liver disease, diabetes or any physical damage and problems and no used any medicinal drugs, dietary supplements, or anabolic steroids within the previous six month. The study design and experimental procedures approved by the Regional Research Ethics Committee of Islamic Azad University, Mahabad Branch and conducted in the laboratory conditions (temperature 22—25◦ C; humidity 50—55%).

Supplementation protocol:

The subjects in the supplemented group were given 200 mg.kg $^{-1}$ of BCAA (comprised of 50% leucine, 25% isoleucine, 25% valine, producted Pooyan Nutrition Company). The supplement was taken directly 30 minutes before the exercise test for supplement. The control group received a placebo of the same form and size of capsule as the omega-3 fatty acid capsules and instructed to ingest these at the same time. Dosages of supplement were based on manufacturer's recommendations and previous human BCAA supplementation studies.

Exercise protocol

Two days prior to initiation of the exercise protocol, all participants underwent a familiarization and correct handling procedure session of the training equipment that would be utilized during the study. Subjects were requested to avoid exercise the day of the test session. The true one repetition maximum (1RM) for the upper and lower extremities was estimated according to the National Strength and Conditioning Association guidelines and calculated using the Brzycki (1995) equation ([1-RM=Weight / (1.0278 - (0.0278 × Number of repetitions)]).The exercise session consisted of seven resistance exercises: high pull, lat pull-down, standing overhead press, leg extension, leg curl, leg press, and bench press. The participants were instructed to lift one warm-up set of 8 repetitions at 80% 1RM, then 1 set of 8 repetitions at 100% 1RM. For the third set, the resistance was the same as the second set (100% 1RM), but the subjects were asked to lift as many repetitions until volitional fatigue.

Blood collection and analysis procedures

Baseline muscle damage and inflammation biomarker was evaluated using the measurements of serum CK and Hs-CRP levels. Venous blood samples were drawn by antecubital venipuncture 30 minutes before the exercise test and at the immediately post-exercise, 1, 2 and 24 hrs after the exercise test. The blood was immediately centrifuged at 1500 RCF for 10 min at 4°C, and the plasma was separated and stored in Eppendorf tubes at -70°C for subsequent use. Plasma levels of C-reactive protein (CRP) were measured by a highly sensitive enzyme linked immunosorbent assay (ELISA) technique as described previously [24]. Afterward, serum was separated by a centrifuge (SAHAND Co) and serum CK activity, as cellular damage indices, determined by commercial kits (Sigma Chemical Co) with automatic analyzers (RA-1000; American TECHNICOM Co).

Statistical Analyses

The Kolmogorov-Smirnov test of normality revealed that none of the variables studied required logarithmic transformation. Values are expressed as the mean ± SD. and were compared with 2-way repeated measure ANNOVA. Bonferroni test was used in order to learn which measurement time the difference comes from. Statistical significance was set at $p<0.05$.

RESULTS

Physiological characteristics of the subjects at the beginning of the research are presented in Table 1.There were no significant differences among groups for age, bodyweight, height, percent body fat or lean mass among two groups. ($p > 0.05$).

Table1. Physiological characteristics of supplement and placebo groups (N = 20)

Characteristics	Supplementation	Placebo	P
Age (yr)	20.8±2	21.2±2	0.5
Height (cm)	177.3±1.1	176.2±1.0	0.8
Weight (kg)	76.1±2.3	75.9±2.6	0.7
% Body Fat	15.1±0.9	15.7±0.7	0.7

Values are mean ±SD

Mean serum CK levels before, immediately post-exercise ,1,2 and 24 hrs after the resistance exercise is presented in Fig1. The serum CK activity before the resistance exercise did not differ between the BCAA and placebo group, and the CK activity significantly increased due to the resistance exercise in both groups at 1,2 and 24 hrs after resistance exercise. However, the serum CK activity after resistance exercise in the BCAA group were significantly lower than those in the placebo group in the 24 hrs after resistance exercise ($P<0.05$).

