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The Effect of Glutamine Supplementation on Immune System in Female Athlete Students of Shahid Chamran University

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Abstract: The purpose of this study was to investigate the effects of glutamine supplementation on immune system of Shahid Chamran University athlete students. 30 volunteers were selected and randomly divided into three groups: glutamine supplementation, placebo and control group. Subjects' entire predetermined immune system factors were measured. All subjects' VO2max was measured by Jostrand test during the pretest. The glutamine supplementation and placebo group exercised up to exhaustion with an ergometer and with 75% of their Vo2max for two hours. 14 gr. glutamine or placebo were used so that each subject took 3.5 gr. glutamine or placebo every 45 minutes in a double-blind way (4 times, 45, 90, 135, 180 minutes, 3.5 gr. every time). The control subjects were asked not to exercise. Two hours after the exercise, blood samples were again gathered in order to measure the research variables for the posttest. Descriptive statistics, dependent and independent T test, 2-way ANOVA and the post hoc Tukey test at α=0.05 were used to test the hypotheses. The findings were as follows: there was a significant decrease in neutrophils of glutamine group (α=0.029). After two hours of training, there was a decrease in CD4 cells of the placebo group when compared with glutamine or control groups. The overall results showed that glutamine supplementation might have a positive effect on athletes' immune systems during intensive training. As a result, athletes are recommended to use glutamine supplementation during intensive training in order to minimize the probable exercise-induced effects.

Key words: Glutamine supplementation · Immune system · Athlete

INTRODUCTION

One of the subjects interesting to be studied by sport medicine is the effect of physical activity on various systems of body such as immune system. Immune system is one of the important systems protecting the body against pathogenic factors. This system is capable of producing cells and molecules which recognize and eliminate all foreign substances [1]. Various researches on the role of physical pressures on the immune system show that training induces much physiological changes in the immune system. Several researches show that the probability of upper respiratory tract infection increases during 1-2 weeks of intensive training to be prepared for endurance competitions [2, 3]. Also, several researchers believe that the body resists infections in regular training and the probability of upper respiratory tract infection decreases [4]. It is reported that intensive training induced neutrophilia (an increase in blood neutrophils) and lymphopenia (a decrease in blood lymphocytes) [5, 1].

Rohde *et al.* as well reported that short intensive training induced leukocytosis (an increase in leucocytes) and it takes about 30 minutes [6].

Other researches show that lymphocyte concentration increases during training, but it is lower after long intensive training than rest and such a reaction was not observed after moderate training [7]. It was also observed that during an intensive training the ratio of CD8 to CD4 decreases [8, 9]. The researches on immunology show that glutamine is used as an important ergogenic source during the training. Therefore, during the last minutes of an intensive training, as the carbohydrates decrease, glutamines and other amino acids (leucine, isoleucine and valine) are used as ergogenic substrate and obtain about 10%-20% of the energy the cells need [10]. Glesson (2002) reported that the normal level of glutamine is essential for some leucocytes such as lymphocytes and macrophages to efficiently operate. After the training, it seems that some weaknesses happen in several operative aspects of the immune system due to a glutamine reduction and when the training is intensive, the function of the immune system will reduce [11].

Many researches show that after repetitive bouts of intensive or constant training, the glutamine concentration reduces to a great extent [12, 11]. Other

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researches show that 4-12 gr. glutamine before and during the training increases the glutamine concentration, consequently increases protein synthesis and improves the immune functions [13, 10]. Recent researches on glutamine show that glutamine supplementation prevents the reduction of glutamine level after the training and does not affect the immune system disorders after the training [14]. There is not much support for the hypothesis that a decrease in glutamine results in a decrease in the immune system function after the training [8]. Therefore, the present research tries to investigate the effect of glutamine on the female athletes' immune systems.

MATERIALS AND METHODS

The present research is quasi-experimental and includes two experimental groups and one non-experimental group. First, the female athletes were invited to participate in the research. Next, 30 healthy females were voluntarily selected for the research. All the subjects were absolutely healthy, had no sickness or infection records and had performed constant physical activity for at least two years. They were randomly divided into three groups: control, placebo and supplementation. Table 1 shows the anthropometric and physiological data of the groups.

