

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

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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.

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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

Exercise and flaxseed on hematological indices

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 CCl₄ (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% V_{max} with 2 repetitions per session, 5 sessions per week, and was gradually increased to 6 repetitions at 90% V_{max} 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	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	9 th
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	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th
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 V_{max}

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 $\pm$ 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

Exercise and flaxseed on hematological indices

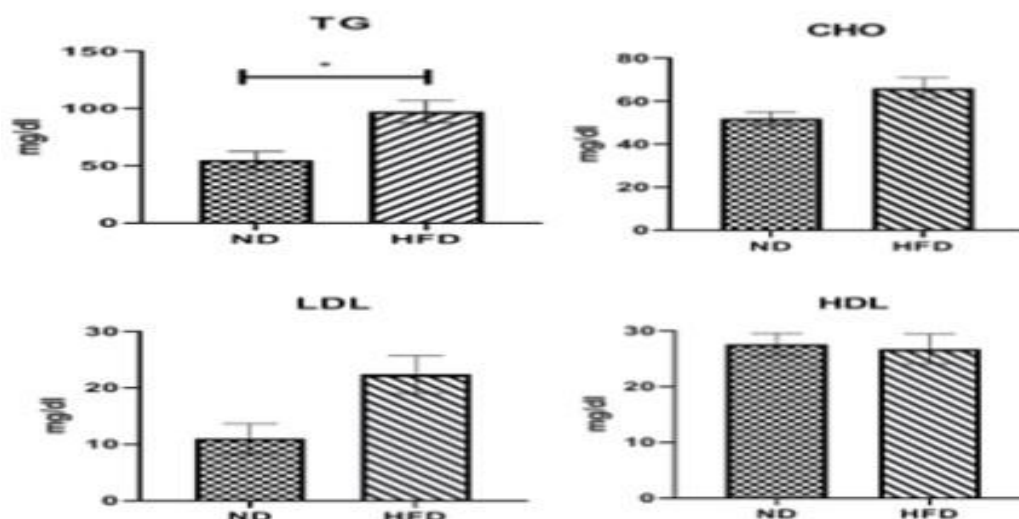


Figure 1. Serum levels of lipids (TG, CHO, LDL, and HDL) in the study groups. ND: Normal diet, HFD: High fat diet. The results are expressed as mean $\pm$ SEM for both groups. The lines connecting the columns indicate that groups differ from each other, and * denotes for $p < 0.05$ compared to ND. For 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.

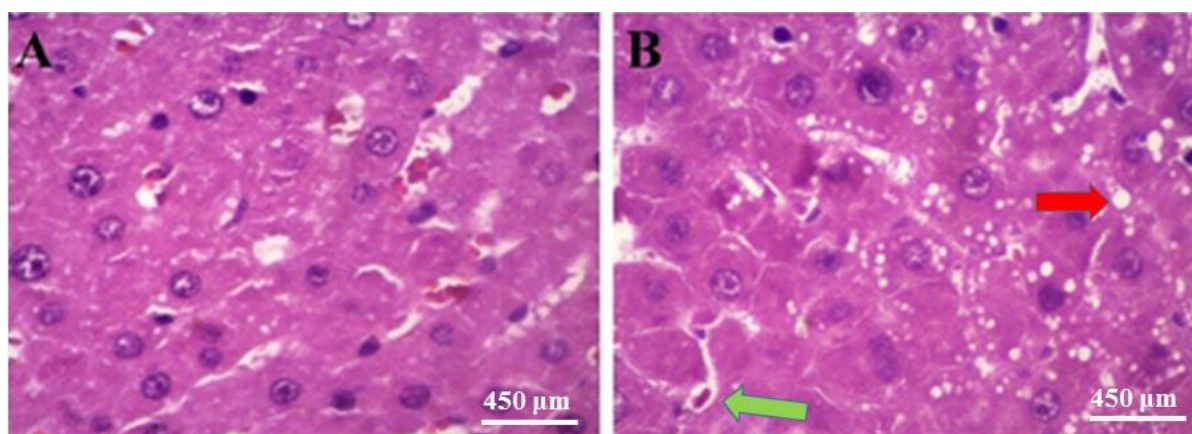


Figure 2. Representative of histological indices (Red: fat droplets, and Green: inflammation and ballooning of the hepatocytes) of H&E staining images of the liver sections in HFD vs ND groups (scale bar= 450 μ m, $\times$ 400 magnification). A) ND and B) HFD. ND: Normal diet, HFD: High fat diet