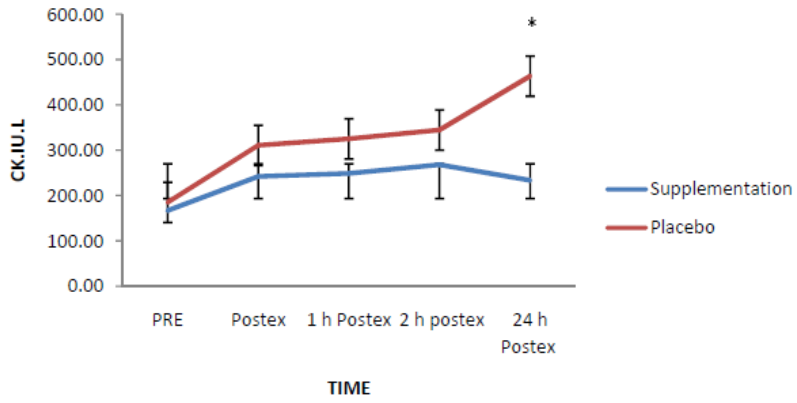

Figure 1: Serum creatine kinase (CK) concentration across the 24 hours.
**Indicated significant ($p < 0.05$) difference vs. baseline*

Mean serum CRP levels before, immediately post-exercise, 1, 2 and 24 hrs after the resistance exercise is presented in Fig 2. The serum CRP levels before the resistance exercise did not differ between the BCAA and placebo group, and the CRP levels significantly increased due to the resistance exercise in both groups at 2 and 24 hrs after resistance exercise. However, the serum CRP level after resistance exercise in the BCAA group were significantly lower than those in the placebo group in the 24 hrs after resistance exercise ($P<0.05$).

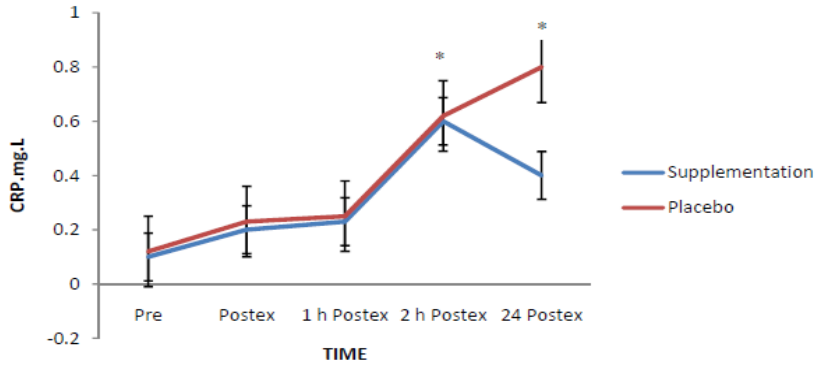

Figure 2: Serum C-reactive protein (CRP) concentration across the 24 hours.
**Indicated significant ($p < 0.05$) difference vs. baseline*

DISCUSSION

In the present study, we found that the BCAA supplementation can attenuate increase in extent of blood CK in young soccer players following acute exercise. Similarly, Coombes et al reported that BCAA supplementation led to lower serum CK activity that were predictor of muscle damage after endurance exercise in comparison to those without BCAA supplementation [25]. Greer et al study also has been shown that acute ingestion of 50g of BCAAs resulted in significantly lower CK levels at 4, 24, and 48 hours following a cycling protocol of 90 minutes at 55% of V02 peak [22]. In addition, Koba et al reported that consuming BCAAs during and in the days following running exercise had effect on CK level [14]. Nevertheless this result is contrast to findings of Jackman et al (2010) who reported that consuming BCAAs during and in the days following unilateral eccentric exercise had no effect on CK levels [19]. Possible explanations for difference between our findings and other published data could include ethnic, age, subjects, protocol exercise, intensity of exercise. Total creatine kinase (CK) levels depend on age, gender, race, muscle mass, physical activity and climatic condition. High levels of serum CK in apparently healthy subjects may be correlated with physical training status, as they depend on sarcomeric damage: strenuous exercise that damages skeletal muscle cells results in increased total serum CK [26, 27].

