

The Effect of the Short-Term Glutamine Supplementation on Exhaustive Exercise-Induced Changes in Immune System of Active Boys

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Abstract: The purpose of this study was to examine the effect of the short-term glutamine supplementation on exhaustive exercise-induced changes in immune system of active boys. Twenty-four active boys (age 18.77 ± 1.2 years, body mass 57.42 ± 4.5 kg, VO_{2max} 42.96 ± 2.31 ml. kg^{-1} . min^{-1}) were randomly divided in a double-blind fashion into either a glutamine treatment group or a placebo group. Subjects performed Bruce maximal test to exhaustion for two separate days with a 4-day interval. L-Glutamine supplement mixed with sugar-free lemonade (0.1 g/kg) or placebo (only sugar-free lemonade) was given during the interval between the tests. One day before the first test and one week after the second test, upper respiratory tract infection symptoms of subjects were assessed. Unstimulating salivary samples were collected after 7 minutes (to evaluate salivary immunoglobulin A concentration, secretory immunoglobulin A and salivary cortisol concentration) and salivary flow rate 20 minutes after performing Bruce maximal test. Statistical analysis ($P < 0.05$) showed the significant effect of short term L-glutamine supplementation on levels of salivary immunoglobulin A concentration ($P = 0.001$) as well as Secretory immunoglobulin A (S-IgA) ($P = 0.001$), but it had no effect on salivary immunoglobulin A ($P = 0.102$) and S-IgA ($P = 0.060$) concentrations when compared with age-matched placebo group. This study does not support that a decrease in plasma glutamine after a bout of exhaustive exertion is related to immune impairment.

Key words: L-glutamine Supplementation % Salivary IgA Concentration % S-IgA % Cortisol % Salivary Flow Rate % URTI % Bruce Test

INTRODUCTION

Various systems affect human body function. Immune system is one of the apparatuses very important for individual health. It involves many changes in response to physical training. Some studies show that physical training with various intensities and durations make various responses in immune system [1]. Light to moderate exercise appears to have a beneficial effect on the immune system, whereas prolonged bouts of exercise and heavy training bouts cause temporary immune impairment [2]. Secretory immunoglobulin A (S-IgA), the predominant immunoglobulin in mucosal secretion, is a major effector of resistance against pathogenic microorganisms causing upper respiratory tract infections (URTI) [3].

The reduction in S-IgA is related to the increase in the risk of URTI in sedentary subjects and elite athletes [4, 5]. S-IgA inhibits bacterial adherence, neutralizes viruses and toxins and prevents the absorption of

antigens through mucosal surfaces [2]. Several studies have shown the significant reduction of S-IgA level after one bout of treadmill maximal test [6], strenuous training [7], interval training [8] and marathon race [9] as a result of overtraining [3]. Krzykowski *et al.* [2] reported a significant reduction of immunoglobulin A (IgA) concentrations and S-IgA after a cycle ergometer exercise for 2 hours at 75% of maximal oxygen uptake in 3 separate days. The duration of the exercise-induced reduction in salivary IgA concentration was found lasted at least one hour and it returned to pre-exercise levels 24 hours after a single bout of severe exercise [10].

In addition to intense exercise, cortisol levels have been associated with immunosuppression [11]. Several reports have shown that cortisol concentrations have negatively correlated with URTI [9, 11]. Heller *et al.* [12] report a significant elevation of cortisol levels 10 minutes after Bruce protocol treadmill test. In addition, O'Connor and Corrigan [13] reported a significant increase in plasma and saliva cortisol level promptly and 15 minutes after an

ergometry at 75% of maximal oxygen uptake when compared with the rest level. However, Hooper *et al.* [14] in a study assessed the stress hormones of fourteen elite swimmers in five points during a 6-month training season. The points included the early-, mid- and late-season, during tapering for national trials and 1-3 days after the trials. No significant differences were seen in cortisol and norepinephrine concentrations at the five sampling points. Although blood has been traditionally used for cortisol assessment, saliva reflects the level of unbound cortisol more accurately than serum total cortisol. Unbound cortisol, as in saliva, gives a direct measurement of the biologically available molecules, whereas bound cortisol, as in serum, is physiologically inactive. In addition, measurement of serum cortisol requires venepuncture, which is associated with negative feelings and could increase cortisol and decrease IgA. On the other hand, saliva flow rate (Saliva-FR), which significantly decreases after exercise, may be the most influential factor in terms of protection against oral pathogens and infections. This can be supported by findings which show that people suffering from xerostomia (dry mouth syndrome) have a substantially increased incidence of oral infections and more pathogenic bacteria in their buccal cavity [15].

