

Effects of high-intensity interval training on the number of white blood cells, Myeloperoxidase, lactoferrin, elastase, and lactate in young college men

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How to Cite: Ghanbari-niaki, A; Soltani-zamani, A; Mirzaei, M* & Tayebi, S-M (2027). Effects of high-intensity interval training on the number of white blood cells, Myeloperoxidase, lactoferrin, elastase, and lactate in young college men, *New Approaches in Exercise Physiology*, 9(18), 106-134.
DOI: 10.22054/nass.2026.95242.1251.

Abstract

Purpose: This study aimed to investigate the effects of high-intensity interval training (HIIT) with varying durations on white blood cell count and neutrophil function markers (myeloperoxidase, lactoferrin, and elastase) in young men.

Method: Thirty-six healthy, untrained males were randomly divided into four groups: control (n = 9), 15-second (n = 9), 30-second (n = 9), and 45-second (n = 9) exercise protocols. The training program consisted of six sessions over two weeks, with 1–2 days of rest between each session. Each session consisted of four to seven "all-out" Wingate tests (15, 30, or 45 seconds) on a cycle ergometer, separated by 4-minute recovery periods. Blood samples were collected before and 48 hours after the final session to measure the mentioned variables. Data were analyzed using ANCOVA and paired t-tests at a significance level of $p < 0.05$.

Results: Results showed that HIIT had no significant effect on total white blood cell or neutrophil counts compared with the control group. However, neutrophil function markers responded differently to training duration: myeloperoxidase and elastase decreased significantly in all training groups, while lactoferrin decreased, especially in the 30- and 45-second protocols.

Conclusion: These findings suggest that longer high-intensity exercise durations may impair neutrophil function by altering the secretion of related enzymes.

Keywords: High-intensity interval training, Myeloperoxidase, Lactoferrin, Elastase.

Introduction

The immune system is a key defensive mechanism that maintains human health. It identifies and eliminates external pathogens while simultaneously monitoring and preserving internal homeostasis. This complex system consists of innate and adaptive immunity, which collectively form the body's first and second lines of defense against infections and diseases (Pradeu et al., 2024; Shi et al., 2025). Modern lifestyle changes, including prolonged sedentary behavior, irregular sleep patterns, and unhealthy diets, have significantly impacted the immune system, leading to immune dysfunction and an increased risk of diseases (Shi et al., 2025). Exercise, as an effective regulatory intervention, has been shown to enhance immune cell activity and restore immune balance, thereby strengthening the body's resistance to diseases (Campbell & Turner, 2018). Consequently, exercise interventions have been proposed as a crucial strategy to address the challenges of modern lifestyles, enhance immune health, and prevent diseases (Boeira et al., 2023).

The immunomodulatory effects of exercise depend on the intensity and volume of training (Simpson et al., 2020). High-Intensity Interval Training (HIIT) is an aerobic exercise method that typically consists of short bouts of high-intensity effort alternated with periods of rest or low-intensity activity. This approach aims to enable a greater total volume of high-intensity work compared to continuous high-intensity exercise (Lee et al., 2023; Seyed Morteza et al., 2024). The beneficial effects of HIIT on human health are well established (Scoubeau et al., 2022). HIIT is a common component of training programs for elite

athletes. Although its potential use to promote health in less-trained individuals is not new, it has gained increasing attention in recent years (Coates et al., 2023). Studies have demonstrated the effectiveness of HIIT in increasing neutrophil count (Lee et al., 2023), improving their function (Fisher et al., 2011; Slusher et al., 2018), activating innate immune responses (Bartlett et al., 2020), and enhancing defense against pathogens (Vidal-Seguel et al., 2023). Moreover, the beneficial effects of HIIT on modulating monocyte immune responses (Durrer et al., 2017; Gabriel et al., 2013) and the balance of monocyte subpopulations (de Matos et al., 2019; Oh et al., 2017) have also been reported. However, despite increasing evidence regarding the effects of HIIT on immune responses, it remains unclear whether the duration of individual high-intensity exercise bouts influences neutrophil function. In particular, limited evidence is available on whether different HIIT bout durations produce different responses in neutrophil-derived markers, including myeloperoxidase, lactoferrin, and elastase.

In addition, the existence of various definitions and forms of HIIT has created ambiguity in reaching a clear and consistent understanding of its overall effects. On the other hand, recent investigations indicate that performing 30-second bouts of maximal effort can fully engage the anaerobic capacity while simultaneously shifting a substantial portion of energy production toward the very short-term aerobic system. Therefore, employing short-term high-intensity interval activities of different durations (15, 30, and 45 seconds) with equal work intensity may help identify which duration is most effective. Based on this perspective, the present study addresses the importance and necessity

of conducting such research to contribute to the optimization of exercise protocols. Accordingly, the primary aim of this study was to examine the effects of High-Intensity Interval Training with different durations on immune and inflammatory indices in young male university students.