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 $\pm$ SEM.

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

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

Variable	ND	HFD	HFD + RT + Flaxseed	HFD + HIIT + Flaxseed	HFD + CT + Flaxseed
Urea (mg/dl)	44.48 $\pm$ 3.09	49.86 $\pm$ 3.26	57.00 $\pm$ 8.94	42.07 $\pm$ 4.67	57.84 $\pm$ 4.31
Cr (mg/dl)	0.78 $\pm$ 0.03	0.83 $\pm$ 0.02	0.79 $\pm$ 0.02	0.79 $\pm$ 0.02	0.79 $\pm$ 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- κ B 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- κ B) 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- α (HIF-1 α) 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 responsiveness 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 (Koliampita et al. 2017) and HIIT activates signaling molecules involved in AMP-activated protein kinase and p38 mitogen-activated protein kinase to promote PGC-1 α 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)- β 1/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.

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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 Animals (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:
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References

- Abe T, Kitaoka Y, Kikuchi D, Takeda K, Numata O, Takemasa T (2015) High-intensity interval training-induced metabolic adaptation coupled with an increase in Hif-1 α and glycolytic protein expression. *J. Appl. Physiol.* 119:jap.00499.2015 doi:10.1152/japphysiol.00499.2015
- Achille I, Luigia De F, Carole B (2009) Molecular basis of inherited microcytic anemia due to defects in iron acquisition or heme synthesis. *Haematologica* 94(3):395-408 doi:10.3324/haematol.13619
- Ahmadizad S, El-Sayed MS (2003) The effects of graded resistance exercise on platelet aggregation and activation. *Med Sci Sports Exerc* 35(6):1026-32 doi:10.1249/01.Mss.0000069406.54766.C6
- Ahmed B, Konje JC (2023) The epidemiology of obesity in reproduction. *Best Pract. Res. Clin. Obstet. Gynaecol.* 89:102342 doi:<https://doi.org/10.1016/j.bpobgyn.2023.102342>
- Al-Madhagy S, Ashmawy NS, Mamdouh A, Eldahshan OA, Farag MA (2023) A comprehensive review of the health benefits of flaxseed oil in relation to its chemical composition and comparison with other omega-3-rich oils. *Eur J Med Res* 28(1):240 doi:10.1186/s40001-023-01203-6
- Al Za'abi M, Ali H, Ali BH (2021) Effect of flaxseed on systemic inflammation and oxidative stress in diabetic rats with or

