



## Effect of intense endurance training with the supplements of egg white powder and wheat germ powder on IL-6, NT-proBNP, and VO2max of endurance runners

**Abdollah Niloufari<sup>1</sup>, Foad Feizolahi<sup>2\*</sup>, Fariba Aghaei<sup>2</sup>, Mahsa Mohsenzadeh<sup>1</sup>**

1. PhD student, Department of Physical Education and Sports Sciences, Islamic Azad University, Karaj, Iran, <https://orcid.org/0000-0001-5386-5639>

2. Assistant Professor/Specialized PhD, Department of Physical Education and Sports Sciences, Islamic Azad University, Karaj, Iran

3. Assistant Professor/PhD, Department of Physical Education and Sports Sciences, Islamic Azad University, Karaj, Iran

4. Assistant Professor/PhD, Department of Physical Education and Sports Sciences, Islamic Azad University, Karaj, Iran

\* Corresponding author: Clinical Care and Health promotion Research Center, Karaj branch, Islamic Azad University, Karaj, Iran

### Abstract

Sports activity reduces significantly the problems caused by cardiovascular and immune system diseases. This study investigated the effect of dietary supplements containing egg white powder and wheat germ powder on increasing immunity, respiratory, and heart electrocardiogram of athletes with intense endurance training. This study randomly divided 24 endurance athletes with an age range of 18 to 30 years and a BMI of less than 25 kg with intense endurance training into two groups: exercise+supplement (12 people) and exercise (12 people). All the conditions for participating in the test were explained to the athletes after they completed the questionnaire. Two groups were tested on IL-6, NT-proBNP, and VO2max indicators at the pre-test stage, and the post-test of the two groups was conducted on IL-6 and NT-proBNP indicators. There was no statistically significant difference. A significant difference was observed between the post-test of the two groups only in the VO2max index. Likewise, the results of the dependent t-test showed a significant difference in the pre-test and post-test measurement stages in the exercise group for IL-6, NT-proBNP, and VO2max indicators. There was also a significant difference in the two stages of pre-test and post-test measurements in the exercise+supplement group for NT-proBNP and VO2max index, but no such results were observed for the IL-6 index.

Keywords: electrocardiogram, endurance exercises, egg white powder, wheat germ powder



### Introduction

Regular sports activities with moderate intensity are beneficial for health. Considerable evidence has shown that intense sports exercises can have essential effects on various aspects of health (1). Endurance training lasts for several hours, and muscle glycogen, along with blood sugar and fat reserves in the muscles, are the main source of fuel for the first 90-120 minutes of training. Increasing muscle glycogen reserves before starting endurance sports and consuming exogenous carbohydrate sources in training and competitions is vital to maintaining optimal performance. Sufficient energy supply is necessary for daily training and preparation for participating in endurance competitions (2). Muscle glycogen reserves may be particularly depleted after a few days of heavy training if proper attention is not paid to dietary carbohydrate intake; it can simultaneously affect various systems, including the immune and cardiovascular systems (1, 2).

Studies that have measured specific cardiac markers have introduced plasma concentration evaluation as a diagnostic index for identifying and predicting heart failure, whose values increase in proportion to the severity of heart failure (3, 4). There are different opinions regarding the response of plasma NT-proBNP to endurance sports activity in athletes. Vidotto, Tschan, Atamaniuk, Pokan, Bachl, Müller (5) and Middleton, Shave, George, Whyte, Forster, Oxborough, et al. (6) have reported, in their pathological evaluation of athletes, an increase in plasma NT-proBNP (N-terminal protein Brain Natriuretic Peptide) levels after intense endurance sports activities (5, 6). Bartek et al. did not report a significant difference between the plasma level of NT-proBNP before and after endurance sports activity (7). Neilan, Januzzi, Lee-Lewandrowski, Ton-Nu, Yoerger, Jassal, et al. (8) reported an increase in NT-proBNP plasma levels of cardiac dysfunction in young athletes. Middleton et al. (9) showed in their research that the increase in NT-proBNP plasma levels in athletes cannot be associated with cardiac dysfunction.

