The effect of BCAA supplementation upon the immune response of triathletes

REINALDO A. BASSIT, LETICIA A. SAWADA, REURY FRANK P. BACURAU, FRANCISCO NAVARRO, and LUI S FERNANDO B. P. COSTA ROSA

Department of Physiology and Biophysics and Department of Histology and Embryology, Institute of Biomedical Sciences, University of São Paulo, BRAZIL; Department of Biodynamic of the Movement of the Human Body, School of Sport and Physical Education, University of São Paulo, BRAZIL; and Laboratory of Human Nutrition for Athletes - CEPEUSP, University of São Paulo, BRAZIL

ABSTRACT

BASSIT, R. A., L. A. SAWADA, R. F. P. BACURAU, F. NAVARRO, and L. F. B. P. COSTA ROSA. The effect of BCAA supplementation upon the immune response of triathletes. Med. Sci. Sports Exerc., Vol. 32, No. 7, pp. 1214–1219, 2000. Introduction: Intense long-duration exercise could lead to immune suppression through a decrease in the circulating level of plasma glutamine. The decrease in plasma glutamine concentration as a consequence of intense long-duration exercise was reversed, in some cases, by supplementing the diet of the athletes with branched-chain amino acids (BCAA). To better address this question, we have evaluated some blood parameters (lymphocyte proliferation, the level of plasma cytokines, plasma glutamine concentration, and \textit{in vitro} production of cytokines by peripheral blood lymphocytes) before and after the São Paulo International Triathlon, as well as the incidence of symptoms of infections between the groups. Methods: Twelve elite male triathletes of mean age 25.5 ± 3.2 yr (ranging from 21.4 to 30.1 yr), weighing 74.16 ± 3.9 kg, swam 1.5 km, cycled 40 km, and ran 10 km (Olympic triathlon) in the São Paulo International Triathlon held in April 1997 and April 1998. In both events, six athletes received BCAA and the others, placebo. Results: Athletes from the BCAA group (BG) presented the same levels of plasma glutamine, before and after the trial, whereas those from the placebo group showed a reduction of 22.8% in plasma glutamine concentration after the competition. Changes in the proliferative response of peripheral blood lymphocytes were accompanied by a reduction in IL-1 production after exercise (22.2%), which was reversed by BCAA supplementation (20.3%), without changes in IL-2 production. Discussion: The data obtained show that BCAA supplementation can reverse the reduction in serum glutamine concentration observed after prolonged intense exercise such as an Olympic triathlon. The decrease in plasma glutamine concentration is paralleled by an increased incidence of symptoms of infections that result in augmented proliferative response of lymphocytes cultivated in the absence of mitogens. The prevention of the lowering of plasma glutamine concentration allows an increased response of lymphocytes to ConA and LPS, as well as an increased production of IL-1 and 2, TNF-α, and IFN-γ, possibly linked to the lower incidence of symptoms of infection (33.84%) reported by the supplemented athletes. Key Words: TRIATHLON, IMMUNE SYSTEM, GLUTAMINE, IMMUNOSUPPRESSION, CYTOKINES, LYMPHOCYTE PROLIFERATION

The triathlon, which comprises sequential swimming, cycling, and running, has retained the attention of many researchers involved in an attempt to characterize the sport and its general features (6,15,25). The growing interest in triathlon is based on the original structure of the sport itself and in the wide variety of distances offered to the participants, ranging from sprint to Ironman distance, lasting about 1 h and 10 h, respectively.

Cardiorespiratory and metabolic responses during Olympic (1.5 km swimming, 40 km cycling, and 10 km running) and long triathlon events have been extensively studied in laboratory and field conditions (7,8,14,15,24,30,32), as well as the changes in the plasma amino acid concentration (10,19,20). The effect of this type of exercise upon the immune system has not, however, been vastly investigated. Rohde and colleagues (31) reported a fall in natural killer-cell and lymphokine activated killer-cell cytotoxic activity and a decrease in serum glutamine concentrations 2 h after the end of a triathlon, but failed to show a correlation between plasma glutamine concentration and lymphocyte proliferative response, as previously proposed by Parry-Billings and coworkers (27,28).
The modulatory effect of exercise upon the immune system is well known. The mechanisms involved, however, are not fully understood, mainly at the level of high top performance (39). Different hypotheses involve the participation of cytokines and/or hormones as mediators of the effects, whereas others suggest that plasma glutamine concentration could be one of the major factors modulating the immune response to exercise. Several studies have demonstrated that glutamine is an important substrate for the cells of the immune system, which utilize this amino acid as a source of energy and precursors for the synthesis of nucleic acids (1,2,27). Based on this hypothesis, Keast and colleagues (17) showed that intense, long-duration exercise could lead to immune suppression through a decrease in the level of plasma glutamine.