- without chronic kidney disease. *PLoS One* 16(10):e0258800
doi:10.1371/journal.pone.0258800
- Arazi H, Mohabbat M, Saidie P, Falahati A, Suzuki K (2022) Effects of Different Types of Exercise on Kidney Diseases. *Sports (Basel)* 10(3) doi:10.3390/sports10030042
- Astorino TA, Allen RP, Roberson DW, Jurancich M (2012) Effect of high-intensity interval training on cardiovascular function, VO₂max, and muscular force. *J Strength Cond Res* 26(1):138-45
doi:10.1519/JSC.0b013e318218dd77
- Bao C, Yang Z, Cai Q, Li Q, Li H, Shu B (2019) Incremental load training improves renal fibrosis by regulating the TGF- β 1/TAK1/MKK3/p38MAPK signaling pathway and inducing the activation of autophagy in aged mice. *Int J Mol Med* 44(5):1677-1686
doi:10.3892/ijmm.2019.4344
- Bettariga F, Taaffe DR, Crespo-Garcia C, Clay TD, Galvão DA, Newton RU (2024) Effects of resistance training vs high intensity interval training on body composition, muscle strength, cardiorespiratory fitness, and quality of life in survivors of breast cancer: a randomized trial. *Breast Cancer Res Treat*
doi:10.1007/s10549-024-07559-5
- Biglari S, Afousi AG, Mafi F, Shabkhiz F (2020) High-intensity interval training-induced hypertrophy in gastrocnemius muscle via improved IGF-I/Akt/FoxO and myostatin/Smad signaling pathways in rats. *Physiol Int* 107(2):220-230
doi:10.1556/2060.2020.00020
- Buettner R, Schölmerich J, Bollheimer LC (2007) High-fat diets: modeling the metabolic disorders of human obesity in rodents. *Obesity (Silver Spring)* 15(4):798-808
doi:10.1038/oby.2007.608
- Committee ADAPP (2023) 8. Obesity and Weight Management for the Prevention and Treatment of Type 2 Diabetes: Standards of Care in Diabetes–2024. *Diabetes Care* 47(Supplement_1):S145-S157
doi:10.2337/dc24-S008
- de Kruijf EFM, Fibbe WE, van Pel M (2020) Cytokine-induced hematopoietic stem and progenitor cell mobilization: unraveling interactions between stem cells and their niche. *Ann N Y Acad Sci* 1466(1):24-38
doi:10.1111/nyas.14059
- Eslami Z, Joshaghani H, Eghbal Moghanlou A, Norouzi A, Mirghani SJ (2025) Atorvastatin and flaxseed dietary treatments improve dyslipidemia and liver injuries in a diet-induced rat model of non-alcoholic fatty liver disease. *AJP* 15(3):1102-1112
doi:10.22038/ajp.2024.25220
- Eslami Z, Mirghani SJ, Moghanlou AE, et al. (2021) An Efficient Model of Non-alcoholic Fatty Liver Disease (NAFLD) Versus Current Experimental Models: Effects of Fructose, Fat, and Carbon Tetrachloride on NAFLD. *Hepatitis Monthly* 21(8)
- Eslami Z, Rezaei Ghomi M, Saidi A, et al. (2022) Endurance Training and Exogenous Adenosine Infusion Mitigate Hippocampal Inflammation and Cell Death in a Rat Model of Cerebral Ischemia/Reperfusion Injury: A Randomized Controlled Trial. *Arch Neurosci* 9(1):e119236
doi:10.5812/ans.119236
- Fellmann N (1992) Hormonal and plasma volume alterations following endurance exercise. A brief review. *Sports Med* 13(1):37-49
doi:10.2165/00007256-199213010-00004
- Gagliano-Jucá T, Pencina KM, Guo W, et al. (2020) Differential effects of testosterone on circulating neutrophils, monocytes, and platelets in men: Findings from two trials. *Andrology* 8(5):1324-1331
doi:10.1111/andr.12834
- Gibala M (2009) Molecular responses to high-intensity interval exercise. *Appl Physiol Nutr Metab* 34(3):428-32
doi:10.1139/h09-046
- Haase VH (2010) Hypoxic regulation of erythropoiesis and iron metabolism. *Am J Physiol Renal Physiol* 299(1):F1-13
doi:10.1152/ajprenal.00174.2010
- Hallund J, Tetens I, Bügel S, et al. (2006) Daily consumption for six weeks of a lignan complex isolated from flaxseed does not affect endothelial function in healthy postmenopausal women. *J Nutr* 136(9):2314-8
doi:10.1093/jn/136.9.2314
- Hornberger TA, Jr., Farrar RP (2004) Physiological hypertrophy of the FHL muscle following 8 weeks of progressive resistance exercise in the rat. *Can J Appl Physiol* 29(1):16-31
doi:10.1139/h04-002
- Hu M, Finni T, Xu L, Zou L, Cheng S (2011) Effects of resistance training on biomarkers

