Avicenna Journal of Phytomedicine (AJP)

Original Research Article

Investigation of the co-administration of flaxseed supplementation with high-intensity interval training, resistance training, or combined training on hematological indices and kidney health in fructose/CCl4-induced NAFLD in rats

Abdorreza Eghbal Moghanlou[1], Zahra Eslami[2], Merve UCA[3], Yusuf Can Gürsoy[4], Seyed Javad Mirghani[5,*]

[1] *Faculty of Sport Science, Istanbul Esenyurt University, Istanbul, Turkey*
[2]*Department of Clinical Biochemistry, School of Medicine, Hamadan university of medical science, Hamadan, Iran*
[3]*Department of Coaching Training, School of Physical Education and Sports, İstanbul Nişantaşı University, Turkey*
[4] *Department of Sports Management, High School of Physical Education and Sports, Istanbul Esenyurt University, Istanbul, Turkey*
[5]*Shahid Mirghani Research Institute, Gorgan, Golestan, Iran*

Article history:
Received: Apr 18, 2025
Received in revised form: Sep 24, 2025
Accepted: Sep 25, 2025
Epub ahead of print

**** Corresponding Author:***
Tel: 989120747177
Fax:08131311148
seyedgavadmirghani@yahoo.com

Keywords:
Flax
Exercise
Kidney
Hematology

Abstract

Objective: Types of exercise training and flaxseed are beneficial approaches to improve non-alcoholic fatty liver disease (NAFLD)-induced hematological abnormalities. We aimed to determine which type of exercise training supplemented with flaxseed is more efficient for the improvement of blood and kidney function and hematological parameters in fructose/CCl4-induced NAFLD in rats.

Materials and Methods: Forty male Wistar rats were divided randomly into five groups (n= 8): 1) ND (normal diet); 2) high-fat diet (HFD); 3) HFD + resistance training (RT) + Flaxseed; 4) HFD + high-intensity interval training (HIIT) + Flaxseed; and 5) HFD+ RT+ HIIT (CT) + Flaxseed. The interventions administered for 8 weeks once daily after NAFLD induction through CCl4 and fructose (15 weeks), and at the end of the 23rd weeks, hematological and renal indices were analyzed.

Results: The blood and liver tissue indices indicated that the HFD group had high triglycerides (TG) and had significant steatosis, inflammation, and ballooning compared to the ND group. Red blood cell (RBC) count (p= 0.039), hemoglobin (Hb) (p= 0.011), and mean corpuscular hemoglobin concentration (MCHC) (p=0.001) in the HFD group were significantly lower, and mean corpuscular volume (MCV) (p=0.003) was higher compared to the ND group. RT+ flaxseed administration led to a decrease in RBC count and hematocrit (HCT) and increased MCH compared to the HFD group. These changes showed similar trends in the HIIT+ flaxseed group. All types of training along with flaxseed caused to decrease MCV (p< 0.005) and elevated MCHC compared to HFD group (p=0.000). Renal markers analysis showed that CT+ flaxseed led to increased urea compared to the HIIT+ flaxseed group (p= 0.018), but there were no significant differences between other groups in urea or creatinine (Cr).

Conclusion: Exercise training when supplemented with flaxseed can improve NAFLD-associated blood abnormalities, while HIIT was more efficient than other modes of training.

Please cite this paper as:
Eghbal Moghanlou A, UCA M, Eslami Z, Gürsoy Y, Fatih Incibas M, Mirghani S.J. Investigating the interplay of flaxseed supplementation, high intensity interval training and resistance training on hematological indices and renal health in high-fat diet rats. Avicenna J Phytomed, 2025. Epub ahead of print.

Introduction

Obesity has become a major Global public health problem, and it is associated with many types of metabolic disturbances such as type-2 diabetes, cardiovascular diseases, and other related conditions (Jin et al. 2023). As reported by the World Health Organization (WHO, 2021), approximately 1.9 billion adults aged 18 and above were identified as overweight, with more than 650 million falling within the obese category up to 2021. Additionally, obesity has significantly increased among younger populations, an estimated 39 million children younger than five have been recognized as having excess weight or obesity (Ahmed and Konje 2023). Obesity has become increasingly prevalent in children and adolescents with an estimated 39 million children under the age of five being categorized as overweight or obese (Ling et al. 2023). Various approaches to treating obesity include lifestyle modification, physical activity, behavioral interventions, pharmacological therapy, herbal medicine, and surgery (Committee 2023). Among these options, flaxseed (*Linum usitatissimum*) has gained attention due to its essential omega-3 lipids, lignan phytochemicals, and high fiber content, which have anti-inflammatory and cholesterol-lowering effects (Al-Madhagy et al. 2023).

