## COACHING \& PHYSIOLOGY

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# Do Continuous and Intermittent Running Exercises Affect Leukocyte Count? Comparison of Acute and Chronic Effects 

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#### Abstract

Background. It is widely accepted that physical exercise can cause changes in the immune system. Acute bouts of exercise can alter the number and function of leukocytes, but the degree of white blood cell increase depends on the intensity and duration of the exercise. Purpose. This aim is to examine the acute and chronic effects the white blood cell count and subsets in the bloodstream of the performance of continuous and intermittent running exercise as well as body composition. Material and Methods. In the adult category, the age, sports age and height of actively engaged in weight sports are respectively; total of 40 top-elite male athletes with CRG: $26.3 \pm 2.67$ years, $9.1 \pm 1.08$ years, and $177.3 \pm 5.06 \mathrm{~cm}$ and IRG: $25.6 \pm 2.79$ years, $8.2 \pm 2.66$ years and $179.9 \pm 6.51 \mathrm{~cm}$ participated. A one-way analysis of variance (ANOVA) with repeated measurements was used to identify differences between measurement points for leukocyte and subset values. The comparison of the groups effect was made by repeated measurements with a two-way (group $x$ time) ANOVA. Results. According to the data obtained; in the CRG group, WBC, lymphocyte, neutrophil, monocyte, eosinophil values and in the IRG group; WBC, lymphocyte, neutrophil and monocyte values were found to be significantly different within the group between weeks. ( $\mathrm{p}<0.05$ ) When the CRG and IRG groups were compared, there was no significant difference between the groups. ( $\mathrm{p}>0.05$ ) Conclusions. Our study reveals that leukocyte and subset values acutely increase after both continuous and intermittent exercises in elite athletes, but when examined from a chronic point of view, blood values after exercise program are similar to resting states. Besides that, continuous and intermittent aerobic running exercises are thought to increase the performance and endurance of athletes.


## Introduction

Lifetime physical activity is a powerful way to reduce the risk of noncommunicable diseases, including cancer, cardiovascular disease, and other chronic inflammatory disorders [Warburton, Bredin 2017]. Evidence also sug-
gests that a physically active lifestyle reduces the risk of contracting a range of infectious diseases, including viral and bacterial infections [Pape et al. 2016; Romaniszyn et al. 2014]. Unlike the widely accepted long-term health benefits achieved with regular physical activity, which implies that immune competence and regulation are
enhanced by frequent bouts of exercise, the effect of a single exercise session on immune function is hotly debated. Undeniably, acute vigorous exercise has a profound effect on the phenotypic structure and functional capacity of the immune system. Indeed, the behavior of nearly all populations of immune cells in the bloodstream changes somewhat during and after exercise [Walsh et al. 2011]. Over the years, these collective observations have come together, leading to the so-called "open window" hypothesis that claims the immune system is compromised in the hours after vigorous exercise and increases the risk of opportunistic infections the athlete in the following days. To this day, despite the existence of conflicting evidence, the "open window" hypothesis continues to be debated [Peake et al. 2017]. However, for decades, it has been widely accepted that these changes cause a temporary decline in immune competence in the hours following exercise. In this article, we re-discuss the available data and try to dispel the misconception that acute exercise is detrimental to immunological health.

Intense training programs or endurance competitions are forms of extreme physical stress and lead to immunosuppression in athletes associated with increased susceptibility to infection, particularly upper respiratory infections [Gleeson et al. 2004; Maughan et al. 2010]. Regular moderate-intensity exercise has beneficial effects that reduce the risk of infection, while prolonged and high-intensity exercise can lead to immunosuppression [Freidenreich, Volek 2012; Masih, Verbeke 2018; Pontzer 2018]. It has adverse effects on acquired immunity such as intense exercise bout, antigen presentation by monocytes/macrophages, T lymphocyte cytokine production and proliferation, and immunoglobulin production by $B$ lymphocytes. This depression is temporary and immune functions usually return to baseline within 24 hours [Walsh et al. 2011]. However, during this so-called "open window" period after intense exercise, many athletes are susceptible to disease, especially upper respiratory symptoms (eg, cough, sore throat, runny nose). Daily training routine and competition performance may deteriorate as an undesirable situation. This is why athletes are interested in various dietary and exercise strategies (particularly endurance exercise types) to maintain immune competence and avoid disease. Athletes may be more vulnerable to infections and illnesses due to the large workloads they are subjected to during intense training [Pedersen, Hoffman-Goetz 2000; Tvede et al. 1993; Chen et al. 2016]. Also, if recovery times between training sessions are insufficient, a temporary decline of some immune factors can become a chronic depression of acquired immunity [Walsh et al. 2011].

In clinical practice, total leukocyte counts and subsets are widely used to confirm acute immune system disorders associated with the inflammatory process associated with the development of some unhealthy conditions [Mochizuki et al. 2012; Manabe 2011]. However,
a complex interaction between various genetic and environmental factors determines interpersonal variability in leukocytes [Mahaney et al. 2005]. A high interpersonal variation in white blood cell (WBC) counts in physically active individuals has also been reported [Nunes et al. 2010; Van den Bossche et al. 2002]. Despite the high interpersonal variation, physical exercise can support changes in the immune system [Navalta et al. 2007; Pedersen, Hoffman-Goetz 2000] and it is widely accepted that it causes major physiological changes [Ihalainen et al. 2014; Timmons et al. 2006]. Even acute bouts of exercise can alter the counts and function of leukocytes. The degree of WBC increase depends on the intensity and duration of the exercise [Edwards et al. 2007]. It has been hypothesized that exercise enhances stress-related changes in the immune-neuroendocrine axis and circulating levels of metabolites that directly affect the function of immune cells [Risøy et al. 2003]. Continuous and intermittent running exercises are specifically designed to maximize neural adaptation, and by placing low metabolic demands (resting intervals or exercise intensities) the inflammatory response after conventional running exercises has been little studied. Although endurance exercise can affect the size of circulating WBC, the exact mechanisms that trigger responses in the immune system during endurance exercises are unknown.

