

Original Article

Effects of Supplementation with BCAA and L-glutamine on Blood Fatigue Factors and Cytokines in Juvenile Athletes Submitted to Maximal Intensity Rowing Performance

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Abstract. [Purpose] This study was conducted to understand the impacts of BCAA (branched-chain amino acid) and glutamine supplementation on the degree of blood fatigue factor stimulation and cytokines along with performance of exercise at the maximal intensity. [Subjects] Five male juvenile elite rowing athletes participated in this study as the subjects; they took 3 tests and received placebo supplementation (PS), BCAA supplementation (BS), and glutamine supplementation (GS). [Methods] The exercise applied in the tests was 2,000 m of rowing at the maximal intensity using an indoor rowing machine, and blood samples were collected 3 times, while resting, at the end of exercise, and after 30 min of recovery, to analyze the blood fatigue factors (lactate, phosphorous, ammonia, creatine kinase (CK)) and blood cytokines (IL (interleukin)-6, 8, 15). [Results] The results of the analysis showed that the levels of blood phosphorous in the BS and GS groups at the recovery stage were decreased significantly compared with at the end of exercise, and the level of CK appeared lower in the GS group alone at recovery stage than at the end of exercise. The level of blood IL-15 in the PS and BS groups appeared higher at the end of exercise compared with the resting stage. [Conclusion] It seemed that glutamine supplementation had a positive effect on the decrease in fatigue factor stimulation at the recovery stage after maximal intensity exercise compared with supplementation with the placebo or BCAA. Besides, pre-exercise glutamine supplementation seemed to help enhance immune function and the defensive inflammatory reaction.

Key words: BCAA, Glutamine, Exercise

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INTRODUCTION

High-intensity exercise can cause temporary fatigue due to ATP depletion and excessive production of hydrogen ions and ammonia. Eventually, the accumulation of fatigue factors can lead to poor exercise performance. In addition, intense exercise causes muscle tissue damage that gives rise to an inflammation response. In these individuals, a higher frequency of lesions, chronic inflammatory processes, reports of muscle pain, and discomfort are observed, and these effects harm athletic performance¹⁾. Exercise with high energy consumption or a high repetition rate, such as rowing, can release intracellular proteins in the blood, such as CK, lactate, and cytokines, independent of the nature of the muscle damage. Activation of the inflammatory mecha-

nism involves the synthesis and release of pro-inflammatory mediators such as cytokines²⁾. The mechanisms involved, however, have not been fully determined and seem to be multifactorial, including such things as training and nutritional status.

BCAA is the only amino acid metabolized in skeletal muscles, and its effects as a precursor for glutamine synthesis have been studied broadly. Regarding the impacts of BCAA supplementation, studies have been reported with contradistinctive results indicating that the BCAA acts as a major energy substrate in the muscle along with the increased contribution of fat as an energy source in accordance with the exhaustion of carbohydrates during long periods of exercise³⁾ while also indicating that the increase in the degree of oxidation of BCAA triggers a gradual decrease in intermediates in the TCA cycle, which could cause fatigue due to exhaustion of energy⁴⁾. However, glutamine is a sort of amino acid found abundantly in human skeletal muscles and blood. It maintains the acid-base balance while acidosis occurs and acts as a nitrogen precursor to synthesize nucleotides, as a fuel in muscles, and as a direct controller for the synthesis and decomposition of protein⁵⁾. Additionally, glutamine has been reported to be an important fuel for immunostimulatory effects⁶⁾. The glutamine

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level, however, is lower after exhaustive exercise, and its provision as an oral supplement after exercise has beneficial effects on infections in athletes⁷. Furthermore, athletes experiencing discomfort from overtraining exhibit lower levels of plasma glutamine. Thus, glutamine supplementation is very important for growing juvenile athletes. However, several studies of oral supplementation of substantial amounts of glutamine in athletes have failed to demonstrate enhancements in immune function or performance parameters. Recently, however, Cruzat et al. demonstrated that a solution containing glutamine represents an effective means of supplementing rats with glutamine, attenuating inflammation markers and plasma CK levels induced by intense exercise⁸.