Understanding the relationship between exercise and infection has potential implications for public health and for the athlete, it may mean the difference between being able to compete or perform at a subpar level or miss the event altogether because of illness. On the other hand, the results of some studies show that glutamine is an important fuel for immune cells and the decreased availability of glutamine to immune cells may be a key factor in immune system suppression after physical activity [16]. Therefore, we hypothesized that glutamine supplementation may be beneficial by maintaining the plasma glutamine concentration and hence preventing the impairment of immune function. Plasma glutamine concentration can decrease in response to prolonged, exhaustive exercise such as a marathon race [17], repeated exercise [18] and during 10 weeks of intensive training [19]. Furthermore, an increased incidence of infection was observed in athletes with decreased plasma glutamine concentration, although this outcome is not consistently reported [16]. Newsholme (1994) postulated that when plasma glutamine concentration decreases below a physiologically normal range of 0.5– 0.9 millimole (mM), limited glutamine availability may impair certain immune cell functions and, in turn, increase an individual's susceptibility to infections such as URTI [20].

Krzywkowski *et al.* [2] reported a 15% decrease in plasma glutamine concentration after 2 hours of physical training at 75% of maximal oxygen uptake in endurance athletes. However, Castell *et al.* [21] showed that glutamine supplementation decreased the incidence of URTI. In contrast, Mackinnon and Hooper [3] reported that swimmers who had overtraining syndromes were compared with swimmer who did not have this syndromes. The first group decreased glutamine concentration but their URTI symptoms were lowered. Hence, various reasons can increase the risk of URTI. Various stressors such as physiological, peripheral, nutritional and exposure to a foreign pathogen increase the incidence of URTI in athletes [22]. It has been proposed that the relationship between exercise and URTI may be modeled in the form of a “J” curve [23]. This model suggests that although the risk of URTI may decrease below that of a sedentary individual when one engages in moderate exercise training, risk may rise above average during periods of excessive amounts of high-intensity exercise [24]. Recently, Matthews *et al.* [25] reported that the regular performance of about 2 hours of moderate exercise per day was associated with a 29% reduction in the risk of picking up a URTI when compared with a sedentary lifestyle.

Linking the possible association between S-IgA [11, 16], IgA concentration [4, 5] and cortisol [9, 11] with URTI on the one hand and glutamine supplementation and URTI [16, 17, 21] on the other led to the hypothesis that short-term glutamine supplementation might affect the immune index of active boys after one bout of exhaustive exercise. Some researchers examined the effect of short-term creatine [26] and chronic glutamine supplementation [16] on immune system, but the effect of short-term glutamine on immune system was not clear. Therefore, the purpose of this study was to determine whether short time, high-dose L-glutamine supplementation would affect S-IgA following the Bruce standard protocol. We hypothesized that supplementation would attenuate a training-induced decrease in secretory IgA concentrations.

MATERIALS AND METHODS

Experimental Design: All experiments on humans were conducted according to Department of Physiology, University of Mazandaran, for experimental purposes. All protocols and written consents were obtained before data collection. Subject characteristics are present in Table 1. Subjects were gathered by an advertisement in

Table 1: Characteristics of subjects

Variable	Glutamine (n = 12)	Placebo (n = 11)	P Value
Age, yr	18.58 ±1.18	18.98±1.26	0.438
Height, cm	172.41 ±4.25	173.63 ± 5.57	0.559
Body mass, kg	56.46 ±4.33	58.46 ±4.77	0.305
Body fat, %	11.92 ±2.42	12.75 ±1.82	0.369
BMI, kg/m	18.99 ±1.37	19.38 ±1.23	0.491
VO _{2max} , ml.min ⁻¹ .kg ⁻¹	44.33 ±3.07	43.1 ±2.36	0.298