## Purpose

In the light of this literature; The aim of this study is to examine the acute and chronic effects of the performance of two different types of strenuous exercise (continuous running exercise and interval running exercise), as well as changes in body composition and circulating white blood cell count and subsets. Since the chronic impact of the immune response to exercise has only been limited to so far, we thought it would be useful to compare the effects of acute and chronic exercise on circulating leukocyte count.

## Material and Methods

## Research Group

In the adult category, the age, sports age, and height of actively engaged in weight sports are respectively; total of 40 (boxer, wrestler, and taekwondo) top-elite male athletes with CRG: $26.3 \pm 2.67$ years, $9.1 \pm 1.08$ years and $177.3 \pm 5.06 \mathrm{~cm}$ and IRG: $25.6 \pm 2.79$ years, $8.2 \pm 2.66$ years and $179.9 \pm 6.51 \mathrm{~cm}$ participated. Based on the weight in international competitions, each group was randomly divided into 2 groups of 20 people. The athletes answered the questions in a form administered before the study, and the athletes who had cardiovascular, neurological, auditory-visual (vestibular-visual) discomfort in the last
year and had no serious injury to their lower and upper extremities in the last 6 months were included in the study. Both protocols applied during the exercise were carried out in a laboratory environment under the control of experts in the field.

## Exercise Protocol

Continuous Running Group (CRG): The athletes started the warm-up run at a speed of $9 \mathrm{~km} / \mathrm{h}$ and were allowed to rest for 1 minute after the warm-up runs were performed at the determined speed within 5 minutes. After the athletes completed the warm-up run, the speed was increased by $1 \mathrm{~km} / \mathrm{h}$ per minute. The increase of $1 \mathrm{~km} / \mathrm{h}$ per minute continued with an increase of $0.5 \mathrm{~km} / \mathrm{h}$ per minute after the completion of the 5th minute. The athletes continued the exercise until they were exhausted. After the exercise was completed, the running speed was reduced to $4 \mathrm{~km} / \mathrm{h}$ and the athletes performed a 5 -minute cool-down run [Midgley et al. 2007].

Intermittent Running Group (IRG): The athletes started the warm-up run at a speed of $9 \mathrm{~km} / \mathrm{h}$ and were allowed to rest for 1 minute after the warm-up runs were performed at the determined speed within 5 minutes. After the athletes completed the warm-up run, the speed was increased by $1 \mathrm{~km} / \mathrm{h}$ every 3 minutes and the athletes had a passive rest for 30 seconds before each speed increase. The athletes rested by putting their feet on the sides of the treadmill during the resting intervals and continued to run again after the rest period ended. The athletes continued the exercise until they were exhausted. After the exercise protocol was completed, the running speed was reduced to $4 \mathrm{~km} / \mathrm{h}$. Athletes performed a 5 -minute cool-down run [Midgley et al. 2007].

## Experimental Design

Hematological baseline measurements were taken at the first visit to the laboratory. Body composition was meas-
ured and $\mathrm{VO}_{2 \text { max }}$ was determined. Training sessions and measurements; It was performed in a temperature-controlled gym $\left(22-24^{\circ} \mathrm{C}\right)$ and applied at the same time of day to avoid any circadian variations. Participants were asked to avoid vigorous exercise 24 hours before the first session and between exercise sessions. All measurements performed were taken at the same time of the day.

## Applied Measurements and Tests

## Age, Height and Body Composition Measurements

The ages of the athletes participating in the study were recorded by obtaining their identity information. Athletes' ages were taken from the athlete declaration and recorded. The height of the athletes; In anatomical stance, bare feet, feet with heels joined, breath-holding, the head was positioned in the frontal plane with the overhead plate touching the vertex point, and it was measured with a portable Seca 216 brand height gauge. Body composition measurement was measured with 0.1 kg sensitive Tanita TBF 300 device while wearing shorts and $t$-shirts, in bare feet and anatomical posture.

## Heart Rate Measurements

Heart rates of the athletes in the research group were monitored by the polar brand M400 GBS sensor attached to the athletes' chest.

## Aerobic Capacity Measurement ( $\mathrm{VO}_{2 \text { max }}$ Measurement)

 20 m Shuttle Run test, one of the field tests, was used to determine $\mathrm{VO}_{2 \text { max }}$. The 20-Meter Shuttle Run starts at 8.5 $\mathrm{km} \cdot \mathrm{s}^{-1}$ ( 9 seconds) and is a 23-level test that results in the running speed increasing by $0.5 \mathrm{~km} . \mathrm{s}^{-1}$ per minute, and completing the 20 -meter distance by round-trip. During the test, the athletes were asked to touch the lines within 20 meters simultaneously with the sound of the signal. The test was terminated for each athlete who could not reach the lines in the 20 -meter range twice in a row with the signal sound. The athletes continued the exercise
: Weekly training program implemented. Hemogram measurements were taken after the 3rd workout of the week.
Figure 1. Application of The Exercise Protocol
until they were exhausted. In converting the obtained results into $\mathrm{VO}_{2 \text { max }}$ values; The method developed by Ramsbottom et al. [1988] was used.

## Hemogram Measurements

Hemogram blood samples from the athletes participating in our study were taken before the exercise program and each week (Monday, Wednesday, and Friday) at the end of the third day and 24 hours after the exercise program. Participants arrived at the laboratory after a 12 -hour fast, having refrained from vigorous exercise for at least 24 hours. On arrival, they were given a simple standardized breakfast, 1.1 MJ ( 250 kcal ) of a commercial liquid meal supplement. While taking blood samples, necessary precautions were taken to prevent the samples from hemolysis. Participants were asked not to engage in any intense physical activity and not to consume alcohol or caffeine products for at least 24 hours before reporting to the laboratory. The samples taken were separated after a 5-minute centrifuge ( 4000 rpm and $4^{\circ} \mathrm{C}$ ) and stored at $-80^{\circ} \mathrm{C}$ until all samples were collected. Blood samples were examined by the specialist physicians according to the complete blood count method. Blood samples $(20 \mathrm{~mL})$ were drawn before the exercise, immediately post, and 24 hours post-exercise from a forearm vein with participants in a seated position. A portion (1-2 mL ) of the blood collected was used to determine the parameters of the complete blood count measured by an automatic hematology analyzer (Roche Sysmex 2000 XLI). Leukocyte, neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts were used in the analysis.

