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ORIGINAL ARTICLE

Effect of exercise on glutamine metabolism in macrophages of trained rats

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Abstract This study investigated the effect of exercise on glutamine metabolism in macrophages of trained rats. Rats were divided into three groups: sedentary (SED); moderately trained (MOD) rats that were swim trained 1 h/day, 5 days/week for 6 weeks; and exhaustively trained (EXT) rats that were similarly trained as MOD for 5 weeks and, in the 6th week, trained in three 1-h sessions/day with 150 min of rest between sessions. The animals swam with a load equivalent to 5.5% of their body weight and were killed 1 h after the last exercise session. Cells were collected, and glutamine metabolism in macrophage and function were assayed. Exercise increased phagocytosis in MOD when compared to SED (34.48 ± 1.79 vs $15.21 \pm 2.91\%$, $P < 0.05$); however, H_2O_2 production was higher in MOD (75.40 ± 3.48 nmol h $\times$ 10^5 cell^{-1}) and EXT (79.20 ± 1.18 nmol h $\times$ 10^5 cell^{-1}) in relation to SED (32.60 ± 2.51 nmol h $\times$ 10^5 cell^{-1}, $P < 0.05$). Glutamine consumption increased in MOD and EXT (26.53 ± 3.62 and 19.82 ± 2.62 nmol h $\times$ 10^5 cell^{-1}, respectively) relative to SED (6.72 ± 0.57 nmol h $\times$ 10^5 cell^{-1}, $P < 0.05$). Aspartate increased in EXT (9.72 ± 1.14 nmol h $\times$ 10^5 cell^{-1}) as compared to SED (1.10 ± 0.19 nmol h $\times$ 10^5 cell^{-1}, $P < 0.05$). Glutamine decarboxylation was increased in MOD (12.10 ± 0.27 nmol h $\times$ 10^5 cell^{-1}) and EXT (16.40 ± 2.17 nmol h $\times$ 10^5 cell^{-1}) relative to SED (1.10 ± 0.06 nmol h $\times$ 10^5 cell^{-1}, $P < 0.05$). This study suggests an increase in macrophage function post-exercise, which was supported by enhanced glutamine consumption and metabolism, and highlights the importance for glutamine after exercise.

Keywords Macrophage · Moderate exercise · Glutamine · Exhaustive training · Macrophage function

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Introduction

Studies have suggested a dual effect of exercise on the immune system. Recently, experimental and epidemiological studies have demonstrated that moderate exercise improves immune function and increases resistance to infections, while exhaustive exercise promotes the opposite effects (Nieman and Pedersen 1999; Nieman 2000; Mackinnon 2000; Sugiura et al. 2002; Nielsen 2003; Peijie et al. 2003; Nieman 2007).

The mechanisms involved in the different effects of exercise on the immune system are not fully understood and seem to be multi-factorial, including endocrine changes and alterations in plasma glutamine concentrations (Mitchell et al. 1996; Nieman and Pedersen 1999; Pedersen and Toft 2000; Bassit et al. 2000; Castell 2002, 2003; Cunha et al. 2003; Costa Rosa 2004).

Several studies have shown a decrease in plasma glutamine concentration after exhaustive exercise in humans and animals (Koyama et al. 1998; Walsh et al. 1998; Bassit et al. 2000; Castell 2002, 2003), as well as in the presence of overtraining syndrome (Parry-Billings et al. 1992; Rowbottom et al. 1995). This decrease in plasma glutamine

correlates with increased symptoms of upper respiratory tract infections, due to partial impairment of immune cell function (Rowbottom et al. 1996; Koyama et al. 1998; Bassit et al. 2000; Castell 2003; Dos Santos Cunha et al. 2004). Despite the claim of Hiscock and Pedersen (2002) that the magnitude of the observed decrease in plasma glutamine concentration was not significant enough to compromise immune cell function, Parry-Billings et al. (1992) suggested that small decreases in plasma glutamine concentration are sufficient to promote immunosuppression.

Macrophages are important T cells in the immune response, since they play a central role in the innate immune response and constitute the first line of defense against external aggression and infectious diseases (Woods et al. 2006). They are mobile cells with phagocytic, cytotoxic and antimicrobial capacities (Stout and Suttles 2005). Macrophages use high rates of glutamine to generate energy and biosynthesis (Newsholme and Calder 1997).