Methods

Participants:

The statistical population of this study consisted of male students residing in Shahid Navab Safavi Dormitory at the University of Mazandaran. The study samples were selected from volunteers who responded to the recruitment announcement and were informed about the study conditions and details. Among 58 individuals who expressed interest in participating, 36 were deemed eligible (non-smokers, non-drug and non-alcohol users, non-athletes with no history of regular physical activity for at least six months, and free from any renal, hepatic, cardiovascular diseases, diabetes, or any physical injury or disorder). Participants were matched based on height, weight, and BMI and were randomly assigned to one of four groups: control ($n = 9$), 15-second HIIT ($n = 9$), 30-second HIIT ($n = 9$), and 45-second HIIT ($n = 9$). A two-week dietary recall was obtained from all participants.

Before the preliminary testing, participants received verbal explanations emphasizing the importance of consistent attendance, maintaining their usual daily activities, and adhering to their regular diet by consuming only meals provided by the University of Mazandaran dining hall. To

ensure their health status and understanding of the study's significance, participants completed a health questionnaire and were informed about the necessity and importance of the research. After obtaining full consent and completing the medical questionnaire, written informed consent was collected from all participants. All experimental procedures were conducted in accordance with the latest revision of the Declaration of Helsinki (Association, 2025).

Training Protocol:

The training protocol was conducted over a period of two weeks, with three sessions per week. In the 15-second group, participants performed four 15-second bouts of maximal cycling on a Wingate ergometer with a resistance load of $0.6 \text{ Nm} \cdot \text{kg}^{-1}$ body mass during the first session, interspersed with 4 minutes of rest between bouts. In the second session, participants completed five 15-second bouts under the same conditions, each separated by 4 minutes of rest. The third and fourth sessions consisted of six 15-second bouts, each with a 4-minute rest interval. The fifth session included seven 15-second bouts, while the sixth session involved four 15-second bouts, both following the same resistance and rest structure.

The 30-second and 45-second groups followed the same protocol, except that the duration of each bout was 30 seconds and 45 seconds, respectively. The recovery time between bouts (4 minutes) and the resistance load ($0.6 \text{ Nm} \cdot \text{kg}^{-1}$ body mass) were kept constant throughout all training sessions (Burgomaster et al., 2008) (Figure 1). The control

group did not perform any exercise training and only continued their normal daily activities.

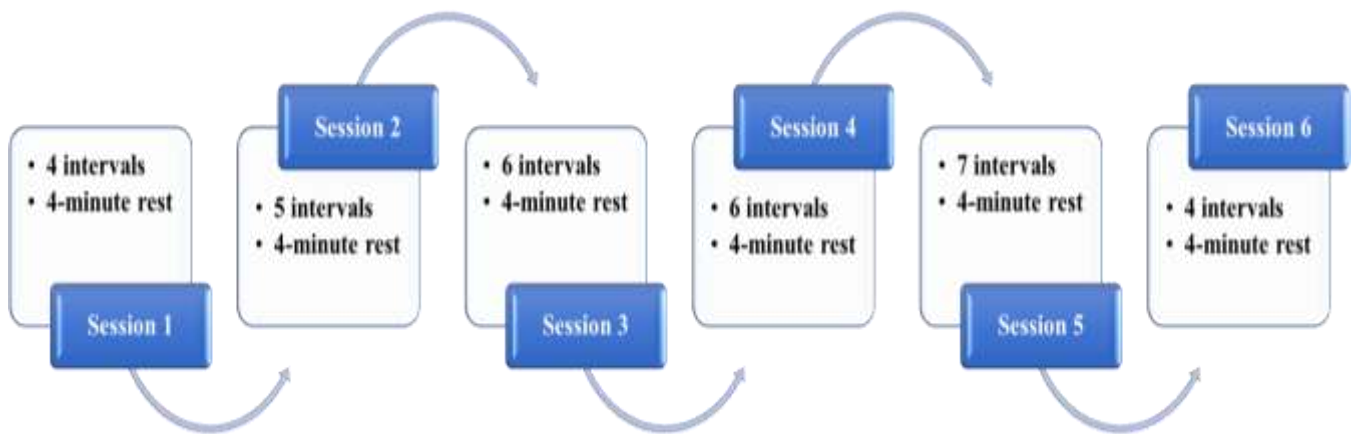


Figure 1. Schematic representation of the training protocol

Measurement Methods:

Blood sampling was conducted in two stages—48 hours before the start of the training program and 48 hours after the final training session. Participants were instructed to fast for 12 hours prior to blood collection. To ensure standardization, participants were required to refrain from using any medications or supplements during the study period, maintain a consistent diet for at least two days prior to testing, avoid any physical activity other than the prescribed training sessions during the study, abstain from long walks for at least 72 hours before testing, and avoid consuming coffee, strong tea, bananas, cereals, and heavy or fatty foods for at least 24 hours before blood collection.