Moreover, exercise in general—and particularly intensive resistance training (IRT) and high-intensity interval training (HIIT) are well known to have benefits for metabolic health and body composition (Kazemi et al. 2023). Resistance training (RT) improves muscular strength and mass, while HIIT is associated with improved cardiorespiratory fitness and fat loss (Bettariga et al. 2024). By combining both modes of training, individuals, particularly those with obesity-related conditions, may benefit synergistically (Visco et al. 2023). The optimal methods for combining dietary interventions with exercise regimens still require clarification. Obesity leads to an increases in blood urea nitrogen (BUN) and creatinine concentrations, potentially show impaired kidney function or reduced renal clearance (Xie et al. 2018). On the other hand, due to inflammatory conditions associated with obesity, some types of white blood cells (WBCs) may be elevated (Visser et al. 2001). Conversely, studies indicate that hemoglobin (Hb) and hematocrit (HCT) levels can also be elevated in certain types of obesity (Hubert Shalanyuy 2024; Jeong et al. 2022).

The present study aims to compare the effects of flaxseed dietary supplementation, along with RT, HIIT, and combined training, on hematological indices for the assessment of renal markers, to explore possible side effects in a rat model of non-alcoholic fatty liver disease (NAFLD) induced by a high-fat diet (HFD).

Materials and Methods

Animals and diet

Forty male Wistar rats were purchased from the Shahid Mirghani Research Institute, and they were randomly divided into five groups (n = 8):

1) ND (normal diet); 2) HFD; 3) HFD + IRT + Flaxseed; 4) HFD + HIIT + Flaxseed; and 5) HFD + combined HIIT-RT (CT)+ Flaxseed. The ND group received a standard chow diet providing 4.30 kcal/g, and containing 3.87% fat derived from soy oil, 17.46% casein-based protein, 68.7% carbohydrates, 8.97% mineral content, and 1% vitamin composition (Zahra Eslami 2023). The HFD groups were fed chow and an HFD comprising 55% fructose solution and 0.1 ml/kg of carbon tetrachloride (CCl4) at a 1:4 volume ratio, diluted in olive oil. All animals had free access to food and water and were maintained under a 12-hr light/dark cycle at a controlled temperature of 20–24°C. Flaxseed was purchased from VERJEN Company (Golestan, Iran) and administered orally as powder (40 g/kg, once daily). Animals were anesthetized at the end of week 23, following 12 hr of fasting, an intraperitoneal dose of ketamine at 50

mg/kg and xylazine at 5 mg/kg (Merck, Germany) was used for anesthesia (Eslami et al. 2022).

Induction of NAFLD

The rats were induced with NAFLD by oral gavage of fructose and injected with 0.1 ml/kg CCl_4 (1:4 v/v, dissolved in olive oil). The fructose solution was prepared at a concentration of 55% in water (Zar Grain Refinery, Alborz, Iran) (Adapted from a recent study (Eslami et al. 2021).

Training protocol

The exercise training protocols (HIIT, RT, and CT) were performed for 8 weeks. One week prior to the commencement of the training protocols, all rats underwent a familiarization phase with the exercise apparatus. Throughout this period, each rat was positioned at the base of a ladder and gently encouraged to climb by applying light physical prompts. This process was repeated until every rat successfully learned to ascend the full length of the ladder. By the end of the familiarization week, all animals were able to climb the ladder independently, without the need for external encouragement. No additional weights or loads were applied during this phase. The RT protocol involved ladder climbing on a structure measuring 1.1 × 0.18 meters, with a 2-cm grid spacing and a 90° incline (Hornberger and Farrar 2004). In the first week, training started with 7 sets of 1 repetition, 5 sessions per week, using an overload (based on a percentage of body weight) of 0–40%. This was gradually increased to 7 sets of 3 repetitions with an overload of 0–60% of body weight per session by the eighth week. The length of the ladder was determined so that the rats completed 18 dynamic movements per climb. Fifty percent of the ladder-climbing time was designated as rest time between climbing bouts. Moreover, HIIT was performed in the first week at 90% Vmax with 2 repetitions per session, 5 sessions per week, and was gradually increased to 6 repetitions at 90% Vmax by the eighth week. The protocols for IRT and HIIT are shown in Tables 1 and 2, respectively (Leandro et al. 2007).