- of bone formation and association with red blood cell variables. *J Physiol Biochem* 67(3):351-8 doi:10.1007/s13105-011-0082-6
- Hubert Shalanyuy D (2024) EFFECT OF OBESITY ON THE HEMOGRAM PROFILE IN PEOPLE ATTENDING THE REGIONAL HOSPITAL BAFOUSSAM, CAMEROON. 9:2455-2631
- Jeong HR, Lee HS, Shim YS, Hwang JS (2022) Positive Associations between Body Mass Index and Hematological Parameters, Including RBCs, WBCs, and Platelet Counts, in Korean Children and Adolescents. *Children (Basel)* 9(1) doi:10.3390/children9010109
- Jin X, Qiu T, Li L, et al. (2023) Pathophysiology of obesity and its associated diseases. *Acta Pharmaceutica Sinica B* 13(6):2403-2424 doi:<https://doi.org/10.1016/j.apsb.2023.01.012>
- Kausar S, Hussain A, Ashraf S, et al. (2024) Flaxseed (*Linum usitatissimum*); phytochemistry, pharmacological characteristics and functional food applications. *Food Chem. Adv.* 4:100573 doi:<https://doi.org/10.1016/j.focha.2023.10.0573>
- Kaweme NM, Butress SMN, Mantina HM (2022) Long-Term Zidovudine Therapy and Whether It is a Trigger of Vitamin B12 Deficiency: A Case Study of Megaloblastic Anemia at the University of Zambia Teaching Hospital. *Case Rep Hematol* 2022:3827616 doi:10.1155/2022/3827616
- Kazemi SS, Heidarianpour A, Shokri E (2023) Effect of resistance training and high-intensity interval training on metabolic parameters and serum level of Sirtuin1 in postmenopausal women with metabolic syndrome: a randomized controlled trial. *Lipids Health Dis* 22(1):177 doi:10.1186/s12944-023-01940-x
- Kim CY, Larsen HJ, Spitalnik SL, et al. (2023) Low-Dose Dietary Fish Oil Improves RBC Deformability without Improving Post-Transfusion Recovery in Mice. *Nutrients* 15(20):4456
- Koch M, Cegla J, Jones B, et al. (2019) The effects of dyslipidaemia and cholesterol modulation on erythrocyte susceptibility to malaria parasite infection. *Malar. J.* 18 doi:10.1186/s12936-019-3016-3
- Koliamitra C, Holtkamp B, Zimmer P, Bloch W, Grau M (2017) Impact of training volume and intensity on RBC-NOS/NO pathway and endurance capacity. *Biorheology* 54(1):37-50 doi:10.3233/bir-16121
- Kurmangaliyeva S, Baktikulova K, Tkachenko A, et al. (2025) Eryptosis in Liver Diseases: Contribution to Anemia and Hypercoagulation. *Med. Sci.* 13(3):125
- Lai CC, Tang CY, Fu SK, Tseng KW, Yu CH, Wang CY (2025) High-intensity interval training attenuates renal injury induced by myocardial ischemia-reperfusion in rats. *J Chin Med Assoc* 88(2):126-137 doi:10.1097/jcma.0000000000001183
- Leandro CG, Levada AC, Hirabara SM, et al. (2007) A program of moderate physical training for Wistar rats based on maximal oxygen consumption. *J Strength Cond Res* 21(3):751-6 doi:10.1519/r-20155.1
- Lee JC, Bhora F, Sun J, et al. (2008) Dietary flaxseed enhances antioxidant defenses and is protective in a mouse model of lung ischemia-reperfusion injury. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 294(2):L255-L265 doi:10.1152/ajplung.00138.2007
- Ling J, Chen S, Zahry NR, Kao TA (2023) Economic burden of childhood overweight and obesity: A systematic review and meta-analysis. *Obes Rev* 24(2):e13535 doi:10.1111/obr.13535
- Longhi R, Almeida RF, Machado L, et al. (2017) Effect of a trans fatty acid-enriched diet on biochemical and inflammatory parameters in Wistar rats. *Eur J Nutr* 56(3):1003-1016 doi:10.1007/s00394-015-1148-y
- Maestroni G (2025) The Sympathetic Nervous Influence on Hematopoiesis Up To Date. *J Neuroimmune Pharmacol* 20(1):61 doi:10.1007/s11481-025-10220-7
- Merle A, Jollet M, Britto FA, et al. (2019) Endurance exercise decreases protein synthesis and ER-mitochondria contacts in mouse skeletal muscle. *J Appl Physiol* (1985) 127(5):1297-1306 doi:10.1152/japplphysiol.00196.2019
- Musso G, Gambino R, Cassader M, Pagano G (2010) A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. *Hepatology* 52(1):79-104 doi:10.1002/hep.23623
- Nieman DC, Pedersen BK (1999) Exercise and immune function. Recent developments. *Sports Med* 27(2):73-80 doi:10.2165/00007256-199927020-00001