The decrease of glutamine concentration in plasma as a consequence of intense long-duration exercise was reversed, in some cases, by supplementing the diet of the athletes with branched-chain amino acids (BCAA) (30). However, this reversal is not fully accepted as a means of restoring immune function in athletes. Rohde and colleagues (30) questioned the statement that maintenance of plasma glutamine concentration in vivo is able to prevent postexercise immunosuppression. To better address this question, we have evaluated the effect of a chronic BCAA supplementation upon the immune system of triathletes after an Olympic triathlon. We have evaluated some blood parameters (lymphocyte proliferation, plasmatic levels of cytokines, plasma glutamine concentration, and in vitro production of cytokines by peripheral blood lymphocytes) before and after the São Paulo International Triathlon. As a mean of evaluating the practical relevance of BCAA supplementation upon athletes health, we addressed, by a questionnaire, the incidence of symptoms of upper respiratory tract between the groups.

MATERIALS AND METHODS

Subjects and protocol. The experimental protocol was approved by the local ethics committee, and after signing an informed consent term, 12 elite male triathletes of mean age 25.5 ± 3.2 yr (range 21.4–30.1 yr) swam 1.5 km, cycled 40 km, and ran 10 km (Olympic triathlon) in the São Paulo International Triathlon held in April 1997 and April 1998.

The athletes were allowed to drink and eat normally but received BCAA or placebo for 30 d before the competition and 1 wk after the event. BCAA was given twice a day, after each training session (6.0 g: 60% L-leucine, 20% L-valine, and 20% L-isoleucine) during the first 30 d, and a single dose of 3.0 g 30 min before the triathlon, as well as a single dose (3.0 g) daily, in the morning, in the first week after the test were administered. On the day of the competition, blood samples were collected (20 mL) from the antecubital vein 45 min before the event and 15 min after the race. During the 37-d period, the athletes answered a questionnaire reporting their health conditions (Table 1).

Incorporation of [2-14C]-thymidine into peripheral blood lymphocytes. Peripheral blood lymphocytes were cultured in RPMI-1640 medium for 24 h at 37°C in an artificially humidified atmosphere of 5% CO₂ in air, under sterile conditions. The cells were cultured in a LAB-LINE Microprocessor CO₂ incubator (Lab Line, Melrose Park, IL) in 96 well plates (Corning, NY), 1 × 10⁵ cells per well (total

![Figure 1](image1.png)

**Figure 1**—Number of marks in the questionnaires filled by the athletes for 1 month before the competition and 1 wk after. The results are expressed as percentile and represent mean ± SEM of 8 athletes in the GP and 11 in the GS. *P < 0.05 for comparison with the placebo group (GP, placebo group; GS, supplemented group).

![Figure 2](image2.png)

**Figure 2**—Plasma glutamine level in athletes before and after an Olympic triathlon. The results are expressed as nmol·L⁻¹ and represent mean ± SEM of 8 athletes in the GP and 11 in the GS. *P < 0.05 for comparison between the values obtained before and after the trial. *P < 0.05 for comparison between GP and GS after the trial (GP, placebo group; GS, supplemented group).
volume, 200 μL). After 24 h in culture, more than 98% of lymphocytes were viable, as measured by Tripian blue exclusion test.

The cells were pulsed with 20 μL of 0.02 μCi [2-14C]-thymidine (sp. Act. 56.0 mCi mM-1) diluted in sterile phosphate-buffered saline (PBS), yielding a final concentration of 1 μg.mL-1. Cells were then maintained under these conditions for an additional 15 h and harvested automatically by a multiple cell harvester onto a filter paper (cat. no. 11731 Skatron Combi, Suffolk, U.K.). The paper disks containing the labeled cells were added to vials containing 5 mL of Bray’s scintillation cocktail (60 g L-1 naphthalene, 4 g L-1 2,5-diphenyloxazole (POPO), 20 mg L-1 1,4-di-[2-(5-phenyloxazolyl)]-benzene - POPOP, 10% methanol (by vol.) and 2% ethylene glycol (by vol.)) in p-dioxane (chromatographic grade) and counted in a Beckman-LS 5000TD liquid scintillator ion counter (Beckman Instruments, Fullerton, CA). All the reagents used in the preparation of the Brays solution were obtained from Sigma (St. Louis, MO) or Merck (Darmstadt, Germany).