Glutamine is a conditionally essential amino acid, which comprises 20% of the total plasma amino acid. It is actively produced in different organs, including skeletal muscle in which a great amount of glutamine is produced and stored (Castell 2003). Many studies have shown that macrophages utilize high rates of glutamine. In addition, macrophages satisfy their energy requirements through partial oxidation of glutamine (Newsholme et al. 1996). In fact, macrophages have elevated glutaminolitic activity; after consumption, the glutamine is coverted to glutamate by glutaminase. The glutamate formed may be transformed to aspartate or NADPH and an acid citric intermediate named oxoglutarate (Costa Rosa et al. 1996; Newsholme et al. 1987, 1996).

Thus, an appropriate glutamine concentration is indispensable and allows for an efficient T-cellular response, since changes in glutamine metabolism can result in a different pattern of phagocytosis, reactive oxygen species (ROS) production, chemotaxis and other functions (Newsholme et al. 1996; Newsholme and Calder 1997).

In light of this, the objective of this study was to investigate the effect of exercise on glutamine metabolism in the macrophages of trained rats. We hypothesized that the enhanced macrophage function such as phagocytosis and hydrogen peroxide production induced by exercise is associated with a higher glutamine demand.

Methods

Ethical principles

In our experiments, protocols were authorized by the Committee for Ethics in Animal Research of the Institute of Biomedical Sciences, in agreement with the ethical principles of animal research adopted by the Brazilian College of Animal Experimentation.

Animals

Three-month-old wistar rats weighing 150–200 g at the beginning of the experiment were obtained from the Biomedical Science Institute of the University of São Paulo, Brazil. Rats were maintained under a constant light-to-dark cycle (12:12 h, lights on at 0700 hours), at 22 ± 2°C and 60 ± 5% humidity. They were kept in collective cages (five animals/cage) and received water and food ad libitum.

Groups and training protocols

Rats were divided into three groups: sedentary rats (SED) did not complete an exercise program ($n = 20$), moderately trained rats (MOD) trained 1 h/day, 5 days/week for 6 weeks ($n = 20$), and exhaustively trained rats (EXT) trained 1 h/day for 5 weeks similar to MOD and, in the 6th week, trained in three 1-h sessions per day with 150 min of rest between sessions ($n = 20$). During exercise bouts, animals swam with an extra load equivalent to 5.5% of their total body weight attached to the tail to enhance aerobic training (Gobatto et al. 2001). At 24 h after the last exercise, ten animals from the MOD and EXT groups were subjected to an exhaustive performance test, while the other animals were killed by decapitation. The training was performed in individual round tanks containing 100 cm depth of water and a water surface area of approximately 1,200 cm^2. The water temperature was maintained constant at 33 ± 1°C. During the training schedule, all exercise was performed at the same time of day. The animals were killed by decapitation 1 h after the last exercise bout.

Macrophage isolation

Phosphate buffered saline (PBS), pH 7.4, was injected (6.0 ml) intraperitoneally. After 30 s, the abdomen was opened and peritoneal macrophages were collected using a plastic Pasteur pipette. Cell viability was confirmed by trypan blue exclusion (>95%). At least 92% of the peritoneal exudate cells were macrophages, as determined by differential counting (Costa Rosa et al. 1996).

Incubation

Macrophages from two different animals from the same experimental group were pooled (10^5 cells) and incubated in 1 ml medium containing 2 mM glutamine, 5 mM glucose, 10% bovine serum albumin (BSA) and PBS for 1 h at 37°C with continuous shaking. After 1 h, the incubation was stopped with the addition of 25% (w V^{-1}) trichloroacetic

acid (TCA). The samples were centrifuged at 8,100 × g for 5 min, and the supernatant was collected. The supernatant was then neutralized with Tris–KOH and stored at −80°C until glutamine consumption; glutamate and aspartate production assays were performed. The glutamine consumption and glutamate and aspartate production were determined by the difference between the final concentration of substances in the supernatant after 60 min of incubation (T_1) and the initial concentration of these substances in T_0, as described by Ardawi and Newsholme (1983).