To control for circadian rhythm variations, all blood samples were collected at 8:00 a.m. in both pre- and post-training stages. Approximately 10 cc of blood was drawn from the antecubital vein while participants were seated, calm, and fasting for 12 hours. The blood samples were immediately centrifuged at 3000 rpm for 10 minutes, and the separated serum was stored at -80°C until analysis. The average room temperature at the time of blood sampling was recorded as 21°C for both stages.

White blood cells are essential and fundamental indicators of the immune response (Cha et al., 2025). Neutrophils, a type of white blood cell, are known as the first immune cells recruited to inflammatory sites (Pham, 2006). Myeloperoxidase and elastase are enzymes secreted by neutrophils (Langhorst et al., 2008; Maruyama et al., 2004), while lactoferrin is an important glycoprotein released from neutrophils (Mayeur et al., 2016). The levels of myeloperoxidase, elastase, and lactoferrin were measured using 96-well ELISA kits (ZellBio GmbH, Germany), and lactate concentration was determined using a standard chemical method.

Statistical Methods:

Descriptive statistics were used to categorize and determine dispersion indices. The Kolmogorov–Smirnov test confirmed that the data followed a normal distribution. To compare pre-test and post-test changes within each group, a paired t-test was applied. Analysis of covariance (ANCOVA) followed by the Bonferroni post hoc test was used to compare differences between groups. Pearson's correlation coefficient

was employed to determine relationships between variables. All data were expressed as mean $\pm$ standard deviation (SD). Statistical analyses were performed using SPSS software, version 20. A significance level of $P < 0.05$ was considered statistically significant.

Results

Statistical analysis related to total white blood cell count revealed no significant differences between groups (neither group effect nor training effect), with p-values of $P = 0.869$ and $F = 0.238$ for the group effect, and $P = 0.505$ and $F = 0.454$ for the time effect (Figure 2).

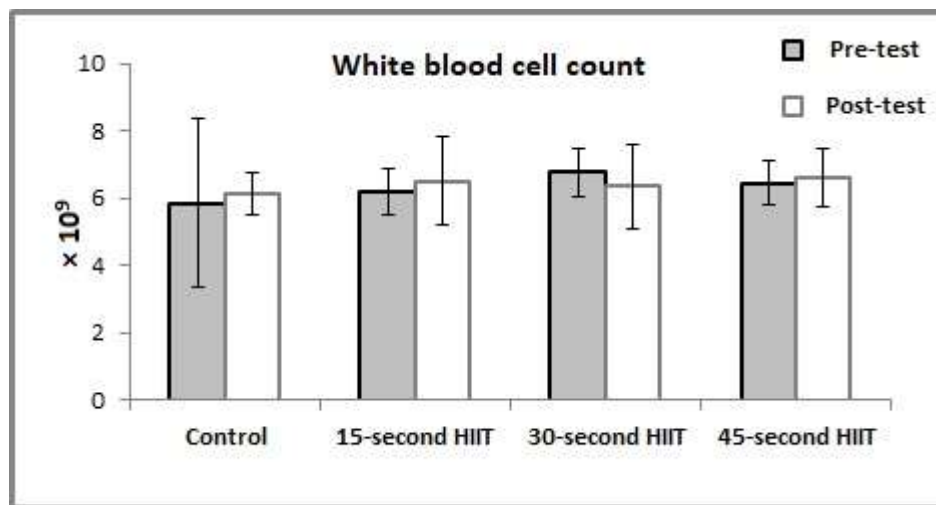


Figure 2. Mean $\pm$ standard deviation of white blood cell counts in the study groups.

The results also indicated that training with different durations had no significant effect on neutrophil count (Figure 3), as well as on eosinophils and monocytes ($P > 0.05$). However, there were

significant differences between groups in lymphocyte count ($P = 0.007$) and basophil count ($P = 0.034$).