Researchers have recently found that an increase in plasma NT-proBNP caused by exercise can result from the release of inflammatory cytokines. They observed that pro-inflammatory cytokines modify the expression of the BNP gene and its secretion (10). Several studies have proven that the increase of cytokines, especially IL-6, strongly modifies cardiac function and can lead to myocyte damage. They consider IL-6 plasma levels to be associated with the severity of left ventricular dysfunction and an important factor in the development of heart failure (11). Nieman, Dumke, Henson, McAnulty, Gross, and Lind (12) have reported a relationship between increased levels of IL-6 and muscle damage during long-term exercise. Yamin, Duarte, Oliveira, Amir, Sagiv, Eynon, et al. (13) also supported the role of cytokines in response to inflammatory processes and muscle damage. However, Stroski et al. (2000) did not report a relationship between the amount of IL-6 and muscle damage in athletes, which is different from the results of previous studies (14).

Studies on the consumption of egg white supplements have shown the importance of the amount of consumed protein during recovery. Protein consumption for endurance athletes is important because high energy consumption during training, besides the need to receive carbohydrates, also requires protein consumption so that the body does not fall into a catabolic state and can return to the anabolic and repair state of damaged tissues during training (9). Other researchers have stated that the importance of the variable of daily protein consumption is greater than the variable of consumption time. In other words, they emphasized the daily consumption until reaching the necessary amounts for endurance athletes to have a greater effect on MPS (15). Therefore, the simultaneous consumption of carbohydrates and protein is



very necessary for endurance athletes, especially those who engage in intense training, and egg whites with wheat germ can be among the most important and consumed food supplements.

### ***Research Method***

The current research is semi-experimental. It included 24 athletes with an age range of 18 to 30 years and a BMI of less than 25 kg in the program of intense endurance training, after coordinating with the Saez Athletics Board, from among the endurance runners with at least one year of experience in endurance running training. They were randomly divided into two groups: exercise+supplement (12 people) and exercise (12 people). All the conditions of participating in the test were explained to the athletes after completing the questionnaire (including personal information, history of sports activity, history of illness, and family history). The subjects, after learning about the method of conducting the research, voluntarily declared their readiness to participate in the research. A written consent form was obtained from the volunteers to conduct the test and blood sampling. Inclusion criteria include participating in endurance running exercises for at least one year and at most for three years, not attending regular continuous and periodic aerobic exercises, not suffering from diseases and physical-muscular complications, not having a history of smoking, alcohol and drugs, absence of complications and history of cardiovascular and respiratory diseases, and having a disease of the immune system or special disease.

### ***Methods of collecting information***

#### ***Body composition***

The subjects' height was measured with a caliper, hip, and waist circumference with a tape measure with a sensitivity of 5 mm, and body fat percentage and weight were measured with an In-body device to evaluate the body composition. All subjects had refrained from eating and drinking for four hours before the test, and their bladder, stomach, and intestines had been emptied as much as possible. A cardiovascular examination, blood pressure measurement, and electrocardiogram recording by a specialist doctor were done and the subjects were allowed to enter the project. Likewise, the blood pressure of each subject was measured before starting physical activity.

#### ***Measuring maximum oxygen consumption***

Bruce's protocol method was used on the treadmill to estimate the maximum oxygen consumption. Bruce's test was performed in ten stages of three minutes. The speed in the first stage was 2.74 km/h with a 10% incline, and both the speed and the incline of the device increased every three minutes until the last stage. The maximum oxygen consumption (in ml/kg/min) was calculated in the Bruce protocol through its special formula (16).