Glutamine decarboxylation

The $^{14}CO_2$ produced from [U-^{14}C]-glutamine was collected as described by Martins et al. (1998). Macrophages (10^5 cells) were incubated in a medium containing 2 mM glutamine, 5 mM glucose, 10% BSA and PBS for 1 h in the presence of one of the radio-labeled substrates in a sealed Erlenmeyer flask (25 ml), presenting one compartment for cell incubation and another for CO_2 collection. Subsequently, the cells were killed with 200 µl 25% perchloric acid (PCA). The labeled CO_2 was collected for 1 h in a solution of phenylethylamine:methanol (1:1) and the radioactivity was counted in Ecolumen, in a Beckman LS 5000TD Liquid Scintilator.

Biochemical determinations

Glutamine consumption in the supernatant was enzymatically assayed in a buffer containing 10 mM KH_2PO_4, 50% glycerol, 4 mM NADH, 10% BSA, 5 U mL^{-1} glutamate dehydrogenase, 4 M 2-oxoglutarate and 5.0 U mL^{-1} asparaginase at pH 8.0 and 20–25°C (Windmueller and Spaeth 1974). In this reaction, the decrease in NADH measured by spectrophotometer at 340 nm is proportional to the amount of glutamine in the medium.

Glutamate production in supernatant was enzymatically assayed in a buffer containing 300 mM glycine, 250 mM hydrazine, 1 mM ADP, 1.6 mM NAD and 4.5 U mL^{-1} glutamate dehydrogenase at pH 9.0 and 20–25°C, according to the method described by Bernet and Bergmeyer (1974). In this reaction, the increase in NADH measured by spectrophotometer at 340 nm is proportional to the amount of glutamate in the medium.

Aspartate production in the supernatant was assayed in a medium containing 60 mM K_2HPO_4, 0.19 mM NADH, 3.1 mM alpha-ketoglutaric acid, 6 U mL^{-1} malic dehydrogenase and 2.5 U mL^{-1} glutamate oxaloacetate transaminases, pH 7.2, according to the method described by Bergmeyer et al. (1974). In this reaction, the decrease in NADH measured by spectrophotometer at 340 nm is proportional to the amount of aspartate in the medium.

Macrophage function

Phagocytosis

As much as 35 mg of zymosan in 100 ml PBS was boiled for 30 min and washed twice with PBS, prior to use. Subsequently, zymosan particles were resuspended in PBS containing Ca^{2+} and Mg^{2+} ions to provide a concentration of 14 mg ml^{-1} and the solution was stored at 4°C. Normal serum used for opsonization was stored at −20°C. For opsonization, 0.5 ml of zymosan particles (14 mg ml^{-1} PBS) was mixed in 0.5 ml rat serum and incubated for 30 min at 37°C. The opsonized zymosan particles were then washed, resuspended in PBS at a concentration of 1 mg ml^{-1} and stored for up to 7 days at 4°C. After zymosan preparation, the macrophages (10^5 cells) were incubated in a medium containing 2 mM glutamine, 5 mM glucose, 10% BSA and 1 ml PBS containing opsonized zymosan for 1 h at 37°C with continuous shaking. After incubation, phagocytosis could be quantified by counting (in a counting chamber) the percentage of cells that had phagocytosed more than three particles of zymosan (Costa Rosa et al. 1996).

H_2O_2 production

The production of H_2O_2 was measured by a modification of the method described by Pick and Mizel (1981). Macrophages (10^5 cells) were incubated in siliconized flasks (25 ml) in 1 ml PBS in the presence of 5 mM glucose, 2 mM glutamine and 10% BSA under an atmosphere of 5% CO_2/95% air at 37°C and in the presence of phorbol–myristate–acetate (PMA) (10 ng ml^{-1}) when indicated. After 1 h incubation, a solution of phenol red and horseradish-peroxidase (HRPO) was added to the medium to quantify the hydrogen peroxide content. After 10 min, the reaction was stopped with 100 µl of 1 N NaOH and the amount of hydrogen peroxide formed was measured spectrophotometrically at 620 nm.

Statistical analysis

All results are presented as mean ± SEM. Data were evaluated using Prisma V Program (GraphPad Software, San Diego, CA, USA). Statistical differences were determined by one-way ANOVA and Tukey post hoc test with a significance level set at $P < 0.05$.