Subjects' heights and weights were measured by a stadiometer and laboratory scale. The 220 – age formula was used to measure the maximal heart rate and the cycle ergometer (Jostrand) was used to measure Vo_{2max}. 2-3cc blood samples were gathered to investigate the subjects' health risks. The blood samples were dyed and the leucocytes were counted by a counter apparatus and a microscope. Also, CD4 and CD8 cells were examined by particular kits and by a flocytometer set. The data obtained from the first sampling were used to compare the three groups in the pretest. Next, each experimental subject exercised with the cycle ergometer for two hours and 75% VO_{2max}. Each supplementation subject took 14 gr. pure glutamine (researches show that this amount of glutamine every 45 minutes is the best way to preserve the glutamine concentration): four times (45-90-135-180 minutes), each time 3.5 gr. powder dissolved in 0.5 liter mineral water during and after training. The training subjects took placebo (maltodextrin) in the same way. The control subjects did not perform any activity and did not take supplementation or placebo. The blood samples were gathered again two hours after the training.

Table 1: The anthropometric and physiological data of the three groups

Variables	Supplementation (n=10) Average	Placebo (n=10) Average	Control (n=10) Average
Age (year)	23.8±1.398	23±1.2472	22.6±0.8432
Height (cm)	162.7±4.738	161.00±4.9888	162.5±3.2403
Weight (kg)	56.64±5.621	54.8±5.5687	54.6±3.2642
Vo_{2max}	37.953±4.299	35.53±4.4174	36.493±3.2092

Statistical Procedures: Two-way variance analysis (ANOVA) and post hoc Tukey test were used to investigate the differences among the three groups. t test was used to investigate the changes of each group in the pretest and posttest. SPSS was used to analyze the data (α=0.05).

RESULTS

The Analysis of the Data Showed the Following Results:

- There was no significant difference in the number of leucocytes among the three groups (consider the significance level in Table 2, α=0.757), but there was a significant increase in the number of leucocytes in the placebo group when compared with the number of leucocytes before the training (α=0.003) and this increase was not significant when compared with the other groups (Table 2 and Figure 1).
- There was not a significant difference between monocytes and lymphocytes of the three groups (Figures 2 and 4).
- There was a significant decrease in the neutrophils of supplementation group when compared with the neutrophils before the training (α=0.029). Tukey test showed this decrease was significant when compared with the control group. The counter-effect of supplementation and training on neutrophils was as well significant (α=0.002) (Tables 2 and 3 and Figure 3).
- There was a significant decrease in the CD4 cells of placebo group when compared with the CD4 cells before the training and when compared with the other groups (α=0.035). The counter-effect of supplementation and training on CD4 cells was as well significant (α=0.041) (Tables 2 and 3 and Figure 5).
- There was a significant increase in the CD8 cells of placebo group when compared with the CD8 cells before the training (α=0.021). This increase was significant when compared with the other groups. The counter-effect of supplementation and training on CD8 cells was as well significant (α=0.017) (Tables 2 and 3 and Figure 6).

Table 2: a comparison of the immune system variables before and after the training in the three groups

	Supplementation group			Placebo group			Control group		
Variables	Before training	After training	Sig.	Before training	After training	Sig.	Before training	After training	Sig.
Leucocytes (no./micro liter)	8130	8990	0.157	7650	9740	*0.003	7610	9077	0.611
Monocytes (%/micro liter)	1.8	1.75	1.00	5	2.33	0.423	3	1.666	0.161
Neutrophils (%/micro liter)	79	68.4	*0.029	65.2	69.5	0.352	61.7	69.22	0.102
Lymphocytes (%/micro liter)	19	25.7	0.167	31.5	27.8	0.450	33.1	29.33	0.798
CD4 (%/micro liter)	59.4	59.3	0.811	57.8	55.2	*0.035	59.4	60.77	0.061
CD8 (%/micro liter)	30	30.7	0.111	31.8	33.9	*0.021	29.8	29.22	0.274

* means that the difference among averages is significant

Table 3: The comparison of the effects of glutamine and training on immune system variables

	Leucocytes (no./micro liter)	Monocytes (%/micro liter)	Neutrophils (%/micro liter)	Lymphocytes (%/micro liter)	CD4 (%/micro liter)	CD8 (%/micro liter)
Supplementation effect	0.757	0.694	0.634	0.289	0.219	0.129
Training effect	0.054	0.559	0.934	0.948	0.907	0.593
Counter-effect	0.438	0.297	*0.002	0.103	*0.041	*0.017