Values are means ± SE

their education department. They were recruited from two boarding high schools in Kerman city. Both high schools had 300 students and at the first phase, 75 students were selected. Inclusion and exclusion criteria were carried out as follows: one week before the first test day, the students were acquainted with the study design and the subjects' profile of mood was assessed by Cattell anxiety and depression test [27]. Also, habitual activities were assessed by health logs and those applicants who had habitual activities below 6 hours per week and suffered from anxiety and depression more than the normal range and, were eliminated from our experimental design. As body fat affects immune system [28], 5 days before the first test day, body fats of 57 remained applicants were assessed and 7 applicants who had body fat more than 20% were eliminated from our experimental trial. Then, these 50 subjects performed the Rockport sub-maximal walking test; 30 subjects who scored higher were selected. 4 days before the first test day, weight, height and body mass index were recorded. Glutamine concentration was assessed and subjects whose glutamine concentrations were lower or higher than the normal range (0.5–0.9 mM) [16] were eliminated from our groups. One day before the first test day, URTI symptoms of subjects were assessed by the questionnaire. Subjects recorded their health problems every day of the first week with codes used by Nieman *et al.* [29]. Finally, 24 remaining subjects were randomly assigned to receive glutamine (n=12) and placebo (n=12). A subject from the placebo group was withdrawn after the first testing day due to self-diagnosed URTI. In two separate days and a 4-day interval, subjects performed Bruce maximal test until exhaustion on a treadmill. Subjects were instructed to refrain from any intense physical activity, dietary supplements and medicines for 24 hours before the test session. Furthermore, tobacco and caffeine consumption were restricted for 12 hours as well as eating for eight hours before testing [15]. On the night preceding each exercise test, each subject slept for a mean (SD) of 7.4 (±1.3)hours.

Supplementation: The study used a randomized, double-blind, placebo-controlled crossover design. During the trial, subjects received 0.1 g/kg of L-glutamine mixed with sugar-free lemonade or a placebo (sugar-free lemonade only) [16] for 4 days between performing of tests. The glutamine or placebo was mixed in 300 ml of 50- 60°C hot water [16]. The glutamine and placebo beverages were identical in appearance and taste when they were mixed. The subjects of both groups began supplementation after lunch in the first day after the test. Subjects were allowed to drink water throughout the study except one hour before the sampling of saliva.

Saliva Collection: Unstimulating saliva samples were collected in pre-chilled and pre-weighed plastic universal containers (20 ml) seven minutes after the test. The saliva samples were obtained according to a standardized procedure, as described by Krzywkowski *et al.* [2].

Blood Sampling: 4 days before the first test day, blood was obtained from an antecubital vein to assess plasma glutamine levels.

Salivary IgA Concentration: Saliva samples were analyzed for IgA concentrations by an enzyme-linked immunosorbent assay (ELISA) according to Krieger *et al.* [16].

Saliva Flow Rate (Saliva-FR): The Saliva-FR was calculated by dividing the sample volume (ml) by the time (min) taken to produce it [15].

IgA Secretion Rate (S-IgA): S-IgA was calculated by multiplying the absolute IgA concentration (mg/ml) by the Saliva-FR (ml/min) [15].

Salivary Cortisol Concentration: Cortisol levels were assessed by a DPC coat-a-count cortisol kit. Total plasma concentrations of cortisol were measured in duplicate by commercial solid-phase¹²⁵I radioimmunoassay kits.

¹²⁵I-labeled cortisol competes for antibody sites for cortisol within the sample. The antibody is bound to the wall of the polypropylene tube, so when the supernatant is decanted, the antibody-bound fraction of the radio-labeled cortisol is still present. The amount of cortisol present in the sample is measured by a gamma counter [11].

Plasma Glutamine Determination: Blood was drawn into glass tubes containing EDTA and was centrifuged at 2,500 g for 15 minutes at 4°C. Plasma was stored at -80°C and analyzed by high-performance liquid chromatography (HPLC) [30].

Statistical Analysis: All values reported are mean (SD). All data were checked for normality and equality of distribution before any analysis was performed. Independent and dependent t-tests were used to analyze the differences between means of variables either in each

group or in the supplementation and placebo groups respectively. All analyses were performed using SPSS 14. In all tests, P#0.05 was considered significant.