Table 1. Protocol of RT

Week	Set	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
1	Repetition	1	1	1	1	1	1	1	-	-
	Overload	0%	20%	30%	40%	30%	20%	0%	-	-
2	Repetition	0	1	2	2	2	1	0	-	-
	Overload	0%	20%	30%	40%	30%	20%	20%	-	-
3	Repetition	0	1	1	1	1	1	1	1	-
	Overload	20%	30%	40%	50%	40%	30%	20%	0%	-
4	Repetition	0	1	1	1	1	1	1	1	1
	Overload	0%	20%	30%	40%	50%	40%	30%	20%	0%
5	Repetition	0	1	1	1	2	1	1	1	1
	Overload	0%	20%	30%	40%	50%	40%	30%	20%	0%
6	Repetition	0	1	1	1	1	1	1	1	1
	Overload	0%	30%	40%	50%	60%	50%	40%	30%	0%
7	Repetition	1	1	1	2	2	1	1	-	-
	Overload	0%	40%	50%	60%	50%	40%	0%	-	-
8	Repetition	1	1	2	3	1	1	1	-	-
	Overload	0%	40%	50%	60%	50%	40%	0%	-	-

Repetition: Number of ladder climbing in each set. Set: Number of bout in each session. Overload: attached weight to tail as a percent of body weight

Table 2. Protocol of HIIT

Week	1st	2nd	3rd	4th	5th	6th	7th	8th
Repetition	2	2	3	4	4	5	6	6
Min	2	2	2	2	2	2	2	2
Overload	90%	90%	90%	90%	90%	90%	90%	90%

Repetition: Number of bout in each set. Set: Number of bout in each session. Overload: Percent of Vmax

Biochemical and blood cell parameters analysis

The rats were anesthetized by an intraperitoneal injection (IP) of ketamine (50 mg/kg) and xylazine (5 mg/kg) (Merck, Germany). Next, blood samples were collected and centrifuged at 3000 rpm for 10 minutes. The serum levels of Urea, creatinine (Cr), and components of the lipid profile—such as triglycerides (TG), total cholesterol (CHO), low-density lipoprotein (LDL), and high-density lipoprotein (HDL)—were measured through enzymatic assay kits provided by Pars Azmoon (Tehran, Iran) and analyzed using a BT 3500 Autoanalyzer (Medsystem, USA). An automated hematology analyzer was employed to assess CBC variables such as red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), Mean Corpuscular Volume (MCV), and platelet levels (Sysmex KX-21N, Japan).

Tissue preparing

At the end of the 23rd week, the rats were sacrificed for pathophysiological evaluations. After collecting blood samples, livers were instantly removed and washed with physiological saline. After collecting the liver tissue samples via incisions, they were kept in 10% buffered formaldehyde and embedded in paraffin for staining and NAFLD grading.

Statistical analysis

All data are presented as the mean ± standard error of the mean (SEM). Statistical evaluations were conducted using IBM SPSS Statistics software. Differences between groups were assessed using one-way analysis of variance (ANOVA) and Tukey's HSD post hoc test.

Results

To confirm the induction of NAFLD by the HFD, we measured serum lipid profile levels and liver tissue histopathological parameters (fat droplets, inflammation, and ballooning of hepatocytes from Hematoxylin-Eosin (H&E)-stained images). Based on the serum lipid analysis, the HFD group showed elevated lipid profile levels relative to the ND group; however, only TG levels demonstrated a statistically significant difference ($p = 0.048$) (Figure 1). Histological assessment of liver tissue revealed that the HFD group presented marked steatosis, inflammation, and hepatocyte ballooning in comparison to the ND group (Table 3 and Figure 2) ((Adapted from recent study, (Eslami et al. 2025)).

