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Research Article

Effect of a session of repeated sprint training (RST) on serum adropin levels, inflammatory, and coagulation factors in healthy young men

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Abstract

Intense and high-speed intermittent training is recognized as an effective method for achieving rapid and extensive physiological and functional adaptations. However, it is important to consider that a single session of such exercises can exert significant physiological stress, potentially leading to adverse effects. Therefore, this study investigated the effects of a repeated sprint training (RSA) session on serum adropin, inflammatory, and blood coagulation factors. Ten healthy young men (age: 23.60 ± 2.01 years, weight: 68.60 ± 7.21 kg, body mass index: 22.00 ± 2.10 kg/m2) participated in this study. Blood samples were collected before, immediately after, and 20 minutes following the exercise protocol for measuring of adropin protein, fibrinogen, CRP, WBC, RBC, Hgb, and Hct levels. The exercise protocol consisted of 20 repetitions of different running speeds in a distance of 20 meters, with a 20-second rest period after each repetition (20*20*20). A significant increase in adropin, fibrinogen, WBCs, RBC, Hgb and, Hct levels was observed immediately after the exercise ($p < 0.05$). But only Adropin levels remained significantly higher than before the exercise after 20 minutes following the exercise. Therefore, even after exercise, adropin levels can remain elevated, allowing for the continuation of its positive effects. However, it is important to note that the increase in fibrinogen levels may require further investigation, particularly in relation to the use of anticoagulant agents. Therefore, when considering the implementation of these intense activities for inactive individuals, it is crucial to exercise caution and carefully assess the potential risks involved.

Key Words: **Adropin, Intensive exercise training, Inflammation, Blood coagulation**

Introduction

High-intensity interval training (HIIT) and sprint interval training (SIT), also known as repeated sprint activity (RSA), have gained popularity due to their ability to enhance cardiovascular fitness, regulate metabolism, and significantly impact various health indicators (Bogdanis et al., 2022; Laursen & Jenkins, 2002; Liang et al., 2024). Repeated sprint activity (RSA) refers to a training method that involves short-duration, maximal effort sprints lasting less than 10 seconds, followed by brief recovery periods of less than 60 seconds (Bishop et al., 2011). This type of exercise training holds significant importance for athletes due to its similarity in metabolic and physiological demands to many team and individual sports. It elicits adaptations such as reduced muscle PH, depletion of creatine phosphate and ATP and addresses the requirements of both the glycolytic and oxidative energy systems (Bishop et al., 2011; Iaia et al., 2017; Impellizzeri et al., 2007). Research has shown that RSA can be effective for various populations in achieving diverse health benefits and improving cardiorespiratory factors (Liang et al., 2024; Martin et al., 2015). Furthermore, these types of exercises require significantly less time (60 to 90% less) than traditional continuous exercises, making them time-efficient and appealing to the different population (Gibala & Little, 2020; Liang et al., 2024). It appears that the intense of this type of exercise may stimulate the secretion of various protective substances in the body, including adropin (Sato et al., 2017). Adropin is a peptide hormone secreted by various tissues, including the liver, brain, heart, and endothelial cells. It seems that adropin has multiple functions, including of regulation of homeostasis, angiogenesis and improving endothelial function. It has been proposed that adropin exert its effects through binding to GPR19, modulating VEGFR2 (vascular endothelial growth factor receptor-2) activity, activating the PI3K-Akt and ERK1/2 pathways, and enhancing endothelial nitric oxide synthase (eNOS) bioactivity (Ali et al., 2022). Researches have indicated that adropin exhibits a negative correlation with inflammatory factors, blood pressure, and oxidative stress. Also, it has gained recognition as a protective diagnostic

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biomarker for cardiovascular diseases (Li et al., 2016; Zhang et al., 2020; Zheng et al., 2019). Therefore, considering the protective role of adropin, increasing its levels is of great importance. In this regard, some studies have reported significant increases in adropin immediately after high-intensity interval training, while others have found no change or a decrease depending on the exercise protocol used and the physiological condition of participants (Azamian Jazi et al., 2022; Ozbay et al., 2020). The variability in adropin levels post-exercise across different studies can indeed be attributed to several methodological differences. Factors such as the intensity and duration of the exercise, the specific population studied (e.g., trained vs. untrained individuals), and the timing of blood sample collection can all influence serum adropin levels. On the other hand, the high intensity of this type of exercise, especially when prescribed to sedentary individuals in the general population, may elicit negative responses in certain body systems, including the inflammatory (Meckel et al., 2009) and coagulation systems (Ali & Hanachi, 2011). Therefore, caution should be exercised when prescribing such activities to inactive individuals (Zwetsloot et al., 2014). For instance, Bizheh et al. observed an increase in C-reactive protein (CRP) levels in sedentary elderly men following a circuit training activity involving ten movements performed at 35% of their maximum strength immediately after the activity (Bizheh & Jaafari, 2011).

