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The effect of high-intensity interval training and vitamin D_3 supplementation on fructose-induced liver inflammation in male Sprague-Dawley rats

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Article

Keywords: Inflammation, Liver M1 macrophages, Liver M2 macrophages, Liver fibrosis, Intrahepatic lipid

Posted Date: July 15th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10120922/v1

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Additional Declarations: No competing interests reported.

Abstract

Fructose consumption leads to fat accumulation and systemic inflammation in the liver, impairing its function and leading to fibrosis. Conversely, physical activity and vitamin D_3 supplementation exhibit anti-inflammatory properties. This study evaluated the impact of High-Intensity Interval Training (HIIT), vitamin D_3 supplementation, and their combined effects on inflammation and liver function in male Sprague-Dawley rats on a high-fructose diet. Forty rats were divided into five groups (n = 8 each): Control with a Standard Diet (CS), Control with a High-fructose Diet (CF), High-fructose Diet with HIIT (FT), High-fructose Diet with Vitamin D_3 (FD), and High-fructose Diet with both HIIT and Vitamin D_3 (FTD). The intervention lasted for 12 weeks. Various assays (ELISA, GOD-POD, auto analyzer, coagulation analyzer) and staining techniques (Oil red O, Masson's trichrome, immunofluorescence) were employed to measure insulin, IL-1β, IL-10, fasting blood glucose (FBG), albumin (ALB), leukocytes, prothrombin time (PT), intrahepatic lipid accumulation, liver fibrosis, and macrophage polarization (M1 and M2). Both HIIT and vitamin D_3 supplementation significantly reduced leukocytes, lymphocytes, neutrophils, monocytes, IL-1β, IL-10, F4/80 + CD206+ and F4/80 + CD11c+ cells, PT, liver fibrosis, hepatic fat accumulation, insulin, and FBG levels while significantly increasing serum ALB levels. HIIT was more effective than vitamin D_3 in lowering cytokine levels, hepatic fat accumulation, PT, insulin, and FBG levels. The combined intervention of HIIT and vitamin D_3 had the most pronounced effect, aligning most marker levels with those of the CS group, except for glucose and PT. Consumption of fructose leads to the development of diabetes and liver inflammation. HIIT and vitamin D_3 supplementation reduce inflammation, prevent diabetes, and improve liver function. The combined effect of the two interventions was more effective than their individual effects.

Introduction

Hepatic inflammation frequently triggers liver disease and is a key factor in hepatic tissue damage [1]. The liver's macrophages, which include resident Kupffer cells, are pivotal to mediating liver inflammation. Upon hepatocyte injury, these cells become activated, producing cytokines [2] that drive inflammatory responses, recruit circulating macrophages, promote the development of fatty liver, and ultimately impair hepatic insulin sensitivity [3]. Broadly, these macrophages can be categorized into M1 and M2 types, with M1 macrophages exhibiting pro-inflammatory activities and M2 macrophages showing anti-inflammatory properties. M1 macrophages upregulate cytokines such as Interleukin-1 beta (IL-1β), while M2 polarization is driven by factors like Interleukin 10 (IL-10) [4]. An imbalance in the M1/M2 polarization ratio can lead to liver inflammation and is often linked to various diseases [1].

Research indicates that fructose can trigger systemic inflammation in the liver [5]. High-fructose consumption has been found to result in the accumulation of lipids in the liver and reduced insulin sensitivity, along with a significant rise in inflammatory markers [6]. Laboratory studies have demonstrated that fructose can directly influence macrophages through the glucose transporter 5 (GLUT5), prompting them to release inflammatory cytokines. This inflammation impairs liver function [7].

Moreover, liver fibrosis can result from hepatic inflammation and the activation of the body's innate immune response [2].

Exercise is known for its anti-inflammatory benefits, which may help protect against diseases associated with chronic inflammation. Specifically, high-intensity interval training (HIIT)—involving periods of near-maximal aerobic activity alternated with lower-intensity exercise—has been shown to affect inflammatory biomarkers [8] and reduce pro-inflammatory indices, thereby benefiting the immune system [9]. Additionally, Vitamin D_3 has been found to exert anti-inflammatory effects on the liver [10] and suppress liver fibrosis, suggesting a potentially valuable nutritional supplement for treating liver disease [11].

Based on the previous studies, our hypothesis is that consumption of a high-fructose diet promotes hepatic inflammation, resulting in the accumulation of fat in the liver. On the other hand, HIIT and vitamin D_3 intake could potentially improve the inflammatory profile. However, previous studies have explored the individual impacts of HIIT [12, 13] and vitamin D3 supplementation [14] on liver inflammation in rats without the presence of fructose. In addition, the studies have investigated the separate effects of vitamin D3 supplementation [15, 16] and HIIT [17] on inflammation markers in the rats consuming a high-fructose diet and the timing of fructose consumption did not align with the administration of these interventions in these studies. It is essential to determine the preventive effect of HIIT and vitamin D_3, as well as the interactive effect of these two interventions on liver inflammation in the rats simultaneously consuming a high-fructose diet. Accordingly, the following research inquiries were formulated and addressed in the present study:

1. Does participating in HIIT and taking vitamin D_3 supplements, while simultaneously consuming a high-fructose diet, aid in lessening the negative consequences of fructose?
2. Which of the training and nutrient interventions is more effective in influencing liver inflammation and function markers?
3. Is it possible for the amalgamation of training and nutrient interventions to produce better outcomes in averting hepatic inflammation in contrast to the separate impacts of both, potentially yielding more advantageous health consequences?

Materials and methods

All animal procedures were reviewed and approved by the Guilan University of Medical Sciences Ethical Committee (Approval ID: IR.GUILAN.REC.1402.024). Although the authors are affiliated with the University of Guilan, ethical review and approval for the animal procedures were obtained from the Guilan University of Medical Sciences Ethical Committee, as the responsible institutional ethics committee providing ethical oversight for this animal study. All methods were performed in accordance with the relevant institutional, national, and international guidelines and regulations for the care and use of laboratory animals. The study was designed and reported in accordance with the ARRIVE guidelines, where applicable.

Experimental animals

Forty mature male Sprague-Dawley rats, aged 7 weeks and weighing 200–250 g, were purchased from the Pasteur Institute of Iran, Tehran, Iran. The animals were housed four per cage in polypropylene cages under controlled environmental conditions, including a temperature of 22 ± 2°C, a 12-hour light/dark cycle, and 50% humidity.

Experimental design

The animals were fed a normal diet for rodents, which included 21.23% protein, 11.95% fat, and 66.82% carbohydrates. This diet followed the recommendations in AIN-93M published by Reeves et al. [18]. Following a week of acclimatization, the rats were distributed randomly into five groups, each consisting of eight rats: Control group with a Standard Diet (CS); Control group with a High-fructose Diet (CF); High-fructose Diet+ HIIT (FT); High-Fructose Diet+ Vitamin D_3 supplementation (FD); and High-fructose Diet+ HIIT+ Vitamin D_3 supplementation (FTD). For a duration of 12 weeks, the fructose-fed groups were given a standard diet with fresh 10% (w/v) fructose solution in their drinking water while the CS group was given standard food and regular water. Every group included in the experiment had unrestricted access to food and beverages.

Exercise performance test

To determine maximum running speed, the rats were placed on a treadmill with a 0° inclination. They began with a 5-minute warm-up at a speed of 6 m/min. Following this, the speed was increased by 2 m/min every 2 minutes until the rats could no longer continue [19]. Exhaustion was identified when a rat stayed on the motivating shockers for over 10 seconds instead of running [20]. Running speed measurements were used to adjust training intensity throughout the regimen [21]. Performance tests were administered to all groups at the beginning and end of the study, with an additional test for the training groups at the 5-week mark to recalibrate training intensity [19].