In the present study, we investigated the effects of supplementation with BCAA and glutamine on blood concentrations of lactate, phosphorus, ammonia, CK, and cytokines in juvenile athletes subjected to maximal intensity rowing exercise.

SUBJECTS AND METHODS

Five male juvenile athletes (mean age (\pm SD), height, weight, and percent body fat were 17.2 ± 1.1 y, 176.2 ± 2.9 cm, 71.7 ± 12.2 kg, and $17.7\pm 5.6\%$) were included in this study. Prior to participation, all subjects provided written informed consent in compliance with the appropriate institutional review board at the university.

The athletes were allowed to drink and eat normally but received a placebo, BCAA, or glutamine for 7 days before the test. BCAA (Spomax, Seoul, Republic of Korea) was given three times a day (25% valine, 50% leucine, 25% isoleucine, 3.15 g/day), and L-glutamine (Optimum Nutrition, Aurora, IL, USA, 6 g/day) was given three times a day. Blood samples were collected from the antecubital vein on the day of testing while resting before the test, immediately at the end of test, and 30 min after the test. All tests were conducted with a 1-week interval to eliminate the potential effects from remaining dosages.

The rowing test was conducted with an indoor rowing machine (Concept2, Morrisville, VT, USA) 3 times each for supplementation with the placebo, BCAA, and glutamine. All the subjects performed a 2,000 m (Olympic single scull race) race at their own individual maximum paces (42–45 pace for 0m–250m, 40 pace for 250m–500m, 36–38 pace for 500m–1,500 m, and over 42 pace for 1,500 m–2,000 m)⁹.

The blood lactate concentration was measured using an Accutrend Plus System (Roche, Indianapolis, IN, USA). Drops of blood were collected from fingertips washed and dried completely using a blood gathering device. Reagent paper was then inserted into the device for 60 seconds, and measurements were obtained thereafter. The blood phosphorous concentration was analyzed using a Hitachi 747 analyzer (Hitachi, Tokyo, Japan) by applying the UV method. A 0.5ml sample of centrifuged blood serum was prepared to develop the color by applying a reagent containing sulfuric acid, 250 μ l of added surfactant, and ammonium molybdate for measurement with a dominant wavelength of

340 nm and sub-wavelength of 505 nm. The blood ammonia concentration was analyzed using a Shimadzu CL-750 spectrophotometer (Poli, Milano, Italy) by identifying the reaction. At first, 1ml of blood was added into 2ml of deproteinization solution to remove enzymatic activity, which could create blood ammonia, and the supernatant was separated after centrifugation. Then 4% of phenol, 0.015% of nitroprusside salt, and 4.1% of KOH were added to make it alkaline, and the color was developed using a reagent containing 28% of potassium carbonate and 3% of potassium chlorate for measurement at a wavelength of 630 nm. CK activity was analyzed with a Hitachi 7600-110 chemistry analyzer (Hitachi, Tokyo, Japan) by applying the UV method. Eight microliters of blood serum was separated to develop the color at 37 °C using 75 μ l of reagent R2 and 300 μ l of reagent R1, and it was measured at a dominant wavelength of 340 nm and sub-wavelength of 405 nm.