#### ***Method of preparing blood samples***

We take five milliliters of blood in a sitting position from a vein of the subject's right arm 48 hours before the start of the training protocol between 8 and 10 in the morning and in a 10-hour fasting state to measure blood samples at the beginning and end of the research period. It is centrifuged, after coagulation, by the device for 15 minutes at a temperature of four degrees Celsius at a speed of 1500 rpm, then the target serum of the blood in the sample tubes without anticoagulant is frozen at minus 80 degrees. We asked the subjects not to do vigorous physical activity and not to use any type of supplements or alcoholic substances on the day before blood sampling. We used a blood sample for measuring the dependent variables to determine the post-test at the end of eight weeks, according to the pre-test sampling time (48 hours after the





last training session). It is noteworthy that the blood samples were collected in the blood laboratory by specialists. The diet was controlled 24 hours before and after sampling. The samples were immediately used to measure plasma concentrations of NT-proBNP and IL-6 at the end of each stage of blood collection.

#### *Method of NT-proBNP measurement*

A blood sample was taken from the anterior vein of the forearm in a sitting position by 5 ccs to measure the plasma level of NT-proBNP, before, immediately, and after the first training session and after 8 weeks of training, and poured into tubes containing EDTA. The blood samples were immediately transferred, at the end of the blood collection phase, to the Razi Saez laboratory to measure the plasma concentration of NT-proBNP. NT-proBNP plasma concentration was measured by means of a Path Fast Immuno Analyzer and using an NTproBNP specialized kit of Mitsubishi brand, made in Japan. The sensitivity of the kit is 125 pg/ml. The subjects were asked to refrain from any intense activity for 48 hours before the start of the test to prevent possible effects of intense activity on the results of the research.

#### *Method of Interleukin-6 measurement*

The level of interleukin-6 was measured by a special human kit (Pharmigen, San Diego, CA, USA) and by the ELISA method. The sensitivity of the measurement method was less than 0.7 pg/ml.

#### *Exercise protocol*

The exercise protocol included high-intensity aerobic endurance exercises for eight weeks and three sessions per week, each session lasting 60 minutes (the total time of the exercise session including warm-up, main body, and cool-down). The aerobic exercise program included running on the treadmill for 21 minutes (the main body of the exercise) with an intensity equal to 70-90% of the reserve heart rate. The exercise intensity was controlled by a heart rate monitor (POLAR/Finland). The subjects' resting heart rate was measured by a heart rate monitor in the morning of the exercise day and after two days without exercise. Exercise sessions took place in the afternoon and around 7 in the evening (16).

#### *Supplementing*

The control group did not receive any nutritional supplements and continued their diet with a placebo. Group 2, who received the food supplement under study in this research, received the food supplement two days before the start of training. Protein supplements included 15 grams of dry egg white powder (75 kcal) and carbohydrate supplements included 17.5 grams of wheat germ powder (78 kcal), without any added flavor. Each supplement was delivered to the subjects as a dry powder in sealed packages with a code to ensure a blinded study. Subjects were asked to take the supplements at approximately 5:00 p.m. each day (approximately two hours before the exercise session on exercise days) by mixing in 200 mL of mineral water, otherwise, they were stored in the refrigerator. Each of the participants consumed the same supplements during the eight-week period and their adherence to the daily diet was controlled (9).

#### *Statistical methods*

We used descriptive statistics to determine the mean and standard deviation and evaluated the data distribution with the Kolmogorov-Smirnov test. An independent t-test was used to examine the difference between the groups' averages, and a dependent t-test to examine the differences between the two stages of pre-test and post-test. It is noteworthy that all findings are at a significance level of 0.05 and were analyzed by SPSS26 software.

Findings

Table 1 shows the individual, anthropometric, and physiological characteristics of subjects in two groups of exercise and exercise+supplement. Table 2 lists the measured values of the indicators under study in the two stages of pre-test and post-test. Likewise, we examined the normal distribution of the data through the Shapiro-Wilk test.