Furthermore, Costello et al. observed an increase in CRP, a pro-inflammatory marker, in trained individuals following intense exercise in hot conditions, attributing the increase to elevated levels of interleukin-6 (IL-6) (Costello et al., 2018). However, Kaspar et al. reported a decrease in CRP levels in healthy untrained subjects after a session of high-intensity interval training (HIIT) consisting of 6 sets of 30 seconds of activity on a bicycle. They found no significant difference between endurance training and HIIT, suggesting that HIIT, with its shorter duration, may be more efficient for achieving health benefits (Kaspar et al., 2016). Similarly, conflicting data have been reported regarding the effect of high-intensity exercise on plasma fibrinogen concentration, a coagulation factor that also plays a role in inflammation. Studies have shown both no change and an increase in fibrinogen levels following exercise (Habibi et al.; Hatamy & Rahmani, 2021; Kaur & Jain, 2022; Plaisance et al., 2007). For instance, plasma fibrinogen levels significantly increased in active women after a session of intensive exercise, which included 6 stages of sprint running over a short distance of 40 yards with 10 seconds of rest between intervals (Ali & Hanachi, 2011). Additionally, various studies have investigated hematological changes, including white blood cell count (WBCs), red blood cell count (RBC), hemoglobin (Hgb), and hematocrit (Hct), in response to exercise (Bogdanis et al., 2022; Jamurtas et al., 2018; Kashef et al., 2022). These changes are influenced by multiple factors, and many studies have indicated an increase in plasma viscosity as a contributing factor (Belviranli et al., 2017). However, there is limited research specifically examining the effects of acute repeated sprint activity (RSA) exercise on hematological responses.

The research aims to answer the following questions:

• Can a single session of repeated sprint activity (RSA) lead to immediate changes in adropin levels, both immediately after the exercise and 20 minutes postexercise?

• Can a single session of RSA exercise induce alterations in inflammatory markers, coagulation factors, immune system indicators, and blood pressure levels, and do these changes persist up to 20 minutes post exercise?

By investigating these questions, the study aims to examine the acute effects of intense repetitive RSA activity on adropin levels, as well as various physiological blood factors related to inflammation, coagulation, immune response, and blood pressure. The results will contribute to a better understanding of the immediate and short-term responses of the body to RSA exercise and shed light on the potential impacts on cardiovascular health and inflammatory processes.

Materials and Methods

Subjects

The present research was conducted in a semiexperimental manner, involving a sample of 10 inactive healthy young men. The selection of this sample size was based on similar studies conducted in the field (Azarbayjani et al., 2014; Belviranli et al., 2017; Kaspar et al., 2016). All participants were assessed for their inactivity using the Goldberg General Health Questionnaire (GHQ-28) (Goldberg & Williams, 1988) and Beck et al.'s 1982 questionnaire (Baecke et al., 1982). The entry criteria for the study included no use of any medications or supplements, including anti-inflammatory drugs, no current diagnosis of a chronic health condition, non-smoking, and being untrained. Prior to the exercise sessions, participants were screened to ensure they met the inclusion criteria and that it was safe for them to undertake a high-intensity exercise program.Prior to the exercise sessions, participants were screened to ensure they met the inclusion criteria and that it was safe for them to undertake a high-intensity exercise programPrior to the commencement of the research, the subjects were provided with a detailed explanation of the research procedure and the potential risks associated with the study. Then they signed informed consent forms. It was made clear to the subjects that they had the right to withdraw from the research at any stage if they desired to do so. Furthermore, the research proposal was submitted to the Ethics Committee of the Research Institute of Sports Sciences of IRAN and was approved

with an assigned ethics code SSRI.REC-2307-2323.