The biochemical analyses for IL-6, IL-8, and IL-15 were conducted with the Bio-Plex Pro Magnetic Cytokine assay (Bio-Rad, Hercules, CA, USA), and it was directly analyzed using a human serum cytokine kit. All components were stored at 4 °C, and a multichannel pipette, reagent reservoir, plate shaker, and vacuum manifold were prepared for the test. The standard, cytokine, and magnetic beads were each diluted, and 50 μ l of diluted standard and diluted samples were sprayed into each of the wells and washed 3 times after 30 minutes of incubation in the shaker. Then, 50 μ l of prepared detection antibody were sprayed into each well and washed 3 times after incubation for 30 minutes in the shaker; using the assay buffer, 50 μ l of prepared streptavidin-PE were sprayed into each well and washed 3 times after incubation for 10 minutes in the shaker. Finally, 125 μ l of assay buffer were sprayed into each well and shook for 30 seconds and the states of calibration and washing were analyzed using the Bio-Plex Manager software (Bio-rad, Hercules, CA, USA). All samples were duplicated.

Data were analyzed using the SPSS 18.0 for Windows computer software package (SPSS, Chicago, IL, USA). Data are expressed as means \pm SD. All the data were tested for normal distribution using the Shapiro-Wilk test. Comparisons of variables between the groups and times of the test were analyzed by two-way mixed model repeated measures ANOVA. In the case of a significant group and time main effect, two-tailed post hoc analysis was performed using Duncan's test. The alpha level was set a priori to $p=0.05$ to determine statistically significant differences.

RESULTS

The blood lactate concentration varied at each stage ($p<0.05$) but was not significantly different between groups. The blood lactate concentration at the end of exercise appeared higher in all 3 groups compared with in the resting stage ($p<0.05$), and those of all 3 groups in the recovery stage were lower than those at the end of exercise. The blood phosphorous concentration differed stage by stage; however, the differences between groups were insignificant ($p<0.05$). The blood phosphorous concentrations of the 3 groups at the end of exercise were all higher than those in

Table 1. Blood fatigue factors

Items	Group	Resting	End of exercise	Recovery
Lactate (mmol/l)	PS	2.26±1.09	8.28±2.92*	4.94±1.79*†
	BS	1.66±0.43	9.24±3.15*	6.28±1.12*†
	GS	2.30±0.37	10.54±0.82*	4.42±1.05*†
Phosphorous (ug/dl)	PS	4.10±0.77	5.62±0.28*	4.42±0.39
	BS	4.52±0.85	5.54±0.46*	4.36±0.52†
	GS	3.96±0.44	5.68±0.41*	3.40±0.27†
Ammonia (mg/d)	PS	88.80±31.99	335.80±85.37*	123.00±56.32†
	BS	111.00±24.24	418.00±60.74*	165.80±57.01†
	GS	129.60±26.17	430.80±102.32*	119.20±25.44†
CK (IU/l)	PS	214.20±78.61	240.40±86.91*	227.40±74.74
	BS	217.40±50.04	258.00±62.72*	235.80±51.09
	GS	203.20±44.40	222.60±45.13*	190.20±42.17†

Mean±SD. PS, Placebo supplementation; BS, BCAA supplementation; GS, Glutamine supplementation

* p<0.05 vs. resting; † p<0.05 vs. end of exercise

Table 2. Blood cytokines

Items	Group	Resting	End of exercise	Recovery
IL-6 (pg/ml)	PS	21.70±20.46	24.76±10.58	18.60±1.14
	BS	14.10±6.53	24.02±10.61	15.36±6.27
	GS	21.76±7.63	22.12±7.59	16.68±6.00
IL-8 (pg/ml)	PS	25.30±10.35	34.16±8.08	29.16±4.02
	BS	27.14±8.37	30.76±7.08	24.22±9.78
	GS	26.12±5.21	25.91±5.09	23.82±6.91
IL-15 (pg/ml)	PS	24.26±6.42	42.66±24.04*	34.96±16.95
	BS	24.26±6.42	41.98±21.89*	19.06±5.56†
	GS	21.36±7.07	29.60±4.87	28.50±10.63

Mean±SD. PS, Placebo supplementation; BS, BCAA supplementation, GS, Glutamine supplementation