Results

Macrophage function is presented in Fig. 1. The rate of phagocytosis was higher in MOD (34.48 ± 1.79%) than in

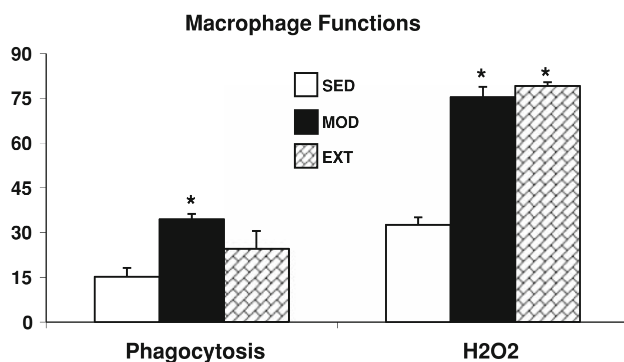

Fig. 1 Rate of phagocytosis (%) and hydrogen peroxide production (H_2O_2) (nmol h × 10^5 cell^{-1}) in macrophages from the sedentary group (*SED*, *n* = 10), moderate training group (*MOD*, *n* = 10) and exhaustive training group (*EXT*, *n* = 10). The values are expressed as mean ± SEM. *$P < 0.05$ difference when compared to SED; $^{\#}P < 0.05$ difference when compared to MOD

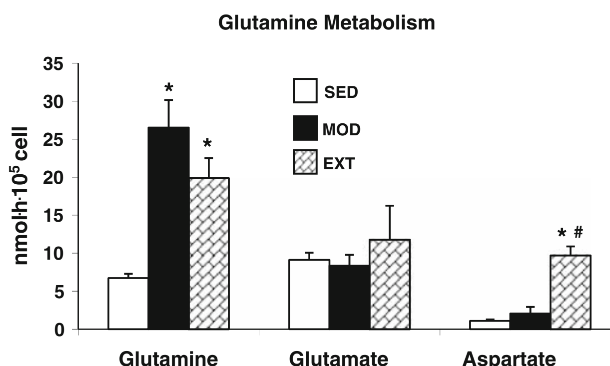

Fig. 2 Consumption of glutamine, and glutamate and aspartate production in macrophages from the sedentary group (*SED*, *n* = 10), moderate training group (*MOD*, *n* = 10) and exhaustive training group (*EXT*, *n* = 10). The values are expressed as nmol h × 10^5 cell and are presented as mean ± SEM. *$P < 0.05$ difference when compared to SED; $^{\#}P < 0.05$ difference when compared to MOD

SED (15.21 ± 2.91%), although there was no difference between SED and EXT (24.60 ± 5.90%) and between MOD and EXT. In contrast, the hydrogen peroxide production increased in trained animals. In the MOD group (75.40 ± 3.48 nmol h × 10^5 cell^{-1}), hydrogen peroxide was higher than in SED (32.60 ± 2.51 nmol h × 10^5 cell^{-1}) (Fig. 1), similar to that observed for EXT (79.20 ± 1.18 nmol h × 10^5 cell^{-1}), showing that this function was higher in trained, in comparison to sedentary, animals.

Figure 2 demonstrates that glutamine consumption was higher in MOD (26.53 ± 3.62 nmol h × 10^5 cell^{-1}) than in SED (6.72 ± 0.57 nmol h × 10^5 cell^{-1}). Similar changes were observed when comparing the SED and EXT groups. Glutamine consumption was higher in animals from the EXT group (19.82 ± 2.62 nmol h × 10^5 cell^{-1}) relative to SED. There was no difference between the MOD and EXT groups. Despite differences in glutamine consumption found in both the MOD and EXT groups, there was no difference in glutamate production among the three groups (Fig. 2). Aspartate production was similar between the SED (1.10 ± 0.19 nmol h × 10^5 cell^{-1}) and MOD (2.07 ± 0.86 nmol h × 10^5 cell^{-1}) groups, although aspartate production was higher in animals from the exhaustive group (EXT) (9.72 ± 1.14 nmol h × 10^5 cell^{-1}) relative to SED (1.10 ± 0.19 nmol h × 10^5 cell^{-1}) and MOD (2.07 ± 0.86 nmol h × 10^5 cell^{-1}), as presented in Fig. 2.