Research and exercise protocol

The participant arrived at the testing and sampling site by 10:00 AM. Before blood sampling, several measurements were taken. The height of the subjects was measured in centimeters using a Seka height measuring device manufactured in Germany. Body weight was measured in kilograms using an electronic scale (SAM-2003D) made in China. Body mass index (BMI) was calculated by dividing body weight (kg) by the square of height (meters). Additionally, waist and hip measurements were taken twice by the same individual by a measuring tape (Table 1). Each person performed three measurements to ensure reliability and accuracy. Furthermore, body fat percentage was assessed using a caliper (Harpenden) made in USA and the 7site method, following the Jackson and Pollock formula. This calculation provided an estimation of the fat percentage for each participant:

Bone density formula = (0.00028826 * age) - (0.00000055 * SUM7²) + (0.00043499 * SUM7) – 1.112

Fat percentage formula = 100 [(4.95/bone density) - 4.5]

At 11:00 a.m., 5 cc blood was taken from the participant's brachial vein in the sitting position before, immediately after and 20 minutes after exercise. The exercise protocol included 20 repetitions of sprint running over a distance of 20 meters and 20 seconds of rest after each repetition (20 x 20 x 20) (Figure 1). The reason for choosing this protocol was the high intensity and appropriate duration of this exercise (approximatley 8 minutes), which had been used in previous studies (Belviranli et al., 2017; Girard et al., 2013). After each repetition, the heart rate was measured by heart rate monitor (Polar) made in Finland, and the rate of perceived exertion (RPE) was recorded to ensure the intensity of the exercise. Immediately after the exercise, 5 cc of blood was taken from the subject's brachial vein, and then the subjects rested passively for 20 minutes. Subjects were only allowed to drink water during rest. After 20 minutes, for the third time, 5 cc of blood was taken. All the stages of blood sampling and exercise are performed under the supervision of a specialist physician to ensure that there is no risk to the subjects (Table 2).

Table 1. Mean ± SD of physical characteristics of the participants

Variable	Mean ± SD
Age (years)	23.60 ± 2.01
Height (cm)	176.30 ± 8.49
Weight (kg)	68.60 ± 7.21
Body Mass Index (kg/m2)	22.00 ± 2.10
Waist Circumference (cm)	80.40 ± 7.02
Hip Circumference (cm)	95.70 ± 6.25

Measurements of variables

In this study, blood samples were collected into tubes containing anticoagulants (trisodium citrate). The serum was separated from the blood samples using centrifuge (Hettich) made in Germany, running at 3000 rpm for 15 minutes. The measurement of serum adropin levels was performed using ELISA kit (Zelbio) made in Germany. The values were reported using a plate reader made in the United States. Fibrinogen levels were measured using a coagulometer and an Immulite device made in Germany. The blood factors of white blood cells, red blood cells, hemoglobin, and hematocrit were measured using a kit (Pars Azmoun Company) made in Iran. The measurements were conducted using the photometric method and autoanalyzer (Mindri model BS380). CRP values were also measured using the photometric method and a kit (Delta Derman Part). All blood measurements were performed at the Navid Pathobiology Laboratory.

Statistical analysis

For statistical analysis, the data were analyzed using SPSS software version 23. The normality of the data was assessed using the Kolmogorov-Smirnov test. The results are reported as the mean ± standard deviation ($P < 0.05$). Since all the data followed a normal distribution, the average factors of adropin, fibrinogen, white blood cells, red blood cells, hemoglobin, hematocrit, and CRP were compared using repeated measures analysis of variance and Bonferroni's post hoc, also for comparing systolic and diastolic blood pressure values, paired t-test was used ($P < 0.05$).

Results

In Table 2, the average size of 7 body sites in millimeters, measured using a caliper, is reported. Additionally, the subjects' fat percentage is reported based on the average size of these 7 body sites. Table 3 presents the heart rate, rate of perceived exertion (RPE), and duration of each repetition, indicated by their mean and standard deviation. As demonstrated in Table 4, both systolic and diastolic blood pressure increased significantly immediately after exercise compared to before training. The increase is statistically significant. Adropin values also showed a significant increase immediately after exercise compared to before exercise, and even after 20 minutes, they remained significantly higher than the pre exercise levels, although they decreased compared to immediately after exercise. However, this decrease is not statistically significant. Blood fibrinogen values exhibited a significant increase immediately after exercise compared to before exercise, and even after 20 minutes, they remained higher than the resting level. Similarly, white blood cell values showed a significant increase immediately after exercise compared to before exercise, but after twenty minutes, their values decreased significantly. C-reactive protein levels also inc-