Table 1: Average and standard deviation of individual indicators

Variable	Exercise	Exercise+supplement
Age (year)	۴۵.۲±۳.۲۲	۴۵.۲±۵۰.۲۲
Weight (kg)	۱۳.۱۰±۷.۷۲	۹۵.۱۰±۷۳
Height (cm)	۸۰.۵±۱۷۵	۱۰.۶±۱۷۶
Fat percentage (%)	۷۲.۳±۲.۱۸	۴±۵.۱۸

Table 2: Average and standard deviation of the dependent indicators under study

Variable	Exercise		Exercise+supplement	
	Pre-test	Post-test	Pre-test	Post-test
IL-6 (Pg/MI)	۳۲.۰±۲۷.۲	۱.۱±۸۴.۲	۱۷.۰±۲۹.۲	۸.۰±۲۴.۳
NT- proBNP	۲۸.۷±۲۸۹.۲۰	۵۲.۸±۹۴۸.۲۳	۸۱.۷±۵۰۴.۲۰	۷۴.۸±۷۲۵.۲۷
VO2max (ml/kg/min)	۳.۲±۱.۵۰	۹.۲±۱.۵۱	۸.۲±۲.۵۱	۵.۱±۵.۵۴

Independent t-test determined the effect of eight weeks of intense endurance exercise with and without simultaneous consumption of egg white and wheat germ supplements on IL-6, NT-proBNP, and VO2max in two groups of exercise and exercise+supplement (Table 3).

Table 3: Differences between groups on IL-6, NT-proBNP, and VO2max in the two groups of exercise and supplement+exercise

Measurement stages		Homogeneity of variance		Difference of the means	Degrees of freedom	T	Sig.
		F	Sig.				
IL-6	Pre-test	۰.۴.۰	۹۵۰.۰	۰.۸۰.۰	۲۲	۲۷.۰	۷۹۱.۰
	Post-test	۸۷۰.۶	۰.۱۶*.۰	۶۴.۰	۲۲	۶۱.۱	۱۲۷.۰
NT- proBNP	Pre-test	۱۹۱.۰	۶۶۶.۰	۲۱۴.۰	۲۲	۰.۷۰.۰	۹۴۵.۰
	Post-test	۰.۵۶.۰	۸۱۶.۰	۷۷۷.۳	۲۲	۱.۰۷۱	۲۹۶.۰
VO2max (ml/kg/min)	Pre-test	۹۰۷.۱	۱۸۱.۰	۰.۲۵.۰	۲۲	۰.۲۵.۰	۹۸۱.۰
	Post-test	۴۵۱.۳	۰.۷۷.۰	۰.۸۹.۳	۲۲	۹۳.۲	۰.۰۸*.۰

\* It shows significance at the level (p>0.05).

The dependent t-test would determine the effect of intense endurance exercise with the simultaneous consumption of egg white and wheat germ supplements on IL-6, NT-proBNP, and VO2max in both groups separately and to determine the effectiveness of exercise and exercise+supplements (Table 4).

Table 4: Intra-group difference in measured IL-6, NT-proBNP, and VO2max values



Pre-test & Post-test		Difference of the means	Standard deviation	Sig.
IL-6	Exercise	۹۸۷.۰	۱۱۲.۱	۰.۱۱*
	exercise+supplement	۲۶۸.۰	۴۳۳.۰	۰.۵۵.
NT- proBNP	Exercise	۶۵۸.۳	۷۶۱.۱	۰.۰۰*
	exercise+supplement	۲۲۱.۷	۱۸۰.۲	۰.۰۰*
VO <sub>2</sub> max (ml/kg/min)	Exercise	۶۸۰.۰	۵۷۹.۰	۰.۰۲*
	exercise+supplement	۷۴۴.۳	۹۸۲.۰	۰.۰۰*

\* It shows significance at the level ( $p > 0.05$ ).

As the independent t-test in the pre-test of the two groups showed, there is no statistically significant difference in IL-6, NT-proBNP, and VO<sub>2</sub>max indices. We observed no significant difference between the post-test of the two groups of exercise and exercise+supplement in the indices of IL-6 and NT-proBNP. Therefore, exercise and exercise+supplement have no significant effect on the values of IL-6 and NT-proBNP indices. We observed a significant difference only in the VO<sub>2</sub>max index between the post-test of the two exercise and exercise+supplement groups. We can conclude that exercise and exercise+supplement have a significant effect on VO<sub>2</sub>max index values.

