Branched-Chain Amino Acid Supplementation and the Immune Response of Long-Distance Athletes

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OBJECTIVE: Intense long-duration exercise has been associated with immunosuppression, which affects natural killer cells, lymphokine-activated killer cells, and lymphocytes. The mechanisms involved, however, are not fully determined and seem to be multifactorial, including endocrine changes and alteration of plasma glutamine concentration. Therefore, we evaluated the effect of branched-chain amino acid supplementation on the immune response of triathletes and long-distance runners.

METHODS: Peripheral blood was collected prior to and immediately after an Olympic Triathlon or a 30k run. Lymphocyte proliferation, cytokine production by cultured cells, and plasma glutamine were measured.

RESULTS: After the exercise bout, athletes from the placebo group presented a decreased plasma glutamine concentration that was abolished by branched-chain amino acid supplementation and an increased proliferative response in their peripheral blood mononuclear cells. Those cells also produced, after exercise, less tumor necrosis factor, interleukins-1 and -4, and interferon and 48% more interleukin-2. Supplementation stimulated the production of interleukin-2 and interferon after exercise and a more pronounced decrease in the production of interleukin-4, indicating a diversion toward a Th1 type immune response.

CONCLUSIONS: Our results indicate that branched-chain amino acid (BCAA) supplementation recovers the ability of peripheral blood mononuclear cells proliferate in response to mitogens after a long distance intense exercise, as well as plasma glutamine concentration. The amino acids also modify the pattern of cytokine production leading to a diversion of the immune response toward a Th1 type of immune response. *Nutrition* 2002;18:376–379. ©Elsevier Science Inc. 2002

KEY WORDS: triathlon, running, immune system, glutamine, immunossupression, cytokines, lymphocyte proliferation

INTRODUCTION

There is a long history of a perceived association between physical activity and health in many cultures. Cowles[1] reported that virtually all cases of pneumonia at a boys' school occurred in athletes after intense exercise and competitive sport. Although not extensively documented, upper respiratory tract infection appears to be the most prevalent infectious illness among athletes.[2] Symptoms of upper respiratory tract infection are generally obtained through self-report, especially in large epidemiologic studies, where it is difficult to obtain medical diagnoses for hundreds to thousands of subjects.

Among different types of exercise, intense long-duration exercise has been associated with immunosuppression, which affects natural killer cells, lymphokine-activated killer cells, and lymphocytes.[3] Whereas a moderate dose of endurance exercise has a beneficial effect on immune responses, more intense and stressful exercise shows an adverse effect.[4] The mechanisms involved, however, are not fully determined and seem to be multifactorial, including endocrine changes and alteration of plasma glutamine concentration.[5–7] Glutamine has been reported as an important fuel for macrophages and lymphocytes, presenting immunostimulatory effects.[6] Plasma glutamine concentration, however, is lowered after the stress of prolonged exhaustive exercise, and its provision, as an oral supplement after exercise, has beneficial effects on the level of subsequent infections in endurance athletes.[6,7]

Therefore, we evaluated the effect of branched-chain amino acid (BCAA) supplementation, precursors for glutamine synthesis,[6] on the immune responses of triathletes and long-distance runners.

MATERIALS AND METHODS

Subjects and Protocol

The experimental protocol was approved by the local ethics committee. After signing an informed consent form, 12 male elite

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triathletes (mean age 25.5 ± 3.2 y, range 21.4–30.1 y) swam 1.5 km, cycled 40 km, and ran 10 km (Olympic Triathlon) in the São Paulo International Triathlon, and marathoners ($n = 24$) ran 30 km in 2 h.

The triathletes were allowed to drink and eat normally but received BCAA or placebo for 30 d before the competition and 1 wk after the event, and runners were supplemented 15 d before the test. BCAA was given twice a day, after each training session, to the triathletes or as a single dose to the runners (6.0 g of 60% L-leucine, 20% L-valine, and 20% L-isoleucine); a single dose of 3.0 g 30 min was given before the triathlon or race; and a single daily dose (3.0 g), in the morning, in the first week after the test were administered, was given only to the triathletes. 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.

Incorporation of [2-^{14}C]-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% CO_2 in air under sterile conditions. The cells were cultured in a Lab-Line Microprocessor CO_2 incubator (Lab-Line, USA) in 96-well plates (Corning, Corning, NY, USA), 1×10^5 cells per well (total volume, 200 μL). After 24 h in culture, more than 98% of lymphocytes were viable, as measured by the Tripan blue exclusion test.