- Obeagu EI, Igwe MC, Obeagu GU (2024) Oxidative stress's impact on red blood cells: Unveiling implications for health and disease. *Medicine* (Baltimore) 103(9):e37360 doi:10.1097/md.00000000000037360
- Okon IA, Beshel JA, Owu DU, Orie NN, Jim AE, Edet LI (2025) Moderate aerobic exercise improves haematological indices without altering cardio-metabolic enzyme activities in sedentary healthy young adults. *BMC Sports Sci Med Rehabil* 17(1):32 doi:10.1186/s13102-025-01080-y
- Okwuegbuna OK, Kaur H, Jennifer I, et al. (2023) Anemia and Erythrocyte Indices Are Associated With Neurocognitive Performance Across Multiple Ability Domains in Adults With HIV. *J Acquir Immune Defic Syndr* 92(5):414-421 doi:10.1097/qai.0000000000003155
- Prasad K (2009) Flaxseed and cardiovascular health. *J Cardiovasc Pharmacol* 54(5):369-77 doi:10.1097/FJC.0b013e3181af04e5
- Proske U, Morgan DL (2001) Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J Physiol* 537(Pt 2):333-45 doi:10.1111/j.1469-7793.2001.00333.x
- Putra KP, Ardha MAA, Kinasih A, Aji RS (2017) Korelasi perubahan nilai VO2max, eritrosit, hemoglobin dan hematokrit setelah latihan high intensity interval training. *J. Keolahragaan* 5(2):161-170
- Reljic D (2025) High-Intensity Interval Training as Redox Medicine: Targeting Oxidative Stress and Antioxidant Adaptations in Cardiometabolic Disease Cohorts. *Antioxidants* (Basel) 14(8) doi:10.3390/antiox14080937
- Rizwan S, Naqshbandi A, Farooqui Z, Khan AA, Khan F (2014) Protective effect of dietary flaxseed oil on arsenic-induced nephrotoxicity and oxidative damage in rat kidney. *FCT* 68:99-107 doi:<https://doi.org/10.1016/j.fct.2014.03.011>
- Sarkar S, Dey SK, Datta G, Bandyopadhyay A (2023) Vitamin C and E supplementation and high intensity interval training induced changes in lipid profile and haematological variables of young males. *SPORTS MED HLTH SCI* 5(2):137-145 doi:<https://doi.org/10.1016/j.smhs.2023.03.006>
- Saud A, Luiz RS, Leite APO, et al. (2021) Resistance exercise training ameliorates chronic kidney disease outcomes in a 5/6 nephrectomy model. *Life Sci.* 275:119362 doi:<https://doi.org/10.1016/j.lfs.2021.119362>
- Skouras AZ, Antonakis-Karamintzas D, Tsantes AG, et al. (2023) The Acute and Chronic Effects of Resistance and Aerobic Exercise in Hemostatic Balance: A Brief Review. *Sports* (Basel) 11(4) doi:10.3390/sports11040074
- Stuglin C, Prasad K (2005) Effect of Flaxseed Consumption on Blood Pressure, Serum Lipids, Hemopoietic System and Liver and Kidney Enzymes in Healthy Humans. *JCPT* 10:23-7 doi:10.1177/107424840501000103
- Varghese J, Varghese JJ, Jacob M (2025a) Effect of a high-fat diet and iron overload on erythropoiesis in mice. *Biochem. Biophys. Rep.* 41:101919 doi:<https://doi.org/10.1016/j.bbrep.2025.101919>
- Varghese J, Varghese JJ, Jacob M (2025b) Effect of a high-fat diet and iron overload on erythropoiesis in mice. *Biochem Biophys Rep* 41:101919 doi:10.1016/j.bbrep.2025.101919
- Velasquez MT, Bhatena SAMJ, Ranich T, et al. (2003) Dietary flaxseed meal reduces proteinuria and ameliorates nephropathy in an animal model of type II diabetes mellitus. *Kidney International* 64(6):2100-2107 doi:<https://doi.org/10.1046/j.1523-1755.2003.00329.x>
- Visco V, Izzo C, Bonadies D, et al. (2023) Interventions to Address Cardiovascular Risk in Obese Patients: Many Hands Make Light Work. *J Cardiovasc Dev Dis* 10(8) doi:10.3390/jcdd10080327
- Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB (2001) Low-grade systemic inflammation in overweight children. *Pediatrics* 107(1):E13 doi:10.1542/peds.107.1.e13
- Wang CY, Babitt JL (2016) Heparin regulation in the anemia of inflammation. *Curr Opin Hematol* 23(3):189-97 doi:10.1097/moh.0000000000000236
- Webb KL, Gorman EK, Morkeberg OH, et al. (2023) The relationship between hemoglobin and [Formula: see text]: A systematic review and meta-analysis. *PLoS*