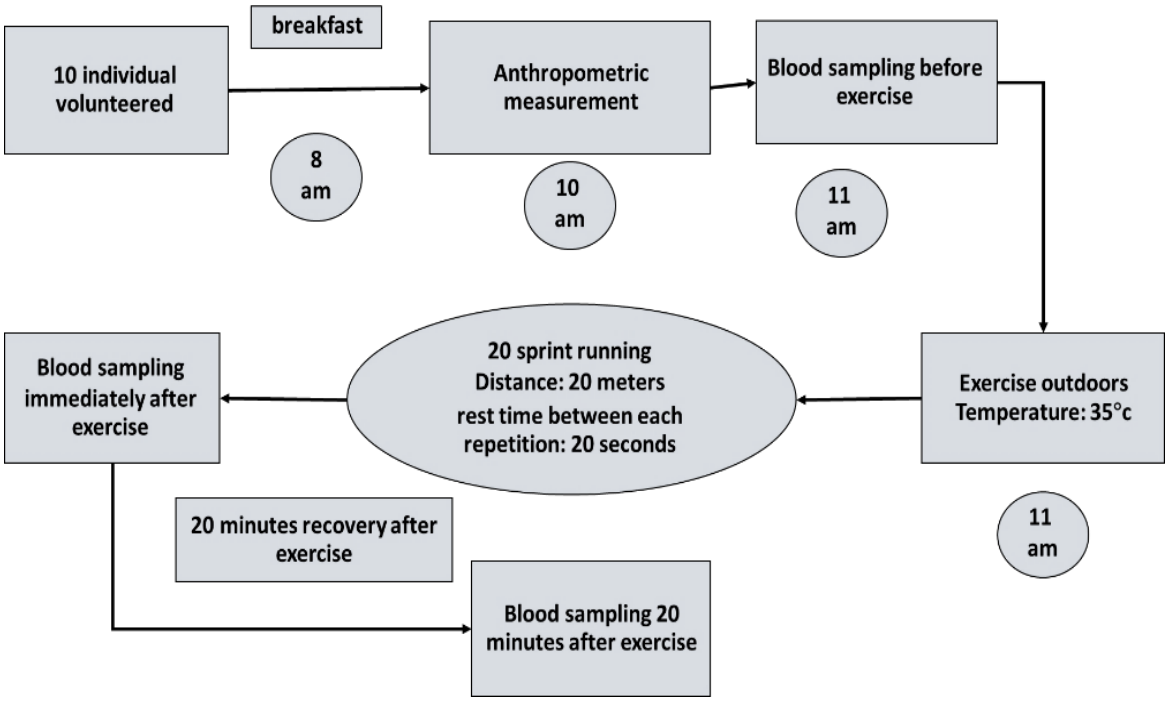

Figure 1. Research process.

-reased significantly immediately after exercise, and after 20 minutes, their values decreased, although they were still higher than the resting levels.

Discussion

The aim of this study was to examine the impact of repeated sprinting training (RST) on blood adropin levels and various inflammatory, coagulation, and immune factors before, immediately after, and 20 minutes postexercise in healthy young men. The results of this study revealed a significant increase in serum adropin levels (84% change) immediately after the exercise session, which consisted of 20 repetitions of sprint runs with 20 seconds of rest between repetitions. Furthermore, 20 minutes after exercise, the adropin level remained significantly higher (52% change) than the resting level.

Table 2. Mean ± SD of 7 site skinfold and body fat percent by Jackson and Pollock's formula

Body Sites	Size (mm)
Chest	7.60 ± 2.63
Abdominal	15.70 ± 4.91
Armpit	12.90 ± 4.28
suprailiac	15.70 ± 5.53
Thigh	14.70 ± 5.65
Subscapular	12.70 ± 3.59
Triceps	10.50 ± 2.71
Seven-Sites fat percentage	12.37%