The cells were pulsed with 20 μL of 0.02 μCi [2-^{14}C]-thymidine (specific activity of 56.0 mCi/nM) diluted in sterile phosphate-buffered saline, yielding a final concentration of 1 μg/mL. Cells were maintained under these conditions for an additional 15 h and harvested automatically by a multiple cell harvester onto a filter paper (catalog no. 11731 Skatron Combi, Suffolk, UK). The paper disks containing the labeled cells were added to vials containing 5 mL of Bray's scintillation cocktail (60 g/L naphathalene, 4 g/L 2,5-diphenyloxazole, 20 mg/L 1,4-di-[2-{5-phenyloxazolyl}]-benzene–POPOP, 10% methanol by volume, and 2% ethylene glycol by volume in *p*-dioxan, chromatographic grade) and counted in a Beckman-LS 5000TD liquid scintillator ion counter (Beckman Instruments, Fullerton, CA, USA). All the reagents used in the preparation of Bray's solution were obtained from Sigma (St. Louis, MO, USA) or Merck (Darmstadt, Germany).

Measurement of Plasmatic Glutamine Concentration

Plasmatic glutamine concentration was measured enzymatically as described by Windmueller and Spaeth.[8]

Determination of Cytokine Concentration

Each 5-mL blood sample was transferred to a glass tube containing 5 μL of heparin (500 IU/mL). The tubes were kept on ice until centrifugation at 2500 rpm for 8 min. The plasma was stored at −80°C. The concentration of cytokines in plasma was measured with a commercially available enzyme-linked immunosorbent assay (Amersham Life Science, Buckimhamshire, UK): interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin 4 (IL-4), interferon-γ (IFN), and tumor necrosis factor-α (TNF).

Cytokines produced by cultivated peripheral blood lymphocytes were also measured. Lymphocytes were prepared by centrifuging the blood with Hystopaque (1.007) for 15 min at 2500 rpm. The mononuclear cells (±97% lymphocytes) were plated (1.0×10^6 cells/mL) onto a plastic Petri dish in the presence of 10 μg/mL of phytohemaglutinin to stimulate IL-2, IL-4, INF, and TNF production or 10 μg/mL of lipopolysaccharide to stimulate IL-1 production. After 48 h, the concentration of the cytokines was measured in the supernatant.

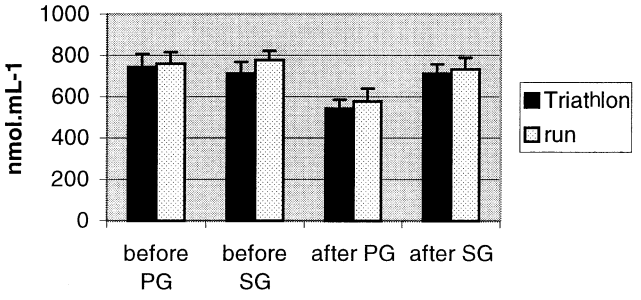

FIG. 1. Plasma glutamine levels in athletes before and after an exercise bout. The results are expressed as nanomoles per milliliter and represent the mean ± standard error of the mean of 12 triathletes and 24 runners (6 and 12 athletes in each group). $P < 0.05$, before versus after the trial. PG, placebo group; SG, supplemented group.

Statistical Analysis

The data obtained in the two events were compared with paired *t* test, and $P < 0.05$ was considered statistically significant. The data are presented as mean ± standard error of the mean.

RESULTS

Triathletes and runners from the placebo group presented, after the exercise bout, a decrease in plasma glutamine concentration (26.6% and 24%, respectively; Fig. 1). That reduction was abolished by BCAA supplementation in both groups (Fig. 1). These changes in plasma glutamine concentration were accompanied by a decrease in the proliferative response of lymphocytes collected after exercise in both groups (34.3%, 35.5%, and 40% for control lymphocytes and those cultivated in the presence of concanavalin A and lipopolysaccharide, respectively; Fig. 2). BCAA supplementation recovered the proliferative response of lymphocytes in response to both mitogens, but only in the control group of runners (Fig. 2). It is interesting to note that BCAA supplementation did not alter the proliferative response of lymphocytes before exercise, i.e., during rest (data not shown).

These changes in the proliferative response of peripheral blood lymphocytes were accompanied by changes in the production of cytokines by mononuclear peripheral blood cells cultivated for 48 h in the presence of lipopolysaccharide (IL-1) or phytohemagglutinin (for IL-2, IL-4, TNF, and INF). Cells obtained from athletes receiving placebo after exercise presented a reduction in the production of TNF (19%); INF (27%), IL-1 (20.6%), and IL-4

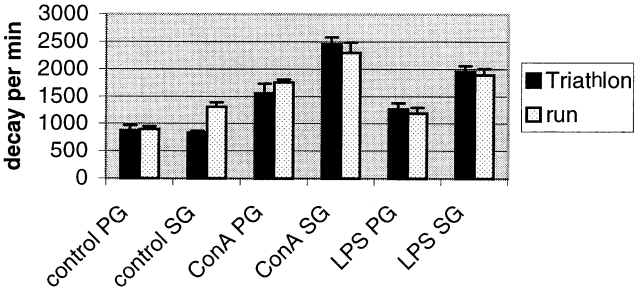

FIG. 2. Proliferative response of peripheral blood lymphocytes obtained from 12 triathletes and 24 runners (6 and 12 athletes in each group). The results are expressed in decay per minute as mean ± standard error of the mean. $P < 0.05$, before versus after the trial. ConA, concanavalin A; LPS, lipopolysaccharide; PG, placebo group; SG, supplemented group.