## Statistical Analysis

All statistical analyzes were performed with SPSS 21 package program (IBM Corp. Released 2012. IBM SPSS Statistics for Windows. Version 21.0. Armonk. NY: IBM Corp). Data are presented as mean $\pm$ standard deviation and pretest-posttest percentile changes. Percentage
change can be defined as the \% change in value due to pretest and posttest changes and values can increase or decrease so that the change can be a positive (+) or a negative value (-). Percent change was calculated according to the formula below.

## Percentage Formula x 100

In addition, in order to evaluate the results in terms of practical significance, the effect size was calculated according to the Cohen $d$ formula and evaluated according to the Hopkins classification. $(\mathrm{d} \leq 0.2$ is insignificant, $\leq 0.6$ is small, $\leq 1.2$ is medium, $\leq 2.0$ is large, $\leq 4.0$ is very large, $\leq 4.0$ the effects is almost perfect) [Hopkins 2002; Cohen 1988]. Kolmogorov-Smirnov and Levene tests were performed to check the normality of the distribution of variables and the homogeneity of variances. Paired sample t-test was applied to determine the difference between pre-test and post-tests of continuous and intermittent exercises. The effect size of the difference between the averages was examined with Cohens'd. The effect size of the difference between the two tests means $\mathrm{ES}<0.20$ small effect, $0.21<\mathrm{ES}>0.50$ medium effect, ES $>0.80$ large effect [Cohen 1988]. A one-way analysis of variance (ANOVA) with repeated measurements was used to identify differences between measurement points for all leukocyte and subset values. Comparison of the effects on the groups was made by repeated measurements with two-way (group x time) ANOVA. Conformity with the sphericity assumption was checked with Maucly's test. Epsilon ( $\varepsilon$ ) values were examined for degrees of freedom in conditions where the assumption of sphericity was not met ( $\mathrm{p}<0.05$ ). Greenhouse-Geisser was applied for $<0.75$, and Huyn-Feldt correction was applied for $\varepsilon>0.75$. Pairwise comparisons between measurements were tested by Bonferroni post-hoc analysis. At the same time, the effect size was calculated with the partial eta square coefficient $\left(\boldsymbol{\eta}_{\mathrm{p}}{ }^{2}\right)$ and was classified as $0.099=$ small, $0.0588=$ medium, $0.1379=$ large effect [Richardson 2011]. The significance level was accepted as $\mathrm{p}<0.05$ for all analyzes.

Table 1. Participants physical, physiological, and body composition characteristics

|  | CRG ( $\mathrm{n}=20$ ) |  | IRG ( $\mathrm{n}=20$ ) |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Variables | Pre-exercise | Post-exercise | \% Change | ES | Pre-exercise | Post-exercise | \% Change | ES |
| Weight | $\begin{gathered} 74.03 \pm \\ 14.82 \end{gathered}$ | $72.97 \pm 14.97 *$ | 1 decreased | 0.10 | $75.25 \pm 13.32$ | $71.10 \pm 14.28^{*}$ | 6 decreased | 0.30 |
| BMI | $22.64 \pm 4.89$ | $21.12 \pm 5.03$ | 7 decreased | 0.30 | $23.62 \pm 2.86$ | $21.30 \pm 3.0^{*}$ | 10 decreased | 0.79 |
| $\begin{aligned} & \hline \mathrm{FM} \\ & (\mathrm{~kg}) \end{aligned}$ | $6.05 \pm 2.84$ | $3.22 \pm 1.43^{*}$ | 47 decreased | 1.25 | $9.52 \pm 4.58$ | $6.60 \pm 4.60 *$ | 31 decreased | 0.63 |
| $\begin{aligned} & \text { FFM } \\ & (\mathrm{kg}) \end{aligned}$ | $67.98 \pm 8.10$ | $69.75 \pm 9.23$ | 3 increased | 0.20 | $65.73 \pm 9.50$ | $64.50 \pm 10.49^{*}$ | 2 decreased | 0.12 |
| $\begin{aligned} & \mathrm{VO}_{2 \max } \\ & \left(\mathrm{ml} . \mathrm{kg} \cdot \mathrm{dk}^{-1}\right) \end{aligned}$ | $44.23 \pm 3.10$ | $49.72 \pm 4.23 *$ | 12 increased | 1.48 | $45.35 \pm 3.17$ | $51.13 \pm 4.23^{*}$ | 13 increased | 1.54 |

CRG: continuous running group, IRG: intermittent running group BMI: Body Mass Index, FFM: Fat Free Mass, FM: Fat Mass ES: Effect Size *: Significantly different according to pre-exercise values ( $\mathrm{p}<0.05$ ).

Tablo 2. CRG and IRG group average, minimum and maximum values of exercise end times and heart rate

