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Comparison of proper nutrition, creatine and glutamine supplements effects on white blood cells

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Abstract

The aim of this study was to investigate the effects of glutamine and creatine supplements and proper nutrition on white blood cells. **Materials and Methods:** The samples for this study consisted of 28 elite wrestlers of Mazandaran province, aged 18 to 25 years old. They were randomly divided into four groups. Proper nutrition with carbohydrate solution made of 5% honey instead of water, creatine supplement group (0.3 g/Kg/ for 15 days), glutamine group (0.3 g/kg for 15 days), control group. Statistical analyses were performed using Excel and SPSS. **Results:** The results of the investigation of the blood leukocytes level in the pre-test showed significant differences ($p < 0.05$). **Conclusion:** Our results revealed that proper nutrition and carbohydrate has positive effects such as prevention of white cells increase after exercise and immune system suppression after exercise.

Keywords: Glutamine, Creatine, White blood cells (WBC).

Introduction

Many studies were carried out on exercise and the immune system. Epidemiological evidence suggests that moderate exercise has a beneficial effect on the human immune system. Moderate exercise has been associated with a 29% decreased incidence of upper respiratory tract infections (URTI). The studies of marathon runners found that their prolonged high-intensity exercise was associated with an increased risk of infection occurrence. Immune cell functions are impaired following acute sessions of prolonged, high-intensity exercise, and athletes are at a higher risk for infections. The immune systems of athletes and nonathletes are generally similar. Athletes may have slightly elevated natural killer cell count and cytolytic action, but these are unlikely to be clinically significant.[1-5]

Leukocytes are cells of the immune system involved in defending the body against both infectious disease and foreign materials. The number of WBCs in the blood is often an indicator of disease. An increase in the number of leukocytes over the upper limits is called leukocytosis, and a decrease below the lower limit is called leukopenia. There are several different types of white blood cells. They all have many things in common, but are all distinct in form and function.[6-7]

Many researchers have carried out in the term the relationship of immune system and exercise. For instance research has been conducted on the number of leukocytes, neutrophils, lymphocytes in sample blood of elite wrestlers and non-athletes before and after an intense exercise. Subsequently, it was ascertained that at the moment of the

body's resting level (except lymphocytes), the combined amounts of leukocytes, neutrophils, monocytes, eosinophils are higher in the blood of wrestlers rather than in non-athletes. But this difference was not statistically significant (P<0,05). That is to say, there was no significant discrepancy between both groups at their moment of rest. However immediately following an incremental exercise to exhaustion on the cycle, the number of leukocytes, neutrophils, and lymphocytes in the blood in both groups had significantly increased (P<0,01).

In blood monocytes, it was significant only in when pertaining to non-athletes in all variables (except the mono-Scythians) and this increase was larger than the wrestlers P<0/05. Examining the effect on the number of leukocytes during activities where the severity and duration of activity were in conjunction with high-intensity activity expended in a shorter period of time has a greater effect on the number of neutrophils. Augmentation in the number of lymphocytes within 30 minutes after conclusion of the activity confirmed that low activity performed over the course of longer durations is more useful in improving safety[8-10] following their investigation, concluded that during the expenditure of strenuous activity, this may cause many changes to the immune system including: decreased lymphocytes, increased neutrophils, decreased lymphocyte proliferation, increased cytokine.[11]

This indicates that the sum of these changes will reduce the body's resistance to infection.[12,13] Risk of upper respiratory tract infection increases during intense activity or a big competition. This issue accepted among athletes, that within 2 weeks immediately following the tournaments, 50-70 percent of athletes reported symptoms of upper respiratory tract infection. Exercise stress increases the likelihood of stress hormones such as cortisol concentration which are associated with effects on safety.

The task of this study was investigation of proper nutrition effect comparison with creatine and glutamine supplements on white blood cells.

Methods and Material

Research population

28 elite wrestlers from Mazandaran province (Iran), aged 18 to 25 years old. Werestling has seven weight groups and that's why we used 7 cases in each study arm. All of our cases were in professional exercise and outstanding wrestlers with international grades were selected to do theire bests. Cases were randomly assigned. Blood samples were evaluated before, immidiatly after and two hours after the exercise.