Exercise training

For 12 weeks, five days a week, the training groups participated in HIIT on a treadmill set at an inclination of 0°. The regimen consisted of ten 4-minute sessions of high-intensity running at 85%–90% of maximum speed, interspersed with 2-minute active rest periods at 50% of maximum speed. The intensity of the intervals gradually increased over the first 10 weeks and was maintained for the final two weeks. Each daily session included 5 minutes of warm-up and cool-down periods at low intensity, and running at 45%–50% of maximum speed [22].

Vitamin D supplementation

Intraperitoneal injection (IP) of 10,000 international units per kilogram (IU/kg) of vitamin D_3 supplementation was administered to the FD and FTD groups of rodents. The full dose was delivered in a single administration on a designated day each week for a total of 12 weeks.

Anthropometric and nutritional measurements

Body length (from nose to anus) was measured after 12 weeks in the anesthetized rats. Body weight was recorded on a weekly basis. waist circumference is measured using metline. These measurements were used to calculate the Lee index, defined as the cube root of body weight (g) divided by nose-to-anus length (cm) [23], and the specific rate of body mass gain (g/kg), defined as dM/M dt, where dM is the gain in body weight during the interval dt = t2 - t1, and M represents the rat's body weight at t1 [24].

Daily monitoring of food and liquid intake was conducted by measuring the difference between the amounts provided and the amounts remaining after 24 hours. This assessment took place consistently between 9:00 and 10:00 AM throughout the whole period. Additionally, energy intake was calculated as the product of average food consumption and dietary metabolizable energy. Feed efficiency was determined using the following formula: (mean body weight gain in grams × 100) / energy intake in kilocalories. These evaluations were based on food and liquid consumption specified in the methodology described by Diniz et al. [25].

Anesthesia, euthanasia, sample collection, and tissue handling procedures

Following a 12-hour fasting period at the end of the intervention period, animals were deeply anesthetized 48 h after the final training session using ketamine hydrochloride and xylazine hydrochloride (60 and 6 mg/kg body weight, respectively, intraperitoneally) to avoid the acute effects of exercise. Adequate depth of anesthesia was confirmed by the absence of pedal withdrawal and corneal reflexes. While under deep anesthesia, blood samples were collected from the inferior vena cava using a sterile syringe and needle, and euthanasia was completed by exsanguination. This procedure represents a chemical anesthesia followed by a physical euthanasia method. Death was confirmed by the cessation of heartbeat and respiration before tissue collection. Blood was collected into two separate tubes, one containing sodium citrate as an anticoagulant and one without anticoagulant. Samples were centrifuged at 3000 rpm for 15 min to obtain plasma and serum. Liver samples were then dissected, collected, and weighed. The right liver lobes were fixed in 10% formalin for subsequent histological and immunohistochemical analyses. Relative liver weight was calculated as the ratio of liver weight to body weight.

Biochemical analysis

Fasting blood glucose (FBG) was measured using commercially available colorimetric diagnostic kits (Pars Azmun Kit, Alborz, Iran) based on the GOD-POD (glucose oxidase-peroxidase) following the provided instructions. Serum insulin levels were determined by a rat ELISA kit (American Laboratory Products Co., New Hampshire, USA, Cat # 80-INSRTH-E01, E10).

Insulin resistance and pancreatic β-cell function

The homeostasis model assessment for β-cell function (HOMA-β) serves as an indicator of insulin secretion, while HOMA-IR is utilized to assess insulin resistance. These indices were computed using the formulas provided by Song et al. [26]:HOMA-IR = (Fasting Insulin μU/ml) × (Fasting Glucose mmol/l) / 22.5HOMA-β = (20 × Fasting Insulin μU/ml) / (Fasting Glucose mmol/l – 3.5)

Measurement of inflammation markers

Serum levels of IL-1β and IL-10 were quantified using enzyme-linked immunosorbent assay (ELISA) with commercially available kits (DY501-05 and DY522-05; R&D Systems, USA), following the protocols provided by the manufacturer. The blood samples were used for the determination of total leukocyte, lymphocytes, neutrophil, monocyte, eosinophil, and basophils counts using a Sysmex xs800i Automated Hematology Analyzer (Sysmex Corporation, Japan).

Inflammatory indexes

The neutrophil to lymphocyte ratio (NLR) was obtained by dividing the neutrophil count by the lymphocyte count. The systemic immune-inflammation index (SII) was calculated by multiplying the NLR with the platelet count. The neutrophil to monocyte ratio (NMR) was calculated by dividing the neutrophil count by the monocyte count. The monocyte to lymphocyte ratio (MLR) was derived by dividing the monocyte count by the lymphocyte count. The platelet to lymphocyte ratio (PLR) was also determined by dividing the platelet count by the lymphocyte count. The leucocyte to lymphocyte ratio (LLR) was derived by dividing the leucocyte count by the lymphocyte count. Prognostic nutritional index (PNI), was calculated using the albumin level (ALB) reflecting nutritional status and lymphocyte count reflecting immune status: [(10 × serum albumin (g/dL)) + (0.005 × total lymphocyte count)]. The Lymphocyte–Albumin–Neutrophil Ratio (LANR) is a novel biomarker was calculated as follows: LANR = ALB × lymphocyte/neutrophil.

Measurement of liver function markers

Serum ALB levels were analyzed using an autoanalyzer following the standard protocols. Prothrombin Time (PT) was determined using an automatic coagulation analyzer. Citrated plasma (50 μL) was incubated at 37°C for one minute, followed by the addition of PT reagent (Neoplastin CI Plus; Diagnostica Stago) warmed at 37°C (100 μL). The time from mixing the plasma with the reagent to clot formation was measured in seconds [27].

Immunohistochemistry staining of macrophages

A double immunofluorescence staining of F4/80 (general macrophage marker), CD206 (marker of M2 macrophages), and CD11c (marker of M1 macrophages) was performed. The methods have been detailed in earlier publications (28). Briefly, Cross-sections were first fixed with 4% paraformaldehyde for 30 minutes, followed by blocking with 10% bovine serum albumin. The conjugated antibodies utilized were Alexa Fluor® 488 anti-mouse F4/80 Antibody (diluted at 1:1000), Alexa Fluor® 647 anti-mouse CD206 (MMR) Antibody (diluted at 1:1000), and Integrin aX CD11c Antibody (D-8): sc-398708 (diluted at 1:1000) which were manufactured by BioLegend, San Diego, California, USA. Cell nuclei were counterstained with DAPI (Vectashield, Vector Laboratories, Burlingame, CA, USA). Imaging of the slides was carried out using a BX50 fluorescence microscope from Olympus-Germany, and image analysis was performed using the Image J software.

Histological analysis

Oil red O staining

Oil red O staining was applied to assess lipid droplet formation in the liver tissues. The animal's liver tissues were first fixed in 4% paraformaldehyde and then embedded in paraffin and sectioned into slices with 6-µm thickness. These sections were then de-waxed in xylene and rehydrated. After rinsing with PBS, the tissue sections were incubated in oil red O reagent for 30 min, followed by hematoxylin counter-staining for 1 min. After washing and dehydration, the oil red O and hematoxylin-stained sections were mounted on fluorescence microscope (BX50, Olympus- Germany, AXIOM) for imaging [29].