* p<0.05 vs. resting; † p<0.05 vs. end of exercise

the resting stage ($p<0.05$), and those of the PS group in the recovery stage were not significantly different from those at the end of exercise. But the concentrations in the BS and GS groups in the recovery stage appeared to be lower than those at the end of exercise ($p<0.05$). The blood ammonia concentration showed different levels in each stage but also did not differ significantly between groups. The blood ammonia concentrations in the 3 groups at the end of exercise all appeared to be higher than those in the resting stage ($p<0.05$), and those of all 3 groups in the recovery stage appeared to be lower than those at the end of exercise. The blood ammonia concentration also varied stage by stage ($p<0.05$), but it also did not differ significantly between groups. The level of blood CK activity at the end of exercise appeared higher in all 3 groups than in the resting stage ($p<0.05$), and from the levels of the PS and BS groups in the recovery stage were not significantly different from those at the end of exercise; however, the level of the GS group appeared to be lower than that at the end of exercise ($p<0.05$) (Table 1).

The results regarding the variation in the blood cytokine concentrations are illustrated in Table 2. IL-6 and IL-8

showed no significant difference among the groups and stages. IL-15 showed differences between stages ($p<0.05$), but the differences between groups were not significant. The blood IL-15 concentrations of the PS and BS groups at the end of exercise were different from those in the recovery stage ($p<0.05$), but there was no significant difference between these stages in the GS group. In the recovery stage, the BS group alone appeared lower than at the end of exercise ($p<0.05$).

DISCUSSION

The enhancement of exercise performance for sports is deeply related to the capability to generate anaerobic ATP continuously along with delaying fatigue. In general, the degree of fatigue depends on external factors (such as exercise intensity, exercising period, etc.) and internal factors (such as muscle mass, muscle fiber type, energy storage, etc.), and the drop in muscle contraction is closely related to the stimulation of fatigue-triggering metabolites such as lactate, phosphorous, and ammonia¹⁰. Thus the proper sup-

ply of ergogenic aids can play a critical role for the enhancement of exercise performance¹¹).

BCAA, an amino acid that can be mobilized in the skeletal muscles, is a nutritive substance that can supplement the energy and help anabolism. Studies on application of BCAA to minimize fatigue substances along with supplementation of required energy sources during long periods of high intensity exercise have been conducted. However, there are disagreements concerning the results from those studies. Some positive results of studies on BCAA application showed that enhancement of exercise performance through an increase in the internal concentration of BCAA led to enhanced ATP resynthesis and that facilitated the anabolic hormone release to stimulate the synthesis of myo-proteins improved the power of the muscles.

Glutamine is an amino acid found in abundance in the human muscles and blood plasma, and it is considered to be essential for proper immune function because it supplies energy for nucleotide biosynthesis. It was reported that in statuses of fasted or prolonged exercise or recovery period followed by high intensity exercises the levels of plasma glutamine dropped¹²). Also, the stressed states caused by exercises demand an increased amount of glutamine for gluconeogenesis, and thus the amount of plasma glutamine is rapidly exhausted. Furthermore, athletes experiencing discomfort from overtraining exhibit lower levels of plasma glutamine. Thus, ingesting a proper amount of glutamine would be critical, especially for juvenile athletes in the growing stage.

From the results of analyses conducted in this study, the blood lactate concentration, one of the fatigue factors, showed differences at each stage; however, those were indifferent to each group that the glutamine supplementation appeared to be ineffective in reducing the level of lactate stimulation during the recovery stage. The rate of lactate production exceeds the rate of removal during high-intensive exercise. The stimulated lactate facilitates acidosis and suppresses the enzymatic activation related to other metabolism and glycolysis, which impedes ATP synthesis and eventually leads to fatigue. According to the results of one study, glutamate transformed into other intermediates in the TCA cycle and the carbon skeleton of α -ketoglutarate, and the glutamate concentration decreased rapidly along with the reduction in glutamine¹³). Eventually, this would cause trouble in energy supply due to the reduction in intermediates in the chained TCA cycle¹⁴). Moreover, since the majority of carbon skeletons required for glutamine synthesis are derived from glycogen or glucose in the muscle, the glycogen saved for glutamine synthesis might be exhausted rapidly along with the practice of exercise. The link between carbohydrate metabolism and glutamine formation is closely connected to the level of postexercise plasma glutamine, so synthesizing and maintaining the intermediates in the TCA cycle by supplying glutamine would decrease the lactate stimulation, giving positive effects to athletes; however, such effects were not clearly observed in this study. In the recovery stage, the level of the glutamine supplementation group was the lowest among the groups, and the difference was not statistically significant, probably due to an