- One 18(10):e0292835
doi:10.1371/journal.pone.0292835
- Xie Y, Bowe B, Li T, Xian H, Yan Y, Al-Aly Z (2018) Higher blood urea nitrogen is associated with increased risk of incident diabetes mellitus. *Kidney International* 93(3):741-752
doi:<https://doi.org/10.1016/j.kint.2017.08.033>
- Yang W, Fu J, Yu M, et al. (2012) Effects of flaxseed oil on anti-oxidative system and membrane deformation of human peripheral blood erythrocytes in high glucose level. *Lipids Health Dis* 11:88
doi:10.1186/1476-511x-11-88
- Yang Y, Bay PB, Wang YR, Huang J, Teo HWJ, Goh J (2018) Effects of Consecutive Versus Non-consecutive Days of Resistance Training on Strength, Body Composition, and Red Blood Cells. *Front Physiol* 9:725
doi:10.3389/fphys.2018.00725
- Zahra Eslami MG, Abdolreza Eghbal Moghanlou, Shohreh Sharifian, Ghaysar Beyshami (2023) The effect of endurance training combined with adenosine on the gene expression of UCP-1 and MAPK p38 in subcutaneous adipose tissue of male Wistar rats fed a high-fat diet. *Yafteh* 25(3)
- Zhao G, Etherton TD, Martin KR, Gillies PJ, West SG, Kris-Etherton PM (2007) Dietary alpha-linolenic acid inhibits proinflammatory cytokine production by peripheral blood mononuclear cells in hypercholesterolemic subjects. *Am J Clin Nutr* 85(2):385-91
doi:10.1093/ajcn/85.2.385
- Zheng L, Qin R, Rao Z, Xiao W (2023) High-intensity interval training induces renal injury and fibrosis in type 2 diabetic mice. *Life Sci* 324:121740
doi:10.1016/j.lfs.2023.121740