| Continuous Running Group <br> Mean (Min-Max) |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1. Seans |  |  | 2. Seans |  |  | 3. Seans |  |  |
| End time | Startup Heart Rate | End Heart Rate | End time | Startup Heart Rate | End <br> Heart Rate | End time | Startup Heart Rate | End <br> Heart Rate |
| 1. 7.49 <br> Week $(4.49-11.19)$ | 128 (120-141) | $\begin{gathered} 185(175- \\ 194) \\ \hline \end{gathered}$ | $\begin{gathered} 8.25 \\ (5.14-11.24) \end{gathered}$ | 133 (124-148) | $\begin{gathered} 186 \text { (183- } \\ 201) \\ \hline \end{gathered}$ | $\begin{gathered} \hline 8.47 \\ (6,17-11,23) \\ \hline \end{gathered}$ | 135 (124-147) | $\begin{gathered} 187 \\ (183-198) \\ \hline \end{gathered}$ |
| 2. 8.44 <br> Week $(5.21-12.01)$ | 131 (121-138) | $\begin{gathered} 189(181- \\ 196) \\ \hline \end{gathered}$ | $\begin{gathered} 9.24 \\ (5.28-12.18) \\ \hline \end{gathered}$ | 134 (125-147) | $\begin{gathered} 191 \text { (182- } \\ 197) \\ \hline \end{gathered}$ | $\begin{gathered} 9.55 \\ (6.18-12.14) \\ \hline \end{gathered}$ | 133 (122-149) | $\begin{gathered} 193 \\ (184-197) \\ \hline \end{gathered}$ |
| 3. 10.48 <br> Week $(7.12-12.14)$ | 132 (120-141) | $\begin{gathered} 194(177- \\ 197) \\ \hline \end{gathered}$ | $\begin{gathered} 10.52 \\ (6.44-12.57) \end{gathered}$ | 136 (124-147) | $\begin{gathered} 194(184- \\ 197) \end{gathered}$ | $\begin{gathered} 11.14(6.56- \\ 13.19) \end{gathered}$ | 133 (125-144) | $\begin{gathered} 195 \\ (187-200) \end{gathered}$ |
| $\begin{array}{cc} \hline 4 . & 11.28(7.14- \\ \text { Week } & 13.53) \\ \hline \end{array}$ | 129 (122-140) | $\begin{gathered} 195(178- \\ 199) \\ \hline \end{gathered}$ | $\begin{gathered} 11.47 \\ (7.41-14.09) \\ \hline \end{gathered}$ | 135 (128-145) | $\begin{gathered} 196(183- \\ 199) \\ \hline \end{gathered}$ | $\begin{gathered} 12.44 \\ (8.11-14.33) \\ \hline \end{gathered}$ | 133 (125-147) | $\begin{gathered} 198 \\ (189-203) \end{gathered}$ |
| Intermittent Running Group Mean (Min-Max) |  |  |  |  |  |  |  |  |
|  | 1. Seans |  |  | 2. Seans |  |  | 3. Seans |  |
| End time | Startup Heart Rate | End <br> Heart Rate | End time | Startup Heart Rate | End Heart Rate | End time | Startup Heart Rate | End <br> Heart Rate |
| 1. 20.12 <br> Week $(14.41-26.43)$ | 127 (121-133) | $\begin{gathered} 187(178- \\ 199) \\ \hline \end{gathered}$ | $\begin{gathered} 22.19 \\ (14.33-27.12) \end{gathered}$ | 129 (122-136) | $\begin{gathered} 190(181- \\ 199) \\ \hline \end{gathered}$ | $\begin{gathered} 23.27 \\ (14.48-28.23) \\ \hline \end{gathered}$ | 130 (123-137) | $\begin{gathered} 191 \\ (183-199) \\ \hline \end{gathered}$ |
| $\begin{array}{cc} \hline \text { 2. } & 24.08 \\ \text { Week } & (14.44-29.51) \end{array}$ | 130 (123-138) | $\begin{gathered} 192 \text { (184- } \\ 199) \end{gathered}$ | $\begin{gathered} 24.49 \\ (14.39-30.13) \end{gathered}$ | 132 (123-139) | $\begin{gathered} 194 \text { (184- } \\ 200) \end{gathered}$ | $\begin{gathered} 25.19 \\ (14.49-30.39) \end{gathered}$ | 133 (122-141) | $\begin{gathered} 195 \\ (186-201) \end{gathered}$ |
| $\begin{array}{cc}3 . & 25.11 \\ \text { Week } & (14.41-30.17)\end{array}$ | 134 (122-141) | $\begin{gathered} 196 \text { (189- } \\ \text { 201) } \end{gathered}$ | $\begin{gathered} 25.57 \\ (14.53-31.04) \end{gathered}$ | 133 (123-140) | $\begin{aligned} & 196(188- \\ & 201) \end{aligned}$ | $\begin{gathered} 27.17 \\ (15.33-32.42) \end{gathered}$ | 136 (127-141) | $\begin{gathered} 197 \\ (190-201) \end{gathered}$ |
| 4. 29.09 <br> Week $(15.57-33.22)$ | 137 (127-141) | $\begin{gathered} 198 \text { (190- } \\ 201) \end{gathered}$ | $\begin{gathered} 30.36 \\ (16.12-33.56) \end{gathered}$ | 136 (129-143) | $\begin{gathered} 198(191- \\ 201) \end{gathered}$ | $\begin{gathered} 32.20 \\ (17.32-35.03) \end{gathered}$ | 135 (128-143) | $\begin{gathered} 199 \\ (190-202) \end{gathered}$ |

Table 3. Participants' leukocytes count before-intervention exchange weekly and 24 hours post-exercise