Masson's trichrome staining

Masson's trichrome is a stain that highlights collagen fibers. Briefly, sections (thickness = 5 µm) of formalin-fixed, paraffin-embedded livers were placed on standard microscopy slides. After deparaffinisation and rehydration, the slides were immersed in Bouin's solution (HT 10132, Sigma-Aldrich) at 56°C for 15 min. Subsequently, the slides were washed with tap water for 5 min. Next, the sections were stained in Weigert's hematoxylin for 5 min, and then washed again with tap water for 5 min and rinsed in distilled water. Next, the slides were stained in Biebrich scarlet-acid fuchsin for 5 min, rinsed in distilled water, incubated in phosphotungstic-phosphomolybdic acid for 5 min, dyed with aniline blue for 5 min, and fixed in 1% acetic acid for 2 min. Finally, the slides were rinsed in distilled water, dehydrated, and mounted [30].

Data analysis

All the data were presented as mean ± standard deviation. The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test on SPSS (version 23.0, IBM Corporation, Armonk, NY, USA). The normal distribution of the data and the homogeneity of variances were assessed using the Shapiro-Wilk test and the Levene's test, respectively. The correlation between the variables was examined using Pearson correlation method.

Results

Anthropometrical determinations

Table 1 presents anthropometric indices in 5 groups of rats. The collected data showed no significant difference in height, initial and final weight, weight gain, waist circumference, and Lee index among the research groups ($p > 0.05$). However, HIIT significantly increased specific rate of body mass gain (g/kg) compared to the CS group. Consumption of a 12-week high-fructose diet resulted in a significant increase in liver weight and relative weight in the CF group compared to the CS group. Conversely, a significant decrease in liver weight and relative weight was observed in the intervention groups compared to the CF group ($p < 0.05$).

Table 1
Anthropometric indices in five groups of rats (CS, CF, FT, FD and FTD)

Parameters	Groups				
	CS	CF	FT	FD	FTD
Body length (cm)	24.1 ± 0.83	24.25 ± 0.88	24.12 ± 0.83	24.50 ± 1.19	23.75 ± 1.83
Initial body weight (g)	256.62 ± 20.49	238.62 ± 6.27	237.37 ± 21.38	244.25 ± 11.28	254.87 ± 21.16
Final body weight (g)	348 ± 39.73	352.37 ± 16.10	361.75 ± 38.69	348.62 ± 43.24	370.75 ± 44.62
Body weight gain (g/day)	91.37 ± 34.30	113.75 ± 11.17	124.37 ± 21.11	104.0 ± 41.85	117.12 ± 25.42
Specific rate of body mass gain (g/kg)	0.35 ± 0.14	0.47 ± 0.03	0.52 ± 0.07 [a]	0.42 ± 0.17	0.45 ± 0.08
Waist circumference (cm)	17.50 ± 0.92	18.87 ± 1.12	17.50 ± 0.92	18.12 ± 1.45	17.37 ± 1.06
Lee index	0.29 ± 0.01	0.29 ± 0.13	0.29 ± 0.11	0.28 ± 0.01	0.30 ± 0.26
Liver weight (g)	8.67 ± 0.39 [b]	12.31 ± 1.92	10.28 ± 0.95 [b]	10.28 ± 1.33 [b]	10.06 ± 0.88 [b]
Relative weight (g/100 g body weight)	2.52 ± 0.36 [b]	3.49 ± 0.56	2.86 ± 0.30 [b]	2.95 ± 0.22 [b]	2.72 ± 0.31 [b]

Values are mean ± standard deviation.

Means with different superscripts differ significantly, $P < 0.05$.

CS: Control group with a Standard Diet; CF: Control group with a High-fructose Diet; FT: High-fructose Diet + HIIT intervention; FD: High-fructose Diet + Vitamin D_3 intervention; FTD: High-fructose Diet + HIIT + Vitamin D_3 interventions.

[a]: significant difference with CS, [b]: significant difference with CF.

Nutritional measurements

According to Table 2, food consumption significantly decreased in fructose-fed rats compared to the CS group ($p < 0.05$). The results did not reveal a significant difference in water/fructose solution consumption between fructose-fed rats and rats on a standard diet ($p > 0.05$). Energy intake in the CF and FTD groups showed a significant decrease compared to the CS group. The results also demonstrated a significant increase in FE in the FTD group in comparison to the CS group ($p < 0.05$).

Table 2
Nutritional indices in the five groups of rats (CS, CF, FT, FD and FTD)

Parameters	Groups				
	CS	CF	FT	FD	FTD
Food consumption (g/day/rat)	22.37 ± 1.73	14.12 ± 1.60 [a]	15.28 ± 2.13 [a]	14.34 ± 1.73 [a]	14.57 ± 2.33 [a]
Water / fructose solution consumption (ml/day/rat)	47.07 ± 7.78	48.04 ± 6.73	48.70 ± 5.45	49.66 ± 4.08	44.12 ± 5.24
Energy intake* (kcal/day)	84.33 ± 6.55	72.47 ± 6.25 [a]	77.09 ± 9.16	73.95 ± 7.02	72.59 ± 10.00 [a]
Feed efficiency (%)	109.40 ± 43.40	157.76 ± 18.85	161.33 ± 20.91	139.60 ± 47.78	164.49 ± 41.65 [a]
Values are mean ± standard deviation.					
Means with different superscripts differ significantly, $P < 0.05$.					
CS: Control group with a Standard Diet; CF: Control group with a High-fructose Diet; FT: High-fructose Diet + HIIT intervention; FD: High-fructose Diet + Vitamin D_3 intervention; FTD: High-fructose Diet + HIIT + Vitamin D_3 interventions.					
* Energy intake from food and fructose solution.					
[a]: significant difference with CS.					

Exercise performance test

Table 3 illustrates the changes in exercise performance of rats over two phases (initial and after 12 weeks) in the CS, CF, and FD groups, and three phases (initial, intermediate, and after 12 weeks) in the training groups. The 12-week HIIT resulted in a significant increase in maximum speed, running time, and running distance in the rats ($p < 0.05$).

Table 3
Exercise performance in the five groups of rats (CS, CF, FT, FD and FTD)

Parameters	Time	Groups				
		CS	CF	FT	FD	FTD
Maximal Speed ($m.min^{-1}$)	Pre	21.50 ± 2.07	22.0 ± 2.39	22.50 ± 2.07	21.50 ± 2.32	22.25 ± 2.71
	Mid	-	-	38.25 ± 4.46 [b]	-	38 ± 4 [b]
	Post	20.50 ± 3.33 [a]	21.25 ± 1.48 [a]	41.62 ± 4.20 [b]	21.50 ± 1.77 [a]	48 ± 3.66 [b]
Running Time (min)	Pre	15.50 ± 2.07	16 ± 2.39	16.50 ± 2.07	15.50 ± 2.32	16.25 ± 2.71
	Mid	-	-	32.25 ± 4.46 [b]	-	32 ± 4 [b]
	Post	14.50 ± 3.33 [a]	15.25 ± 1.48 [a]	35.50 ± 4.10 [b]	15.50 ± 1.77 [a]	35.50 ± 3.66 [b]
Running Distance (m)	Pre	231 ± 50.58	244 ± 58.35	255 ± 51.12	232 ± 56.93	250.50 ± 66.75
	Mid	-	-	758.50 ± 169.35 [b]	-	747 ± 156.17 [b]
	Post	212 ± 81.33 [a]	244.50 ± 34.89 [a]	890 ± 182.59 [b]	231 ± 42.26 [a]	888.50 ± 158.36 [b]

Values are mean ± standard deviation.