RESULTS

There were no statistically significant differences between groups found in physical characteristics (Table 1). Table 2 show plasma glutamine concentration, salivary IgA concentration, Saliva-FR, S-IgA, cortisol concentration and number of days with URTI symptoms in glutamine and placebo groups and in 2 stages of pre and post supplementation. Significant effects of treatment on salivary IgA concentration (P=0.001; Fig. 1B), Saliva-FR (p=0.001; Fig. 1C), S-IgA (p=0.001; Fig. 1D) and cortisol concentration (p=0.001; Fig. 2A) were found in both groups of glutamine and placebo and significant effects of treatment on plasma glutamine concentration were found in glutamine group (p=0.016), but not in

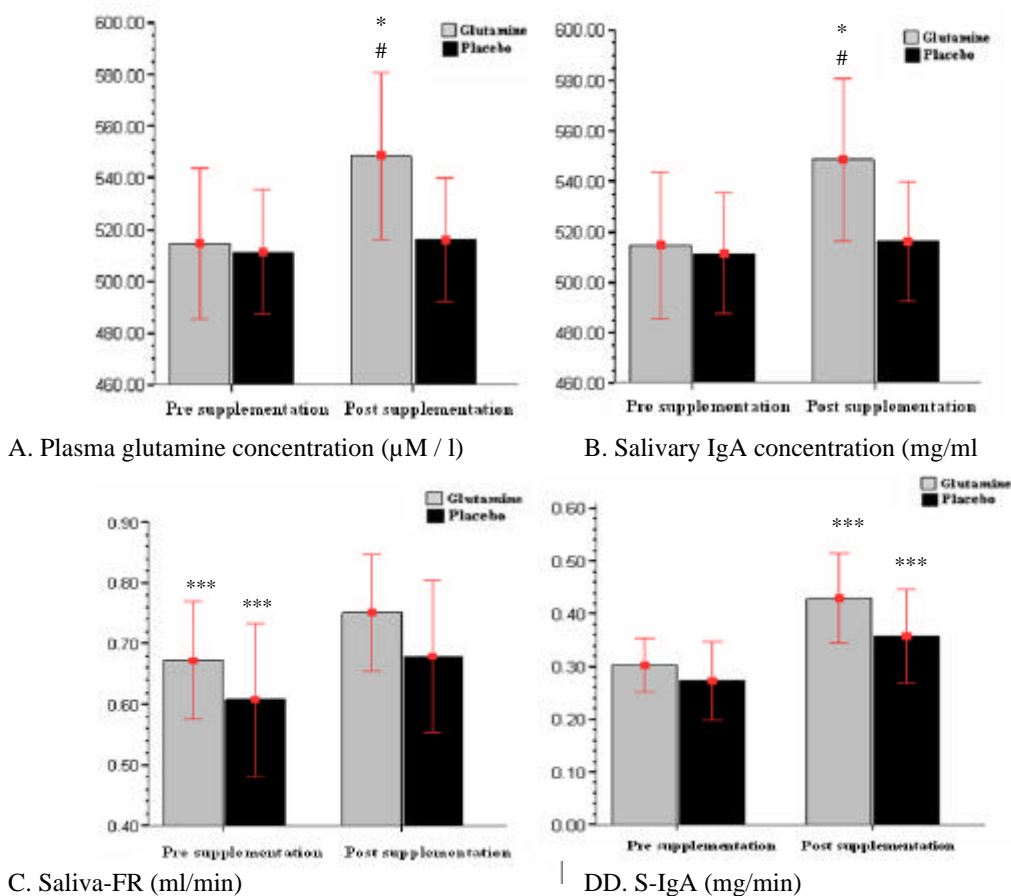


Fig. 1: Plasma glutamine concentration (A), Salivary IgA concentration (B), Saliva-FR (C), and S-IgA (D) in the glutamine and placebo groups. Values are means ± SE. Significantly different from pre-supplementation: *p<0.05; **p<0.01; ***p<0.001. Significantly different from placebo group: #p<0.05.