|  |  | Baseline | 1.Week | 2.Week | 3.Week | 4.Week | 24 hours Post-exercise | F | p | $\eta_{p}{ }^{2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\begin{gathered} \text { WBC } \\ \left(10^{3} / \mu \mathrm{L}\right) \end{gathered}$ | $7.47 \pm 1.73$ | $12.5 \pm 1.94{ }^{\text {a }}$ | $12.4 \pm 2.35^{\text {a }}$ | $13.26 \pm 1.76^{\text {a }}$ | $12.25 \pm 1.78{ }^{\text {a }}$ | $7.96 \pm 1.85{ }^{\text {abcde }}$ | 72.394 | <0.001 | 0.792 |
|  | $\begin{aligned} & \text { LYMPH } \\ & \left(10^{3} / \mu \mathrm{L}\right) \end{aligned}$ | $2.41 \pm .55$ | $5.39 \pm .84^{\text {a }}$ | $5.54 \pm .99^{\text {a }}$ | $5.62 \pm .79^{\text {a }}$ | $5.30 \pm 1.13^{\text {a }}$ | $2.8 \pm .59$ abcde | 160.062 | <0.001 | 0.894 |
|  | $\begin{aligned} & \text { NEUT } \\ & \left(10^{3} / \mu \mathrm{L}\right) \end{aligned}$ | $3.95 \pm 1.20$ | $5.26 \pm 1.30^{\text {a }}$ | $5.79 \pm 1.02^{\text {a }}$ | $5.40 \pm 1.12^{\text {a }}$ | $5.29 \pm 1.26^{\text {a }}$ | $5.09 \pm .68{ }^{\text {a }}$ | 9.116 | <0.001 | 0.324 |
|  | $\begin{aligned} & \mathrm{MONO} \\ & \left(10^{3} / \mu \mathrm{L}\right) \end{aligned}$ | $.53 \pm .16$ | $.95 \pm .13^{\text {a }}$ | $.94 \pm .20^{\text {a }}$ | . $88 \pm .23{ }^{\text {a }}$ | $.92 \pm .29^{\text {a }}$ | $.58 \pm .18$ | 23.017 | <0.001 | 0.548 |
|  | $\begin{gathered} \text { BASO } \\ \left(10^{3} / \mu \mathrm{L}\right) \end{gathered}$ | . $031 \pm .01$ | . $034 \pm .01$ | . $035 \pm .01$ | . $033 \pm .01$ | . $031 \pm .01$ | . $033 \pm .01$ | 1.114 | 0.358 | 0.055 |
|  | $\begin{gathered} \text { EO } \\ \left(10^{3} / \mu \mathrm{L}\right) \end{gathered}$ | $.13 \pm .09$ | $.23 \pm .13^{\text {a }}$ | $.21 \pm .16^{\text {a }}$ | $.23 \pm .14^{\text {a }}$ | $.19 \pm .14^{\text {a }}$ | . $14 \pm .08^{\text {bcde }}$ | 13.353 | <0.001 | 0.413 |
|  | $\begin{aligned} & \text { WBC } \\ & \left(10^{3} / \mu \mathrm{L}\right) \end{aligned}$ | $8.11 \pm 1.92$ | $12.71 \pm 1.64{ }^{\text {a }}$ | $12.38 \pm 2.70^{\text {a }}$ | $14.17 \pm 1.98^{\text {ab }}$ | $11.79 \pm 1.35^{\text {ad }}$ | $8.97 \pm 2.12^{\text {abcde }}$ | 50.526 | <0.001 | 0.727 |
|  | $\begin{aligned} & \text { LYMPH } \\ & \left(10^{3} / \mu \mathrm{L}\right) \end{aligned}$ | $2.87 \pm .72$ | $5.94 \pm 1.26^{\text {a }}$ | $5.67 \pm 1.13^{\text {a }}$ | $6.04 \pm .83^{\text {a }}$ | $5.45 \pm 1.06^{\text {ad }}$ | $3.11 \pm .74{ }^{\text {abcde }}$ | 102.349 | <0.001 | 0.843 |
|  | $\begin{gathered} \text { NEUT } \\ \left(10^{3} / \mu \mathrm{L}\right) \end{gathered}$ | $4.68 \pm 1.45$ | $5.49 \pm 1.08^{\text {a }}$ | $6.45 \pm .95^{\text {ab }}$ | $6.68 \pm 1.63{ }^{\text {ab }}$ | $5.90 \pm .93{ }^{\text {a }}$ | $5.28 \pm .93{ }^{\text {d }}$ | 11.442 | <0.001 | 0.376 |
|  | $\begin{aligned} & \mathrm{MONO} \\ & \left(10^{3} / \mu \mathrm{L}\right) \end{aligned}$ | $.57 \pm .15$ | $.91 \pm .28^{\text {a }}$ | $1.00 \pm .31^{\text {a }}$ | $1.16 \pm .44^{\text {a }}$ | $.87 \pm .32^{\text {a }}$ | . $57 \pm .12^{\text {bcde }}$ | 25.344 | <0.001 | 0.572 |
|  | $\begin{gathered} \text { BASO } \\ \left(10^{3} / \mu \mathrm{L}\right) \end{gathered}$ | . $042 \pm .01$ | . $045 \pm .02$ | . $047 \pm .02$ | . $046 \pm .02$ | . $050 \pm .02$ | . $051 \pm .03$ | 2.378 | 0.079 | 0.111 |
|  | $\begin{gathered} \mathrm{EO} \\ \left(10^{3} / \mu \mathrm{L}\right) \end{gathered}$ | $.14 \pm .05$ | $.18 \pm .05$ | $.19 \pm .07$ | $.17 \pm .06$ | $.18 \pm .09$ | $.16 \pm .05$ | 2.394 | 0.081 | 0.112 |

CRG: continuous running group. IRG: intermittent running group. WBC: white blood cell. LYMPH: lymphocyte. NEUT: neutrophil. MONO: monocyte. BASO: basophil. EO: eosinophil. a ${ }^{\text {a }}$ Significantly different according to the baseline values. ${ }^{\text {b: Significantly }}$ different according to the 1 . week values. c: Significantly different according to the 2 . week values. d: Significantly different according to the 3 . week values. e: Significantly different according to the 4 . week values ( $\mathrm{p}<0.05$ ).

## Results

This study was conducted to examine the effects of two different exercise protocols on body composition and leukocyte cells and subsets. Two different exercise protocols (CRG: continuous running group, IRG: intermittent running group) were applied in the study. These different exercise protocols have been grouped and presented using charts and figures for the relationships between baseline and week-long and leukocyte cell responses 24 hours after exercise.The mean and standard deviation values of the descriptive findings belonging to the CRG and IRG groups are shown. Measurements taken before and after exercise were compared with paired samples t-test. While significantly lower body weight and FM scores were obtained in the CRG and IRG groups after exercise, significantly higher scores were obtained in VO2max values. There was a significant difference in BMI and FFM values after exercise in the IRG group ( $\mathrm{p}<0.05$ ). There was no significant difference between continuous and intermittent exercise groups in comparison of the difference values before and after exercise in all variables ( $\mathrm{p}>0.05$ ) (Table 1).