Table 3. Histological scoring of NAFLD in the HFD vs ND group, and the components of NAFLD activity score (NAS)

NAS activity component	ND	HFD
Steatosis	0	2
Lobular inflammation	0	2
Hepatocyte ballooning	0	1
SCORE	0	5

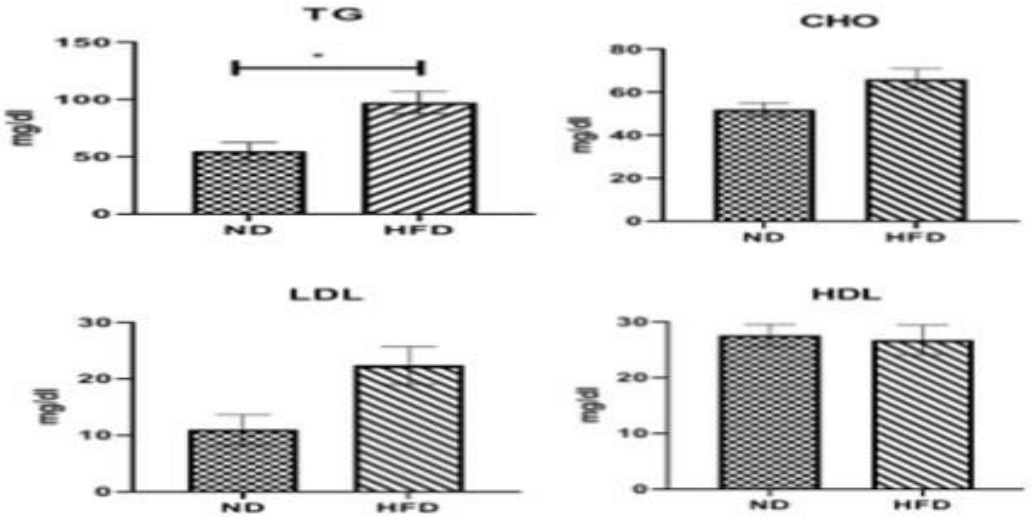

Figure 1. Serum levels of lipids (TG, CHO, LDL, and HDL) in the study groups. ND: Normal diet, HFD: High fat diet. The results are expressed as mean ± SEM for both groups. The lines connecting the columns indicate that groups differ from each other, and * denotes for $p<0.05$ compared to ND. For group comparisons, we used One-Way ANOVA and Tukey's HSD post hoc test (eight rats per group). CHO: Cholesterol, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, TG: Triglyceride.

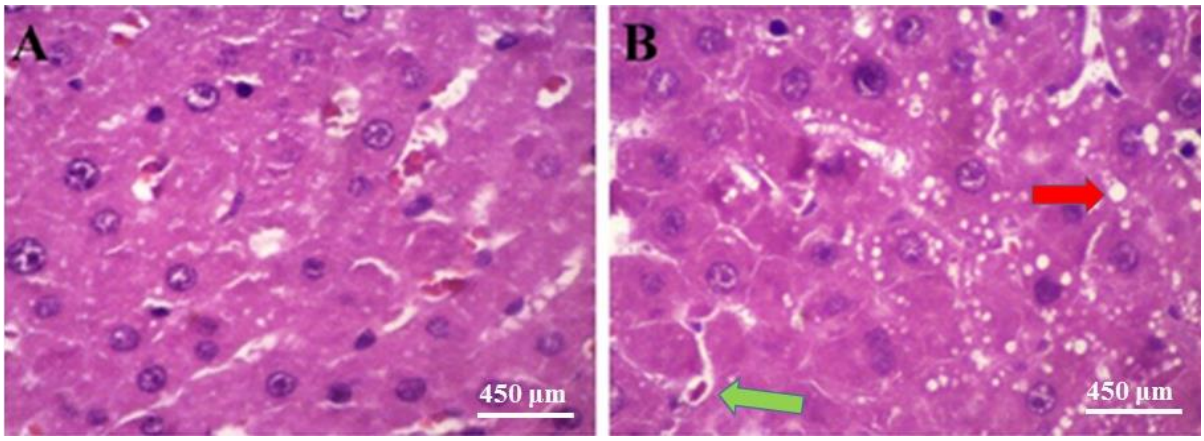

Figure 2. Representative of histological indices (Red: fat droplets, and Green: inflammation and ballooning of the hepatocytes) of H&E staining images of the liver sections in HFD vs ND groups (scale bar= 450 µm, × 400 magnification). A) ND and B) HFD. ND: Normal diet, HFD: High fat diet

