

### **Short Communication**

## **Effect of Carbohydrate Supplementation On The Immune System of Futsal Players**

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### **ABSTRACT**

One of the effective supplements in proper functioning of immune system is carbohydrate supplements which can have a positive effect on athletes. An athlete who continues his training in carbohydrate depletion, experience more increase in circulating stress hormones in the blood, and more disorders in various indicators of safety performance. The aim of the present study is to investigate the effect of 4 periodic 90-minute sessions during carbohydrate supplementation on the response of immune system cells among futsal players in Iran. For this purpose, 12 professional futsal players were selected, divided into three groups who (1) consuming carbohydrates, (2) consuming placebo as well as (3) control group. Collected data were analyzed using SPSS. The results of descriptive statistics showed that the indicators of immune systems among athletes consuming carbohydrate and placebo were increased remarkably at the end of second and fourth sessions (after supplementation) compared to the time before implementation, but on the other hand, immune systems indicators of control group at the end of second and fourth sessions had no significant changes compared to the time before implementation. Also there was not any significant difference in the number of white blood cells, neutrophil, and cortisol among the groups but a significant increase was observed in glucose in first group compared to the second group.

**Keywords:** Carbohydrate supplements, immune system, periodic physical activity, futsal players

### **1. INTRODUCTION**

Sports nutrition is a sub-branch of the sports science, scientifically studies the athletes' nutrition, and offers guidelines for nutrition before, during, and after physical activity. Such researches are along with new findings day by day, and will lead to the growing development of professional sport, and further success in sports competitions. According to the conducted researches on the professional athletes, who participated in heavy, breathtaking training programs and sports, it was concluded that they are exposed to dangerous, infectious diseases due to the temporary disruption of the immune

system during the sports activities (Mackinnon et al, 1993). The risk of developing respiratory infections increases in the following days of intense training. So, athletes may start next period of their exercise trainings or professional sports without returning to the normal state of their immune system. In some cases, this results in suppressed immune function of body and causes some risks (Haffman, 2002). Poor or inadequate nutrition can multiply the negative effects of the heavy or prolonged trainings, because it can increase human body needs during the exercise ( Aoi, 2009).

Considering the relationship between stress hormones and immune response to the long-term intense activities, consuming carbohydrates can maintain plasma glucose level, reduce the increase of stress hormones, and thus make change in the immune system. In addition, lack of carbohydrate oxidation during the exercise can create some problems for the athlete (Edward, 1992); Therefore, it seems that consuming carbohydrates during the exercise minimizes or decreases the regulating hormones disorder and indicators of immune such as the number of cells and the T-lymphocyte function (Katherine et al, 2003). The effect of one exercise session on the immune system was investigated in the previous researches but few researches have been carried out on immunological changes, which occur after a period of increasing the amount or intensity of the exercise (Malm et al, 2004). The results of researches, which were conducted on the effect of the reduced training intensity on the physiological changes, have shown that although the disorders induced by hard exercise and training are temporary; lack of returning of the immune system to its initial state can cause disease. Exposing to such conditions frequently and in a short-term period can lead to further occurrence of stress responses, and different qualitative, quantitative responses in comparison with further pressures (Ronsen et al, 2002). Many researchers have shown that carbohydrate consumption can lead to the drop in the immune system after many complex physical activities by reducing the release of cortisol. According to the results of these researches, the group which consumed carbohydrate experienced a decrease in cortisol response, and less disorder of immune cells in comparison with the group which consumed placebo (Gleeson et al, 1998). Vigorous physical activity triggers the activation of the hypothalamus, pituitary and adrenal, and finally leads to adrenal cortex hormone secretion. In other words, vigorous physical activity triggers adrenal cortex gland to produce and secrete

cortisol. For achieving this goal, lowering blood glucose is another stimulus. It is a way in which carbohydrate affects the immune function (Shi and Gisolfi, 1998). Therefore, with regard to the relationship between stress hormones and immune response to the long-term strenuous exercise, eating carbohydrates can maintain the plasma glucose levels, and reduce the increasing stress hormones and consequently the immune system changes as well (Pedersen et al, 1997). Based on what was said, it seems that no clear answer has been given to the effect of carbohydrate supplementation on the athletes' immune system since now. Although, according to the most of the researches, the influence of consuming carbohydrate on the immune system response is observed after doing long-term physical activity, this influence is also experienced by a large number of athletes after doing short-term physical due to the intensity of the activities. Given the above, in this paper we want to give a clear answer to the function of the immune system cells during a severe 90-minute periodic protocol for athletes particularly futsal players.