As a result of one-way ANOVA analysis performed between measurements; in the CRG group. WBC $\left(10^{3} /\right.$ $\mu \mathrm{L})\left(\mathrm{F}=72.394 . \mathrm{p}<0.001 . \eta_{\mathrm{p}}^{2}=0.792\right)$. LYMPH $\left(10^{3} /\right.$ $\mu \mathrm{L})\left(\mathrm{F}=160.062 . \mathrm{p}<0.001 . \eta_{\mathrm{p}}^{2}=0.894\right)$. NEUT $\left(10^{3} /\right.$ $\mu \mathrm{L})\left(\mathrm{F}=9.116 . \mathrm{p}<0.001 . \eta_{\mathrm{p}}{ }^{2}=0.324\right)$. MONO ( $10^{3} /$ $\mu \mathrm{L})\left(\mathrm{F}=23.017 . \mathrm{p}<0.001 . \eta_{\mathrm{p}}{ }^{2}=0.548\right)$ ve $\mathrm{EO}\left(10^{3} / \mu \mathrm{L}\right)$ ( $\mathrm{F}=13.353 \cdot \mathrm{p}<0.001 . \eta_{\mathrm{p}}^{2}=0.413$ ) variables were found to be statistically significant, while there were no statistically significant difference in BASO $\left(10^{3} / \mu \mathrm{L}\right)(\mathrm{F}=1.114$. $\mathrm{p}>0.358$. $\left.\eta_{\mathrm{p}}^{2}=0.055\right)$ variables. At the same time, Bonferroni post-hoc analysis was applied to determine which measurements the difference occurred. According to the scores of the WBC $\left(10^{3} / \mu \mathrm{L}\right)$ ve LYMPH $\left(10^{3} / \mu \mathrm{L}\right)$ baseline measurements at all weeks and 24 hours after exercise. again in the measurements taken 24 hours after the exercise. significantly lower scores were detected compared to the scores obtained in all weeks. Significantly lower scores were detected in the NEUT $\left(10^{3} / \mu \mathrm{L}\right)$ baseline measurement compared to the scores obtained in all weeks and 24 hours after exercise. Significantly lower scores were detected in the MONO $\left(10^{3} / \mu \mathrm{L}\right)$ baseline measurement compared to the scores obtained in all weeks. Significantly lower scores were detected in the EO $\left(10^{3} / \mu \mathrm{L}\right)$ baseline measurement compared to the scores obtained in all weeks. Again, in the measurement taken 24 hours after the exercise, significantly lower scores were found compared to the scores obtained in all weeks ( $\mathrm{p}<0.05$ ).

As a result of one-way ANOVA analysis performed between measurements; in the IRG group. WBC $\left(10^{3} /\right.$ $\mu \mathrm{L})\left(\mathrm{F}=50.526 . \mathrm{p}<0.001 . \eta_{\mathrm{p}}^{2}=0.727\right)$. LYMPH $\left(10^{3} /\right.$ $\mu \mathrm{L})\left(\mathrm{F}=102.349 . \mathrm{p}<0.001 . \eta_{\mathrm{p}}^{2}=0.843\right)$. NEUT $\left(10^{3} /\right.$ $\mu \mathrm{L})\left(\mathrm{F}=11.442 . \mathrm{p}<0.001 . \eta_{\mathrm{p}}^{2}=0.376\right)$. MONO $\left(10^{3} / \mu \mathrm{L}\right)$ ( $\mathrm{F}=$ 25.344. $\mathrm{p}<0.001 . \eta_{\mathrm{p}}^{2}=0.572$ ) variables were found to be


Figure 2. Graphical representation of $\mathrm{WBC}\left(10^{3} / \mu \mathrm{L}\right)$ measurement values within and between groups in different exercise protocols. Note: Between groups; $\mathrm{F}=1.666$. $\mathrm{p}>0.172 . \eta_{p}^{2}=0.042$. ( $\mathrm{p}>0.05$ ).


Figure 3. Graphical representation of LYMPH $\left(10^{3} / \mu \mathrm{L}\right)$ measurement values within and between groups in different exercise protocols. Note: Between groups; $\mathrm{F}=0.828 . \mathrm{p}>0.496 . \eta_{p}^{2}=0.021$. ( $\mathrm{p}>0.05$ ).


Figure 4. Graphical representation of NEUT $\left(10^{3} / \mu \mathrm{L}\right)$ measurement values within and between groups in different exercise protocols. Note: Between groups; $\mathrm{F}=1.697 . \mathrm{p}>0.159 . \eta_{p}^{2}=0.043$. ( $\mathrm{p}>0.05$ ).
statistically significant. while there was no statistically significant difference in BASO $\left(10^{3} / \mu \mathrm{L}\right)(\mathrm{F}=2.378 . \mathrm{p}>0.079$. $\left.\eta_{\mathrm{p}}^{2}=0.111\right)$ ve $\mathrm{EO}\left(10^{3} / \mu \mathrm{L}\right)\left(\mathrm{F}=2.394 . \mathrm{p}>0.081 . \eta_{\mathrm{p}}^{2}=0.112\right)$ variables. At the same time, Bonferroni post-hoc analysis was applied to determine which measurements the difference occurred. According to the scores of the WBC $\left(10^{3} / \mu \mathrm{L}\right)$ and LYMPH $\left(10^{3} / \mu \mathrm{L}\right)$ baseline measurements at all weeks and 24 hours after exercise, again in the measurements taken 24 hours after the exercise. sig-


Figure 6. Graphical representation of BASO $\left(10^{3} / \mu \mathrm{L}\right)$ measurement values within and between groups in different exercise protocols. Note: Between groups; $\mathrm{F}=1.723$. $\mathrm{p}>0.149 . \eta_{\mathrm{p}}{ }^{2}=0.043$. ( $\mathrm{p}>0.05$ ).


Figure 5. Graphical representation of $\mathrm{MONO}\left(10^{3} / \mathrm{LL}\right)$ measurement values within and between groups in different exercise protocols. Note: Between groups; $\mathrm{F}=4.057 . \mathrm{p}>0.429 . \eta_{p}{ }^{2}=0.017$ ( $\mathrm{p}>0.05$ ).