These findings are in line with the research conducted by Sato et al. in 2017, which demonstrated increased adropin levels in rats with streptozotocin-induced diabetes following a session of aerobic exercise (Sato et al., 2017). Hence, it is plausible that the mechanical and metabolic stress induced by exercise contributes to the elevation of adropin levels. Some studies have reported a positive correlation between exercise training and adropin (Fujie et al., 2017). However, the association between exercise and adropin increase is not consistent. For instance, Alizadeh et al. did not observe significant changes in adropin levels and insulin resistance in overweight women after a session of aerobic exercise at maximal fat oxidation intensity. The researchers suggested that insufficient duration and intensity of exercise might have contributed to the absence of change (Alizadeh et al., 2018). In contrast, the exercise protocol employed in the current study was rigorous, as indicated by the data in Table 3 (RPE: 16.92 ± 1.19, HR: 173.82 ± 34.55), suggesting that the exercise regimen used was intense enough to induce this increase. However, Sánchez Gomar et al. also did not observe significant changes in serum adropin levels in football players, possibly due to already elevated resting levels of adropin in athletes (Sanchis-Gomar et al., 2015). In our study, the participants were inactive individuals who likely had lower adropin levels prior to exercise compared to athletes, leading to significant changes. Nevertheless, this is not always the case, as Hashemi et al. observed an increase in adropin levels in athletes after an exercise training session, whereas no change was observed in nonathletes (Hashemi et al., 2021). Similarly, Ozbay et al. did not find significant alterations in serum adropin levels following acute

exercise in healthy men (Ozbay et al., 2020). Hence, it appears that the changes in adropin levels due to exercise are influenced by various factors, and due to limited research on the effects of acute exercise on adropin, a definitive conclusion cannot be drawn. Additionally, the exact half-life of adropin has not been cl--early defined, which may contribute to conflicting results observed in different studies. However, in this study, even 20 minutes after exercise, adropin levels remained elevated compared to the baseline level. This finding further supports the notion of increased adropin levels following intense exercise, wh-

Table 3. Mean ± SD of heart rate, RPE and duration of each repetition of RSA exercise.

Repetition	Heart Rate	Rate of Perceived Pressure (RPE)	Duration of each repetition
Rest	69.12 ± 6.32	------	--------
First repetition	151.70 ± 48.89	15.95 ± 2.50	4.77 ± 0.7
second repetition	150.65 ± 48.03	16.05 ± 2.80	4.93 ± 0.60
third repetition	149.65 ± 46.61	16.50 ± 2.62	4.82 ± 0.51
4th repetition	153.10 ± 49.12	16.45 ± 2.56	5.01 ± 0.41
5th repetition	146.90 ± 49.09	16.55 ± 2.18	5.47 ± 0.33
6th repetition	162.45 ± 49.12	16.20 ± 2.56	4.87 ± 0.59
7th repetition	153.25 ± 47.20	15.35 ± 2.52	4.74 ± 0.69
8th repetition	168.00 ± 43.60	14.90 ± 2.63	4.99 ± 0.55
9th repetition	158.70 ± 41.93	15.65 ± 2.99	5.54 ± 0.28
10th repetition	169.40 ± 42.53	16.45 ± 2.08	4.89 ± 0.56
11th repetition	175.67 ± 41.32	16.58 ± 1.52	4.99 ± 0.30
12th repetition	177.94 ± 43.84	16.69 ± 1.32	5.31 ± 0.46
13th repetition	185.76 ± 48.62	16.98 ± 2.84	5.62 ± 0.37
14th repetition	196.45 ± 10.51	17.24 ± 2.94	5.31 ± 0.48
15th repetition	199.47 ± 8.65	17.54 ± 1.36	5.69 ± 0.94
16th repetition	208.12 ± 3.71	17.84 ± 1.39	5.32 ± 0.14
17th repetition	215 ± 2.36	18.14 ± 2.67	5.87 ± 0.78
18th iteration	217.66 ± 4.75	18.75 ± 1.91	6.01 ± 0.98
19th repetition	220.68 ± 4.31	19.36 ± 1.11	6.23 ± 0.87
20th repetition	220.54 ± 3.67	19.13 ± 1.80	6.45 ± 0.74
Total mean	215.91±32.21	16.91±2.45	4.79±0.56

Table 4. Mean ± SD of blood indices measured in the research.

Variable	Before exercise	Immediately after exercise	20 minutes after exercise	P value	F
SBP (mmHg)	109.00 ± 3.16	* 177.00 ± 8.23	-	0.001	-
DBP (mmHg)	76.50 ± 4.74	* 91.00 ± 3.16	-	0.001	-
Adropin (ng/l)	39.48 ± 14.80	* 66.98 ± 16.93	* 55.58 ± 12.91	0.000	13.68
Fibrinogen (mg/dl)	199.90 ± 19.34	* 234.70 ± 47.13	215.70 ± 32.56	0.005	7.12
RBC (million/µl)	5.31 ± 0.38	* 5.56 ± 0.27	5.41 ± 0.36	0.002	9.26
WBC (1000/ µl)	5.95 ± 1.03	* 8.64 ± 1.54	■ 5.73 ± 1.07	0.000	37.47
Hct (%)	44.85 ± 1.57	* 47.14 ± 1.86	■ 45.20 ± 1.70	0.000	14.98
Hgb (g/dl)	15.48 ± 0.85	* 16.11 ± 0.96	■ 15.53 ± 0.71	0.002	9.48
CRP (mg/l)	2.44 ± 0.61	*2.70 ± 0.54	2.59 ± 0.48	0.01	5.98