Means with different superscripts differ significantly, $P < 0.05$.

CS: Control group with a Standard Diet; CF: Control group with High-fructose Diet; FT: High-fructose Diet + HIIT intervention; FD: High-fructose Diet + Vitamin D_3 intervention; FTD: High-fructose Diet + HIIT + Vitamin D_3 interventions.

[a]: Significant difference with FT and FTD.

[b]: Significant difference with Pre Time.

Inflammation markers

As a result of consuming fructose for 12 weeks, a significant increase was observed in the levels of leukocytes, lymphocytes, platelet, IL-1β and IL-10 in the CF, FT, and FD groups, and neutrophils as well as monocytes in the rats of the CF group, compared to the CS group ($p < 0.05$). Conversely, no significant difference was discovered in the levels of eosinophils and basophils among the groups ($p > 0.05$). HIIT

and vitamin D_3 supplement interventions caused decreased levels of leukocytes, lymphocytes, neutrophils, monocytes, platelet, IL-1β, and IL-10 compared to the CF group ($p < 0.05$). There was no significant difference between HIIT and vitamin D_3 supplementation in reducing leukocytes, lymphocytes, neutrophils, and monocytes ($p > 0.05$). However, HIIT was more effective than vitamin D_3 supplementation in reducing platelet, IL-1β and IL-10 levels ($p < 0.05$). Additionally, the interaction between vitamin D_3 supplementation and HIIT led to no significant difference in the levels of leukocytes, lymphocytes, platelet, IL-1β, and IL-10 between the CS and FTD groups of rats ($p > 0.05$). The levels of leukocytes, IL-1β and IL-10 are depicted in Fig. 1. No significant difference was also found in the NLR, NMR, MLR, LLR and IL-1β/IL-10 ratio among the research groups ($p > 0.05$). However, SII revealed a significant increase in the CF group compared to the other groups. PLR showed a significant decrease in the CS and FTD groups in comparison to the CF group. In addition, a significant decrease was observed in PNI and LANR in the CF, FT, and FD groups compare to the CS group. The PNI index showed a significant increase as a result of HIIT and vitamin D_3 supplementation compared to the CF group. ($p < 0.05$) (see Table 4).

Table 4
Inflammatory indexes in the five groups of rats (CS, CF, FT, FD and FTD)

Parameters	Groups				
	CS	CF	FT	FD	FTD
Platelets	94.49 ± 17.50	131.09 ± 46.34 [a]	40.30 ± 14.25 [a b]	121.86 ± 43.08[abc]	33.05 ± 11.68 [b d]
NLR	0.24 ± 0.08	0.29 ± 0.05	0.25 ± 0.04	0.23 ± 0.02	0.26 ± 0.05
NMR	2.89 ± 1.41	2.59 ± 0.99	3.94 ± 2.45	3.82 ± 2.48	4.28 ± 2.48
MLR	0.09 ± 0.32	0.12 ± 0.04	0.08 ± 0.04	0.07 ± 0.032	0.07 ± 0.035
PLR	101.65 ± 15.85 [b]	123.50 ± 17.24	105.72 ± 7.62	115.22 ± 13.21	102.39 ± 6.71 [b]
SII	175.38 ± 67.08 [b]	363.59 ± 92.96	227.92 ± 33.34 [b]	243.11 ± 28.95 [b]	201.12 ± 45.71 [b]
LLR	1.38 ± 0.29	1.43 ± 0.05	1.36 ± 0.11	1.32 ± 0.06	1.35 ± 0.10
PNI	54.53 ± 2.50	36.42 ± 4.13 [a]	43.41 ± 2.61 [a b]	41.60 ± 0.71 [a b]	50.66 ± 2.61 [b d]
LANR	24.71 ± 8.88	12.90 ± 3.36 [a]	17.18 ± 3.13 [a]	17.78 ± 2.27 [a]	19.64 ± 3.19
IL-1β/IL-10	0.52 ± 0.11	0.55 ± 0.08	0.54 ± 0.02	0.58 ± 0.06	0.52 ± 0.01

Values are mean ± standard deviation.

Means with different superscripts differ significantly, $P < 0.05$.

CS: Control group with a Standard Diet; CF: Control group with a High-fructose Diet; FT: High-fructose Diet + HIIT intervention; FD: High-fructose Diet + Vitamin D_3 intervention; FTD: High-fructose Diet + HIIT + Vitamin D_3 interventions.

NLR: The neutrophil to lymphocyte ratio, NMR: The neutrophil to monocyte ratio, MLR: The monocyte to lymphocyte ratio, PLR: The platelet to lymphocyte ratio, SII: The systemic immune-inflammation index.

[a]: Significant difference with CS, [b]: Significant difference with CF, [c]: Significant difference with FT, [d]: Significant difference with FD and FT.

Immunofluorescence of M1 and M2 macrophages

Double immunofluorescence results showed that, in response to a 12-week high-fructose diet, F4/80 + CD11c+ cells (Figs. 2) and F4/80 + CD206+ cells (Fig. 3) increased significantly in the liver of CF, FT, and FD groups of rats as against the CS group. HIIT and vitamin D_3 supplementation significantly decreased

the percentage of M1 and M2 macrophages compared to the CF group ($p < 0.05$), with no significant difference observed between HIIT and vitamin D_3 supplementation ($p > 0.05$). Additionally, the results revealed no significant difference in the percentage of M1 and M2 macrophages between the CS and FTD groups due to the interactive effect of vitamin D_3 supplementation and HIIT ($p > 0.05$).

Liver function markers

The obtained results regarding liver function markers are shown in Fig. 4. Changes in ALB levels and PT after 12 weeks of fructose consumption were associated with a decrease in ALB in the CF, FT, and FD groups, as well as an increase in PT in fructose-fed rats compared to the CS group. In contrast, HIIT and vitamin D_3 supplementation interventions were able to increase ALB levels and decrease PT compared to the CF group, with the difference being that HIIT was more effective than vitamin D_3 supplementation in reducing PT ($p < 0.05$). Furthermore, no significant difference was observed in the increase in ALB levels between the training and vitamin D_3 supplementation groups. Additionally, the results were indicative of no significant difference in ALB levels between the CS and FTD groups as a result of the combined effect of vitamin D_3 supplementation and HIIT ($p > 0.05$). The reduction in PT was significantly greater in the FTD group compared to the separate training and supplementation groups ($p < 0.05$).

Hepatic fat accumulation

As illustrated in Fig. 5, the rats in the CF, FT, and FD groups exhibited significantly higher intrahepatic lipid compared to the CS group after 12 weeks of a high-fructose diet. Conversely, both HIIT and vitamin D_3 supplementation were able to reduce liver fat accumulation in the rats compared to the CF group; HIIT was found to be more effective than vitamin D_3 supplementation ($P < 0.05$). Additionally, the findings indicated that the combined effect of vitamin D_3 supplementation and HIIT produced no significant difference in hepatic fat accumulation between the CS and FTD groups of rats ($p > 0.05$).

Liver fibrosis

The results related to liver fibrosis indicated that in response to a 12-week high-fructose diet, the rats in the CF, FT, and FD groups had significantly higher liver fibrosis compared to the CS group. Conversely, HIIT interventions and vitamin D_3 supplementation decreased the percentage of liver fibrosis compared to the CF group ($p < 0.05$); no significant difference was observed between HIIT and vitamin D_3 supplementation in this respect. Additionally, the results indicated no significant difference in liver fibrosis between the CS and FTD groups ($p > 0.05$) due to the interactive effect of vitamin D_3 supplementation and HIIT. Images related to liver fibrosis and its quantification are provided in Fig. 6.