Participation in the study was completely voluntary. At first, medical history and health conditions of each of participants were evaluated through special questionnaires. Furthermore, related data about the samples' recent nutritional diets and also their meals within the last 24 hours were collected. Following the general assessment, the participants were informed clearly about the aims and experimental protocol of the research, as well as potential dangers of the experiments. Then, the volunteers signed a written consent to participate in all stages of the study.

The subjects received the glutamine and creatine supplements (0.3 g/kg of body weight of each subject) for 15 consecutive days. For glutamine supplementation, the daily dose was administered in two equal parts (morning and night) and the creatine powder was consumed with 250 cc water in 5 consecutive days per a week (4 times in each day).[14-17] These typs of food were selected based on nutritional values and acceibilty and price according to advanced sport nutrition guidlines but each athlete used himself amount according to his weight and daily requirements (all of the werslers used the same food in sports camp).

The program for proper nutrition which should contain caloric energy and macro nutrients was calculated by special software which was invented for this purpose. Nutritional plan in this category included: 55-60% carbohydrates, 25-30% fats, 10-15% protein. The rest of the groups had almost a same nutrition diet but because of the similarity of diets, they were not involved in the calculation of this test except in the first group which instead of water was used soluble carbohydrate concentration of 5% with honey. In preparation of the food, steaming and boiling had been utilized, so that the nutritional value could be preserved as much as possible. The amount of the input caloric energy of subjects was calculated according to their weight and its variances and their activities so that even the most miniscule variation in their weights could be a probable perspective[17-19]

To determine intra-group differences independent t-tests were performed and for inter-group analysis, one-way ANOVA test was performed and if the results were significant, Tukey test was performed to clarify the difference between each group. Statistical analyses were performed using SPSS (version 15; SPSS, Inc., Chicago,

Ill.) and the significance level of 0.05 was used for all statistical tests.

Laboratory Tests

The blood sampling consisted of 2 levels, each of which included 2 shots and were carried out before and after the main test. Within two weeks time, this procedure was duly repeated after the proper administration of supplementary food additives. In each shot, the laboratory technician drew 7ml blood from each participant's brachial before breakfast. Blood samples were subsequently transferred to the laboratory in an expedited fashion and were preserved in special test tubes containing EDTA, which is an anticoagulant element, in order to measure the blood parameters including leucocytes. 20 M of EDTA solution was blended with 2cc blood, and then this mixture was put in the rotator for 5 minutes so that a perfect liquefied fluid without any kind of coagulation could be the result.

For cell counting, an automated cell counter (Abbott Cell-Dyn 1600 Automated Cell Counter) was used. The unique mechanism of the device was based upon electrical resistance. Each shot bore a complete blood sample, intermingled with the anticoagulant EDTA-K3. Additionally, the device was equipped with isotone and sysmex mindrey diatron; which are considered to be the necessary solutions during the cell counting. The device had two channels, one of which was used separately for WBC counting. Receptacle consisted of two capsules; the left one was designated for compete mingling of WBC with diluted hemoglobin and the other one acted as a barrier, preventing counted cells to mix up in the counting capsule with others.

There were two resistant electrodes with positive and negative electrical charges, engendering direct current which made cells flow through the counting aperture. In the other channel, hemoglobin was measured with the evolved technology of cyanmethemoglobin through the 450 nanometer wavelength. The third channel counted simultaneously RBC (Red Blood Cells) and platelets. Receptacle consisted of two capsules; the left one was designated for the compete mingling of RBC and platelets and the other one acted as a barrier, preventing platelets to mix up in the counting capsule with RBC. There were two resistant electrodes in each of the receptacles.[20]

Results

Leukocytes variable between groups in pre and post test

The results of the investigation of the blood leukocytes level in the pre-test showed significant differences before, immediately and after two hours of activity (at rest) in correct nutrition and control groups ($p < 0.05$) (Table 1).