## 2. MATERIALS AND METHODS

### 2.1. Literature Review

The hypothesis of the influence of carbohydrate supplementations on the immune system of body was first investigated by Nieman (1998) in relation to the immune response to 2.5 hours of run among a group of 30 marathon runners. Following this research, the effect of consuming carbohydrate on immune responses was examined among 10 triathletes by 2.5 hours running and cycling on treadmill or bicycle ergometer at 75%  $VO_{2max}$  speed (Nieman et al, 1998). In both studies, a drink containing carbohydrates before and after 2.5 hours of physical activity as well as during the competition or physical activity led to the higher level of plasma glucose, and decrease in cortisol response and disorder of immune system. They

concluded that a part of the mechanism of the responses and decrease in cortisol responses are due to maintain the level of plasma glucose concentration.

In 1999 Nieman et al examined the influence of the consuming carbohydrate and placebo beverages on the immune responses to normal rowing training sessions among 15 elite female rowers. Metabolic measures indicated that training was performed at moderate intensities, with some high intensity intervals interspersed throughout the sessions. It was concluded that glucose and insulin were significantly lower after two hours of rowing with drinking placebo beverage comparing with carbohydrate beverage. The patterns of change in cortisol, growth hormone, epinephrine, and nor epinephrine as well as the patterns of changes in blood lymphocyte and lymphocyte subset counts, and lymphocyte proliferative responses, except for a slight difference in NK cell counts and activity did not differ between placebo and carbohydrate groups. In the same year, Bishop et al (1999) investigated the effect of carbohydrates versus placebo beverage consumption on the immune response to a soccer-specific exercise protocol. The players received carbohydrates or placebo beverage before, during, and after two 90-min soccer-specific exercise bouts (3 days apart). After 90 minutes of exercise, plasma glucose concentration was lower among a group of players, who received placebo beverages than the other group of players, who received the carbohydrate beverages but the pattern of change in cortisol and circulating lymphocyte count did not significantly differ between the carbohydrate and placebo trials (Bishop et al, 1999). These findings seem logical, because the cells of the immune system greatly require blood glucose as an energy substrate, and carbohydrate consumption can prevent from decreasing in the efficiency of the immune system after the physical activity by reducing cortisol release.

Van Hall et al (1998) examined the influence of carbohydrate supplementation on plasma glutamine during prolonged exercise and recovery. *Muscle glycogen* and *glucose* have been suggested to be carbon-chain precursors for glutamine synthesis in skeletal muscle. Therefore, the aim of the present study is to investigate whether carbohydrate supplementation affects plasma glutamine and other amino acids during exercise and 7 h of recovery. Eight well-trained subjects cycled at an alternating workload of 50 and 80%  $W_{max}$  until exhaustion (59 to 140 min). During the exercise bout the subjects received either water (control) or a carbohydrate (CHO) drink (83 g CHO x l(-1), 2 ml x kg(-1) per kg body weight every 15 min). Plasma glutamine concentration appeared not to be affected by exercise, as a significant increase was only observed at some points in time during the control test. During recovery, however, plasma glutamine concentration decreased from 682 $\pm$ 24 and 685 $\pm$ 19 micromol x l(-1) at exhaustion to 552 $\pm$ 19 and 534 $\pm$ 12 micromol x l(-1) after 2 h of recovery for the control and CHO test, respectively. Plasma glutamine concentration returned to pre-exercise values after 7 h of recovery. Alanine concentration increased during exercise in both tests. During the recovery period the concentration of alanine (48%), and total amino acids (23%) decreased below the pre-exercise level. The plasma alanine and the total amino acid concentration were still suppressed after 7 h of recovery. In conclusion, carbohydrate supplementation had an effect neither during exercise nor during recovery on the concentration of plasma glutamine or other amino acids. Exercise, however, causes a substantial decrease in the plasma amino acid concentration during recovery. Ji et al (1993) studied the influence of blood glutathione during exercise, and the following results were achieved. Blood glutathione status and activities of antioxidant enzymes have been investigated during