Evaluation of CBC parameters revealed significant alterations in the HFD group relative to the ND group, with notable differences observed in RBC count ($p = 0.039$), Hb levels ($p = 0.011$), MCHC ($p = 0.001$), and MCV ($p = 0.003$). No significant changes were detected in the remaining hematological indices. More specifically, the HFD group demonstrated reductions in RBC, Hb, and MCHC, along with an elevation in MCV compared to ND. On the other hand, WBC ($p = 0.001$) and platelet (Plt) ($p = 0.005$) showed significant changes in the HFD + RT + Flaxseed group compared to HFD alone. Additionally, WBC levels were lower in the HFD + HIIT + Flaxseed ($p = 0.012$) and HFD + CT + Flaxseed ($p = 0.015$) groups compared to the HFD + RT + Flaxseed group. Furthermore, RBC count decreased following the RT + Flaxseed intervention compared to both the HFD group ($p = 0.015$) and the HFD + CT + Flaxseed group ($p = 0.048$). Intervention with RT ($p = 0.001$) and HIIT ($p = 0.017$) combined with flaxseed consumption led to a decrease in HCT, and HCT levels in the HFD + RT + Flaxseed group were lower than those in the

HFD + CT + Flaxseed group (p = 0.028). Conversely, RT (p = 0.033) and HIIT (p = 0.009) led to an increase in MCH compared to HFD. Moreover, all training types when combined with flaxseed supplementation resulted in decreased MCV (p < 0.005) and increased MCHC (p = 0.000). In the HFD + HIIT + Flaxseed group**,** Plt count was significantly lower compared to the HFD + RT + Flaxseed group (p = 0.016) (Table 4).

The analysis of renal toxicity indices showed that HFD led to an increase in urea and creatinine compared to ND, but this elevation was not statistically significant**.** Additionally, HFD + CT + Flaxseed caused an increase in urea levels compared to HFD + HIIT + Flaxseed (p = 0.018). Moreover, there were no other significant differences among the intervention groups in urea and creatinine levels (Table 5).

Table 4. Mean value of hematological parameters in the study groups. Data are expressed in mean ± SEM.

Variable	ND	HFD	HFD + RT + Flaxseed	HFD + HIIT + Flaxseed	HFD + CT + Flaxseed
WBC (10^3/μl)	6.86 ± 0.49	6.36 ± 0.68	10.57 ± 1.89	7.05 ± 0.36	7.54 ± 0.31
RBC (10^6 /μl)	8.48 ± 0.17	7.86 ± 0.19	6.96 ± 0.45	7.60 ± 0.13	7.70 ± 0.20
Hb (mg/dl)	15.05 ± 0.25	13.56 ± 0.42	12.62 ± 0.87	13.95 ± 0.42	13.82 ± 0.36
HCT (%)	46.15 ± 0.80	45.51 ± 0.97	37.25 ± 2.39	40.20 ± 1.55	42.24 ± 1.65
MCH (pg/cell)	17.73 ± 0.19	17.22 ± 0.30	18.10 ± 0.33	18.32 ± 0.35	17.84 ± 0.06
MCHC (mg/dl)	32.62 ± 0.33	29.83 ± 0.91	33.85 ± 0.19	34.75 ± 0.29	33.78 ± 0.13
MCV (fl)	54.38 ± 0.34	57.95 ± 1.19	53.52 ± 0.89	52.82 ± 1.44	52.82 ± 0.22
Plt (10^3/μl)	911.0 ± 42.30	764.25 ± 49.42	1136.75 ± 140.53	777.25 ± 171.38	970.0 ± 66.62

Table 5. Mean value of kidney markers in the study groups. Data are expressed in mean ± SEM

Variable	ND	HFD	HFD + RT + Flaxseed	HFD + HIIT + Flaxseed	HFD + CT + Flaxseed
Urea (mg/dl)	44.48 ± 3.09	49.86 ± 3.26	57.00 ± 8.94	42.07 ± 4.67	57.84 ± 4.31
Cr (mg/dl)	0.78 ± 0.03	0.83 ± 0.02	0.79 ± 0.02	0.79 ± 0.02	0.79 ± 0.01

Discussion

This study demonstrates that flaxseed supplementation combined with structured exercise interventions, IRT, HIIT, and CT, exerts significant protective effects against HFD-induced metabolic and hematological disturbances. We observed that HFD feeding led to elevated TG, hepatic steatosis, inflammation, and ballooning, alongside anemia-like changes in RBC indices. Notably, modulation of CBC parameters, including reductions in MCV and elevations in MCHC occurred in all our interventions, suggesting enhanced erythrocyte quality and systemic resilience. Furthermore, our findings reveal that interventions influence renal markers through distinct molecular pathways involving inflammation, oxidative stress, and protein turnover. Unlike most studies, our study uniquely evaluates how flaxseed and incremental exercise impact CBC indices, offering insight into erythropoietic and hemorheological adaptations. By directly comparing exercises, we adopted an experimental design that nicely enhances our understanding of the interaction between exercise types and flaxseed on systemic physiology.

