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Effect of repeated bouts of exercise and carbohydrate supplementation on serum interleukin-6

Seyed Hoseini MohammadAli, Farbod Maryam, Daraei Shokrabad Firooz, Imanipour Vahid

Department of Physical Education and Sport Science, Parand Branch, Islamic Azad University, Parand, Iran

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ABSTRACT

The human immune system is affected by exercise and physical activity. Plasma concentration of IL-6 increases more than other cytokines through exercise. Each of the training methods together with nutritional supplementation has its own specific effect on serum IL-6 expression. The purpose of this study is to confirm the impact of training bouts and CHO supplementation on serum IL-6. 19 male subjects with a mean age of 37.2 ±24.89, BMI 6.97 ±72. 25 kg, height 176.1 ±4.60 cm, fat percentage, ±5. 80 1.18 kg and Vo2max 56.40 ±1.74 kg liters per minute were present in two PLA or CHO groups. The test was completed in two days. Each of the subjects ran 60 minutes each day on the treadmill, with 80% MHR. On each day blood samples were collected from subjects 10 minutes before the exercise and then immediately after the exercise. The subjects drank either PLA or CHO drinks during exercise. The analysis of the samples was done by ELISA method. Data analysis was done in the form of descriptive and inferential statistical methods. To make a decision as to acceptance or rejection of hypotheses, the significance level of 0.05 was considered. IL-6 levels increased significantly in both groups under the influence of exercise and this increase was higher in PLA than in CHO; moreover their difference was significant. 24 hours after exercise, IL-6 serum concentrations in the two groups differed but the difference was not significant. Also under the influence of the second bout of exercise IL-6 serum in PLA group had a significant increase compared with the first day before the exercise ($p < 0.05$). According to the findings of the research CHO supplementation during 60 minutes of running with less than 80% MHR causes less increase in the incidence of serum IL-6 in a delayed manner and immediately after exercise. Although by consumption of CHO, IL-6 levels increased after the first bout like Pla group, its amount was a smaller; also in repeated bout of exercise, serum IL-6 increase was not significant in CHO group, while this increasing trend in Pla group also continued after the second bout of exercise.

Key words: interleukin-6, carbohydrate supplements, continuous sub-maximal exercise

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INTRODUCTION

Sports trainers and physicians believe that the athletes are prone to infectious diseases during intense exercise and after tough competitions. Research findings indicate that 47% of runners after a 56-km ultramarathon were afflicted

by URTI [1]. Although these statistics have been subject to tiny variation, they have been by and large confirmed by other researches as the risk of URTI after a week of marathon racing and rowing has been reported 50%. [2]

At the same time the positive impact of exercise on the functioning of cardiovascular system, hormones, recovery from certain diseases and acquisition of general health can not be denied. Apparently cytokines' response to exercise is complex and depends on such variables as sport, exercise history, location of measurement (tissue, blood, urine) and the measurement method [3]. During exercise plasma concentrations of IL-6 increases more than other cytokines, eg IL-6 plasma concentrations increase 100-fold after a marathon [4, 5, 6], while under the influence of eccentric sub-maximal activity the increase of IL- 6 levels has also been reported [7]. Previous studies show that under the influence of certain training programs, changes in plasma IL-6, sometimes linger on a few hours or few days after the exercise [8]. A study looked into the effects of oral carbohydrate supplementation during a second 90-minute bout of cycling. Venous blood samples were taken from both groups 5 minutes before the exercise, immediately after training and 18 hours after the second bout. The main finding of this study was that eat a carbohydrate meal, compared with eating placebo during the second bout of exercise, maintained plasma glucose concentration at a better level [9]. In another research, 10 trained endurance runners ran on the treadmill 2.5 hours with 75% maximum oxygen consumption. Plasma IL-6 concentrations increased 30 minutes after the beginning of the exercise and this increase continued until the end of exercise. At this moment the amount of IL-6 came to a peak compared with its amount before the exercise. After running, IL-6 plasma levels uniformly decreased but after 6 hours of rest, it was still higher than the time before the exercise. All IL-6 measured data significantly increased compared to the time before the exercise except on the second and fifth days after running [10]. In another study, the subjects practiced five bouts of eccentric exercise "on one foot". IL-6 plasma concentration reached a peak 90 minutes after exercise and remained elevated for 4 days [11].