prolonged exercise with or without carbohydrate (CHO) supplementation. Eight subjects cycled at approximately 70% of maximal oxygen uptake to fatigue [134 +/- 19 (SE) min] on the first occasion (control, CON) and at the same work load and duration on the second occasion but with CHO ingestion during exercise. Blood reduced glutathione (GSH) concentration increased from 0.55 +/- 0.05 mM at rest to 0.77 +/- 0.09 mM after 120 min of exercise during CON ( $P < 0.01$ ) but remained constant during CHO exercise. Blood glutathione disulfide (GSSG) levels were unchanged during CON and CHO exercise. Blood GSH + GSSG content and GSH/GSSG ratio were also significantly ( $P < 0.05$ ) elevated during CON but not during CHO exercise. The increases in GSH and GSH + GSSG in CON were associated with decreases in plasma glucose and insulin levels. Activities of blood GSH peroxidase, GSSG reductase, and glucose-6-phosphate dehydrogenase were significantly increased during the CHO exercise, whereas only GSSG reductase activity was elevated during the CON ride.

It is concluded that blood GSH increases during prolonged exercise and that CHO supplementation may prevent blood GSH increase possibly because of its inhibitory effects on hepatic hormonal releases, which stimulate GSH output. Haff et al (1999) conducted a research on the effects of carbohydrate (CHO) supplementation on multiple sets of resistance training exercise during the second training session on a given training day. The subjects participated in a randomized, counter balanced, double-blind protocol with testing days separated by at least 1 week. A CHO supplement consisting of 0.3 g/kg bodymass<sup>-1</sup> or placebo (P) was ingested during the morning training session, 4 hours of recovery, and the sets of squats performed to exhaustion (STE). STE consisted of sets of 10 repetitions of squats performed at 55% of 1 repetition maximum, with a 3-minute rest between sets, performed to muscular failure.

Performance measured in number of sets was statistically different between the CHO and P trials.

The results suggest that CHO supplementation enhances the performance of multiple STEs during the second workout on a given day.

## 2.2. Research methodology

In this study, statistical population was consisted of professional futsal players in Iran. Among this, 12 futsal players were selected as the study sample by random sampling technique. Data collection tool was a researcher-made questionnaire. First, the questionnaires were distributed among them, and after collecting, data were analyzed using SPSS.

## 3. RESULTS AND DISCUSSION

### 3.1. Descriptive statistics

In this section, descriptive statistics related to participants and study variables are presented in tables 1-4.

**Table 1.** Mean and standard deviation related to participants in terms of age, weight, and body masses in three groups

Study groups		N	Mean	SD
Carbohydrate group	Age	4	21.7500	1.35680
	Weight	4	71.5250	.39590
	Body mass	4	24.1125	.80551
Placebo group	Age	4	23.1667	1.94625
	Weight	4	76.6750	1.38638
	Body mass	4	24.7450	.78583
Control group	Age	4	24.5833	1.50504
	Weight	4	75.7750	1.11854
	Body Mass	4	24.3725	.81107

According to table 1, mean and standard deviation values of athletes' ages, weights, and body masses are calculated in all the three groups including carbohydrate, placebo, and control groups. As it can be observed, mean values of the carbohydrate group athletes' ages, weights, and body masses are respectively equal to 21.75±1.35, 71.52, and 24.11±0.805. Also the

mean values of placebo group athletes' age, weights, and body masses are  $24.58 \pm 1.50$ ,  $75.77 \pm 1.11$ , and  $24.37 \pm 0.811$  respectively. weight, and mass body are respectively equal to  $23.16 \pm 1.94$ ,  $76.67 \pm 1.38$ , and  $24.74 \pm 0.785$ . in control group, the average of athletes' ages,

**Table 2.** Mean and standard deviation of immune system indicators in carbohydrate group in three periods

Sessions	Indicators	Mean	SD
Before the first session	White blood cells	6.8175	.67549
	Neutrophil	3.5000	.60072
	Lymphocytes	2.5000	.29439
	The ratio of CD4 to CD8	1.4025	.30576
	Glucose	95.2500	5.25198
	Cortisol	11.4375	1.02744
Second session	White blood cells	11.5775	.63757
	Neutrophil	6.1275	.25316
	Lymphocytes	3.9250	.26338
	The ratio of CD4 to CD8	1.8850	.12689
	Glucose	94.2500	1.70783
	Cortisol	18.4575	.38526
Fourth session	White blood cells	11.5150	.63710
	Neutrophil	5.5725	.79450
	Lymphocytes	4.6700	.72236
	The ratio of CD4 to CD8	1.6250	.18930
	Glucose	92.0925	4.19693
	Cortisol	16.7025	.54286

Based on table 2, mean and standard deviation values of indicators of immune system among the carbohydrate group athletes are calculated in three periods of time. As it can be seen, their indicators of immune system have been increased considerably at the end of second and fourth sessions in comparison with the time before the first session.