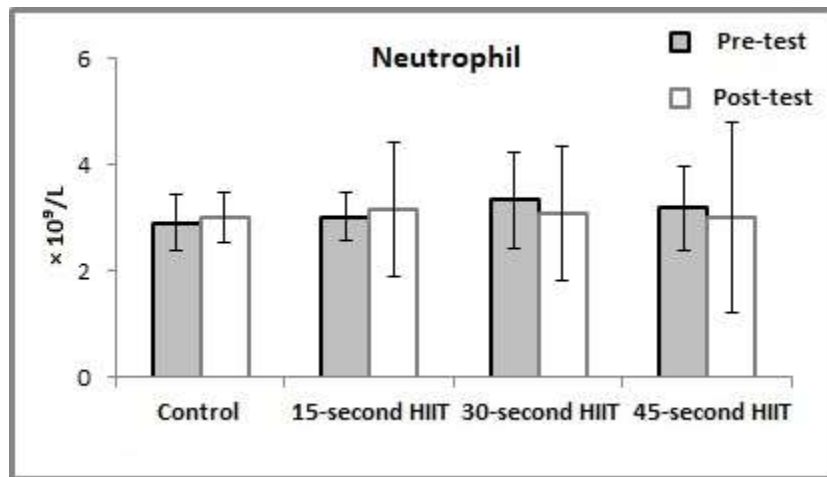


Figure 3. Mean $\pm$ standard deviation of neutrophil counts in the study groups.

The statistical analysis of myeloperoxidase enzyme levels indicated a significant between-group difference (both group and training effects). This significance was observed for the group effect ($P = 0.004$, $F = 5.332$) and the training effect ($P = 0.001$, $F = 13.224$) (Figure 4). Despite a significant increase in pre- and post-test values within the control group, plasma myeloperoxidase levels in the 15-second training group did not reach statistical significance. Although slight variations in the control group differed in direction compared to the training groups, statistical analysis revealed no significant differences among the training groups themselves.

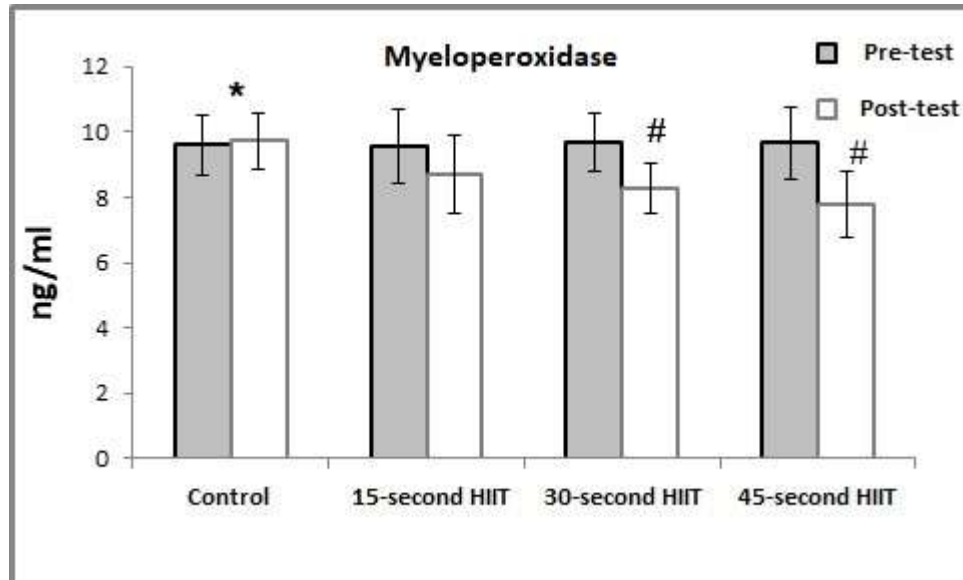


Figure 4. Mean $\pm$ standard deviation of myeloperoxidase levels in the training and control groups.

* Indicates a significant difference compared with the 15-, 30-, and 45-second training groups (between-group difference).

Indicates a significant difference compared with pre-test values.

The statistical analysis of elastase enzyme levels revealed a significant between-group difference (both group and training effects). This significance was observed for the group effect ($P < 0.001$, $F = 13.278$) and the training effect ($P < 0.001$, $F = 23.665$) (Figure 5). Despite a significant increase in plasma elastase levels from pre- to post-test in the control group, those in the 15-second training group did not reach statistical significance. Although minor variations in the control group were opposite in direction to those in the training groups, the statistical analysis revealed significant differences among the training groups.