It was shown that in pre-test subgroup of correct nutrition group, there is a significant difference between before, immediately after and 2hr after activity subgroups. As we can see in table 1, there is a strong tendency of increasing of the data in after activity subgroup compare with before activity subgroup (about 3,16 k/µl followed with further increasing of the data in 2r after activity subgroup, which is about 2,28 k/µl compare with after activity subgroup, and about 5,44 k/ul compare with before activity subgroup.

However when we compare the differences of leukocytes data between the same subgroups (before, immediately after and 2hr after activity) we see that as in post-test group there occurs the process of increasing of the data in after activity subgroup compare with before activity subgroup, which is significant. Followed then with decreasing of the investigated data in 2hr after activity subgroup compare with immediately after activity subgroup, practically reached to the data obtained before activity.

Compare obtained data of pre- and post-test subgroups in correct nutrition group, between all 3 subgroups (before, immediately after and 2hr after activity) we indicate the following changes of leucocytes data. As we can see in table 1 in pre- and post-test subgroups there is a tendency of increasing data in before and after activity subgroups, while in 2hr after activity the decreasing process is observed, respectively).

In control group the same tendency as in correct nutrition group was observed. It was shown that in pre- and post-test subgroups occurs increasing tendency in immediately after activity subgroup and decreasing process in 2hr after activity subgroup, respectively (Table 1).

However compare pre- and post-test subgroups in control group we can see that there is a tendency to increase of the data in all investigated subgroups. So we can see that in post-test compare with pre-test subgroup in before activity subgroup the data increase, in immediately after activity subgroup, and in 2hr after activity subgroup, respectively.

So our data indicate that correct nutrition (specific diet include honey) lead to decreasing of leukocytes in the rest period, compare with control group.

The results of the investigation of the blood leukocytes level in the pre-test showed significant differences before, immediately and after two hours of activity (at rest) in creatine supplement administration and control groups ($p < 0.05$) (Table 2).

Table 1: Leukocytes variable immediately and 2 hours after activity in correct nutrition and control group

The experimental groups	Test procedures Subgroup	Leukocytes Mean±SD (pre test) k/µl	Leucocytes Mean± SD (post test) k/µl
Correct Nutrition	Before the activity	7.44±1.25	7.87±1.24
	After the activity	12.60±1.40* p <0.05	12.27±1.32* p <0.05
	2 hr after activity	12.88±1.76[+] p <0.05	11.85±0.71[+] p <0.05
Control	Before the activity	8.14±1.22	8.60±1.25
	After the activity	9.64± 2.90* p <0.05	10.51± 1.36* p <0.05
	2 hr after activity	9.57± 2.13	10.37± 1.48

*Significant difference between before and immediately after activity, + significant difference between immediately and 2 hours after activity

Table 2: Leukocytes variable immediately and 2 hours after activity in creatine supplement administration

The experimental groups	Test procedures Subgroup	Leukocytes Mean±SD (pre test) k/µl	Leukocytes Mean± SD (post test) k/µl
Creatine	Before the activity	7.65±1.01	8.01±1.01
	After the activity	12.10±1.20* p <0.05	12.60±1.37* p <0.05
	2 hr after activity	12.97±2.92[+] p <0.05	12.65±0.81[+] p <0.05
Control	Before the activity	8.14±1.22	8.60±1.25
	After the activity	9.64± 2.90* p <0.05	10.51± 1.36* p <0.05
	2 hr after activity	9.57± 2.13	10.37± 1.48

*Significant difference between before and immediately after activity, + significant difference between immediately and 2 hours after activity

Here as well as in correct nutrition group we observe the same tendency. So, it was shown that in pre-test subgroup of creatine supplementation group, there is a significant difference between before, immediately after and 2hr after activity subgroups. As we can see in table 2, there is a strong tendency of increasing of the data in after activity subgroup compare with before activity subgroup, followed with further increasing of the data in 2r after activity subgroup, compare with after activity subgroup.

However when we compare the differences of leukocytes data between the same subgroups (before, immediately after and 2hr after activity) in post-test group we see that here occurs the process of increasing of the data in after activity subgroup compare with before activity subgroup, which is significant. Followed then with decreasing of the investigated data in 2hr after activity subgroup compare with immediately after activity subgroup.