**Table 3.** Mean and standard deviation of immune system indicators in placebo group in three periods

Sessions	Indicators	Mean	SD
Before the first session	White blood cells	7.6625	.68908
	Neutrophil	4.3075	.79584
	Lymphocytes	2.6000	.24495
	The ratio of CD4 to CD8	1.3450	.18646
	Glucose	91.7500	3.68556
	Cortisol	11.5150	.68379

Second session	White blood cells	12.3750	.45538
	Neutrophil	7.2375	.44327
	Lymphocytes	3.8750	.85391
	The ratio of CD4 to CD8	1.8075	.07500
	Glucose	82.8650	4.74390
	Cortisol	20.1000	1.12057
Fourth session	White blood cells	11.4750	1.01632
	Neutrophil	6.5100	.46137
	Lymphocytes	4.6050	.47142
	The ratio of CD4 to CD8	1.6500	.23678
	Glucose	91.7825	3.52220
	Cortisol	19.4250	1.03488

As it is presented in table 3, mean and standard deviation values of indicators of immune system among athletes in placebo group have been calculated in three periods of time. Based on table 3, the indicators of immune system among athletes in placebo group have been significantly increased at the end of second and fourth sessions comparing before the first session.

**Table 4.** Mean and standard deviation of immune system indicators in control group in three periods

Sessions	Indicators	Mean	SD
Before the first session	White blood cells	7.3525	.59818
	Neutrophil	3.6675	.53119
	Lymphocytes	2.6850	.33441
	The ratio of CD4 to CD8	1.1925	.16460
	Glucose	87.2500	3.30404
	Cortisol	12.4675	.52627
Second session	White blood cells	6.4075	.34635
	Neutrophil	3.8150	.25053
	Lymphocytes	2.5350	.27683
	The ratio of CD4 to CD8	1.5200	.20720
	Glucose	95.3975	3.38963
	Cortisol	14.8825	1.16743
Fourth session	White blood cells	6.5800	.60997
	Neutrophil	3.2850	.39946
	Lymphocytes	2.3625	.22750
	The ratio of CD4 to CD8	1.3625	.18733
	Glucose	91.0525	4.32894
	Cortisol	15.5050	.68908

As it is presented in table4, mean and standard deviation values of control group athletes' indicators of immune system have been estimated in three periods of time. Based on table 4, control group athletes' indicators of immune system have not been changed considerably at the end of second and fourth sessions compared to before the first session.

### 3.2. Inferential statistics

Now we compare three groups with each other at the time before the first session, second session, and fourth sessions. Results are shown in tables 5-7.

**Table 5.** Comparison of three study groups before the first session

Indicators	Study groups		Mean difference	p-value
White blood cells	Carbohydrate group	Placebo group	-.84500	.102
		Control group	-.53500	.278
	Placebo group	Control group	.31000	.520
Neutrophil	Carbohydrate group	Placebo group	-.80750	.114
		Control group	-.16750	.725
	Placebo group	Control group	.64000	.199
Lymphocytes	Carbohydrate group	Placebo group	-.10000	.641
		Control group	-.18500	.396
	Placebo group	Control group	-.08500	.692
The ratio of CD4 to CD8	Carbohydrate group	Placebo group	.05750	.729
		Control group	.21000	.224
	Placebo group	Control group	.15250	.368
Glucose	Carbohydrate group	Placebo group	3.50000	.265
		Control group	4.00000	.164
	Placebo group	Control group	4.50000	.161
Cortisol	Carbohydrate group	Placebo group	-.07750	.891
		Control group	-1.03000	.093
	Placebo group	Control group	-.95250	.116

Table 5 shows that there is not any significant difference among carbohydrate, placebo, and control groups before the first session.