Significant differences in elastase levels were observed between the control group and the 15-second training group ($P = 0.013$), the 30-second training group ($P < 0.001$), and the 45-second training group ($P < 0.001$). Furthermore, significant differences were found between the 15-second training group and the 30-second ($P = 0.02$) and 45-second ($P = 0.005$) training groups. However, no significant difference was observed between the 30-second and 45-second training groups ($P = 0.582$) (Figure 5).

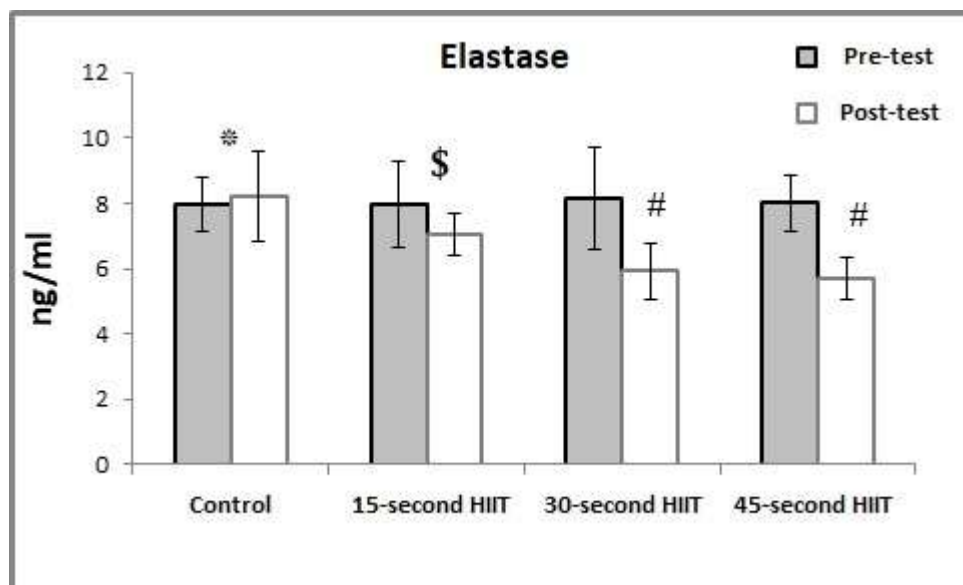


Figure 5. Mean $\pm$ standard deviation of elastase levels in the training and control groups.

* Indicates a significant difference compared with the 15-, 30-, and 45-second training groups (between-group difference).

Indicates a significant difference compared with pre-test values.

\$ Indicates a significant difference compared with the 30- and 45-second training groups (between-group difference).

The statistical analysis of plasma lactoferrin concentration indicated a significant between-group difference (both group and training effects). This significance was observed for the group effect ($P < 0.02$, $F = 3.781$) and the training effect ($P = 0.007$, $F = 8.437$) (Figure 6). Despite a significant increase in plasma lactoferrin concentration from pre- to post-test in the control group, the 15-second training group did not reach statistical significance. Although slight variations in the control group were opposite in direction compared with the training groups, the statistical analysis revealed significant differences among the training groups.

Lactoferrin concentration in the control group was significantly higher compared to the 30-second training group ($P = 0.041$) and the 45-second training group ($P = 0.002$). However, there was no significant difference between the control and 15-second training groups ($P = 0.092$). Furthermore, no significant differences were observed among any of the training groups themselves ($P > 0.05$) (Figure 6).

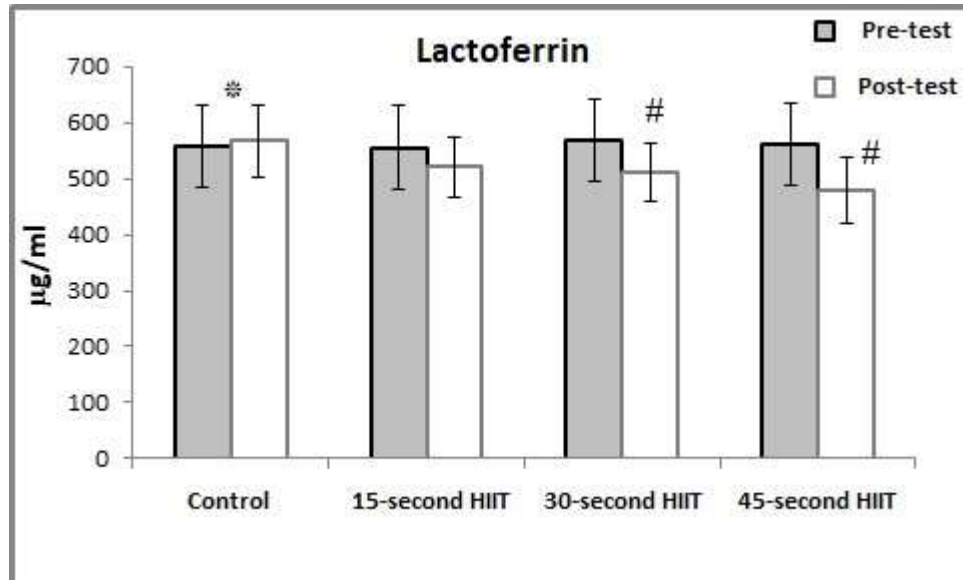


Figure 6. Mean $\pm$ standard deviation of lactoferrin levels in the training and control groups.