Compare obtained data of pre- and post-test subgroups in correct nutrition group; between all 3 subgroups (before, immediately after and 2hr after activity) we indicate the following changes of leucocytes data. As we can see in table 2 in pre- and post-test subgroups there is a tendency of increasing data in before and after activity subgroups, while in 2hr after activity the decreasing process is observed, respectively).

So, we can conclude that compare with control, creatine administration lead to decreasing of the leukocytes level in a rest period.

For understanding the influence of glutamine supplementation on the leukocytes level changes we have to compare the obtained data in pre- and post-test subgroups, as well as in all investigated subgroups separate (before, immediately after and 2hr after the activity).

The results of the investigation of the blood leukocytes level in the pre-test showed significant differences before, immediately and after two hours of activity (at rest) in glutamine supplement administration and control groups ($p < 0.05$) (Table 3).

Table 3: Leukocytes variable immediately and 2 hours after activity in glutamine supplement administration and control group

The experimental groups	Test procedures Subgroup	Leukocytes Mean±SD (pre test) k/µl	Leukocytes Mean± SD (post test) k/µl
Glutamine	Before the activity	6.61±1.73	8.02±1.10
	After the activity	12.11±1.60* $p < 0.05$	12.08±1.05* $p < 0.05$
	2 hr after activity	12.73±1.54 $p < 0.05$	12.17±0.74[+] $p < 0.05$
Control	Before the activity	8.14±1.22	8.60±1.25
	After the activity	9.64± 2.90* $p < 0.05$	10.51± 1.36* $p < 0.05$
	2 hr after activity	9.57± 2.13	10.37± 1.48

*Significant difference between before and immediately after activity, + significant difference between immediately and 2 hours after activity

As we can see in table 3, there is a significant difference between before, immediately after and 2hr after activity subgroups in pre-test subgroup of glutamine supplementation group. A tendency of increasing of the data occurs in after activity subgroup compare with before activity subgroup followed with further increasing of the data in 2hr after activity subgroup, compare with after activity subgroup.

However in post-test group there occurs the process of increasing of the data in after activity subgroup compare with before activity subgroup, which is significant. Followed then with decreasing of the investigated data in 2hr after activity subgroup compare with immediately after activity subgroup.

Compare obtained data of pre- and post-test subgroups in correct nutrition group, between all 3 subgroups (before, immediately after and 2hr after activity) we indicate the

following changes of leucocytes data. As we can see in pre- and post-test subgroups there is a tendency of increasing data in before and after activity subgroups, while in 2hr after activity the decreasing process is observed).

Our data indicate that glutamine supplementation was less effective compare with other treatments (correct nutrition and creatine supplementation) as well as with control group.

As have shown our results Leucocytes level in plasma of wrestlers were significant different in all groups of treatment in pre and post test periods. It was shown that in pre test period Leucocytes level before, after and 2hr of activity were different. Immediately after activity were registered the increasing of leucocytes level with its following decreasing 2 hr after activity.

These tendencies were observed practically in all investigated groups except Glutamine treatment group, where the number of leucocytes 2hr after activity decreased, but lesser then control.

As we can see in Pic. 1 In a rest period (2hr after activity) there is a significant difference between leukocytes level in wrestler's blood in pre- and post-test groups at the administration of creatine, glutamine supplementation, correct nutrition and control groups.

As we see in Pic. 1, in a rest period leukocytes level decreased significantly in correct nutrition and in creatin supplement administration groups, while in glutamine and control groups this decreasing process was no significant.

Discussion

Nutrition is an important aspect of an athlete's training program. Although exercise and athletic training is considered to increase nutrient needs in some athletes, a balanced diet with adequate calories can potentially provide the necessary nutrients.[21,22] Today, the supplement industry is an international market worth billions of dollars. In 2000, it was reported that sales of dietary supplements in the US reached US$17.1 billion with an annual increase in consumer spending of more than 10%. Although many individuals use supplements, those engaged in sport and physical activity represent a substantial portion of the population purchasing supplements.[23]

Nutrition is one of the factors, which is in close relation with output and enhancement of athletes` performance. Ever-increasing development in aspects of championship sport, its position in different societies and its relationship with physical and physiological health of individual in on hand, and determination of nutrition's important role in life and sport, particularly championship sport, relationship between proper nutrition and enhancement of athlete's performance on the other hand, has brought this belief to researchers and hard-working scholars who deal with championship sport field and sport nutrition, that nutrition is one of the most important factors of sport particularly championship sports. In other words, proper nutrition is a necessary precondition for athletes` success.