Glutamine decarboxylation in macrophages from the three groups is demonstrated in Fig. 3. Decarboxylation was higher in MOD (12.10 ± 0.27 nmol h × 10^5 cell^{-1}) than cells from sedentary animals (1.10 ± 0.06 nmol h × 10^5 cell^{-1}) (Fig. 3) while the EXT group (16.40 ± 2.17 nmol h × 10^5 cell^{-1}) had glutamine decarboxylation rates higher than both the SED (1.10 ± 0.06 nmol h × 10^5 cell^{-1}) and MOD (12.10 ± 0.27 nmol h × 10^5 cell^{-1}) groups.

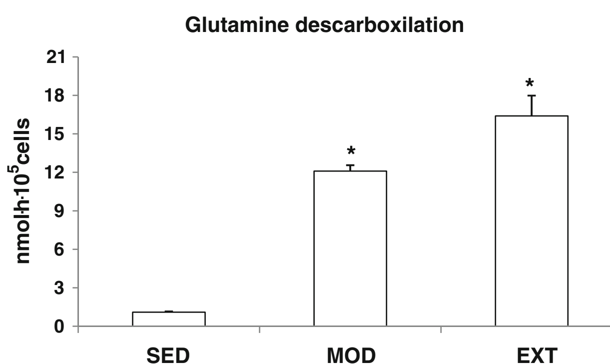

Fig. 3 Glucose and glutamine decarboxylation in macrophages from the sedentary group (*SED*, *n* = 10), moderate training group (*MOD*, *n* = 10) and exhaustive training group (*EXT*, *n* = 10). The values are expressed as nmol h × 10^5 cell and are presented as mean ± SEM. *$P < 0.05$ difference when compared to SED

Discussion

Recent studies suggest that moderate exercise enhances the immune response. On the other hand, exhaustive exercise can promote immunosuppression and increase susceptibility to infections and disease. It is also clear that the mechanisms responsible for the relationship between exercise and immunology are poorly understood (Peijie et al. 2003).

Macrophages play an important role in the immune and inflammatory response. They are the first line of defense against external aggression and infections, because of their phagocytic (the principal function), cytotoxic and intracellular killing capacities (Woods et al. 2006). In addition, macrophages, when stimulated, can release several molecules including cytokines and display antigen presenting activity and phenotypes (Stout and Suttles 2005).

With respect to the effect of exercise on macrophages, several studies have demonstrated exercise-induced increases in function, such as chemotaxis, adherence, respiratory burst and phagocytosis (Woods et al. 2006). However, there have been few studies on human macrophages, likely due to technical difficulties associated with their collection. Hence, most studies use animal models, i.e., rats or mice, and many of them use peritoneal cells for ease of handling and access (Ortega 2003).

Our results confirm previous studies that observed enhanced exercise-induced macrophage function. Some studies have suggested that the increased macrophage function (i.e., H_2O_2 production) is independent of exercise duration, while other functions, such as phagocytosis, are more sensitive to exercise load and recovery periods.

At 1 h after exercise, the macrophages of exercised animals were more active when compared to those of sedentary animals. The macrophage phagocytic capacity is considered the most important function for these cells. In addition, there is some evidence that the effect of exercise on rodent macrophages reflects similar responses in humans (Ortega 2003). In our study, macrophages from the MOD group showed increased phagocytosis relative to the SED group, which has been confirmed in other studies (Fehr et al. 1988; De La Fuente et al. 1990; Sugiura et al. 2001; Ortega 2003), but not in the EXT group, which did not differ when compared to the SED group.

With regards to other macrophage functions assayed, both exercise groups showed increased H_2O_2 production, confirming previous studies with similar results (De La Fuente et al. 1990, 1993; Woods et al. 1994; Ortega et al. 1996; Sugiura et al. 2001). These results indicate that exercise increases hydrogen peroxide production by peritoneal macrophages, independent of the exercise load, since rats from the MOD group were trained for 1 h, while exhaustively trained rats performed 3 × 1-h sessions with 150 min of rest between sessions.

Many studies have shown that macrophages utilize high rates of glucose and glutamine (Newsholme et al. 1987). Peritoneal macrophages satisfy their energy requirements through anaerobic glycolysis and aerobic oxidation of glutamine and fatty acid (Costa Rosa et al. 1996); therefore, macrophage metabolism is markedly modified by diet composition and the presence of adequate substrate.