MATERIALS AND METHODS

Subjects: The statistical population of the study consisted of male students in undergraduate programs in Physical Education at the University of Imam Hussein (AS) in the second semester of the academic year 2008-2009 and the total number of them was 37 with a mean age was 24.89±2.37 years, BMI 72.25 ± 6.97 kg, height 176.1± 4.60 cm, fat percentage, 5.80 ± 1.18 kg and Vo2max 56.40 ± 1.74 kg of liters per minute.

Exercise program: before conducting the test, the subjects in both CHO and Pla groups were briefed on the objective and the method of study. The subjects were prohibited from doing any heavy exercise 24 hours before each test and they were advised strictly only to attend theoretical classes. The subjects were advised to use only the dormitory food, from 24 hours before the first bout of running until the end of the second bout, and take their night rest from 22.00 to 06.00. After 10 to 12 hours of night fasting the subjects participated in the test from 8 to 9:30 AM and 9 to 10:30 AM in form of groups of two. After a 10-minute break the first blood sampling was done by collecting 5 cc of blood from the brachial vena cava of the subjects and after 5 minutes they warmed up for 10 minutes which included 5 minutes of walking with 50% maximum heart rate and 5 minutes of running on the treadmill with 60% Maximal heart rate and after the warm-up the subjects an on the same treadmill for 60 minutes with 80% maximum heart rate. All the way through the exercise, subjects' heart rate was controlled by a heart rate meter manufactured by Polar Company, China. Subjects each drank 0.8 liters of beverages with a temperature of 3 to 5°C given to them four times; respectively at minutes 0, 15, 30, 45 of the exercise and the volume of each drink was about 0.2 liters. The CHO group drank 6% carbohydrate solution containing 48 grams of pure glucose with GIBCO brand produced in Canada and 0.02 ± 0. 75 liters of RO water treated at "Zamzam Tehran" Company which contained 20 ppm soluble solids and while the Pla groups only drank 0.8 liter of RO water. Immediately after the exercise, a second blood sample was taken from the subjects to compare changes in plasma IL-6 levels in the two groups; 24 hours later after identical eating and resting conditions, the subjects attended the test place and the test protocol of the day before was repeated. During the tests the ambient temperature was 21±2 and the humidity was 24±1.5.

Blood samples were centrifuged for 10 minutes at the rotation speed of 4000 to 4500 rpm. Then the samples were poured in small 1cc tubes and were kept at -80 degrees centigrade until all samples were collected.

Statistical methods:

To ensure normal distribution of data and to evaluate the impact of changes of time and the groups, IL-6 variable Kolmogrov-Smirnov test was used. Paired t-test with Bonferroni adjustment was used for two-by-two comparison of times. Also, independent t-test was used for comparing the groups at any test point of time. The correlation of IL-6 variables in different time scales was tested with Pearson correlation test and Spearman's rank correlation [12]. To decide about affirmation or rejection of hypotheses, the significance level of 0.05 was considered. SPSS 15 software was used for data analysis and Microsoft Excel 2007 software was used for drawing the diagrams.