* Indicates a significant difference compared with the 15-, 30-, and 45-second training groups (between-group difference).

Indicates a significant difference compared with pre-test values.

\$ Indicates a significant difference compared with the 30- and 45-second training groups (between-group difference).

For plasma lactate, a significant group effect ($F = 19.439$, $p < 0.001$) and a significant time effect ($F = 30.450$, $p < 0.001$) were observed. Plasma lactate concentrations were significantly higher in the control group than in the 15-, 30-, and 45-s HIIT groups. The 15-s group also had significantly higher lactate concentrations than the 45-s group ($p = 0.004$). No significant differences were observed between the 15- and 30-s groups ($p = 0.999$) or between the 30- and 45-s groups ($p = 0.051$).

Blood samples were collected 48 h after the final training session; therefore, these results represent resting plasma lactate concentrations measured after the intervention. (Figure 7).

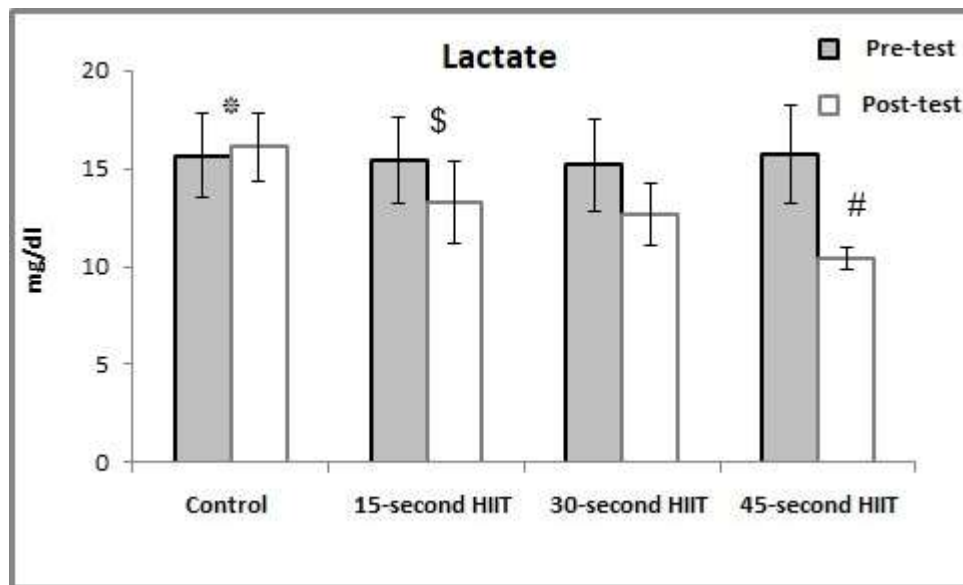


Figure 7. Mean $\pm$ standard deviation of lactate levels in the training and control groups.

* Indicates a significant difference compared with the 15-, 30-, and 45-second training groups (between-group difference).

Indicates a significant difference compared with pre-test values.

\$ Indicates a significant difference compared with the 30- and 45-second training groups (between-group difference).

Additionally, regarding plasma volume, the obtained P-value was 0.769, indicating that six sessions of high-intensity interval training with varying durations had no significant effect on plasma volume levels.

Furthermore, the Bonferroni post hoc test showed no significant differences between groups ($P > 0.05$). The paired t-test used to assess within-group changes revealed no significant differences between pre-test and post-test values in any of the groups ($P > 0.05$) (Figure 8).