*: Significant changes compared to before training. ■: Significant changes compared to immediately after training

-ich may have a positive impact on blood pressure, glucose metabolism, and lipid metabolism during the half-life period. It is likely that the metabolic stress induced by exercise triggers the expression of the adropin gene in tissues, although more time is needed for the translation of the gene into protein. Regardless, considering the significance of adropin in preventing fat accumulation, reducing insulin resistance, improving glucose tolerance, and its role in cardiovascular health and regulation, further studies should be conducted in this field.

Blood pressure is an important indicator of cardiovascular health, and chronic high blood pressure is associated with various diseases and an increased risk of premature mortality (Kokubo & Matsumoto, 2017). However, an excessive increase in blood pressure during and after exercise can also have negative consequences. The results of this study demonstrated that acute intense exercise led to a significant increase in systolic blood pressure by 68 ± 7.5 mmHg (62% change) and diastolic blood pressure by 14.5 ± 5.7 mmHg (19% change) immediately after exercise. This increase falls within the normal range of blood pressure elevation during exercise, as reported by Kim et al. (Kim & Ha, 2016). Similar findings were observed by Heidari et al., who reported an increase of approximately 40 mmHg in systolic blood pressure and 4 mmHg in diastolic blood pressure at the conclusion of exercise (Heidari et al., 2021). Additionally, Shakoor et al. observed an increase of approximatly 22 mmHg in blood pressure immediately after HIIT in patients with first-degree hypertension. However, in their research the blood pressure levels significantly decreased after 30 and 60 minutes compared to pretraining levels and compared to the endurance training group (Shakoor et al., 2020). It is worth noting that their exercise intensity was lower due to the patient population, resulting in a lesser increase in blood pressure. Freitas et al. also reported an increase in blood pressure in postmenopausal women following a HIIT session (de Freitas et al., 2022). The rise in blood pressure during and immediately after exercise is considered a natural phenomenon in healthy individuals due to the activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS) (Kim & Ha, 2016). Indeed, some studies have shown a decrease in blood pressure during the recovery period after exercise. Palomo et al. demonstrated that acute intense interval training resulted in a greater reduction in blood pressure (14 mmHg) compared to 45 minutes postexercise (4 mmHg) in patients with metabolic disease, as compared to pre exercise levels (Morales-Palomo et al., 2017). It appears that intense interval training has a stronger impact on parasympathetic modulation after exercise, leading to a decrease in blood pressure during the recovery period compared to pre exercise levels (Roque Marçal et al., 2022). Santiago et al. also reported a decrease in blood pressure after exercise and up to 30 minutes post-HIIT session in patients with type 2 diabetes (Santiago et al., 2018). The decrease in blood pressure following exercise is closely related to the reduction in blood pressure immediately after a single exercise session. Additionally, high-intensity exercises that stimulate cardiovascular function can cause vasodilation for up to one hour after exercise, leading to a decrease in blood pressure. Neural, hormonal, and hemodynamic factors play a role in the changes observed in blood pressure during and after exercise. The increased blood flow to the muscles and the pressure exerted on the vessel walls stimulate the release of nitric oxide (NO), which reduces vascular resistance and ultimately leads to a decrease in blood pressure (de Oliveira Sant'Ana et al., 2023). Studies that have reported a decrease in blood pressure after exercise have typically examined the period of one hour to 24 hours post exercise. In this study, blood pressure was measured immediately after the exercise session, and it is likely that subsequent measurements would have shown a decrease in blood pressure during the recovery period.