BCAA administration leads to protein synthesis, particularly Leucine demonstrates a direct anabolic effect. On the other hand, Maclean et al has shown that skeletal muscle protein degradation decrease after a 77 mg/kg weight BCAA supplementation before and during cycle exercise [28]. Although the mechanisms responsible for this improvement are not entirely clear, but it has been suggested that the 2 g of BCAA intake at the onset of exercise can effectively suppress skeletal muscle proteolysis induced by endurance exercise at moderate intensity [29]. As a result, BCAA administration may be beneficial in preventing muscle protein degradation, Because, Muscle proteolysis during exercise has been shown to be associated with muscle damage [13]. Moreover, BCAA oral supplementation before exercise in human has been reported to induce an increase in the BCAA concentration in the muscle fiber, thereby suppressing the protein breakdown of the muscle fiber during exercise, and suppressing the increase in serum CK activity induced by exercise [14].

To our knowledge this is the first study that has investigated the effects of BCAA supplementation on CRP following resistance exercise bout. The result of this study showed that BCAA supplementation decreased indictor of inflammation following resistance exercise. The mechanical damage can result in a variety of inflammatory responses, initially due to infiltrating cells, such as neutrophils and macrophages within the muscle that result in increased muscle,

blood, and even brain concentrations of inflammatory cytokines. These responses can have profound effects on both physical and mental function (fatigue, perceived discomfort, impaired mood, and perhaps other cognitive deficits [31].

Several investigations have examined the ability of nutritional intervention to attenuate the post-exercise inflammatory response. Carbohydrate ingestion and a vitamin E and omega-3 fatty acid combination have been successful in attenuating the IL-6 response to exercise [31]. BCCA supplementation has been shown to alter the acute inflammatory response following exercise protocols that produce muscle damage [32]. Bassit et al. (2002) reported that BCAA supplementation stimulated the production of interleukin-2 and interferon-c and a suppression of interleukin-4 following a triathlon. These results indicated that BCAA supplementation is effective in keeping plasma glutamine concentration constant after a triathlon and long-distance run, and that this procedure is important for maintaining the Th1 cell response after exercise [32].

We observed a considerable increase in he-CRP concentration after the resistance exercise. It has reported that the acute exercise results in a first, rapid and profound neutrophilia (increase in blood neutrophil count) which is linked to intensity and duration of exercise. This increase is likely due to demargination caused by shear stress and catecholamine's [33]. CRP is another acute phase reactant which is released in response to infection, surgical trauma, and tissue injury resulting from intense exercise. CRP is a factor produced by liver in acute phases of infection. Its evaluation is a suitable index for assessment of infection progression or severity. CRP levels rise considerably in intense and long exercise. An intensive exercise may increase the primary level of CRP [34].

Local response to an infection or injury requires cytokine production which is released at the site of inflammation. The local inflammatory response accompanies a systemic response known as the acute phase response, involving production of many acute phase proteins such as CRP, macroglobulin α2 and transferin. Fitness level is also an important factor in CRP changes and for this reason; exercises of smaller intensity and duration are able to induce acute phase response in non-athlete individuals [34, 35]. Inflammation is known to be a critical component during the muscle repair and regeneration periods [36], but the effect of BCAA supplementation on these processes requires further investigation.

CONCLUSION

Taken together, our data led us to conclude that resistance exercise program induced increased Muscle damage, the leakage of protein into blood from skeletal muscle, and an inflammatory response. However, The BCAA supplementation may ameliorate these markers after acute resistance exercise in young soccer players, and therefore can an effective devise to preventing of Muscle damage and inflammatory response in young athletes.

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