Figure 7. Graphical representation of $\mathrm{EO}\left(10^{3} / \mu \mathrm{L}\right)$ measurement values within and between groups in different exercise protocols. Note: Between groups; $\mathrm{F}=0.353$. $\mathrm{p}>0.556 . \eta_{p}^{2}=0.009$ ( $\mathrm{p}>0.05$ ).
nificantly lower scores were detected compared to the scores obtained in all weeks. Significantly lower scores were detected in the NEUT $\left(10^{3} / \mu \mathrm{L}\right)$ baseline measurement compared to the scores obtained in all weeks. A significant difference was found in the scores obtained in the 2 nd and 3rd weeks compared to the 1st week and
in the score value obtained after 24 hours compared to the 3rd week. Significantly lower scores were detected in the MONO $\left(10^{3} / \mu \mathrm{L}\right)$ baseline measurement compared to the scores obtained in all weeks. Again, in the measurement taken 24 hours after the exercise. significantly lower scores were found compared to the scores obtained in all weeks ( $\mathrm{p}<0.05$ ).

## Discussion

It is a controversial issue that continuous and intermittent running methods have a great impact on both aerobic capacity and complete blood count. With this study, we reviewed this controversial issue again. To compare these two separate running methods, we decided to have standard protocols. Because these running methods are a method that can be easily applied by athletes who are engaged in indoor sports (boxing, wrestling, taekwondo, etc.), we preferred it to be the standard protocol. For this reason, we included athletes interested in indoor sports as a branch, while creating a training limitation. We believe that this article is of great importance for athletes engaged in indoor sports. Apart from indoor branches, we believe that it is important for athletes who want to do endurance training in intense winter conditions.

Leukocytes go into action with exercise. This condition leads to circulating and tissue lymphocytes-mediated leukocytosis [Prestes et al. 2008]. The changes that occur depend on the intensity and duration of the exercise [Pedersen, Hoffman-Goetz 2000]. Considering the findings of the study, an increase in leukocyte and subset counts was observed in blood samples taken immediately after continuous and intermittent exercises. According to the data obtained; in the CRG group, WBC, lymphocyte, neutrophil, monocyte, eosinophil values and in the IRG group; WBC, lymphocyte, neutrophil, and monocyte values were found to be significantly different within the group between weeks ( $\mathrm{p}<0.05$ ). When the CRG and IRG groups were compared, there was no significant difference ( $\mathrm{p}>0.05$ ).

In our study, it was determined that continuous and intermittent intensive treadmill running training methods applied three days a week for four weeks were effective on body mass index, body weight, body fat percentage and $\mathrm{VO}_{2 \text { max }}$ and created a significant difference between pre and post tests. Body fat percentage decreased and weight loss occurs due to the burning of high amounts of calories with training [Stamford 1983]. In our study, the reduction in body fat percentage and weight loss is in parallel with this principle. It is also important for the applicability of these running methods that the athletes participating in the study are weight athletes and have to constantly control their weight. However, losses of more than $5 \%$ of body weight can reduce the working capacity up to $30 \%$, and it negatively affects
the performance of the athlete due to dehydration (fluid loss) and electrolyte loss [Dorfman 2011]. Also, training is beneficial as long as it forces the body to adapt to high levels of stress. If the load is not enough to create a change in the body, adaptation will not occur [Bompa 2003]. Therefore, these types of exercise models should be carried out in accordance with the purpose.

As the intensity of exercise increases, so does the increase in circulating levels of epinephrine and norepinephrine followed by mobilization of immune cells [Natale et al. 2003]. Several studies have reported that high-paced cardiorespiratory exercises cause an increase in circulating leukocyte counts of approximately $50 \%$ to 100\% [Jenkins et al. 2020; Patlar, 2010; Nieman, 1996; Suzuki et al. 1995]. Our findings show that there is a significant increase in leukocyte counts in both exercise groups between the resting state and just after the exercises. Significant increases in total WBC count after exercise indicate that exercise and stress are associated with both hemoconcentration and hormonal modifications [Morgan et al. 2004; Sodique et al. 2000]. It is known that the increase in the number of leukocytes in the circulation due to increased blood flow during exercise may result from demargination. Demargination reduces leukocyte accumulation in the blood endothelial wall due to stress hormones and thus increases the total leukocyte count [Sodique et al. 2000; Gleeson 2007].

Although the health benefits of exercise are well known, participation in aerobic exercise and endurance activities is not without risk. Due to the increase in the number of recreational athletes who do not have sports experience and participate in endurance activities at a much higher intensity than the international health organizations recommend, it has been of practical importance to investigate risk factors on health [Nikolaidis et al. 2020]. However, the circulating numbers and functional capacity of leukocytes can be reduced by repetitive, intense and prolonged bouts of exercise. This is probably due to increased stress hormone levels during exercise and less mature leukocytes, particularly neutrophils, entering the circulation from bone marrow. Both acute administrations of glucocorticosteroids [Moynihan et al. 1998] and exercise [Lancaster et al. 2004; Northoff et al. 1998] are known to temporarily inhibit interferon (IFN)-g production by T lymphocytes, and it has been suggested that immune cell function may be an important mechanism in exercise-induced depression [Northoff et al. 1998]. In the study, although there was a difference in leukocyte and subset blood values obtained from athletes after 4 weeks of exercise, the results obtained from the measurements show that there was an approximation with the values before the exercise program.

In studies examining the effects of exercise on WBC values in athletes; Hazar, Akyol [2019] stated that there was no difference in leukocyte values of runners and cross-country skiing, whose blood samples were taken
every one year. Toklu [2018] stated that there is no differentiation in the pre- and mid-season WBC values of the soccer players. Gencer et al. [2018] found no significant changes in leukocytes and subsets in blood samples taken from basketball players before and after a competition. Cakmakci [2009] stated that the leukocyte values of the elite taekwondo athletes preparing for the European and World Championships did not differ before and after the 4 -week camp. Yeh et al. [2006] reported that 14 male and 23 female athletes who exercised regularly for 12 weeks did not find a significant change in WBC levels in blood samples taken before and after 12 weeks. Banfi et al. [2006] reported that the WBC levels of 19 male rugby players before and after the camp were similar, and there was no differentiation. Ergun et al. [2006] reported that no significant increase in leukocyte levels could be detected in the blood samples taken at the end of 2 weeks in middle-aged men who did regular aerobic exercise for 2 weeks. The fact that 4 weeks of chronic exercise did not cause a significant change in leukocyte levels in the study is also supported by the above studies.