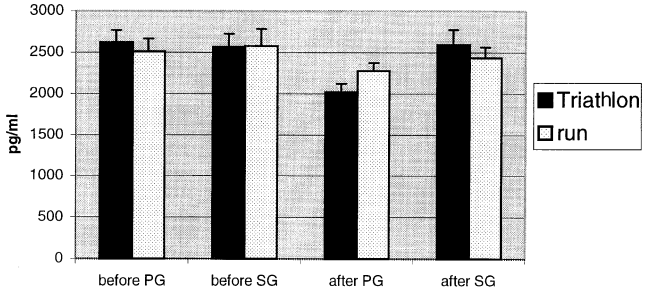

FIG. 3. Production of tumor necrosis factor alpha by peripheral blood mononuclear cells cultivated for 48 h with 10 μg/mL of phytohemagglutinin before and after the trial. The results are expressed as picograms per milliliter and represent the mean ± standard error of the mean of 12 triathletes and 24 runners (6 and 12 athletes in each group). $P < 0.05$, before versus after the trial. PG, placebo group; SG, supplemented group.

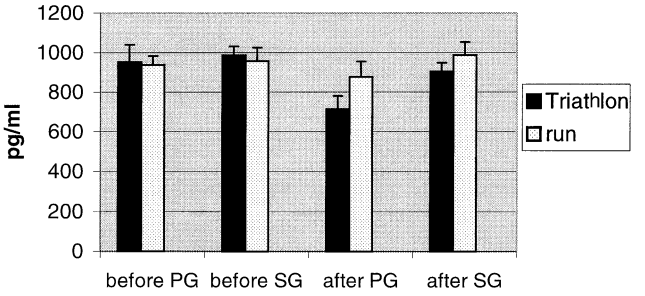

FIG. 5. Production of interleukin-1 by peripheral blood mononuclear cells cultivated for 48 h with 10 μg/mL of lipopolysaccharide before and after the trial. The results are expressed as picograms per milliliter and represent the mean ± standard error of the mean of 12 triathletes and 24 runners (6 and 12 athletes in each group). $P < 0.05$, before versus after the trial. PG, placebo group; SG, supplemented group.

(18.7%) in comparison with the production of these cytokines from cells harvested before the exercise bout (Figs. 3, 4, 5, and 7). There was, however, an increase in IL-2 production (48%; Fig. 6). BCAA supplementation restored the production of TNF and IL-1 (Figs. 3 and 5) and increased that of INF and IL-2 (Figs. 4 and 6). It is interesting to note, however, that cells obtained from the supplemented group presented an even greater reduction in IL-4 production after exercise (49%; Fig. 7).

DISCUSSION

Long-distance high-intensity exercise has been related to an increased risk of upper respiratory tract infections and to overtraining. Among the mechanisms involved, some investigators have proposed changes in stress hormones,[9] cytokine production,[10] natural killer cell activity,[11] and plasma glutamine concentration.[7,12] In fact, all these changes occur at the same time, and a cooperative mechanism is likely the major cause for the immunesupression detected in such athletes. Therefore, we investigated the effect of BCAA supplementation on the immune response of athletes, triathletes, and runners to elucidate in which aspect of the response BCAA would take part.

The supplementation of the athletes with BCAA before competition, 30 d for triathletes and 15 d for runners, was very efficient in keeping plasma glutamine concentration constant after exhaustive exercise. BCAA supplementation increased the circulating levels of these amino acids and their metabolization to glutamine in the skeletal muscle, leading to greater muscle ammonia production.[13] Ammonia is derived from the transamination with 2-oxoglutarate, which forms glutamate and branched-chain oxo-acids, through the reaction catalysed by BCAA aminotransferase. Glutamate can be oxidatively deaminated by glutamate dehydrogenase by releasing NH_3 and reforming 2-oxoglutarate. The ammonia produced in such a pathway is released in the form of glutamine. This mechanism is reinforced by the fact that the rate-limiting step in BCAA catabolism involves the non-reversible decarboxylation of the branched-chain oxo-acids by branched-chain oxo-acid dehydrogenase, which is activated during exercise and is responsive to an increase in the intracellular concentration of the oxo-acids.[13,14]