Numerous studies have demonstrated that intense interval training can help control and reduce inflammatory factors (Reljic et al., 2022; Vigriawan et al., 2022). However, the effect of acute intense exercise can vary and sometimes lead to an increase in inflammation and systemic inflammatory responses similar to injury, involving the immune system (Meckel et al., 2009; Zwetsloot et al., 2014). In the current study, CRP, an inflammatory marker, exhibited a significant increase (13% change) immediately after exercise. However, it decreased after 20 minutes and approached the initial level. CRP is normally present at very low levels in healthy individuals but can increase up to 1000 times during the acute phase. In pathological conditions, CRP levels can increase up to 25% (Gabay & Kushner, 1999). Therefore, the increase in CRP observed in this study was not pathological and remained within the physiological range. The changes in CRP during intense interval training are still a topic of debate, as some studies have reported an increase, while others have reported a decrease or no change in CRP levels (Garcia-Hermoso et al., 2016; Kaspar et al., 2016; Rohnejad & Monazzami, 2023). It appears that CRP increases due to the secretion of pro-inflammatory cytokines such as IL-6 and IL-1β following intense exercise (Kaspar et al., 2016). Additionally, hypoxia resulting from exercises such as high-intensity interval training and resistance training (RST) may contribute to increased CRP levels. In general, exercises that induce greater levels of hypoxia tend to stimulate higher CRP secretion (Rohnejad & Monazzami, 2023). Also, metabolic stress induced by RST may activate signaling pathways such as AMP-activated protein kinase (AMPK) and nuclear factor kappa B (NF-κB), which are known to play roles in inflammation and coagulation responses. On the other hand, metabolic stress is one of the factors that increase adropin levels. Elevated adropin levels may interact with these pathways, potentially modulating inflammatory responses and influencing coagulation factor levels

(Le Scouarnec et al., 2022; Thurlow et al., 2024). Exercise can have diverse effects on blood components and cells depending on the intensity and duration of the exercise (El-Sayed, 1998; Wardyn et al., 2008). Blood, through its cells and substances, performs various tasks, such as oxygen and nutrient transport, temperature regulation, pathogen defense, coagulation, buffering, homeostasis regulation, and maintenance of the body's internal environment (El-Sayed, 1998; Heidari et al., 2016). In the present study, there was a significant increase in red blood cells (5% change), hemoglobin (4% change), and hematocrit (5% change) immediately after exercise, which is consistent with the findings of Kashef et al. (Kashef et al., 2022), Minuzzi et al. (Minuzzi et al., 2017), and Belviranli et al. (Belviranli et al., 2017). belviranli et al. and Minuzzi et al. also observed a decrease in red blood cells, hematocrit, and hemoglobin levels 1 to 24 hours after exercise. However, in this study, after 20 minutes of recovery, the levels of hematocrit and hemoglobin remained significantly higher than immediately after exercise but were not significantly different from before exercise. The level of red blood cells was still elevated after 20 minutes, but the difference was not significant. It appears that changes in plasma volume are a crucial factor in the alterations observed in blood cells after exercise. Kashef et al. observed a decrease in red blood cells, hematocrit, and hemoglobin when adjusting for changes in blood and plasma volume, suggesting that the initial increase in these factors after exercise is due to a decrease in plasma volume (Kashef et al., 2022). Although changes in plasma volume were not accounted for in this study, given the short duration of exercise (between 8 and 9 minutes), significant changes in plasma volume are unlikely. Following the release of various factors and the activation of blood cells, the immune system becomes active. For instance, white blood cells, which are involved in the immune response, can increase by up to 100% after an anaerobic exercise session and remain elevated for up to half an hour after exercise (Heidari et al., 2016). Indeed, studies have consistently shown an increase in white blood cells following intensive HIIT sessions (Bogdanis et al., 2022; Jamurtas et al., 2018). The present study also demonstrated a significant increase in WBC (47% change) immediately after exercise, which aligns with previous research (Bogdanis et al., 2022; Jamurtas et al., 2018). For instance, Brancaccio et al. observed an increase in WBC following HIIT (Brancaccio et al., 2010). Additionally, in this study, the WBC count returned to pre-exercise levels after 20 minutes of recovery. Sheykhlouvand et al. reported a similar increase in WBC following an intensive interval rowing session (Sheykhlouvand et al., 2018). The increase in white blood cells, which are part of the immune system, and inflammatory factors such as CRP, can be attributed to several factors. These include the release of hormones such as catecholamines, small muscle injuries incurred during exercise, increased oxidative stress, and elevated lactic acid levels. These factors prompt the recruitment of WBCs to the site of injury or stress (Kashef et al., 2022).