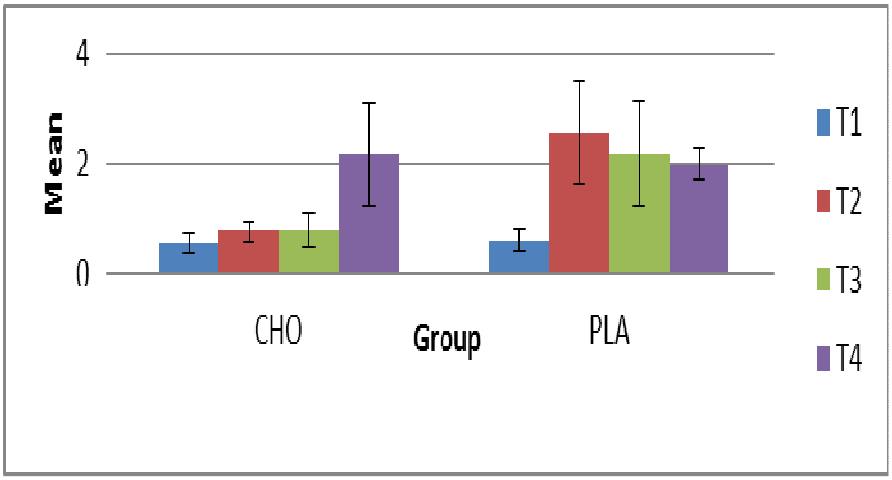
RESULTS

60 minutes of running with 80 percent of maximum heart rate increases concentration of serum IL-6. This increase was significant in both CHO and PLA groups (P<0.05), but in the PLA group it had a greater increase and IL-6 concentration difference in between the two groups was significant (P<0.05). IL-6 concentrations were still higher than baseline concentrations in both groups until 24 hours after the first exercise and this difference was reported to be lower in CHO group, but the difference between the two groups was not significant (P>0.05). After the second bout of exercise, there was no significant difference IL-6 serum concentration in CHO and Pla groups (P>0.05) but the IL-6 of PLA group significantly increased after the second bout of exercise compared with that of before the the first bout while this amount was not significant in the CHO group. Table 1 shows the changes in serum concentrations of IL-6 of the two groups.

Table 1), IL-6 serum concentrations in four instances of blood sampling in the CHO and Pla groups

Standard deviations	Maximum *pg ml-1*	Minimum *pg ml-1*	Average *pg ml-1*	Group	Period of Blood Sampling
0.21	1.6	0	0.64	CHO	IL6_T1
0.19	1.8	0	0.55	PLA	*(Pg/ml.)*
0.14	1.8	0	0.60	CHO	IL6_T2
0.21	2.7	0	0.86	PLA	*(Pg/ml.)*
0.33	3	0	1.63	CHO	IL6_T3
0.28	2.4	0	1.24	PLA	*(Pg/ml.)*
0.12	2.2	0.24	0.77	CHO	IL6_T4
0.22	2.9	0.6	1.22	PLA	*(Pg/ml.)*

** Data are reported based on the mean and standard deviation.*



DISCUSSION AND CONCLUSION

Therefore on the impact of a one-session exercise on IL-6 serum concentration among the researches conducted with respect to sports immunology, in a study the effects of concentric exercise on levels of plasma cytokines were explored [13]. Healthy young men with moderate exercise pedaled on ergometer for one hour with 75% of maximum oxygen uptake. IL-6 plasma levels significantly increased during exercise which is consistent with the results of this study. In contrast, other researchers in a study involving 12 subjects with mean age 30 years showed

after performing one bout of low-intensity continued exercise, despite the increase in muscle tissue IL-6, plasma concentration of IL-6 had no significant changes [14]. The difference between the findings of the said study and those of this study could result from differences in the intensity of exercise used in the two studies.

Also about the effects of two bouts of exercise on serum concentrations of IL-6, some researches showed that repetitive bouts of exercise would have significant effects on plasma levels of IL-6 and IL-1ra that is consistent with the findings of this research. In this study they compared the effect of repeated prolonged cycling exercise with the effect of one bout of long biking exercise on plasma IL-6. Nine trained men participated in four different tests that were 12 to 17 days apart. The first test involved a two-phase exercise and the first phase was performed from 8:00 to 9:30 AM and the second phase would start within 6 hours in the afternoon of the same day and would continue for 90 minutes. The second test also had two phases the first one of which was from the 11:00 AM to 12:30 PM and the second phase was performed with a 3-hour interval in the afternoon of the same day. In the third test only one session was performed in the afternoon and in the fourth test subjects only rested. All bouts of exercise were performed on ergometer bike with 75% Vo2max. In the end it was concluded that two bouts of endurance training on the same day compared with one bout of similar exercise, would bring about significant increases in IL-6 and IL-1ra which may be associated with muscle glycogen depletion [15]. The results of another study, nonetheless, showed that despite increased strength and Vo2max and decreased C reactive protein, 12 weeks of exercise would have no significant effect on IL-6 serum which is incongruous with the results of this study. The said study aimed to evaluate the impact of 12 weeks of combined training program (aerobic - resistance) with low intensity on the inflammatory cytokines concentration and CRP. The subjects were divided into two groups: exercise and no exercise. A Blood sample were collected before and after the training period and it was shown that serum concentrations of IL-6 and IL-1 would not significantly alter under the influence of exercise which is inconsistent with the findings of this research [16]. Possible reasons for this discrepancy could be the duration and intensity of exercise, because in the present study, serum concentrations of IL-6 were examined during two bouts of 60-minute running with 80 percent of maximum heart rate, but in the former study, IL-6 resting levels was evaluated after 12 weeks of combined (aerobic-resistance) exercise.