Biochemical analysis, insulin resistance, and pancreatic β-cell function

The results pointed to a significant increase in FBG and a significant decrease in the HOMA-β index in the rats fed with fructose solution compared to the CS group. Furthermore, FBG levels in the CF group of rats surpassed 13.8 mmol/L, signifying the beginning of diabetes in these subjects (31). Serum insulin levels and insulin resistance (HOMA-IR) significantly increased in the CF, FT, and FD groups compared to the CS group. The impact of HIIT and vitamin D_3 supplementation significantly reduced FBG, insulin levels, and resistance in comparison with the CF group, with HIIT being more effective than the vitamin D_3 supplementation. The combined effect of HIIT and vitamin D_3 supplementation in reducing FBG and insulin levels was greater than the individual effect of each intervention. Further, a significant increase in the HOMA-β index was observed in the FT and FTD groups compared to the CF group ($p < 0.05$); no significant difference was discovered between them in this respect ($p > 0.05$) (Fig. 7).

Correlation between variables

Table 5 illustrates the Pearson correlation between the variables (IL-1β, IL-10, insulin, glucose, leukocyte, ALB, and PT) across all groups. As presented in Table 5, there was a significant correlation between IL-1β and leukocyte in the CF ($r = 0.73$, $P = 0.03$) and FD ($r = 0.72$, $P = 0.43$) groups. A significant correlation was observed between IL-10 and insulin ($r = 0.7$, $P = 0.05$) and ALB ($r=-0.81$, $P = 0.01$) in the CF group as well as leukocyte ($r=-0.77$, $P = 0.25$) in the FTD group. A significant correlation also existed between IL-10 and IL-1β ($r = 0.77$, $P = 0.25$) in the FTD group. Insulin and glucose were significantly correlated in the FT ($r = 0.71$, $P = 0.04$) and FTD ($r = 0.82$, $P = 0.01$) groups. Finally, a significant correlation was found between PT and insulin ($r=-0.79$, $P = 0.01$) in the CF group and glucose ($r=-0.72$, $P = 0.04$) in the FD group.

Table 5
Pearson's correlation between variables in the five groups of rats (CS, CF, FT, FD and FTD)

CS	IL-1β (1)	IL-10 (2)	Glucose (3)	Insulin (4)	Leukocyte (5)	Albumin (6)	Prothrombin Time (7)
1		0.35	-0.12	-0.28	0.33	-0.18	0.10
2			-0.15	-0.24	-0.39	-0.19	0.49
3				**0.91***	-0.19	0.09	-0.13
4					-0.25	0.15	0.06
5						0.48	-0.33
6							0.17
CF							
1		-0.14	0.60	0.23	**0.73***	0.40	-0.19
2			-0.77	**-0.70***	-0.32	**-0.81***	-0.37
3				0.18	0.29	0.07	-0.34
4					0.24	-0.56	**-0.79***
5						0.57	-0.27
6							0.23
FT							
1		-0.58	0.33	0.22	-0.39	0.02	0.06
2			-0.43	-0.53	-0.13	0.24	0.26
3				**0.71***	-0.08	0.44	-0.14
4					0.48	0.07	0.15
5						0.03	0.31
6							0.25
FD							
1		-0.05	0.08	0.58	**0.72***	0.15	-0.09
2			0.07	-0.04	-0.004	-0.18	0.27

CS: Control group with a Standard Diet; CF: Control group with a High-fructose Diet; FT: High-fructose Diet + HIIT intervention; FD: High-fructose Diet + Vitamin D_3 intervention; FTD: High-fructose Diet + HIIT + Vitamin D_3 interventions. *: Significant correlation.

CS	IL-1β (1)	IL-10 (2)	Glucose (3)	Insulin (4)	Leukocyte (5)	Albumin (6)	Prothrombin Time (7)
3				0.48	0.36	-0.13	**-0.72***
4					0.61	-0.15	-0.07
5						-0.48	-0.42
6							0.14
FTD							
1		**0.77***	0.15	-0.13	-0.63	-0.05	0.31
2			0.11	-0.001	**-0.77***	-0.25	-0.13
3				**0.82***	0.16	0.38	0.12
4					0.41	0.19	0.05
5						0.09	0.15
6							-0.18

CS: Control group with a Standard Diet; CF: Control group with a High-fructose Diet; FT: High-fructose Diet + HIIT intervention; FD: High-fructose Diet + Vitamin D_3 intervention; FTD: High-fructose Diet + HIIT + Vitamin D_3 interventions. *: Significant correlation.

Discussion

Regarding the first research question, based on the findings, 12 weeks of fructose consumption led to hepatic fat accumulation, fibrosis, and inflammation in the rats independently of body weight, waist circumference, Lee index, and caloric intake. On the other hand, HIIT and vitamin D_3 supplementation interventions counteract the adverse effects of fructose.

Fructose intake triggers liver inflammation [32]. Fructose-activated carbohydrate-responsive element-binding protein (ChREBP) and peroxisome proliferator-activated receptor gamma coactivator-1β (PGC-1β) are believed to function as transcriptional coactivators for sterol regulatory-element binding protein 1c (SREBP-1c). This interaction results in the enhanced expression of enzymes crucial for de novo lipogenesis (DNL) [33]. DNL is a process of converting surplus dietary carbohydrates into fatty acids [34]. The accumulation of fatty acids in the liver leads to hepatic lipotoxicity, initiating the release of inflammatory cytokines, activation, and infiltration of macrophages [35]. External stimuli such as lipopolysaccharide (LPS) or interferon-gamma (IFNγ) activate Toll-like receptors (TLR) of macrophages to secrete inflammatory cytokines [36]. Pro-inflammatory cytokines exacerbate hepatic insulin sensitivity by disrupting insulin signaling. This creates a vicious cycle of inflammation by inducing further release of inflammatory cytokines [37]. Together, these processes cause hepatic inflammatory injury, insulin

resistance, and fibrosis [34]. Furthermore, Excessive fructose consumption also promotes the expansion of visceral adipose tissue, where hypertrophic adipocytes secrete pro-inflammatory cytokines [35]. Additionally, the metabolites generated from fructose metabolism predominantly originate from the small intestine and liver before entering the systemic circulation. Elevated levels of fructose metabolites like uric acid, free fatty acids, and lactate are closely linked to local tissue and organ inflammatory responses [38].

Concerning the second research question, HIIT was more effective than vitamin D_3 supplementation in reducing inflammatory markers and improving liver function.