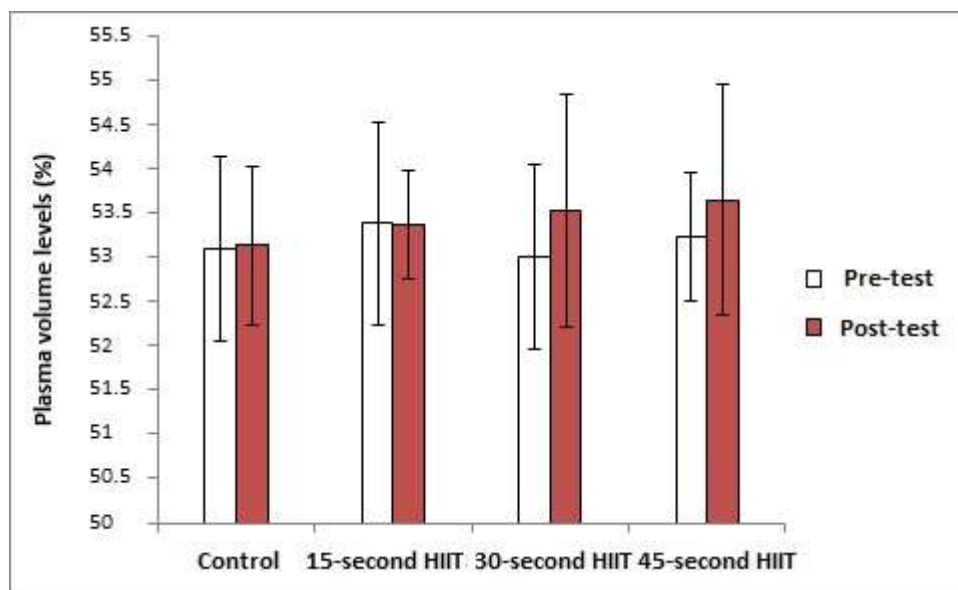


Figure 8. Plasma volume values in the experimental groups.

Discussion

The present study aimed to investigate the effects of different durations of short-term high-intensity interval training (six sessions over two weeks) on total leukocyte count, neutrophils, and neutrophil function markers. The results showed that six sessions of intermittent high-intensity training did not induce leukocytosis, and no significant

differences were observed between groups after training. However, changes were observed in leukocyte composition, particularly in lymphocytes, such that lymphocyte counts decreased in the 30-second training group but increased in the 15-second and 45-second training groups. Additionally, basophil counts increased in the 15-second training group. Among the training groups, a significant difference in lymphocyte count was found only between the 30-second and 45-second groups. In contrast, no significant changes were observed in other leukocyte subtypes compared with the pre-test.

The present findings are consistent with the study by Child et al. (Child et al., 2013), who reported that two weeks of high-intensity interval training had no significant effect on total white blood cell, monocyte, or neutrophil counts. However, some discrepancies were observed in the results for basophils and lymphocytes. The trafficking of white blood cells is dependent on both exercise intensity and duration. Exercise induces changes in the neuroendocrine-immune axis, increasing circulating metabolites that, in turn, affect immune cell function (Risøy et al., 2003). Higher exercise intensity is associated with increased secretion of epinephrine and norepinephrine, which stimulate the mobilization of immune cells. These hormones act through beta-adrenergic receptors to facilitate the redistribution of monocytes and lymphocytes (Neves et al., 2015). The elevation of these cells is typically transient, and high-intensity interval training does not exert a lasting effect on circulating leukocyte counts (Robson-Ansley et al., 2007).

In the present study, the lack of change in total leukocyte counts may be attributed to the transient effect of each training session, as values

returned to baseline after the intervention period. However, exercise may still influence specific leukocyte subtypes, such as memory or immature cells. The significant reduction in lymphocyte counts in the 15-second and 45-second training groups is likely due to exercise-induced apoptosis (Tuan et al., 2008). During high-intensity activity, decreased glutamine availability in immune tissues, such as the spleen and thymus, may also impair leukocyte function (Pithon-Curi et al., 2003). Overall, two weeks of high-intensity interval training led to increased lymphocyte apoptosis, resulting in a reduction in their number.

Among leukocyte subtypes, neutrophils are of particular interest due to their role in producing reactive oxygen species, inflammatory mediators, and activating the myeloperoxidase pathway (Cooper et al., 2007; Klebanoff, 2005). Neutrophils infiltrate damaged muscle tissue, and inhibiting their production of reactive oxygen species can help reduce tissue injury (Toumi et al., 2006). High-intensity exercise activates neutrophils and increases lactoferrin levels, which serve as a marker of this activation (Toumi et al., 2006). The present study demonstrated that 30-second and 45-second training sessions led to a reduction in lactoferrin concentration compared with the control group, consistent with the findings of Tsai et al. (Tsai et al., 2011).

In addition to lactoferrin, elastase and myeloperoxidase are also indicators of neutrophil function that typically increase in response to high-intensity activity. In this study, both myeloperoxidase and elastase significantly decreased in the training groups, likely due to the higher training volume in the 30-second and 45-second groups. Previous studies have suggested that changes in these enzymes may be associated with

alterations in neutrophil degranulation or the recruitment of neutrophils with different functional characteristics (Robson-Ansley et al., 2007). However, these mechanisms were not directly assessed in the present study and therefore remain speculative.