Table 2: Comparison of salivary IgA concentration or output, cortisol concentration, Saliva-FR and number of days with URTI symptoms in glutamine and placebo groups

Variables	Glutamine		Placebo		P Values
	Pre supplementation	Post supplementation	Pre supplementation	Post supplementation	
Plasma glutamine concentration ($\mu\text{M} / \text{l}$)	514.75 \pm 29.180	548.58 \pm 32.320	511.36 \pm 24.010	516.09 \pm 23.730	0.013*
Salivary IgA concentration (mg/ml)	0.460 \pm 0.075	0.578 \pm 0.083	0.456 \pm 0.058	0.529 \pm 0.047	0.102
Saliva-FR (ml/min)	0.672 \pm 0.090	0.751 \pm 0.090	0.607 \pm 0.120	0.679 \pm 0.120	0.132
S-IgA (mg/min)	0.302 \pm 0.050	0.429 \pm 0.080	0.272 \pm 0.070	0.357 \pm 0.080	0.060
Cortisol concentration (ng / ml)	46.175 \pm 2.407	33.166 \pm 6.210	45.363 \pm 3.714	34.009 \pm 6.250	0.749
Number of days with URTI symptoms (day)	0.41 \pm 0.5100	0.50 \pm 0.5200	0.54 \pm 0.6800	0.63 \pm 0.6700	0.488

Significantly different: * p<0.05

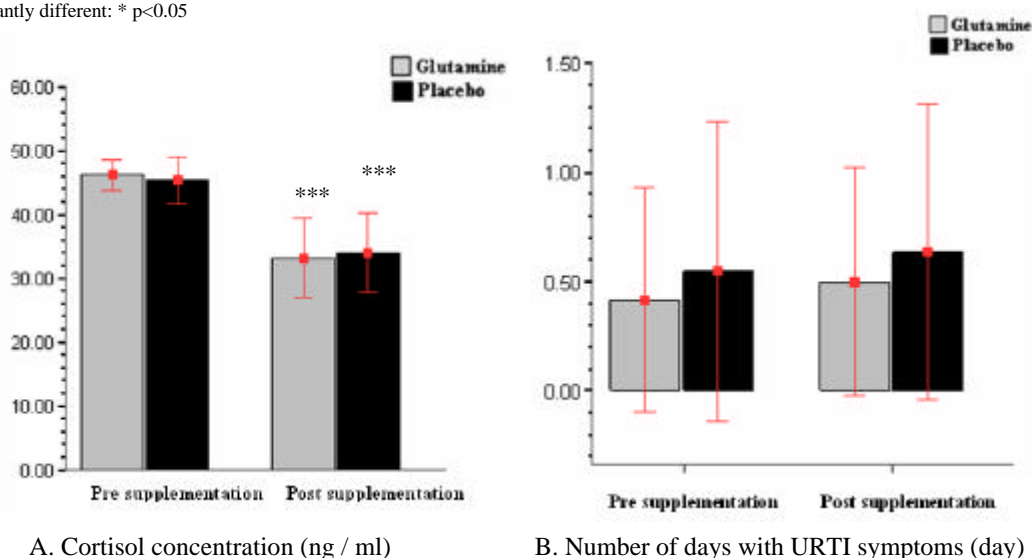


Fig. 2: Cortisol concentration (A), and number of days with URTI symptoms (B) in the glutamine and placebo groups. Values are means \pm SE. Significantly different from pre-supplementation: ***p<0.001.

the placebo group ($p=0.096$; Fig. 1A). Also, no significant effects in the number of days with URTI symptoms were found in glutamine ($p=0.723$) and placebo group ($p=0.756$; Fig. 2B). In addition, no Significant effects were found between groups on salivary IgA concentration ($p=0.102$; Fig. 1B), Saliva-FR ($p=0.132$; Figure 1C), S-IgA ($p=0.060$; Fig. 1D), cortisol concentration ($p=0.749$; Fig. 2A) and URTI symptoms ($p=0.488$; Fig. 2B), but significant effects were found between groups on plasma glutamine concentration ($p=0.013$; Fig. 1A).

DISCUSSION

The main finding of the present study was that none of the salivary parameters evaluated showed an effect of supplementation compared with the placebo group, although glutamine supplementation attenuated the exercise-induced decline in glutamine concentration. As shown in Table 2, short-time, high-dose L-glutamine

supplementation was not able to attenuate the exercise-induced reduction in salivary IgA. This finding is consistent with Krzywkowski *et al.* [2] and Yalcin *et al.* [31], but in conflict with the findings of Lai *et al.* [32] and Krieger *et al.* [16]; these findings show a decrease in salivary IgA concentration. This difference may have many reasons. One of them is related to methods and data collection styles. Lai *et al.* assayed IgA levels in plasma. Observed differences between studies may be due to the determination of IgA levels in plasma vs. saliva, which makes the comparison of studies difficult. Chwalbinska-moneta *et al.* [33] reported that adaptation to physical training caused a decrease in cortisol levels. This researcher believes that the lowered cortisol level in the posttest is attributed to subjects' acquaintance with the test. Therefore, perhaps, this factor in the present study caused a decrease in subjects' cortisol concentration and an increase in IgA concentration and S-IgA after supplement in both groups. Some studies confirm this