But the results of previous studies on the effects of carbohydrate supplementation during one bout of exercise on the concentration of IL-6 serum have shown that carbohydrate ingestion during exercise would slow down the increase of IL-6 serum in humans [17], which is consistent with the findings of this study. In that study, 7 moderately trained men, in four random groups performed a 60-minute exercise based on their individual lactate threshold; 2 groups on ergometer bike and 2 by treadmill running. One of two groups used carbohydrate beverages during exercise. The venous blood samples were collected at rest, 30 minutes after exercise (during exercise) and at the end of exercise. The research data showed that carbohydrate ingestion during both running and pedaling prevented further increase of IL-6 plasma concentrations. In contrast, certain findings indicated that CHO intake would have no significant impact on preventing IL-6 increase or decrease which differs from the findings of this study [18]. In that study, 30 power trained subjects performed weight training for 2 hours in two CHO and Pla groups. In the first set the exercise was performed with 40% of one maximum repetition and the next set with 60 percent of one maximum repetition and included 10 moves in 4 sets and each set of 10 repetitions with 2 to 3 minutes intervals. The CHO group subject consumed 10 ml.kg-1.h-1 of 6% carbohydrate drink during exercise. Although in comparison blood samples before and after exercise, a significant difference was observed in serum IL-6 levels in both groups, but there was no significant difference in IL-6 increased between CHO and Pla groups.

Considering the effect of carbohydrate supplementation during two bouts of exercise on serum concentrations of IL-6, a study was conducted with eight male subjects and looked into the effect of consuming carbohydrate beverage during 3 hours of cycling on serum IL-6 concentration. IL-6 serum concentrations increased in response to exercise, but carbohydrate intake, significantly retarded the increase of IL-6 serum levels in CHO group than in Pla group until the end of the exercise [19]. Also in other study the effect of carbohydrate supplementation during a two-stage pedal continuative exercise on immune system responses. The first phase of exercise was performed at 09.00 and the second phase at 13:30. Each stage consisted of 90 minutes was pedaling with 60% maximum oxygen consumption. Among the findings of the said study was that the ingestion of CHO compared with Pla ingestion slowed down the increase in serum IL-6 in the second phase of exercise that is consistent with the findings of this research [9].

Based on the above discussion it can be concluded that IL-6 serum concentration varies under the influence of intensity, duration and type of exercise. Based on findings of this study, following 60 minutes of running on a treadmill with 80% of maximum heart rate, IL-6 serum concentration would increase significantly in both CHO and PLA groups, with the difference that increased IL-6 concentration in Pla group was significantly greater than that of CHO group. Thus, CHO supplementation is effective in reducing the concentration of IL-6 serum. Also 24 hours after the first 60-minute bout of running with 80% of maximum heart rate, although IL-6 serum levels decreased in comparison with the peak time, it still remained higher than the pre-exercise level. The second 60-minute bout of running with 80 percent of maximum heart rate, resulted in the relative increase in IL-6 serum concentrations in both groups, yet this increase was not significant, while at the end of the second bout of exercise, the concentration of IL-6 levels significantly increased in the Pla group compared with that of before the first bout, this change was not significant in the CHO group.

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