HFD-mediated hepatic steatosis is an important feature of NAFLD which is caused by excessive fat storage, mitochondrial dysfunction, and oxidative stress. Our histological findings were consistent with a study which showed that saturated fat consumption resulted in hepatocyte lipid accumulation and the initiation of proinflammatory signaling cascades via NF-κB and JNK pathways (Musso et al. 2010). Similarly, HFD administration led to steatosis, lobular inflammation and ballooning degeneration

in the hepatocytes. These changes are often coupled with elevated TG concentrations (as observed in our study) indicative of an inability to adequately clear lipids and increase hepatic production of VLDL (Longhi et al. 2017). Other study indicated a higher degree of inflammation from diets rich in saturated fat than from diets rich in unsaturated fatty acids, suggesting that not all HFDs are equally toxic to the liver (Buettner et al. 2007). The lowered values of RBC count, Hb, and MCHC along with elevated MCV reflect a macrocytic anemia pattern that may be due to chronic inflammation, abnormal iron metabolism, and liver dysfunction resulting in disrupted erythropoiesis (Okwuegbuna et al. 2023). Increases in MCV often indicate disrupted DNA synthesis in erythroid precursors resulting from folate or B12 deficiency (Kaweme et al. 2022), or increases in MCV may be due to oxidative damage to the RBC membranes (Obeagu et al. 2024). Chronic inflammation and liver stress may inhibit bone marrow function and contribute to this anemia-like pattern (Achille et al. 2009). The previous finding of lowered RBC counts and Hb levels in the HFD group is in line with Varghese et al. who documented that diet-induced obesity manipulation exhibited a decrease in erythrocyte parameters with increased MCV, possibly secondary to impaired erythropoiesis, RBC turnover, and oxidative stress (Varghese et al. 2025b). Similarly, a decrease in Hb and altered RBC indices suggested a link between hepatic lipid accumulation and anemia of chronic disease in NAFLD patients (Kurmangaliyeva et al. 2025). Noteworthy, other studies have not seen any significant alterations in RBC indices between HFD feeding vs controls (Koch et al. 2019; Varghese et al. 2025a) which may be related to differences in diet make-up, duration of feeding, or potential strain differences.

Flaxseed promotes nuclear factor E2 related factor 2 (Nrf2) activation leading to an increase of antioxidant enzymes gene expression (Lee et al. 2008), which protects RBC membranes and renal tubular cells from oxidative damage (Rizwan et al. 2014). Concurrently, lignans and alpha-linolenic acid (ALA) suppress nuclear factor kappa B (NF-κB) to decrease pro-inflammatory cytokines (TNF-α, IL-6) that hinder erythropoiesis (Zhao et al. 2007). As a result, reduced inflammation stimulates hepcidin downregulation and improves iron accessibility for hemoglobin synthesis (Wang and Babitt 2016), which confirmed the elevation level of MCH and MCHC in our study. On the other hands, by mitigating inflammation and oxidative damage, flaxseed may help decrease urea production linked to the breakdown of amino acids and also stabilize creatinine levels by preventing muscle wasting (Al Za'abi et al. 2021). Previous research identified that flaxseed improved lipid profiles, decreased steatosis, modulated oxidative stress (Prasad 2009), and protected erythrocytes from oxidative damage, and the polyunsaturated fatty acids may alter the fluidity of membranes and RBC deformability (Yang et al. 2012). However, the current finding of a reduction in RBC count with the co-administration of flaxseed and incremental exercise training, contrasts with prior research which did not identify significant changes in RBC parameters with flaxseed alone (Stuglin and Prasad 2005). Additionally, it has been reported that CBC parameters were stable in postmenopausal women following flaxseed intake, possibly due to low baseline inflammation (Hallund et al. 2006). These findings suggest that combination of incremental exercises and flaxseed may have resulted in greater erythrocyte turnover.