claim. Also, the difference of IgA concentration and S-IgA in the present study can be attributed to subjects' age and fitness when compared with Krieger [16]. Miletic *et al.* [34] reported that Saliva-FR and S-IgA level is significantly lower in the elderly than the youth. Considering that S-IgA is affected by Saliva-FR and salivary IgA concentration, perhaps, lower age and fitness level of our subjects partly responsible for the observed differences between the present study and Krieger [16].

Also, type [7], intensity [6] and duration [3] of physical activity affect immune system. Krieger *et al.* (2004) investigated the effects of chronic glutamine supplementation on salivary and nasal IgA concentration. Subjects participated in interval training protocol for 9–9.5 days and twice per day. Morning sessions were conducted between 6 and 9 AM and included 15 × 1-min periods separated by 2 min of recovery, where subjects were permitted to walk briskly. Afternoon sessions were conducted between 4 and 8 PM and involved 10 × 1-min run periods separated by 1 min of recovery. The training period was followed by a 5-7 days recovery period. The treatment and training protocols did not significantly alter salivary IgA concentration. But mean nasal IgA across the study period was greater in runners receiving glutamine vs. placebo. These results differ from the present study and several explanations such as type, intensity and duration of physical activity are possible. Another case that can explain the observed difference between studies is the difference in sampling time. In our study, samples were gathered 7 minutes after the training protocol. This method was used by other researchers, but Krieger *et al.* (2004) gathered saliva samples between 6 - 8 a.m. and during interval training for 9–9.5 days followed by a recovery (5–7 days) [16]. Also, Dimitriou *et al.* [15] studied circadian effects on the acute responses of salivary cortisol and IgA in well-trained swimmers and reported S-IgA and Saliva-FR levels measured before exercise to be higher in the evening than in the morning (in contrast with IgA concentration). This may be explained by sympathetic nerve activity, which is higher in the morning than at other times of the day. Increased sympathetic activity decreases Saliva-FR whereas parasympathetic activity increases it [15]. Furthermore, many steroid hormones such as cortisol have been found to affect the composition - for example, IgA, electrolytes - and secretion rate of saliva. Cortisol has been shown to peak in the morning and to fall in the evening [15]. Furthermore, salivary cortisol is an immunosuppressive hormone that plays an important role

in the function of some immune system cells, especially B-lymphocytes [1]. IgA concentrations produced by B-lymphocytes [22], change in response to a reduction or weakening function of these cells.

Carbohydrate and immune system reciprocal effect is one of the major cases that must be examined. During the past few years, attempts have been made to identify nutritional supplements that could attenuate exercise-induced immune changes. Some studies have shown that carbohydrate adjusts the effect of physical activity on IgA levels [9, 35, 36]. Costa *et al.* [36] observed a significant increase in IgA concentration of subjects with hyper-carbohydrate diet (12 g/kg) after physical training. Considering that the rate of carbohydrate in our study is very lower than the rate used by Costa *et al.* [36], probably IgA concentration and S-IgA changes are related to carbohydrate consumption.

Costa *et al.* [36] reported that carbohydrate supplementation affects cortisol concentration. In the present study, the glutamine supplementation was compared with carbohydrate-containing placebo. Thus, the effect of glutamine supplementation on cortisol might have been confounded by the carbohydrate in the placebo. The change in cortisol is not related to carbohydrate consumption because carbohydrate rate is the same in both groups. Also, Volek *et al.* [37] reported that protein and fat in diet are related to pre-training levels of testosterone but this nutrition variable is not related to cortisol concentration. Boarding high school students in both glutamine and placebo groups had similar scheme of dieting and it is not possible that the diet might have had an effect on the cortisol concentration.