Following an acute exercise period changes in circulating leukocyte counts and function normally return to pre-exercise values within 3-24 hours. Cross-sectional studies comparing leukocyte counts and functions in blood samples taken from athletes 24 hours after their last training session with inactive individuals generally reported little difference. Therefore, in a true resting state, the immune function appears to be generally similar in athletes compared to sedentary individuals. Although circulating leukocyte count and neutrophil function are generally lower, most athletes have clinically normal levels at rest compared to sedentary people [Gleeson 2006]. There is a weak suggestion of slightly elevated natural killer cell count and cytolytic effect in trained individuals. However, these effects are small and unlikely to have any clinical significance [Gleeson 2006].

It is known that regular exercise positively affects general health, cardiovascular functions, and metabolic systems, $\mathrm{VO}_{2 \text { max }}$ which includes definitions such as the amount of $\mathrm{O}_{2}$ that tissues and muscles can use in one minute has been accepted by the majority of researchers as the best indicator of an organism's aerobic capacity as well as an athlete's physical capacity [Ranković et al. 2010]. With its definition of maximal oxygen usage capacity in the field, endurance creates different needs according to the branches. The durability feature varies according to the nature of the branch and the dominance of the energy system used. A strong specific strength is due to a solid overall strength. Boxing, wrestling, taekwondo, judo etc. $\mathrm{VO}_{2 \text { max }}$ capacity plays an important role in the development of the recovery capacity, delaying the fatigue between sessions, sets, periods, rounds and cycles, and reducing the technical and tactical errors in eliminating the fatigue [Karatosun 2010]. For this reason, it
is very important to improve the aerobic capacity of the athletes (boxer, wrestler, and taekwondo) in our working group through continuous and intermittent running.

Although there is no difference between the groups, it is very clear that both training methods improve the $\mathrm{VO}_{2 \text { max }}$ level of the athletes. Many studies have stated that running methods improve the aerobic capacity of athletes [Helgerud et al. 2007; Gunay 1998]. However, these running, which are generally carried out in outdoor environments. contain limiting factors for the athletes in case of long and cold harsh winters where the terrestrial climate dominates the athletes' lives. For this reason, treadmill running methods can be considered as alternative training methods in terms of improving the aerobic capacities of these athletes and providing a controlled speed increase while doing this.

## Conclusion

Our study reveals that leukocyte and subset values acutely increase after both continuous and intermittent exercises in elite athletes but when examined from a chronic point of view, blood values after the exercise program are similar to resting states. It is thought that continuous and intermittent aerobic running exercises will increase the performance and endurance of athletes.

## Financial Resource

During this study, any pharmaceutical company that has a direct connection with the subject of the research, a company that provides and/or produces medical tools, equipment and materials, or any commercial company, during the evaluation process of the study, financial and / or no moral support was received.

## Conflict of Interest

Regarding this study, the authors and/or their family members do not have a scientific and medical committee membership or relationship with their members, consultancy, expertise, working status in any company, shareholding or similar situations that may have a potential conflict of interest.

## Ethical considerations

Informed consent form was obtained from the athletes included in the study. This study was approved by the Ataturk University Faculty of Sport Sciences Ethics Committee (with the decision dated 17/02/2021 and numbered 70400699-050.02.04-E.2100048684) and was
carried out in Ataturk University Athlete Performance Measurement Center in accordance with the current version of the Helsinki Declaration.

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## Czy ciągłe i przerywane ćwiczenia biegowe wpływają na liczbę leukocytów? Porównanie efektów ostrych i przewlekłych

Słowa kluczowe: liczba leukocytów, wysiłek ciągły, wysiłek przerywany, odporność

## Streszczenie

Tło. Powszechnie przyjmuje się, że ćwiczenia fizyczne mogą powodować zmiany w układzie odpornościowym. Gwałtowne okresy wysiłku fizycznego mogą zmienić liczbę i funkcję leukocytów, ale stopień wzrostu białych krwinek zależy od intensywności i czasu trwania ćwiczeń.
Cel. Celem było zbadanie gwałtownego i przewlekłego wpływu liczby i podzbiorów białych krwinek w krwiobiegu na wykonywanie ciągłych i przerywanych ćwiczeń biegowych, a także na skład ciała.
Materiał i metody. W kategorii osób dorosłych aktywnie uprawiających sporty siłowe (odpowiednio wg wieku, wieku sportowego i wzrostu) uczestniczyło łącznie 40 czołowych sportowców o CRG: 26,3 $\pm 2$,67 lat, $9,1 \pm 1,08$ lat i $177,3 \pm 5,06$ cm oraz IRG: $25,6 \pm 2,79$ lat, $8,2 \pm 2,66$ lat i $179,9 \pm 6,51 \mathrm{~cm}$. W celu określenia różnic pomiędzy punktami pomiarowymi dla wartości leukocytów i podzbiorów zastosowano jednokierunkową analizę wariancji (ANOVA) z powtarzanymi pomiarami. Porównanie efektu grup zostało dokonane przez powtarzane pomiary z dwukierunkową analizą ANOVA (grupa x czas).
Wyniki. Zgodnie z uzyskanymi danymi; w grupie CRG stwierdzono, że wartości WBC, limfocytów, neutrofili, monocytów, eozynofili, a w grupie IRG; wartości WBC, limfocytów, neutrofili i monocytów różniły się istotnie w obrębie grupy pomiędzy tygodniami. ( $\mathrm{p}<0,05$ ). W przypadku porównania grup CRG i IRG nie stwierdzono istotnej różnicy pomiędzy grupami ( $\mathrm{p}>0.05$ ).
Wnioski. Niniejsze badania wykazały, że wartości leukocytów i podzbiorów krwi gwałtownie wzrastają zarówno po ćwiczeniach ciągłych, jak i przerywanych u elitarnych sportowców, ale badane z przewlekłego punktu widzenia, wartości krwi po programie ćwiczeń są podobne do tych w stanie spoczynku. Poza tym uważa się, że ciągłe i przerywane ćwiczenia aerobowe mogą zwiększyć wydajność i wytrzymałość sportowców.