The maintenance of plasma glutamine concentration after exercise allowed the athletes from the supplemented group to present an increased proliferative response to concanavalin A and lipopolysaccharide, mitogens to T and B lymphocytes, respectively, indicating a possible increase in their function that could be related to a decrease in the symptoms of upper respiratory tract infection reported elsewhere.[7] It is a fact, however, that the immune response is strictly regulated, and cytokines play a major role in this action. This group of intercellular signaling proteins regulates local and systemic inflammatory and immune responses and presents such overlapping effects that can produce a cascade of biological

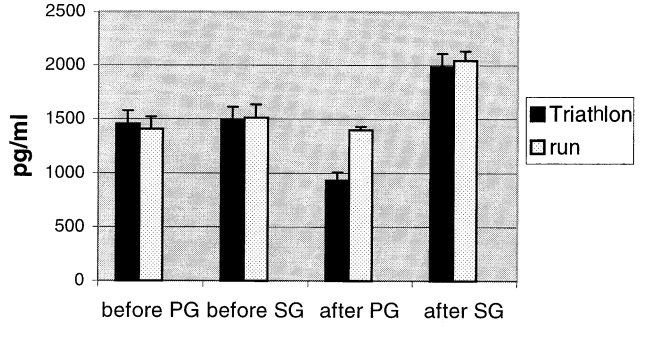

FIG. 4. Production of interferon-γ by peripheral blood mononuclear cells cultivated for 48 h with 10 μg/mL of phytohemagglutinin before and after the trial. The results are expressed as picograms per milliliter and represent the mean ± standard error of the mean of 12 triathletes and 24 runners (6 and 12 athletes in each group). $P < 0.05$, before versus after the trial. PG, placebo group; SG, supplemented group.

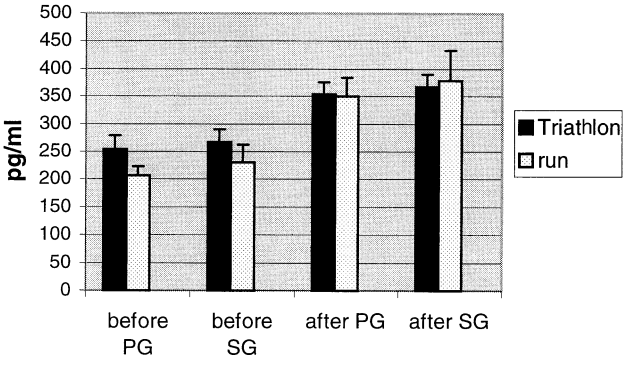

FIG. 6. Production of interleukin-2 by peripheral blood mononuclear cells cultivated for 48 h with 10 μg/mL of phytohemagglutinin before and after the trial. The results are expressed as picograms per milliliter and represent the mean ± standard error of the mean of 12 triathletes and 24 runners (6 and 12 athletes in each group). $P < 0.05$, before versus after the trial. PG, placebo group; SG, supplemented group.

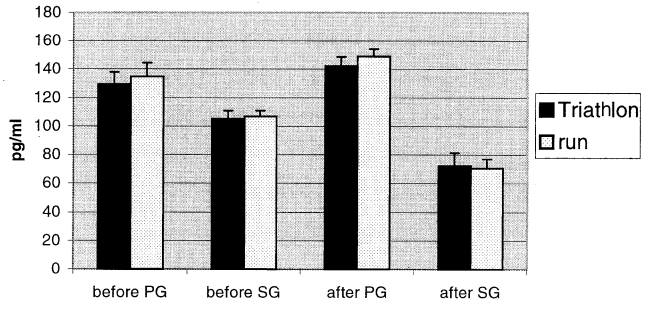

FIG. 7. Production of interleukin-4 by peripheral blood mononuclear cells cultivated for 48 h with 10 μg/mL of phytohemagglutinin before and after the trial. The results are expressed as picograms per milliliter and represent the mean ± standard error of the mean of 12 triathletes and 24 runners (6 and 12 athletes in each group). $P < 0.05$, before versus after the trial. PG, placebo group; SG, supplemented group.

effects. Many research groups have evaluated the effect of exercise on the cytokine profile[10,15,16] and found no effects or changes in one or two differet types of cytokine. In our study we evaluated the production of cytokine by cultivated peripheral blood mononuclear cells in response to immunologic stimulli, to better address the effect of BCAA supplementation on the control mechanims of the immune response, because we, like others,[10] did not find changes in plasma cytokine concentrations before and after high-intensity long-duration exercise (data not shown).

Peripheral blood monuclear cells produced, after exercise, less TNF (19%), IL-1 (20.6%), IL-4 (18.7%), and INF (27%) and 48% more IL-2, suggesting that the inflammatory and immunologic responses are compromised. BCAA supplementation, however, stimulated the production of IL-2 and INF after exercise and a more pronounced decrease in the production of IL-4, indicating a diversion toward a Th1 type of immune response. 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. The precise mechanism involved and the other effects of the supplementation regimen have to be clarified.

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