insufficient number of subjects.

An increase in phosphorous, which is another fatigue substance, decreases the ratio of cross-bridges in muscle fibers, and hinders the catalysis of ATPase, and hydrogen ion stimulation would form an inorganic phosphate ($H_2PO_4^-$), which leads to a decrease in power eventually. The results of our analysis showed that the levels of blood phosphorous in the BS and GS groups in the recovery stage were decreased significantly compared with at the end of exercise ($p < 0.05$). The results of the present study showed that the phosphorous concentrations in the BS and GS groups were significantly lower than that in the PS group at the end of exercise. As discussed previously, it was presumed that the BCAA or glutamine could have contributed in maintaining the intermediate energy substrate in the TCA cycle, which enabled the preservation of ATP that might have reduced the creation of inorganic phosphates.

The magnitude of amino acid metabolism may increase due to sharp rise in the level of blood ammonia during high-intensity exercise, but the majority of the increase in ammonia during the high-intensity exercise is created in the PNC cycle to maintain the total amount of adenine nucleotides; thus, the amount of ammonia created by amino acid metabolism is estimated to be slight¹⁵). Glutamine may discharge NH_4^+ through glutaminase catalysis in the oxidative deamination of protein or may transform itself into glutamine by accepting the NH_4^+ from glutamate, and this role of ammonium ions is crucial in amino acid metabolism. Problems with internal energy supply could be triggered by conditions involved with inactive metabolites, hypoxia, stresses and dietary restriction. The level of glycogen is decreased by high-intensity exercise along with the decrease in the level of α -ketoglutarate which is the intermediate in the TCA cycle. Glutamate also decreases in accordance with the decrease in α -ketoglutarate, which lowers the creation of glutamine, and accordingly, the increase in ammonia from by the exercise hinders glutamine transformation through the glutamine synthetase reaction; eventually the increased level of ammonia would trigger central fatigue and degrade exercising capability. In this study, it was not possible to identify the effects of internal BCAA presumed to be decreased by the exercise and glutamine supplementation because the observed ammonia concentration among the groups and per stage did not differ significantly from each other.

The CK, an enzyme decomposing creatine phosphate to generate anaerobic ATP during high-intensity exercise, is closely related to energy metabolism and is used as a marker for tissue damage due to exercise stresses. The results of the present study showed that the levels of CK in the GS group at the end of exercise and in the recovery stage were lower than those in the PS and BS groups, which might represent the effects of energy supplementation from glutamine supply, which activated as a fuel in the muscle and as a nitrogen precursor for nucleotide synthesis; it could also be presumed that there was a positive effect in protecting against tissue damage due to exercise stresses.

Intensive exercise not only causes fatigue but also causes tissue damage triggering inflammatory responses

and changes in cytokines. Until now, pro- and anti-inflammatory cytokines have been considered part of the acute phase response to an infection or tissue injury¹⁶). Thus, cytokines are released at the site of inflammation. IL-6 has been classified as a pro- and anti-inflammatory cytokine, and recently, its main anti-inflammatory effects have been a concern. IL-6 directly inhibits the expression of TNF- α and IL-1 β , and furthermore, IL-6 is a potent inducer of the IL-1 receptor antagonist (IL-1ra), which exerts anti-inflammatory activity by blocking IL-1 receptors and thereby prevents signal transduction of pro-inflammatory IL-1¹⁶). Some studies have shown that several cytokines can be detected in plasma during and after strenuous exercise^{17, 18}). That is, as the pro-inflammatory cytokines increase rapidly, anti-inflammatory cytokines such as IL-6 also increase accordingly to bring about balance in cytokine secretion.