Moreover, the results of the dependent t-test showed a significant difference in the pre-test and post-test measurement stages in the exercise group for IL-6, NT-proBNP, and VO<sub>2</sub>max indicators. Likewise, the results of the dependent t-test showed a significant difference in the two stages of pre-test and post-test measurement in the exercise+supplement group for NT-proBNP and VO<sub>2</sub>max indicators, but there was no significant difference in the two stages of measurement for the IL-6 index.

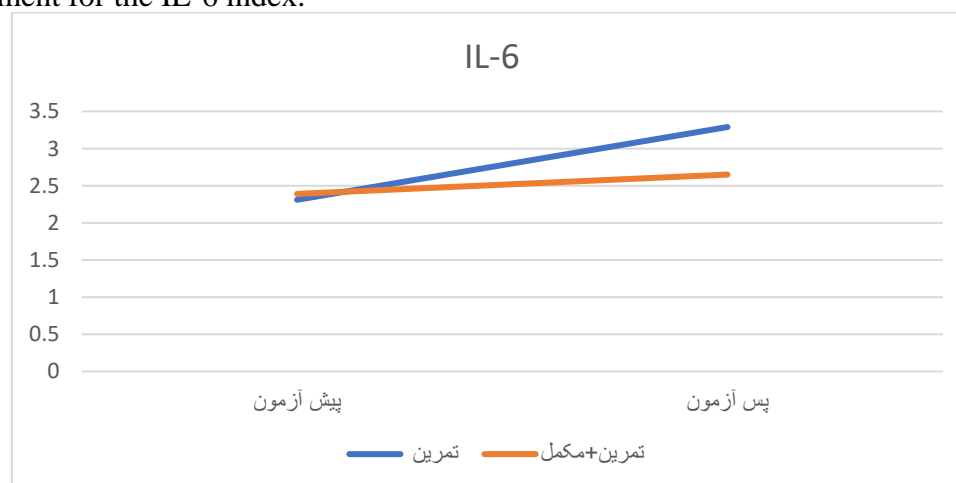


Figure 1: IL-6 changes in the two pre-test-post-test stages in two groups of exercise and exercise+supplement

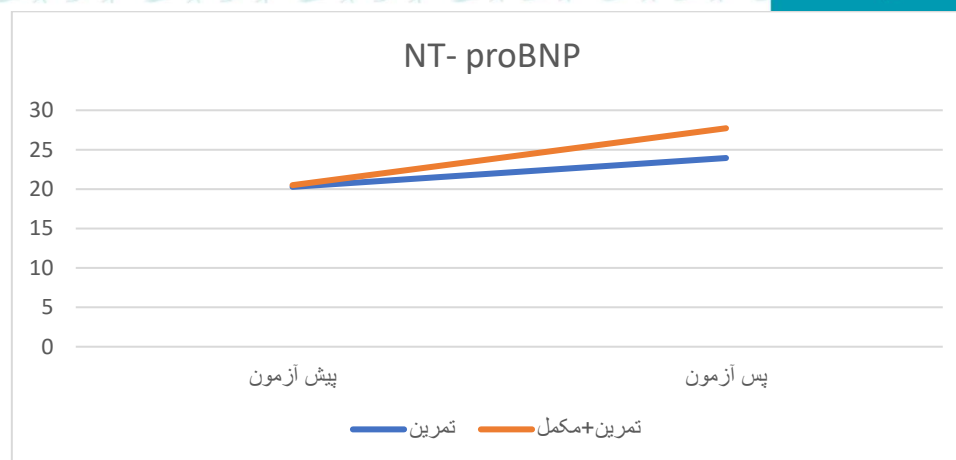


Figure 2: NT-proBNP changes in the two pre-test-post-test stages in two groups of exercise and exercise+supplement