HIIT offsets the detrimental pro-inflammatory state through several mechanisms. HIIT enhances the activation of the serine-threonine protein kinase Akt and improves insulin sensitivity in the liver. The main downstream effector of insulin signaling in the liver is Akt. [37]. It is known that Akt plays a role in promoting insulin resistance by inhibiting glucose release from hepatocytes. Akt can induce numerous downstream molecules by facilitating the translocation of GLUTs to the cell membrane, thereby increasing glucose uptake [39]. Our study demonstrated a significant reduction in FBG, insulin levels, and HOMA-IR as well as a significant increase in HOMA-β after 12 weeks of HIIT. Additionally, HIIT boosts AMP-activated protein kinase activation (AMPK), which leads to increased expression of PGC-1α. The exercise-induced PGC-1α functions to suppress SREBP-1c [37], resulting in a decrease in the expression of lipogenic genes, reduced hepatic DNL, and lower lipid content in the liver [40]. Correspondingly, various animal and human studies have shown that HIIT diminishes hepatic fat content and aids in the management of fatty liver disease [41, 42]. Consistently, in our current investigation, intrahepatic lipid content notably decreased following 12 weeks of HIIT. Furthermore, HIIT produces anti-inflammatory effects by curtailing macrophage recruitment. Since activated macrophages secrete detrimental cytokines like IL-1β, HIIT shields the liver by attenuating both the accumulation and activation of monocyte-derived macrophages, while also steering macrophages toward a less inflammatory state [43]. At the molecular level, PGC-1α inhibits NF-κB transcriptional activity regarding its target genes, including those that encode pro-inflammatory cytokines [44]. The downregulating of NF-κB activity leads to the regulation of IL-10 secretion via monocytes and T cells through the Th2 pathway [45]. Another potential mechanism governing IL-10 secretion after regular exercise is the equilibrium between Th1- and Th2-secreted cytokines; regular exercise can potentially enhance the production of cytokines released by Th2 cells while regulating the relative reduction in the cytokines secreted by Th1 cells. Ultimately, this orchestrated process leads to the adjustment of inflammatory cytokines [46]. In the present study, HIIT significantly reduced the percentage of F4/80 + CD11c+ M1 macrophages and F4/80 + CD206+ M2 macrophages in the liver tissue as well as IL-1β and IL-10 in the serum of the rats fed a high-fructose diet. These discoveries underscore the efficacy of HIIT in modulating IL-1β and IL-10 levels in the serum of rats.

Liver function can be assessed by its capacity to produce ALB and vitamin K-dependent clotting factors [47]. Therefore, in the current study, the common tests ALB and PT were used to assess liver function,

which showed that serum ALB levels and PT were significantly higher and lower, respectively, in the training groups, compared to the CF group, suggesting that liver function is better preserved by HIIT.

Vitamin D_3 influences hepatic inflammation and liver function through various pathways:1. Vitamin D_3 diminishes both adipose tissue release of lipid droplets and hepatic DNL [48]. Notably, in our study, we observed a substantial reduction in intrahepatic lipid levels following 12 weeks of vitamin D_3 supplementation in the rats; 2. Vitamin D_3 regulates metabolic pathways that are linked to insulin sensitivity and glucose-insulin balance. It promotes glucose uptake in the muscle cells by enhancing the intracellular expression of the insulin receptor substrate IRS-1. Additionally, it facilitates the expression of the insulin-dependent GLUT-4 on adipocytes. [48]. Consistently, our study revealed reductions in FBG, insulin levels, and HOMA-IR after 12 weeks of weekly vitamin D_3 intake; 3. Vitamin D_3 exhibits anti-fibrotic properties in the liver by impeding the proliferation of hepatic stellate cells and suppressing the expression of pro-fibrotic mediators like platelet-derived growth factor (PDGF) and transforming growth factor β (TGF-β) [48]. Masson's trichrome staining in our investigation also exhibited a notable decrease in liver fibrosis percentage in the rats as a consequence of vitamin D_3 supplementation; 4. Liver macrophages have the highest expression of vitamin D receptors (VDRs) among nonparenchymal cells. Stimulating these VDRs with vitamin D ligands alleviates liver inflammation, steatosis, and insulin resistance [3]. Our research demonstrated that vitamin D_3 supplementation notably decreased the proportion of F4/80 + CD11c+ M1 macrophages and F4/80 + CD206+ M2 macrophages in the liver tissue, accompanied by reduced levels of IL-1β and IL-10 in the serum of the rats consuming a high-fructose diet.

The findings related to the third research question also revealed that the greater combined effect of HIIT and vitamin D_3 supplementation on liver inflammation and function markers compared to their individual effect. Based on our findings, given the independent mechanisms of HIIT and vitamin D_3 involved in improving liver inflammation and function, a combination of these two interventions can potentially have a greater impact on these changes.

Conclusion

Briefly, the consumption of a high-fructose diet leads to hepatic fat accumulation, the onset of diabetes, liver inflammation, and fibrosis. However, the simultaneous use of HIIT and vitamin D_3 supplementation can potentially prevents the negative consequences of a high-fructose diet and improves liver function. These findings highlight the potential benefits of HIIT and vitamin D_3 supplementation in mitigating the adverse effects of a high-fructose diet on liver health.

Declarations

Authors' contributions

The study was conceived, developed and designed by HM, BS and JM. The experiments were performed and data was collected and subsequently analysed and interpreted by HM and BS. Manuscript preparation was undertaken by HM and BS. All authors approved the final version of the article.

Data availability statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

The author received No Funding for this work.

Ethics approval and consent to participate

Guilan University of Medical Sciences Ethical Committee approved all the procedures pertaining to animals with Approval ID IR.GUILAN.REC.1402.024.