**Table 6.** Comparison of three study groups after second session

Indicators of immune	Groups		Mean difference	p-value
White blood cells	Carbohydrate group	Placebo group	-.79750*	.049
		Control group	5.17000*	.000
	Placebo group	Control group	5.96750*	.000
Neutrophil	Carbohydrate group	Placebo group	-1.11000*	.001
		Control group	2.31250*	.000
	Placebo group	Control group	3.42250*	.000
Lymphocytes	Carbohydrate group	Placebo group	.05000	.899
		Control group	1.39000*	.005
	Placebo group	Control group	1.34000*	.007
The ratio of CD4 to CD8	Carbohydrate group	Placebo group	.07750	.474
		Control group	.36500*	.007
	Placebo group	Control group	.28750*	.022
Glucose	Carbohydrate group	Placebo group	11.38500*	.001
		Control group	-1.14750	.655
	Placebo group	Control group	-12.53250*	.001
Cortisol	Carbohydrate group	Placebo group	-1.64250*	.039
		Control group	3.57500*	.001
	Placebo group	Control group	5.21750*	.000

Table 6 shows that there is a significant difference among carbohydrate, placebo, and control groups in terms of the number of white blood cells. It should be stated that the number of white blood cells has been increased significantly in carbohydrate group comparing with control group, and decreased significantly

in comparison with placebo group. Also in terms of neutrophil indicator, there is difference among three groups. Neutrophil has been increased significantly in carbohydrate group in comparison with control group, and decreased significantly compared to the placebo group. Also, in terms of lymphocytes and the ratio of CD4 to CD8, there is a significant difference between carbohydrate and placebo groups as well as between carbohydrate and control groups, and no significance difference has been observed between placebo and carbohydrate groups. In terms of the indicator of glucose, carbohydrate group experienced a significant increase in comparison with placebo group, and a significant reduction in comparison with control group. Also, there is a significant reduction in glucose in placebo group comparing with control group. A significant difference in cortisol is observed among each of three groups. Cortisol in control group has been increased significantly compared to the control group, and decreased significantly comparing with placebo group.

**Table 7.** Comparison of three study groups after fourth session

Indicators	Study groups		Mean difference	p-value
White blood cells	Carbohydrate group	Placebo group	.04000	.944
		Control group	4.93500*	.000
	Placebo group	Control group	4.89500*	.000
Neutrophil	Carbohydrate group	Placebo group	-.93750*	.048
		Control group	2.28750*	.000
	Placebo group	Control group	3.22500*	.000
Lymphocytes	Carbohydrate group	Placebo group	.06500	.862
		Control group	2.30750*	.000
	Placebo group	Control group	2.24250*	.000
The ratio of CD4 to CD8	Carbohydrate group	Placebo group	-.02500	.867
		Control group	.26250	.105
	Placebo group	Control group	.28750	.080
Glucose	Carbohydrate group	Placebo group	.31000	.916
		Control group	1.04000	.724
	Placebo group	Control group	.73000	.804
Cortisol	Carbohydrate group	Placebo group	-2.72250*	.001
		Control group	1.19750	.059
	Placebo group	Control group	3.92000*	.000

Table 7 shows that there is significant difference in the indicator of white blood cells between carbohydrate and placebo groups, and control group, and in indicator of neutrophil among all the three groups. It should be mentioned that neutrophil has been significantly increased in carbohydrate group comparing with control group, and decreased in comparison with placebo group. There is a significant difference in lymphocytes between carbohydrate and placebo groups, and control group, and there is not any significant difference between placebo and carbohydrate groups in this regard. None of the groups significantly differ from each other in the ratio of CD4 to CD8 and glucose. The indicator of cortisol decreased significantly in carbohydrate group comparing the placebo

group, but no significant difference was observed in the indicator of cortisol in carbohydrate group comparing with control group. Cortisol significantly increased in placebo group in comparison with control group.

#### 4. CONCLUSION

This paper conducted to analyze the effect of 4 periodic 90-minute sessions during carbohydrate supplementation on the response of immune system cells among futsal players in Iran. In the present study, Athletes were divided into three groups including carbohydrate, placebo, and control groups. The results of descriptive statistics showed that the indicators of immune system among carbohydrate and placebo groups were increased significantly at the end of second

and fourth sessions (after supplementation) rather than before the first session, but minor changes were made in the indicators of immune system among control group athletes at the end of second and fourth session comparing before the first session. The results showed that there was not any significant difference between carbohydrate and placebo groups, and control group before the first session. A significant difference was observed in the indicator of the white blood cells. The white blood cells were significantly increased among carbohydrate group comparing the control group, and decreased in comparison with placebo group. Also, there was a significant difference among all of the three groups over the indicator of Neutrophil, and carbohydrate group experienced a significant increase in Neutrophil comparing the control group, and a significant decrease in comparison with placebo group. There was also a significant difference in the indicators of lymphocyte and the ration of CD4 to CD8 between carbohydrate group and placebo groups as well as carbohydrate and control groups. In terms of the indicator of glucose, a significant increase was only occurred among the carbohydrate group comparing to the placebo group.

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