HIIT incorporates both intensive bursts of anaerobic stress and lower intervals of aerobic exercise, resulting in a rapid metabolic switch and improved oxidative stress that can affect erythrocytes (Reljic 2025). Intermittent hypoxia during HIIT may stabilize hypoxia-inducible factor 1-alpha (HIF-1α) signaling to ultimately increase erythropoietin (EPO) synthesis

(Abe et al. 2015). Increases in MCH and MCHC lead to modest decreases in MCV, relating to an increase in the production of normocytic RBC. This suggests that HIIT drives marrow responsivity through AMPK activation in concert with declining tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) due to myokine release. Recent evidence showed that HIIT promotes activation of RBC-nitric oxide synthase, RBC nitrite concentration, and RBC deformability, which means that chronic HIIT improves endurance capacity (Koliamitra et al. 2017) and HIIT activates signaling molecules involved in AMP-activated protein kinase and p38 mitogen-activated protein kinase to promote PGC-1α expression (Gibala 2009). Furthermore, HIIT increases muscle hypertrophy by modulating the IGF-I/Akt/FoxO and myostatin/Smad pathways, causing increased expression of IGF-I, Akt, and follistatin while decreasing myostatin and Smad2/3 expression (Biglari et al. 2020). These changes together enhance oxidative capacity and muscle hypertrophy following HIIT. Based on a study, an increase in Hb and HCT levels occur followed by HIIT that are due to improvement and an increase in erythropoiesis. Hence, erythropoiesis and VO2 max or impaired oxidative function are likely contributing to this response and improving athletic performance (Webb et al. 2023). In contrast, Sarkar et al., reported reductions in RBC, Hb, and HCT following HIIT (Sarkar et al. 2023) and an 8-week HIIT program led to increased VO2 max without any changes in Hb, RBC or HCT (Putra et al. 2017) which were consistent with Astorino et al findings (Astorino et al. 2012). These conflicting findings suggest that the effects of HIIT on RBC parameters may depend on the duration of training, intensity of training, and personal characteristics of the subjects.

Our findings showed that combination of IRT and flaxseed led to decreased RBC count, Hb, HCT, and MCV. Several mechanisms may underpin the reduction of RBC counts observed in RT + flaxseed, such as: RT can stimulate an increase in plasma volume, particularly in conjunction with elements of endurance or anti-inflammatory agents. The resulting hemodilution can decrease apparent RBC count and HCT even though total RBC mass stays the same or is increasing (Fellmann 1992) RT involving eccentric loading and/or high mechanical strain may induce mild hemolysis from repeated muscle contractions and capillary strain (Proske and Morgan 2001). Additionally, the membrane fluidizing omega-3 in flaxseed may improve RBC deformability, while also making them more susceptible to being ripped under mechanical strain (Kim et al. 2023). Besides flaxseed contains phytates and lignans which can bind minerals such as iron and zinc, both of which are important for synthesizing hemoglobin (Kauser et al. 2024).

Hypoxia within muscle tissue, along with muscle mechanical overload, can stimulate EPO production, further producing janus kinase 2 and signal transducer and activator of transcription 5 (JAK2/STAT5) activation in the bone marrow that may increase erythroid progenitor proliferation (Haase 2010), leading to elevation of MCH and MCHC. It has showed that prolonged IRT in previously sedentary women reduced RBC, Hb, HCT, and MCV (Okon et al. 2025). Likewise, Hu et al. reported that IRT increased RBC turnover with changes in MCHC in men (Hu et al. 2011). The increase in MCHC found in our training groups, is parallel to the findings of Yang et al. that showed MCHC elevated through stimulation of the production of younger erythrocytes rich in hemoglobin (Yang et al. 2018). Moreover, the observed concurrent decrease in MCV implicated a response towards erythrocyte populations with normocytic or microcytic erythrocyte populations. These changes may also suggest increased utilization of iron for hemoglobin production, and decreased hepatic inflammation that may be

associated with incremental training and/or flaxseed generally may exacerbate these results. Furthermore, Nieman and Pedersen observed limited changes to CBC parameters in young male after IRT, which highlights that both duration and intensity may be important for hematological adaptation (Nieman and Pedersen 1999). A single session of IRT can elicit a transient increase in platelet count, possibly due to a release of platelets from either the spleen or bone marrow (Ahmadizad and El-Sayed 2003). Stimulation of bone marrow microenvironment, induction of releasing cytokines which stimulate hematopoietic stem cells (HSCs) and megakaryocyte differentiation (de Kruijf et al. 2020), activation of sympathetic nervous system which can mobilize platelets from the spleen into circulation (Maestroni 2025), and elevation of testosterone, growth hormone, and cortisol (Gagliano-Jucá et al. 2020) which they influence hematopoiesis led to increase platelet activation followed by IRT. But a study reported that long-term IRT significantly decreases platelet activation regardless of the form of exercise performed (Skouras et al. 2023). Factors, like intensity and volume, potentially dictate the effects of RT on platelets. RT can incur larger fibrinolytic responses when compared to non-incremental RT. The acute physiological response to RT can also vary based on age, health, and pre-existing ailments. Therefore, we can conclude that a significant contributor to the increase in platelet count was in fact the progressive increase in intensity applied to the RT.