However, it is worth noting that Azarbaijani et al. did not observe any changes in WBC levels after an exercise session and suggested that the increase in WBC following exercise is due to a decrease in plasma volume (Azarbayjani et al., 2014). According to their findings, if plasma volume remains unchanged after exercise, there may be no significant alteration in the number of blood cells Overall the increase in WBC following intensive exercise, including HIIT, appears to be a physiological response to the metabolic and physiological demands placed on the body during such activities.

The present study observed a significant increase in fibrinogen immediately after exercise (17% change), and although it remained higher than before exercise (8% change) after 20 minutes of recovery, the difference was not significant. These findings are consistent with some previous studies (Eriksson-Berg et al., 2002). However, it is important to note that other studies have reported no change or a decrease in fibrinogen levels following intensive exercise, which is inconsistent with the results of this study (El-Sayed et al., 2004; Martin et al., 1985). Overall, the effects of intensive exercise on plasma fibrinogen concentration have yielded conflicting results in the literature (Habibi et al.; Plaisance et al., 2007). Fibrinogen is the largest plasma protein and plays a role as a cofactor in platelet aggregation and coagulation. It is also involved in leukocyte adhesion and increases during inflammation, thus playing an important role in homeostasis due to its various functions (Kaur & Jain, 2022). Fibrinogen is considered a risk factor for cardiovascular diseases due to its involvement in atherogenic and thrombogenic processes (Ernst, 1993). The increase in blood fibrinogen levels during inflammation is likely attributed to the elevation of plasma IL-6 and IL-1. The varying results observed in studies examining the effects of exercise on fibrinogen levels may be due to differences in exercise intensity, duration, and type. Other factors, such as the release of fibrinogen from the liver or temporary increases in fibrinogen concentration in the blood, as well as the timing of fibrinogen measurement, can also influence the results (El-Sayed, 1998; El-Sayed et al., 2004). Given the increase in inflammatory factors observed in this study, it appears that the increase in fibrinogen is associated with inflammation. It is important to acknowledge some limitations of the current research, including the small number of subjects and the lack of control over their nutrition prior to the test. Although the subjects were instructed to consume their usual diet, the influence of nutrition could not be fully controlled. Additionally, the motivation level of participants during exercises such as HIIT and RST could not be controlled, and subjects were simply instructed to perform the activities with maximum speed and intensity.

Conclusion

Based on our results, a high-intensity training session elicits varying responses in normal and healthy individuals. However, the crucial point is that all the changes, such as the elevation of adropin, fibrinogen, and white blood cells, occur within the physiological range, indicating that the body is adequately prepared to respond to the stimulus. In this context, exercise plays a significant role and does not appear to pose any risks for healthy individuals. In fact, these individuals can reap the advantages of such activities, particularly in terms of increasing plasma atropine levels. However, we must emphasize that the level of physical fitness of individuals can be of significant importance in the application of this type of training. Also, we suggest that future research could include exploring different exercise intensities, durations, or populations to better understand the relationship between RST and adropin levels.

What is already known on this subject?

Repeated Sprint Training (RST) has been studied extensively for its effects on physical fitness like maximal oxygen consumption, repeated-sprint ability, Countermovement jump, Change of direction. Also, Studies have shown that RST can lead to an increase in inflammatory markers such as interleukin-6 (IL-6) post-exercise and RST can induce oxidative stress, indicated by increased levels of markers like serum myoglobin and lipid peroxidation.

What this study adds?

Specific studies on the effect of RST on serum adropin levels are scarce. Also, there is limited research specifically focusing on the effects of RST on coagulation factors.

Organ Cross-Talk Tips:

- High-intensity training sessions stimulate muscle-derived cytokines like IL-6, which play a crucial role in mediating immune responses and metabolic changes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures in this study were carried out under the Helsinki Statement regarding human research. The proposal for this project was presented to the Ethics Committee of the Research Institute of Physical Education and Sports Sciences of Iran and approved (ethics code SSRI.REC-2307-2323).

Informed consent Participants signed an informed consent form prior to participation in the study.

Author contributions

Conceptualization: S.S.Z., H.R., M.A.F.; Methodology: A.A., H.A., H.R.; Software: A.A.; Validation: H.R., Formal analysis; Investigation: S.S.Z.; Resources: M.A.F.; Data curation: H.R.; Writing - original draft: A.A.; Writing – review & editing: H.R.; Visualization: M.M.A.; Supervision: H.R. Project administration: H.R.; Funding acquisition: A.A.

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