Measurement of plasmatic glutamine concentration. Plasmatic glutamine concentration was measured enzymatically as described by Windmueller and Spaeth (40).

Determination of cytokines concentration. Each 5-mL blood sample was transferred to a glass tube containing 5 μL of heparin (500 IU mL-1). The tubes were kept on ice until centrifuging at 2500 rpm for 8 min. The plasma was stored at -80°C. The concentration of cytokines in plasma was measured using commercially available ELISA kits (Amersham Life Science, Clearbrook, IL): interleukin-1 (IL-1), interleukin-2 (IL-2), γ-interferon (IFN), and tumor necrosis factor-α (TNF).

Cytokines produced by cultivated peripheral blood lymphocytes were also measured. Lymphocytes were prepared by centrifuging the blood in the presence of Hystopaque (1.007) for 15 min at 2500 rpm. The mononuclear cells (± 97% lymphocytes) were plated (1.0 × 10⁶ cells mL−1) onto a plastic Petri dish in the presence of phytohemagglutinin (PHA) 10 μg mL−1 to stimulate IL-2, INF, and TNF production or lipopolysaccharide (LPS) 10 μg mL−1 to stimulate IL-1 production. After 48 h, the concentration of the cytokines was measured in the supernatant.

Statistical analysis. The data obtained in the two events were compared using paired t-test, and the level of significance of P < 0.05 was chosen for all statistical comparisons. The data are presented as mean ± SEM.

RESULTS

Athletes participating in the Olympic triathlon answered a questionnaire concerning the incidence of infection symptoms before (1 month) and after (1 wk) the competition (18; Table 1). Supplementation with BCAA induced a decrease in the symptoms of infection reported (33.84%) when the answers were compared with those of the group receiving placebo (PG) (Fig. 1).

Athletes from the BCAA group (BG) presented the same plasma glutamine level, before and after the trial (Fig. 2), whereas those from the placebo group showed a reduction of 22.8% in plasma glutamine concentration after the competition (Fig. 2). This change in glutamine concentration was accompanied by increased lymphocyte proliferation obtained from resting subjects (Table 2). In such athletes, we observed a systematic decrease in lymphocyte proliferation that was, however, not significant (Table 2). Lymphocytes obtained from subjects of the BG, on the other hand, presented a smaller index of proliferation before the test (repsresented a smaller index of proliferation (36.2% as compared with PG), which was slightly increased by 12.6% after the competition (Table 2). The effects of BCAA supplementation appeared when the cells were stimulated by mitogens. In PG lymphocytes the presence of concanavalin A (ConA) or lipopolysaccharide (LPS) in the culture medium induced an increase in the proliferative response (79.9% and 41.1%, respectively) that was amplified in the BG (3.34-fold and 2.35-fold, respectively, Table 2). We have also observed in the BG triathletes a
greater lymphocyte proliferative response after the test when compared with the changes observed in the placebo group after the triathlon (27.4% and 17.2% for ConA and LPS, respectively).

These changes in the proliferative response of peripheral blood lymphocytes were accompanied by changes in the production of IL-1 and IL-2, TNF-α, and INF-γ by cells cultivated for 48 h in the presence of LPS (IL-1) or PHA (IL-2, TNF and INF). The athletes from PG presented a reduction in IL-1 production after exercise (22.2%), which was reversed by BCAA supplementation (20.3%, Fig. 3). There were no changes in IL-2 production induced by the exercise per se (Fig. 4), but we could notice an increase in the production of this cytokine in the supplemented group before (47.9%) and after the trial (84.8%, Fig. 4). The production of TNF and INF presented the same pattern of changes as that of IL-1. Cells from PG, when harvested after exercise, showed a decreased production of these cytokines (35.1% for IFN and 16.9% for TNF, Figs. 5 and 6, respectively). In the cells obtained from BG this decrease in cytokines production after exercise was abolished (Figs. 5 and 6).

**DISCUSSION**

Participation in triathlon is continuously growing. This sport, which began in the mid-1970s, includes three sequentially performed endurance events: swimming, cycling, and running. The distance of each segment varies substantially, so that the total time of competition ranges from 30 min to several hours (25). The physiological effects of a triathlon have been extensively studied (5,7,8,12,14,19,24,26,35,41). It is interesting to note that the physiological and energetic demands of such a sequence of exercise are unique and require the triathletes to develop a blend of characteristics seen in endurance swimming, cycling, and running (33). As a consequence of these elevated demands, triathletes presented with some side-effects such as glomerular damage (41) and the presence of oxidized DNA bases in urine (12) as well as cellular dehydration and a decrease in serum amino acids, reflecting a catabolic state (19).