Oral glutamine supplementation has been shown to elevate glutamine uptake by skeletal muscle, small intestine and the splanchnic area. In response to acute, exhaustive exercise, oral glutamine supplementation attenuates exercise-induced decreases in plasma glutamine concentration. The mechanisms behind the reduction in plasma glutamine concentration in response to training are not currently known but may include renal uptake of glutamine to maintain acid-base balance under conditions of exercise-induced acidosis [16, 38]. Results of the present study about plasma glutamine levels showed an insignificant difference in plasma glutamine levels between the supplemented and placebo groups in pretest, but a significant difference in plasma glutamine levels at the end of the supplementation period. Also, during recovery from exercise, plasma glutamine concentration declined in the placebo group, whereas plasma glutamine concentration was maintained in the

glutamine supplementation group. However, glutamine supplementation did not have a significant effect on immune indexes. In the present study, exhaustive exercise can not deplete the body's glutamine stores. Perhaps for this reason, glutamine supplementation can not affect immune indexes such as cortisol. Differences may also in part be explained by the difference in time of saliva collection. Time of saliva collection in our study was 7 minutes after the test. This method was used by other researchers [15], but Krieger *et al.* [16] collected the saliva between 6:00 and 8:00 a.m. Another case that must be considered is the consumption rate and duration of supplementation in studies. Some investigators examined the effect of chronic, high-dose glutamine supplementation [16]. For example, duration of supplementation in the study of Krieger *et al.* [16] was four times daily for 14 days. Furthermore, subjects received 0.1 g/kg of L-glutamine supplement mixed with sugar-free lemonade that was the same as our study. Krieger *et al.* [16] reported that chronic glutamine supplementation does not affect salivary IgA concentration or output; it has been shown in the present study as well.

Other result of this study was an insignificant change of Saliva-FR between supplemented and placebo groups. Dimitrio *et al.* [15] reported that circadian effects are a major factor that can affect Saliva-FR. These researchers showed that Saliva-FR significantly decreased after physical training and it was higher in the evening than in the morning. Therefore, circadian effects may be responsible for an observed difference between studies. In the present study, the Saliva-FR unstimulating samples were collected 20 minutes after the training protocol. This method was used by other researchers [2]. Krieger *et al.* [16] collected saliva samples between 6:00 and 8:00 a.m. and during the interval training for 9–9.5 days followed by 5–7 days of recovery. It should be noticed that, however, a difference in saliva sampling style (rest saliva, whole saliva stimulation and parotid stimulation) in various studies makes it difficult to compare studies. Many studies show that saliva flow stimulation by chewing a single Parafilm (5 cm²) section for 1 minute increases epithelial cell transcytosis of IgA into salivary fluid and may have masked the effects of glutamine supplementation on basal IgA secretion in unstimulated samples [16]. In our study, saliva samples have been collected by unstimulated method, but Krieger *et al.* [16] collected them by stimulated salivary method.

Epidemiological and anecdotal evidences suggest that athletes engaged in high-volume and/or high-intensity trainings are at an increased risk of upper respiratory tract infections (URTI) when compared with athletes engaged in more moderate forms of training [16]. For example, some studies show that symptoms of URTI such as sore throat and flu symptoms are prevalent in athlete when compared with non-athletes [39]. Sometimes, this little sickness in training or race may change the result of race from win to no-win. The mechanisms behind these clinical manifestations are not clear but may result from training-related changes in immune indexes, including decreased salivary IgA concentration and secretion rates [16, 40, 41]. The result of this study showed an insignificant change of URTI between supplemented and placebo groups. Some studies reported that a decrease in S-IgA is related to an increase in the risk of URTI trial in sedentary and elite athletes. Therefore, the lack of a significant effect of URTI symptoms may be related to insignificant effect of glutamine supplementation on IgA concentration and S-IgA. Furthermore, Saliva-FR was recognized as an important factor to protect against infections and oral diseases. Therefore, perhaps an insignificant difference in URTI symptoms may be related to any significant change in Saliva-FR of our subjects.

CONCLUSION

In conclusion, this study showed that short-time, high-dose L-glutamine supplementation did not have a significant effect on salivary IgA concentration or output, cortisol concentration, Saliva-FR and the number of days with URTI symptoms in active boys of the present study, but glutamine supplementation attenuated the exercise-induced decline in glutamine concentration. Therefore, the available data of active boys do not support the contention that the post-exercise change in some immune indices is caused by a decrease in plasma glutamine concentration.

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