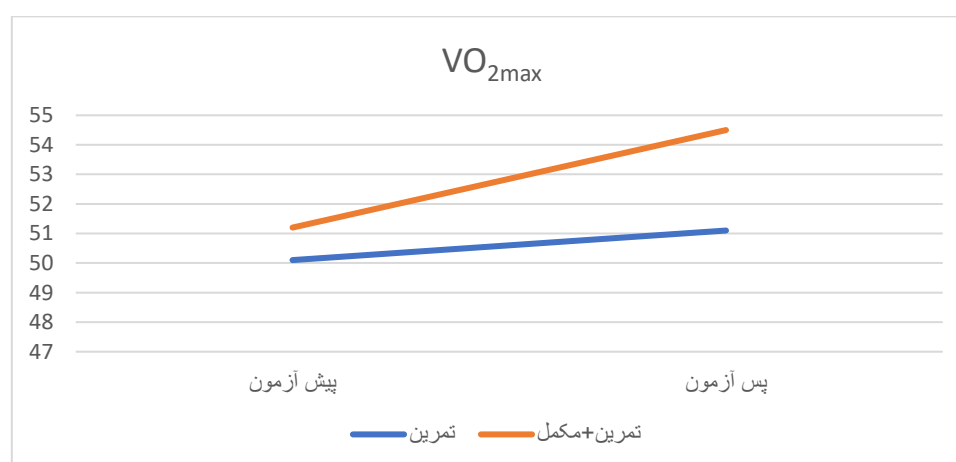


Figure 3: Changes in VO2max in the two pre-test and post-test stages in the two groups of exercise and exercise+supplement

### Discussion

As the results showed, the values of the IL-6 index in the pre-test of the two groups were not significantly different from each other. No significant difference was between the two groups of exercise and exercise+supplement after the test. Therefore, exercise and exercise+supplement have no significant effect on IL-6 levels. Likewise, a significant difference was observed for the IL-6 index in the two stages of measurement in the exercise group. Therefore, intense endurance exercise without taking supplements leads to a significant difference between the pre-test and post-test levels of IL-6. No significant difference existed in the two stages of measurement in the exercise+supplement group for the IL-6 index. So intense endurance exercise without supplementation does not lead to a significant difference between the pre-test and post-test levels of IL-6. These results show that there is a significant difference between the exercise group and the exercise+supplement group. Thus, IL-6 levels in the group of intense endurance exercises without taking supplements show a statistically significant increase compared to the group of intense endurance exercises with simultaneous consumption of egg white and wheat germ supplements.





The results are in line with the studies of Scharhag, Meyer, Auracher, Müller, Herrmann, Gabriel, et al. who reported an increase in IL-6 levels after endurance sports activities (4). However, some recent findings support the role of intensity and periods of sports activity in the expression of the IL-6 gene in all types of tissues. Studies have shown an increase of IL-6 plasma concentration undoubtedly under the influence of intense activity and a decrease during long-term activities. Pederson and Toft (17) measured IL-6 mRNA in marathon runners after the race and showed that IL-6 is mainly produced and secreted in response to intense sports activities and muscle damage caused by the activity. Nobar et al. (18) also reported the increase of IL-6 by investigating the signs of muscle damage and the systemic end response in athletes. Therefore, the relationship between the severity of injury and inflammation shows that severe contractions can affect the mechanism of production and secretion of IL-6. So damage caused by sports activity is a primary trigger of IL-6 production.

Our results are inconsistent with the findings of Broadbent, Rousseau, Thorp, Choate, Jackson, Rowlands (19), Gielon, Adam, Mobius Winkler, Linke, Erbs, Ya, et al. (20) and Su, Tian, Zhang, Zhang (21) who reported a decrease in IL-6 levels in the blood samples of athletes.

Kokhal et al. (2007) investigated the effect of regular exercise on IL-6 in athletes and non-athletes. Their results revealed that athletes showed the least amount of change in plasma IL-6 following long-term sports activity compared to non-athletes. As they stated, regular training reduces the immune response to sports activity. A high amount of sports activity is associated with a decrease in IL-6 plasma levels. Therefore, the increase in muscle IL-6 receptor expression in individuals with high aerobic capacity, as a result of exercise, seems to be able to justify the decrease in plasma IL-6 (22).