* means that the difference among averages is significant

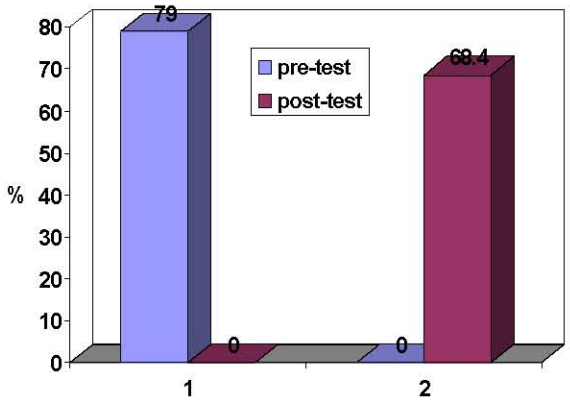

Fig. 1: The comparison of leucocytes in the 3 groups

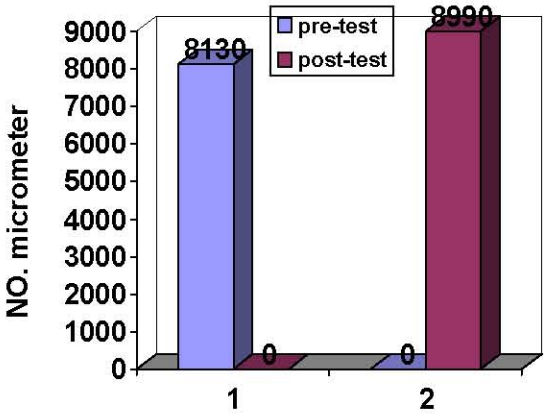

Fig. 3: The comparison of neutrophils in the 3 groups

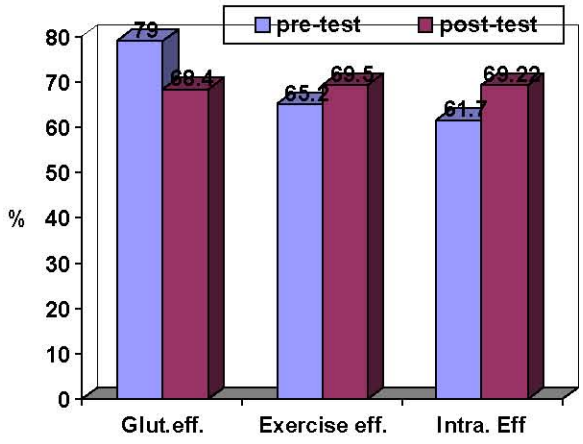

Fig. 2: The comparison of monocytes in the 3 groups

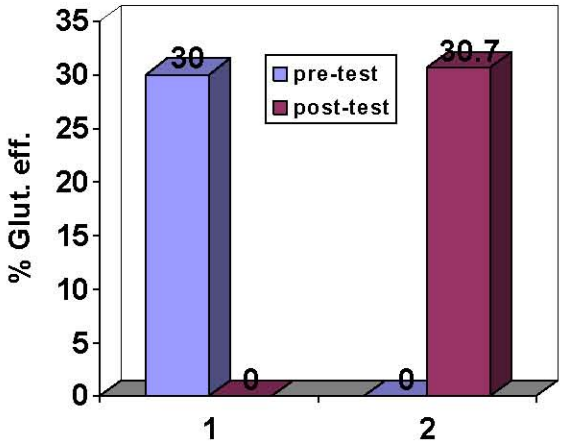

Fig. 4: The comparison of lymphocytes in the 3 groups

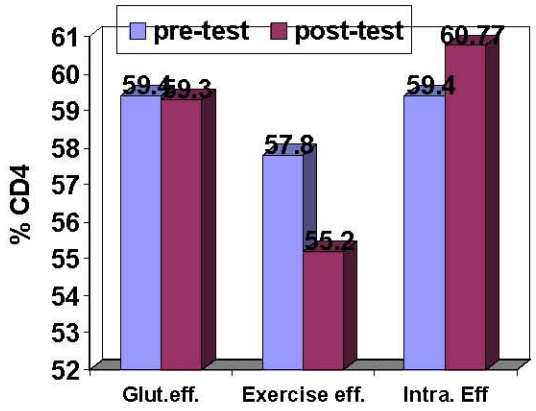

Fig. 5: The comparison of CD4 cells in the 3 groups

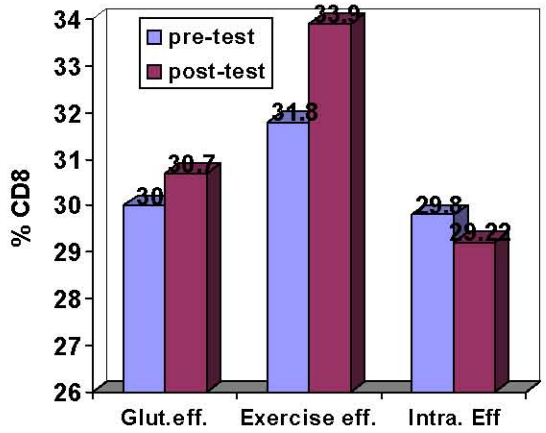

Fig 6: The comparison of CD4 cells in the 3 groups

DISCUSSION AND CONCLUSION

The present results showed that the glutamine supplementation slightly increased the percentage of leucocytes after the training. Krzywkowski *et al.* (2001) [5], Castell and Newsholme (1998) [15] and Rohde *et al.* (1998b) [6] support the present results.