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Figures

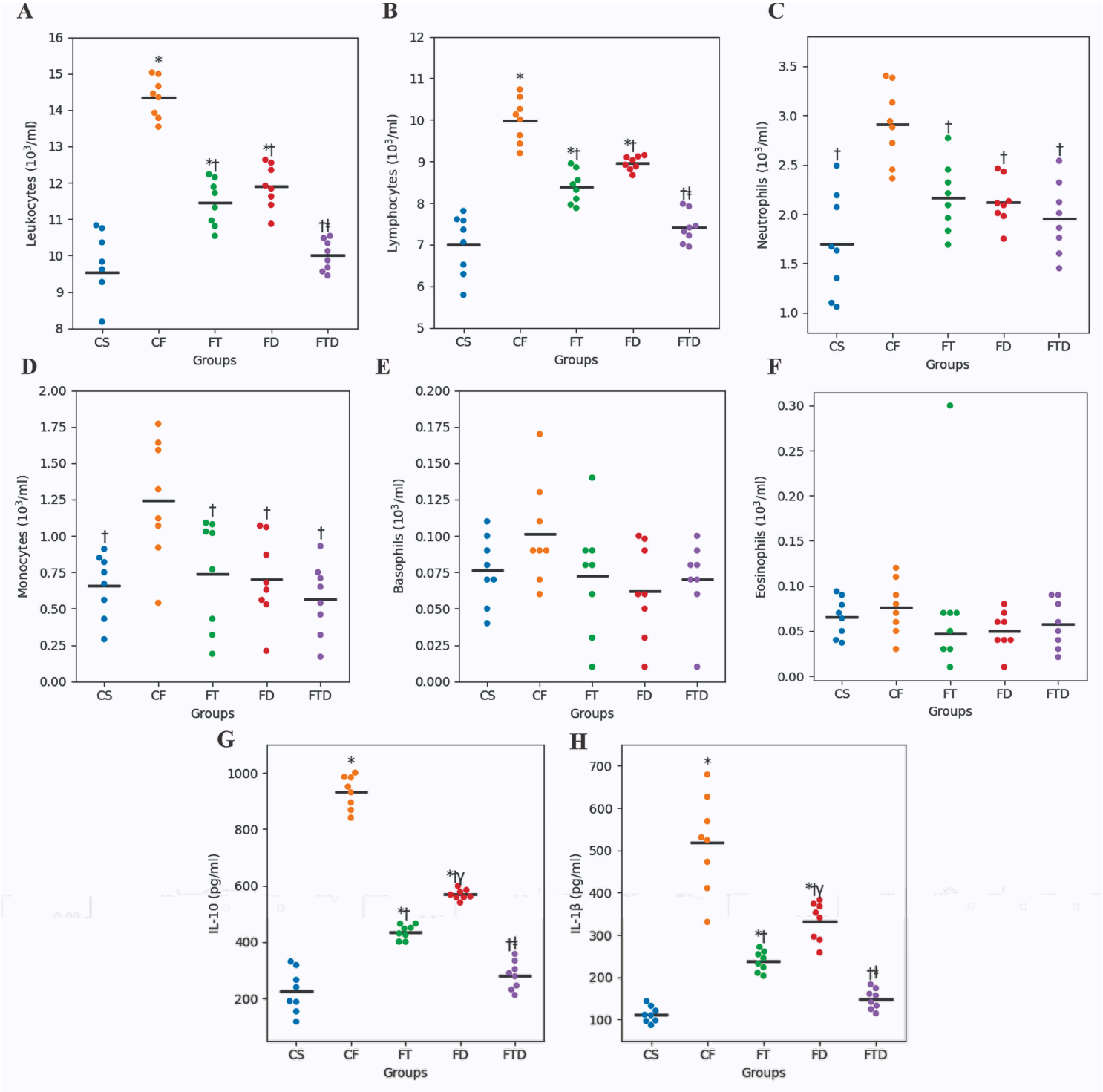


Figure 1

Blood profiling to detect inflammation. (A) Leucocytes, (B) Lymphocytes, (C) Neutrophils, (D (Monocytes, (E) Basophils, (F) Eosinophils, (G) IL-10 and (H) IL-1β in rats of groups: CS: Control group with a Standard Diet; CF: Control group with a High-fructose Diet; FT: High-fructose Diet + HIIT intervention; FD: High-fructose Diet + Vitamin D_3 intervention; FTD: High-fructose Diet + HIIT + Vitamin D_3 interventions. Means with different superscripts are significantly different ($P < 0.05$) (n = 8 in each group). *: significant

difference with CS. †: significant difference with CF. ‡: significant difference with FD and FT. §: significant difference with FT.

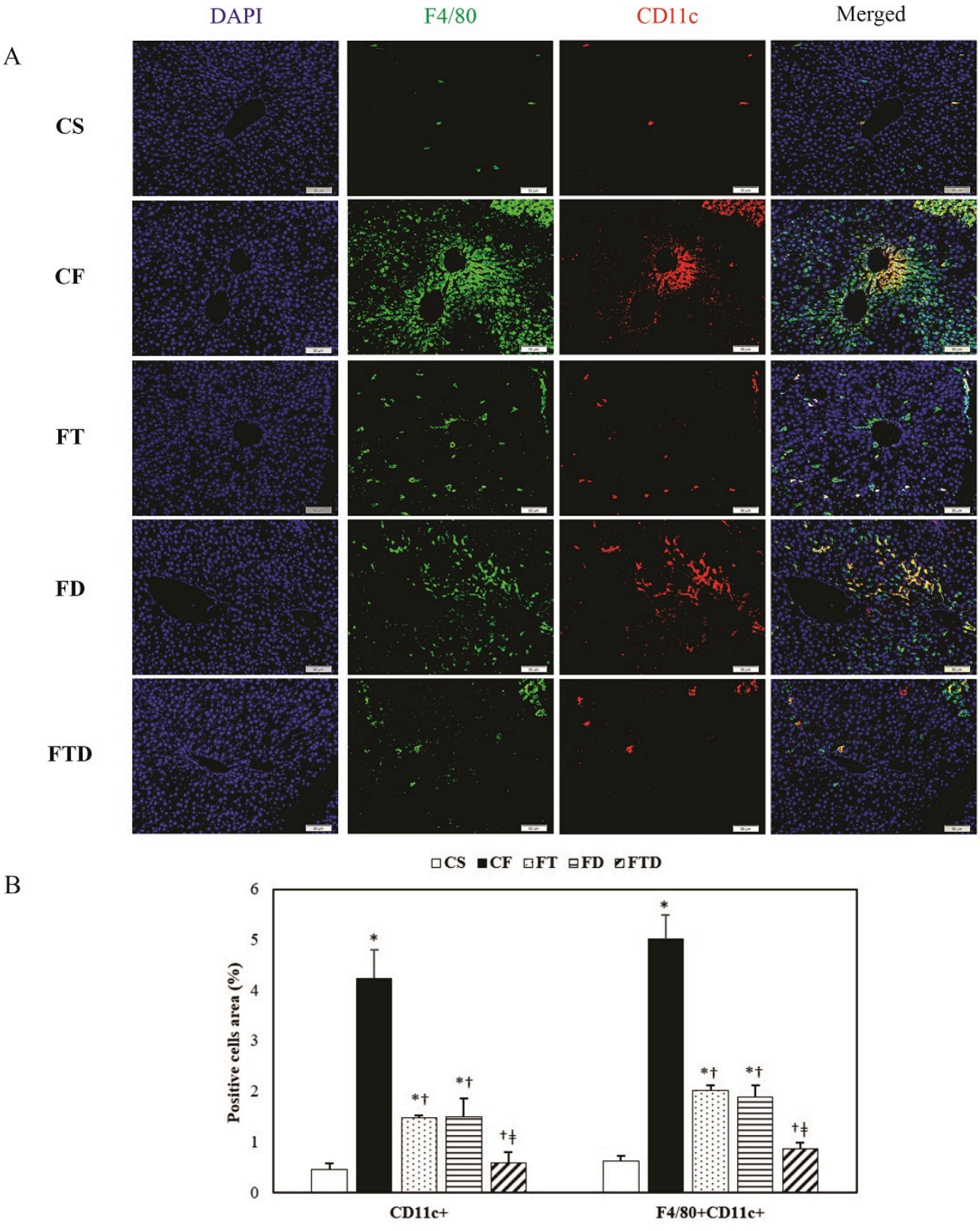


Figure 2

(A, B) Double immunofluorescence staining and statistical analysis of F4/80+CD11c+ for M1 macrophage in liver tissues of rats in groups: CS: Control group with a Standard Diet; CF: Control group

with a High-fructose Diet; FT: High-fructose Diet + HIIT intervention; FD: High-fructose Diet + Vitamin D_3 intervention; FTD: High-fructose Diet + HIIT + Vitamin D_3 interventions. Scale bar, 50 μm. Values are presented as mean ± standard deviation. Means with different superscripts are significantly different ($P < 0.05$) (n = 5 in each group). *: Significant difference with CS. †: Significant difference with CF. ‡: Significant difference with FD and FT.