The present study did not show a statistically significant difference between the two groups in the pre-test and post-test. Therefore, exercise and exercise+supplement have no significant effect on NT-proBNP values. Likewise, we observed a significant difference in the NT-proBNP index in both the exercise and exercise+supplement groups. Intense endurance exercise with and without supplementation leads to a significant difference between the pre-test and post-test values of NT-proBNP. Therefore, intense endurance exercise alone and with the simultaneous consumption of egg white and wheat germ supplements have a significant effect on NT-proBNP.

Our result was consistent with those of the studies of Middleton et al. (6) and Vidotto et al. (5). They also reported a significant increase in plasma levels of NT-proBNP after activities such as marathon running, cycling, and triathlon. Scharhag et al. (4) have reported an increase in plasma NT-proBNP because of exercise as a result of increased heart pressure caused by the stretching of myocytes during endurance exercise, which can be attributed to the positive correlation between the duration of endurance exercise and the level of NT-proBN plasma concentration in their research. Although Tomas, Janes, Elizabeth, Thang-Thao, Danita, Davinder, et al. (23) have reported an exercise-induced increase in NT-proBNP plasma levels for cardiac dysfunction in young athletes, this finding was not in agreement with the results of Banfi, Milqiorian, Dolici, Nosed, Scapellato, Franzini (24) and Bartek, Stejskal, Lancnk, Jurakava (7). They have reported a significant decrease and no change in plasma levels of BNP or NT-proBNP during endurance activities such as professional cycling and endurance triathlons, respectively.

Since the primary purification and destruction of plasma BNP and NT-proBNP occurs through neutral endopeptidase and the filter of AC and glomeruli, the speed of purification of BNP and NT-proBNP has been different. Considering the high molecular weight of plasma NT-proBNP





and its long half-life (60 minutes) compared to BNP (22 minutes), the increase in the plasma concentration of NT-proBNP after exercise occurs supposedly in response to the decrease in the purification rate of its primary secretion from the kidneys. This delay in purification can also be caused by changes in the permeability of kidney cells or damage to their removal, which affects the speed of kidney extraction (7).

Moreover, as for VO<sub>2</sub>max, the results did not show a significant difference in VO<sub>2</sub>max index values in the pre-test of the two groups. However, a significant difference was observed between the post-test of the two exercise and exercise+supplement groups. Therefore, exercise and exercise+supplement have a significant effect on VO<sub>2</sub>max values. There was also a significant difference in the VO<sub>2</sub>max index in the two measurement stages in the exercise and exercise+supplement groups. Thus, intense endurance exercise without taking supplements leads to a significant difference between pre-test and post-test VO<sub>2</sub>max values.

Our results were consistent with those of the studies of Park, Park, Kwon, Yoon, and Kim (2003). They showed an increase in the maximum amount of oxygen consumed in both groups, which caused the adaptation of individuals during this period (25). The results of Saberi et al. were inconsistent with ours. They did not observe any significant increase in the pre-test and post-test after eight weeks of endurance and resistance training for elite runners in two separate groups (16). In contrast, we observed a significant increase in both exercise and exercise+supplement groups after eight weeks of training. This increase was greater in the exercise+supplement group than in the exercise group.

One main and popular indicator in endurance sports is to examine the VO<sub>2</sub>max level with the strenuous increasing test, the result of which has a clinical correlation with long-term and high-intensity activities. Athletes suffering from non-functional fatigue experience a drop in VO<sub>2</sub>max. Takarda Y. and Ishii N. believe that the increase in muscle endurance is initially caused by adaptation in the muscle through an increase in oxidative energy metabolism, acid buffering capacity, and an increase in resistance to fatigue in the nervous system (26).

### **Conclusion**

As the findings revealed, intense endurance training inherently leads to the improvement of IL-6, NT-proBNP, and VO<sub>2</sub>max values of endurance runners. It is also possible to supplement egg white and wheat germ. However, more studies are necessary, in conditions without corona, in different age groups, and with different levels of physical fitness. Therefore, endurance athletes should benefit from intense continuous training with egg white powder and wheat germ powder to improve their cardio-respiratory indicators and immune system. Future studies should be done on subjects with different age groups, genders, physical fitness levels, and different sports to observe the effects of egg white powder and wheat germ supplementation along with intense endurance exercises.

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