On the other hands, our findings showed that co-administration of CT and flaxseed led to increase in urea compare to HIIT , while there were no between-group differences in creatinine concentrations. Increased urea following by CT and flaxseed may also indicate differences in protein catabolism for energy purposes or divergence in nitrogen metabolism associated with the training-type. IRT mitigated renal injury, hypertension and inflammation potentially by modulating the Akt/mTOR signaling pathway (Saud et al. 2021). Moreover, incremental load training mitigated renal fibrosis through modulation of the transforming growth factor (TGF)-β1/TAK1/MKK3/p38MAPK signaling pathway as well as activating autophagy (Bao et al. 2019). AMPK is strongly activated by endurance exercise, stays elevated after combined protocols, and acts as a suppressor of protein synthesis (Merle et al. 2019). It seems that training's volume/intensity may affect catabolism and the interaction of RT and HIIT has been associated with increased catabolism. Previous research has indicated renoprotective effects of flaxseed in models of chronic kidney disease (Velasquez et al. 2003), but it is unclear if the same would hold true in healthy or HFD-fed animals. Our observations were also inconsistent with Al Za’abi et al. who found that flaxseed treatment caused to decrease urea and creatinine (Al Za'abi et al. 2021). This may be explained by differences in baseline renal status and exercise-time interaction. As regards, a research has demonstrated that HIIT improves renal function due to the maximally increased cardiac outputs related to maximal efforts (Arazi et al. 2022). However, recent evidence showed that HIIT has heterogeneous renal functionality in different conditions and improved glucose homeostasis but also caused renal injury and renal fibrosis (Zheng et al. 2023). In contrast, in myocardial ischemia-reperfusion rats, HIIT attenuated renal injury caused by the ischemia-reperfusion through the down-regulation of inflammatory markers (Lai et al. 2025). Overall, it is indicated that while HIIT may have renoprotective effects in some conditions, in other diseases, it may have potential adverse renal outcomes.

The limitations of the NAFLD model include the possible mortality of animals during the intervention, the inability to use imaging techniques to verify the induced model, and variability in assessing serum indices between animal species.

The individual and combined effects of HIIT and IRT have enhanced the multifactorial benefits of flaxseed in improving health and fitness parameters. Hematological parameters—namely, increased RBC counts and Hb levels—contribute to improved fitness performance and recovery. These results underscore the integrative potential of nutritional and physical interventions in mitigating HFD-induced systemic dysfunction. In contrast to the most of studies that primarily examine lipid or liver parameters, we are one of the first to evaluate the influence of co-administration of flaxseed with exercise interventions, on the CBC indices, highlighting important erythropoietic and hemorheological adaptations. By directly comparing RT, HIIT, and combined exercises, we adopted an experimental design that nicely enhances our understanding of the interaction between exercise types and flaxseed on systemic physiology. Although future studies should clarify the optimal dosage of flaxseed supplementation and its long-term impact on performance and health across diverse populations.

Acknowledgment

The authors thank the staff of Shahid Mirghani Research Institute and Kavosh laboratory in Golestan province.

Conflicts of interest

There are no conflicts of interest.

Ethical Considerations

All experimental procedures adhered to the NIH Guide for the Care and Use of Laboratory Anim als (Publication No. 85–23, revised 1996) and.

Code of Ethics

The protocol was approved by the institutional ethics committee (IR.GOUMS.REC.1397.274).

Authors' Contributions

Conception and design of the study: Zahra Eslami and Seyed Javad Mirghani

Analysis and interpretation of data: Abdorreza Eghbal Moghanlou, Merve UCA and Yusuf Can Gürsoy.

Drafting the article: Abdorreza Eghbal Moghanlou, Zahra Eslami

Revising the article: Zahra Eslami, and Mehmet Fatih Incibas

Supervisor the article: Seyed Javad Mirghani

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