Glutamine supplementation did not significantly change the percentage of monocytes. As monocytes secrete substances such as cytokines, an increase in their concentration is due to their insertion into blood circulation during training. Krzywkowski *et al.* (2001) [5], Nieman *et al.* (1995) [8], Shephard *et al.* (1995) [12] and Mackinnon *et al.* (1992) [1] support the present results.

There was a significant decrease in the percentage of neutrophils of supplementation group when compared with that of the control group. Krzywkowski *et al.* (2001) [5], Castell and Newsholme (1998) [15], Castell *et al.* (1997) [11], Nieman *et al.* (1997) [16] and Rohde and Maclean (1998a) [14] did not s upport the present results. All these researches reported a significant increase in neutrophils. The reasons were their subjects' fitness level, gender, heredity and nutrition of training protocol and training type.

The percentage of lymphocytes did not significantly change as well. Researchers believe that the lymphocyte concentration increases during the intensive exercise and it reaches under the baseline two hours after the training. The present results support this condition and this decrease was observed in the training group who did not receive any supplementation. But the lymphocyte concentration stays high in the supplementation group due to the glutamine expenditure. As this group used glutamine, the result can be attributed to glutamine. Krzywkowski *et al.* (2001) [5], Castell and Newsholme (1998) [15], Rohde *et al.* (1998b) [6] and Rohde and Maclean (1998a) [14] support the present results.

Glutamine supplementation significantly decreased the percentage of CD4 cells of placebo group. This decrease was significant when compared with the other groups. It means that training significantly decreased the percentage of CD cells. This decrease was too slight in the supplementation group due to the glutamine. Therefore, it can be concluded that glutamine prevented the reduction of CD4 cells; so, it can prevent the immune system attenuation. Shek *et al.* (1995) [17], Shephard *et al.* (1995) [12], Castell and Newsholme (1998) [15] and Krzywkowski *et al.* (2001) [5] support the present results.

Glutamine expenditure showed a significant increase in the percentage of CD8 cells of the placebo group. This increase was significant when compared with the other groups. This change shows the effect of intensive training on the percentage of CD8 cells. This effect was to the extent that the percentage of these cells stayed high even two hours after the training. But, it can be mentioned that the glutamine slightly prevented an increase in this variable. The evidences show that CD8 cells are used more than CD4 cells during intensive training. Therefore, these two variables show a decrease. Shek *et al.* (1995) [17], Shephard *et al.* (1995) [12], Krzywkowski *et al.* (2001) [5] and Castell and Newsholme (1998) [15] who reported an increase in the percentage of these cells, support the present result. The percentage of these cells slightly increased in the supplementation group due to the glutamine expenditure. Krzywkowski *et al.* (2001) [5], Rohde *et al.* (1998b) [6] and Rohde and Maclean (1998a) [14] support the present result.

The probable factors affecting the mentioned changes are as follows: 1- physiological factors (an increase in body temperature during the training and the metabolic substances resulted from the activity) 2- neural-hormonal factors 3- glutamine level.

During intensive training, the cells' need to produce energy and excrete waste matter is so high that stress hormones such as cortisol, adrenaline, epinephrine and norapinephrine increase. The primary increase in these hormones can recall all leucocytes subsets especially neutrophils from the blood and blood vessels and create an increase in the cells. When returning to the original status, the fast excretion of epinephrine can decrease leucocytes. In the next phases, cortisol slowly returns to the normal level and prompts the leucocytes to stay at a high level for several hours after the training. Neutrophils help a primary decrease in lymphocytes through the production of prostaglandins, but a primary increase in adrenaline increases the lymphocytes during intensive training. In the next phases, prostaglandins prompt the lymphocytes to reach under the baseline.

Another effective factor is the levels of glutamine. During intensive training, cellular respiration becomes more intensive and free radicals produce oxygen. Over-concentration of these radicals injures the cellular membrane especially muscular cells. Glutamine functions as a prostaglandin inhibitor as well as an antioxidant and ergogenic source for immune cells. Therefore, any change in glutamine concentration indirectly affects and attenuates the immune system function. Consequently, glutamine supplementation may balance such changes.

Generally, the present results showed that glutamine prevents excessive increase or decrease in some immune system variable and it decreased the training harmful effects. Therefore, glutamine has advantageous effects and prevents the attenuation and damage of the immune system by training.

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