The effect on the number of leukocytes during activities where the severity and duration of activity were in conjunction with high-intensity activity expended in a shorter period of time has a greater effect on the number of neutrophils. Augmentation in the number of lymphocytes within 30 minutes after conclusion of the activity confirmed that low activity performed over the course of longer durations is more useful in improving safety . This indicates that the sum of these changes will reduce the body's resistance to infection.[23,24] The risk of upper respiratory tract infection increases during intense activity or a big competition.

It has been shown that carbohydrate deficiency exposes athletes to the danger of cortisol effects such as reduction in antibody production, reduction of cytokine exercise and natural killer cells and increase in lymphocytes reproduction.[25] Numerous studies in past years have shown that immune system is affected by intense exercises. For example, deficiency of carbohydrate in athletes exposes them to danger of cortisol effects such as reduction in antibody production, reduction of natural killer cytotoxic exercise and reproduction of lymphocytes. Blood glucose maintenance prevents stress-induced hormones rise such as cortisol and Interlukin-6 release and also restrains immune suppression. Glucose decrease is a major factor in cortisol release, before, during and after physical exercise. Consuming carbohydrate maintains the normal blood glucose and therefore prevents the increase of cortisol and Epinephrine during and after exercise. Consumption of carbohydrate drink increases the level of glucose, improves endurance performance and reduces the level of cortisol and subclasses of circulating leukocytes.[26]

Our finding showed that utilizing proper nutrition and carbohydrate has positive effects on factors under study in this research which can comply with obtained results of who have asserted that carbohydrate usage can prevent white cells increase after intense physical exercise and immune system suppression after exercise.[27,28] On the contrary, it is inconsistent with viewpoints of that we may attribute this inconsistency to different levels, concentration, and the steps of usage and carbohydrate type and time difference.[29] In this study, the level of cortisol and leukocytes in proper nutrition group had significantly decreased as compared to other groups. Therefore, it seems that proper nutrition can be used instead of the mentioned supplements.

Kreider RB *et al*[30] in their study evaluated the Effects of creatine supplementation on body composition, strength, and sprint performance. In their research football players were matched-paired and assigned to supplement their diet for 28 d during resistance/agility training. Before and after

supplementation, fasting blood samples were calculated; total body weight, total body water, and body composition were determined; subjects did a maximal repetition test on the isotonic bench press, squat, and power clean; and subjects performed a cycle ergometer sprint test. They results showed that Hematological parameters remained within normal clinical ranges for active individuals with no side effects reported. But Statistical analysis of the current study showed leukocytes values between groups in post test measurements has significant difference immediately and 2 hours after exercise in the proper nutrition, as compared to other groups. They concluded the addition of creatine to the glucose/taurine/electrolyte supplement promoted greater gains in fat/bone-free mass, isotonic lifting volume, and sprint performance during intense resistance/agility training. Likewise, our investigation revealed that Creatine supplement has a positive effect on the power and capacity of upper and lower limbs, but it is possible to reach the same results as if we use the supplements itself. In fact, the protocols which were used in this study were unique and we can't find the same study as our study to compare the outcoms but alike studies indicated the same results.

So our data indicate that correct nutrition (specific diet include honey) lead to decreasing of leukocytes in the rest period, compare with control group.

These results are in common with the research results of some authors.[31,32] Also results that obtained by, which reported the administration of carbohydrate can reduce the levels of leukocytes.

Conclusion

Statistical analysis of leukocytes values between groups in post test measurements showed significant difference immediately and 2 hours after exercise in the proper nutrition, as compared to the three other groups. Our finding showed that utilizing proper nutrition and carbohydrate has positive effects such as prevention of white cells increase after exercise and immune system suppression after exercise.

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