Neutrophil degranulation induced by high-intensity exercise typically leads to a reduction of intracellular proteins and an increase in their plasma levels. However, the mechanisms underlying the observed decrease in plasma concentrations of these proteins remain unclear. Repeated neutrophil stimulation and subsequent changes in responsiveness have been proposed as possible explanations in previous research (Prasad et al., 1991). Additionally, the recruitment of immature neutrophils with lower phagocytic capacity may occur (Yang & Hill, 1991). Therefore, the reductions in lactoferrin, myeloperoxidase, and elastase observed in the 30-second and 45-second training groups may reflect alterations in neutrophil-derived mediator release following repeated high-intensity exercise. However, the specific mechanisms responsible for these changes cannot be determined from the present data and require further investigation using direct measures of neutrophil activation, degranulation, and functional responsiveness.

Plasma lactate concentrations were lower in the HIIT groups than in the control group 48 h after the final training session. However, because blood samples were collected 48 h after exercise, these findings reflect resting plasma lactate concentrations rather than the acute lactate response to HIIT. Therefore, the lower lactate concentrations in the training groups should be interpreted cautiously and may reflect adaptations to repeated HIIT rather than an acute exercise effect.

Lactate accumulation is associated with decreased pH and inhibition of calcium release from the sarcoplasmic reticulum (Pilegaard et al., 1999). High-intensity exercise enhances the capacity for lactate and hydrogen ion (H^+) uptake by increasing the expression of monocarboxylate transporters (MCT1 and MCT4) (Pilegaard et al., 1999). It has been reported that high-intensity interval training reduces lactate release and arteriovenous lactate differences in exercised muscles (Juel et al., 2004). Additionally, through increased MCT1 and MCT4 content, it improves lactate/ H^+ transport capacity (Pilegaard et al., 1999; Seyedi et al., 2024). These transporters facilitate lactate exchange between glycolytic and oxidative fibers, enhancing its mitochondrial utilization (Brooks, 1986). Increased MCT1 expression is associated with elevated citrate synthase activity and mitochondrial mass (Dubouchaud et al., 2000). Short-term high-intensity interval training enhances muscle oxidative capacity, increases resting glycogen and GLUT4 content, and promotes fat oxidation and PGC-1 α expression (Burgomaster et al., 2008; Burgomaster et al., 2005). Therefore, the observed reduction in lactate in this study is likely due to increased oxidative capacity, glycogen sparing, and improved lactate transport and oxidation, particularly in the 45-second training model.

Conclusions

The results of the present study indicated that six sessions of intermittent high-intensity training, with varying durations over two weeks, had no effect on total white blood cell count but did influence leukocyte composition. These training sessions, however, were

associated with changes in circulating biomarkers related to neutrophil activity. Although neutrophil count was not altered, high-intensity interval training of different durations was associated with changes in circulating neutrophil-related biomarkers. Specifically, compared with the 15-second training group and the control group, the 30-second and 45-second training groups showed decreased levels of elastase, lactoferrin, and myeloperoxidase, which are biomarkers associated with neutrophil activity. These findings suggest that six sessions of high-intensity interval training with longer durations (30 and 45 seconds) were associated with greater changes in circulating biomarkers related to neutrophil activity. However, direct neutrophil functional capacity and susceptibility to infection were not assessed in the present study. Furthermore, the results showed that although high-intensity interval training of various durations reduced resting lactate concentrations compared with the control group, only the longer-duration (45-second) training produced a significant reduction in lactate relative to pre-test values. Overall, the findings suggest that exercise duration during high-intensity activity is a critical factor influencing neutrophil-derived mediators, and longer durations at high intensity are associated with greater impairment of neutrophil function as reflected by these secreted markers.

Conflicts of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Informed consent

Informed consent has been obtained from all individuals included in this study.

Institutional Review Board Statement:

Not applicable.

Informed Consent Statement:

Not applicable.

Acknowledgments:

The authors are especially grateful to the participants in this study.

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How to Cite: Ghanbari-niaki, A; Soltani-zamani, A; Mirzaei, M* & Tayebi, S-M (2027). Effects of high-intensity interval training on the number of white blood cells, Myeloperoxidase, lactoferrin, elastase, and lactate in young college men, *New Approaches in Exercise Physiology*, 9(18), 106-134.

DOI: 10.22054/nass.2026.95242.1251.



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