In this study, the level of IL-6 in each group and in each stage did not appear to differ significantly from each other. The stagnant level of IL-6 during the intensive exercise might be regarded as indicating that glutamine supplementation could have prevented the increase in inflammation induced by exercise; however, it would be difficult to draw a clear conclusion based on those insignificant results. Besides, it has been reported that IL-6 is discharged from the muscle to maintain glucose homeostasis and that the IL-6 created in skeletal muscle activates AMP-kinase, which subsequently stimulates intramuscular glucose uptake and fat oxidation¹⁹). Thus, the increase in IL-6 secretion might not occur due to supplementary effects on energy from glutamine supplementation however, given the insignificant results obtained in the present study, it would be difficult to insist that there were clear effects related to glutamine supplementation.

It is known that IL-8 causes inflammation by activating inflammatory cells and that it is a chemokine produced by macrophages and other cell types such as epithelial cells. This chemokine is one of the major mediators of the inflammatory response. It serves as a chemical signal that attracts neutrophils at the site of inflammation, and therefore is also known as neutrophil chemotactic factor. IL-15, a sort of cytokine related to immune function, is created in the mononuclear phagocyte, macrophage, and muscle to trigger the creation of natural killer cells²⁰).

The results of the present study showed that there were no significant differences in IL-8 among the 3 groups in the resting stage or at the end of exercise; however, in the case of IL-15, the PS and BS groups showed differences, and the GS group did not. The results regarding IL-8 obtained in this study turned out to be different from those of another study reporting that the concentration of the chemokine IL-8 was increased¹⁸), and this was probably due to the difference in the level of exercise intensity and duration, which would have been insufficient to stimulate the IL-8 reaction. Fischer reported that the blood chemokine concentrations would increase little or remain stagnant unless a sufficient muscle mass is not mobilized and maintained at a certain level of intensity sufficiently²¹); thus, it seems that changing the blood chemokine concentrations requires performance of exercise at a comparatively high-intensity

for a long duration. Besides, differences in the blood IL-15 concentration were apparent in the PS and BS groups in the resting stage and at the end of exercise except in the GS group. This shows that IL-15 has a more sensitive reaction than IL-8 to exercise, and it seems that the glutamine supplementation caused an effective increase in the amount of saved glutamine. As the amount of glutamine application decreases, the cell proliferation rate of lymphocytes decreases, and antioxidants, peptides, oxidative stress, amino sugars related to cell resistance against and apoptotic processes, purines, and synthesis of key molecules such as pyrimidines also decrease accordingly²²). Moreover, along with the decrease in the saved amount of glutamine, neutrophils and macrophages, which are crucial to immune function and the inflammatory response, and the protective activity related to apoptosis of lymphocytes might also decrease²³). So, the IL-15 concentrations observed in the PS and BS groups were increased as a compensational effect corresponding to the decrease in the glutamine level, and in the GS group, the IL-15 concentration was not increased because the immune function and inflammatory defense reaction were not diminished.

Compared with the group supplemented with the placebo, the groups supplemented with BCAA or glutamine showed a lower level of blood phosphorous during the recovery stage after maximal intensity exercise. Regarding blood CK, the groups supplemented with glutamine alone appeared to have a lower concentration than the other groups, which suggests positive effects on reduction of fatigue factor stimulation. Differences in measurements of blood IL-6 and IL-15 were found between the resting stage and the end of exercise in the PS group and BS group, but not in the GS group. Thus, glutamine supplementation could be helpful for enhancement of immune function and the defensive inflammatory reaction after exercise.

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