The imbalance in plasma amino acids concentration could be related to the increased risk of upper respiratory tract infections that follows intense long-duration exercise (13,23,29) by inducing, in the host, a suppression of natural killer and lymphokine activated cells activity and lymphocyte proliferation (36,37). Glutamine decrease seems to be the main factor causing immunosuppression, because this amino acid is essential for lymphocyte and macrophage metabolism (2,27,30) and its concentration is reduced in the plasma of athletes after long-term, strenuous exercise (17,28). Rohde and colleagues (30) showed that after long-term intense exercise (a triathlon consisting of 2.5 km swimming, 81 km cycling, and 19 km running), there was a reduction in NK and LAK cell activities paralleled by a decrease in serum glutamine concentrations.

In this study, we have evaluated the effect of BCAA supplementation (BG) upon peripheral blood lymphocyte proliferation, serum glutamine concentration, and the production of IL-1, IL-2, TNF-α, and INF-γ, as well as by a questionnaire, in the incidence of infections.

As previously reported (30), the athletes presented reduced serum glutamine concentration (22.8%) after the triathlon, which is very similar to the observed by Parry-Billings and colleagues (28) after a marathon (a reduction of 16%) and by Rhode and colleagues (30) after another triathlon: 2.5 km swimming, 81 km cycling, and 19 km running (reduction of 32%). The supplementation of the athletes with BCAA restored serum glutamine concentration to values similar to those found before the competition. In fact, it is known that supplementation with BCAA is able to increase the circulating levels of these amino acids and their metabolization to glutamine in the skeletal muscle (21) leading to a greater muscle NH₃ production (21). NH₃, in such model, is derived from the transamination with 2-oxoglutarate, which forms glutamate.
and branched-chain oxo acids, a reaction catalyzed by BCAA aminotransferase. Glutamate can then be oxidatively deaminated by glutamate dehydrogenase, releasing the NH₃ and reforming 2-oxoglutarate (22). The NH₃ produced in such a pathway, during exercise, is released in the form of glutamine (21). This mechanism is reinforced by the fact that the rate limiting step in BCAAs catabolism involves the nonreversible decarboxylation of the BCOAs by branched-chain oxo acid dehydrogenase, which is activated during exercise (11,16,38) and is responsive to an increase in the intracellular concentration of BCOAs (21).

It is interesting to note that in the athletes of the PG, who showed a reduction in serum glutamine concentration after the triathlon, an increased proliferative response of peripheral blood lymphocytes, harvested before and after the exercise session, cultivated without mitogens was also observed. These cells, however, showed a reduced response to ConA and LPS, mitogens for T and B cells, respectively, when compared with those obtained from BG athletes, who presented a normal serum glutamine concentration after the triathlon.

An important aspect of immune response concerns the production of cytokines by immune cells. This group of intercellular signaling proteins regulates local and systemic immune and inflammatory responses, as well as other biologic processes. Their effect often overlap considerably and one cytokine may induce the secretion of others, producing a cascade of biological effects. We have evaluated the effect of BCAA supplementation upon cultivated blood mononuclear cells cultured for 48 h in the presence of phytohemagglutinin 10 μg (mL), before and after the trial. The results are expressed as pg mL⁻¹ and represent the mean ± SEM of 8 samples in the placebo group (GP) and 11 in the supplemented group (GS). *P < 0.05 for comparison with the placebo group.

Figure 6—Production of TNF-α by peripheral blood mononuclear cells cultivated for 48 h in the presence of phytohemagglutinin 10 μg (mL), before and after the trial. The results are expressed as pg mL⁻¹ and represent the mean ± SEM of 8 samples in the placebo group (GP) and 11 in the supplemented group (GS). *P < 0.05 for comparison with the placebo group.

We gratefully acknowledge Twinlab® for kindly provide the amino acids for this project. The work was supported by FAPESP 98/07141-7.

Address for correspondence: Dr. Luis F. B. P. Costa Rosa, Departamento de Histologia e Embriologia, Instituto de Ciências Biomédicas I, Universidade de São Paulo, Av. Lineu Prestes, 1524, 05508–900, Butantã, São Paulo, SP, Brasil; E-mail: ggrosa@icb.usp.br.

REFERENCES