Despite of the role of glutamine for maintenance of macrophage function and energy production, the effect of exercise on glutamine metabolism is unknown. This was the first study that assayed macrophage glutamine metabolism in exercised animals. We found exercise-induced increases in glutamine consumption in both the MOD and EXT trained groups, suggesting that increased macrophage functionality is supported by high glutamine consumption. Glutamine provides nitrogen for transamination reactions, either directly or via glutamate. In addition, glutamine is a precursor of purine and pyrimidine synthesis for the maintenance of high transcription rates (Newsholme et al. 1996).

Despite increased glutamine consumption in the MOD and EXT groups, glutamate production was unchanged. Interestingly, in sedentary animals, almost 100% of glutamine consumption by macrophages was converted to glutamate, while this fraction was altered in the trained animals. In the MOD group, about 35% of glutamine was transformed into glutamate, while 55% was transformed in the EXT group. With respect to aspartate production, exercise in the EXT group increased aspartate production relative to the SED and MOD groups. Additionally, 40% of glutamine was used for aspartate synthesis in contrast to other groups that observed a conversion of about 2%. The high conversion of glutamine to aspartate can suggest an elevated rate of mRNA to satisfy the need for biosynthesis during increase in macrophage activity. In fact, aspartate may be a source of nitrogen for the synthesis of purine and pyrimidine nucleotides. The nucleotides are needed for the synthesis of new DNA, RNA, mRNA synthesis and DNA repair in macrophages; however, new studies are needed to confirm this hypothesis (Castell 2003; Rowbottom et al. 1996; Newsholme et al. 1985).

In addition to the high rate of purine and pyrimidine synthesis, these metabolites are necessary for enhanced energy production to support macrophage function. In fact, glutamine oxidation by macrophages in the MOD group was 12 times greater relative to the sedentary animals, while, in the EXT group, glutamine oxidation was enhanced 16-fold.

This study also demonstrated that higher macrophage function was possible, because there was an increase in glutamine consumption and metabolism. But, our study was performed in vitro and the cells were incubated in a medium under ideal conditions and sufficient glutamine concentrations, which are different from those of in vivo conditions. In addition, in vivo, other mechanisms, such as hormone concentrations, especially cortisol and catecholamines (Forner et al. 1995; Ortega et al. 2001, 2005), may influence macrophages during and after exercise.

As described in literature, there is a decrease in glutamine plasma concentration after exercise. It is possible that, in the in vivo setting, the decrease in glutamine plasma concentration after exercise may contribute to immunosuppression after exhaustive exercise during the open window period.

Several studies have shown a decrease in glutamine plasma concentration after exhaustive exercise in humans and animals (Castell et al. 1996; Koyama et al. 1998; Walsh et al. 1998; Bassit et al. 2000) This decrease in plasma glutamine correlates with increased symptoms of upper respiratory tract infections (Bassit et al. 2000; Castell 2003), due to impairment of the immune system cells function, such as

suppressed mitogen-induced lymphocyte proliferation response and change in the production of cytokines and antibodies (Koyama et al. 1998; Dos Santos Cunha et al. 2004). Parry-Billings et al. (1992) suggested that a small decrease of about 10% in plasma glutamine concentration may impair immune response. On the other hand, the maintenance of plasma glutamine concentration has been associated with a decrease in upper respiratory tract infections (URTI) and an improvement in immune parameters after exhaustive exercise (Koyama et al. 1998). Literature shows that glutamine consumption by immune system cells depends on glutamine concentration (Castell 2003; Costa Rosa 2004). So, the maintenance of plasma glutamine concentration close to resting levels maintains the function and metabolism of immune system cells (Costa Rosa 2004). This observation was supported recently in our laboratory while 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. BCAA supplementation reversed the reduction in plasma glutamine concentration after exercise, allowed the athletes from the supplemented group to present an enhanced production of cytokines, increased proliferative response to concanavalin A and lipopolysaccharide, mitogens to T and B lymphocytes, respectively, and a decrease in the symptoms of upper respiratory tract infection reported elsewhere (Bassit et al. 2000).

In conclusion, our results suggest that the increase in macrophage function induced by exercise is supported by enhanced glutamine consumption and metabolism, which highlights the importance of this amino acid in the modulation of macrophage function during and after exercise.

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