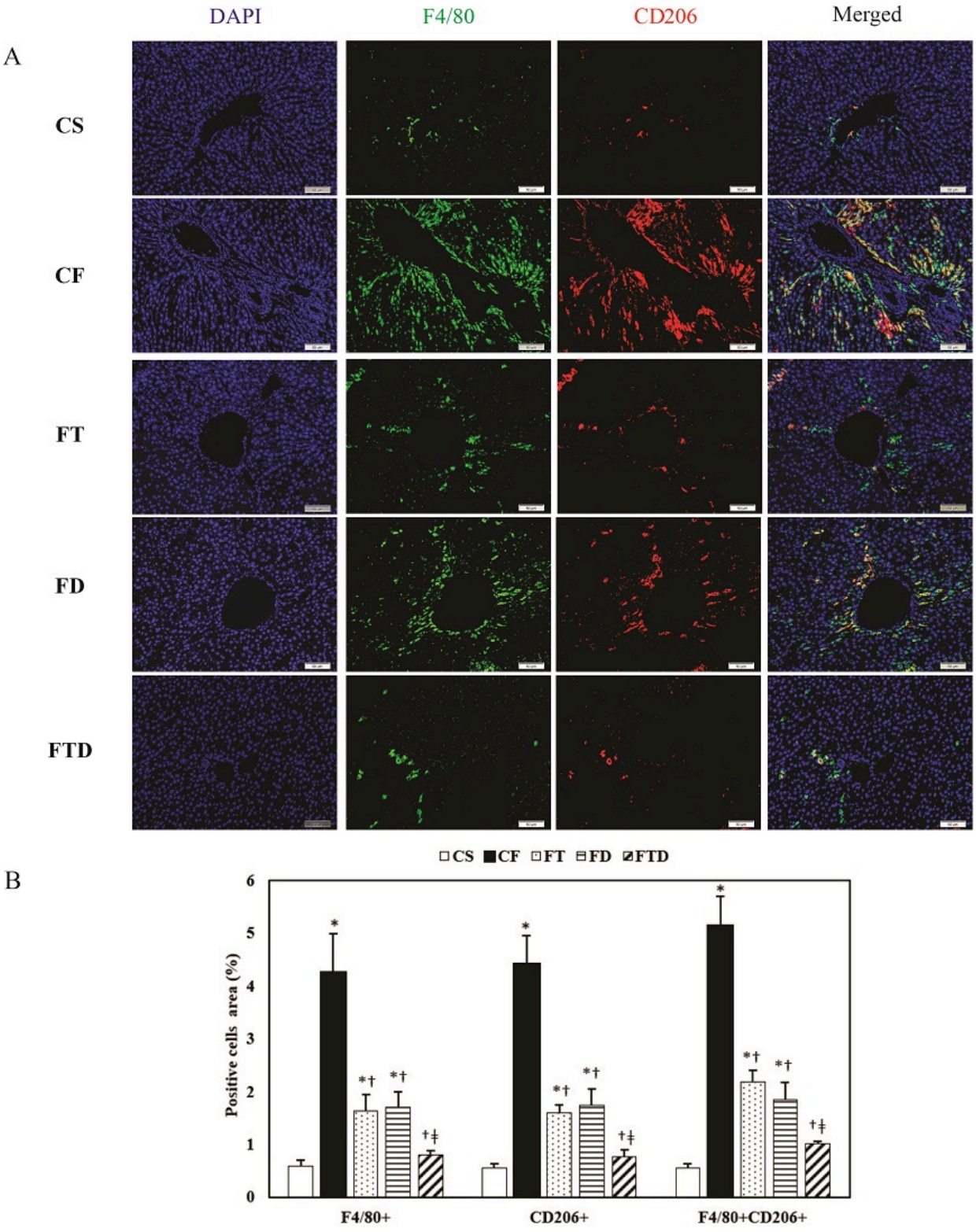


Figure 3

(A, B) Double immunofluorescence staining and statistical analysis of F4/80+CD/206+ for M2 macrophage in liver tissues of rats in groups: CS: Control group with a Standard Diet; CF: Control group with a High-fructose Diet; FT: High-fructose Diet + HIIT intervention; FD: High-fructose Diet + Vitamin D_3 intervention; FTD: High-fructose Diet + HIIT + Vitamin D_3 interventions. Scale bar, 50 μm. Values are presented as mean ± standard deviation. Means with different superscripts are significantly different ($P < 0.05$) (n = 5 in each group). *: Significant difference with CS. †: Significant difference with CF. ‡: Significant difference with FD and FT.

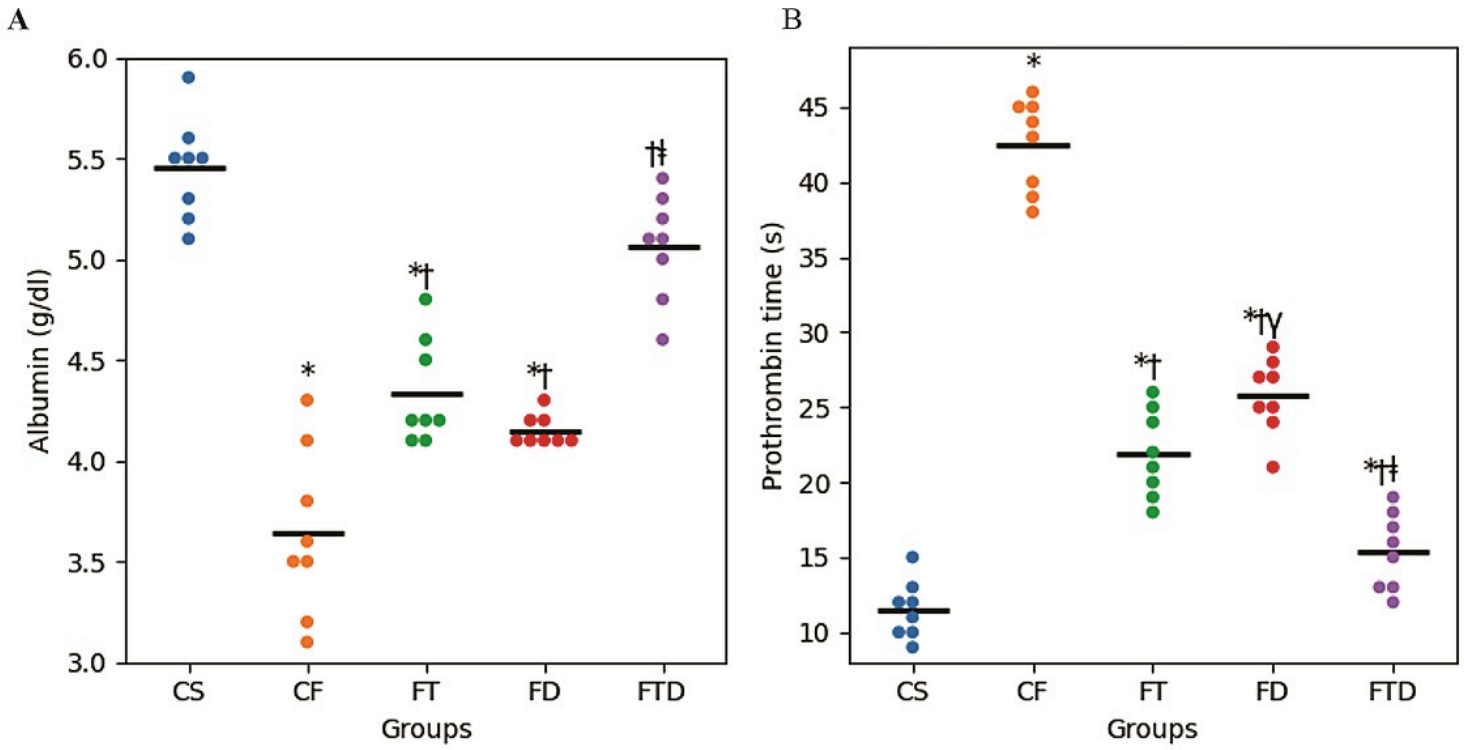


Figure 4

Comparison levels of (A) Albumin and (B) prothrombin time in in groups of rats CS: Control group with a Standard Diet; CF: Control group with a High-fructose Diet; FT: High-fructose Diet + HIIT intervention; FD: High-fructose Diet + Vitamin D_3 intervention; FTD: High-fructose Diet + HIIT + Vitamin D_3 interventions groups. Means with different superscripts are significantly different ($P < 0.05$) (n = 8 in each group). *: significant difference with CS. †: significant difference with CF. ‡: significant difference with FD and FT. ¥: significant difference with FT.

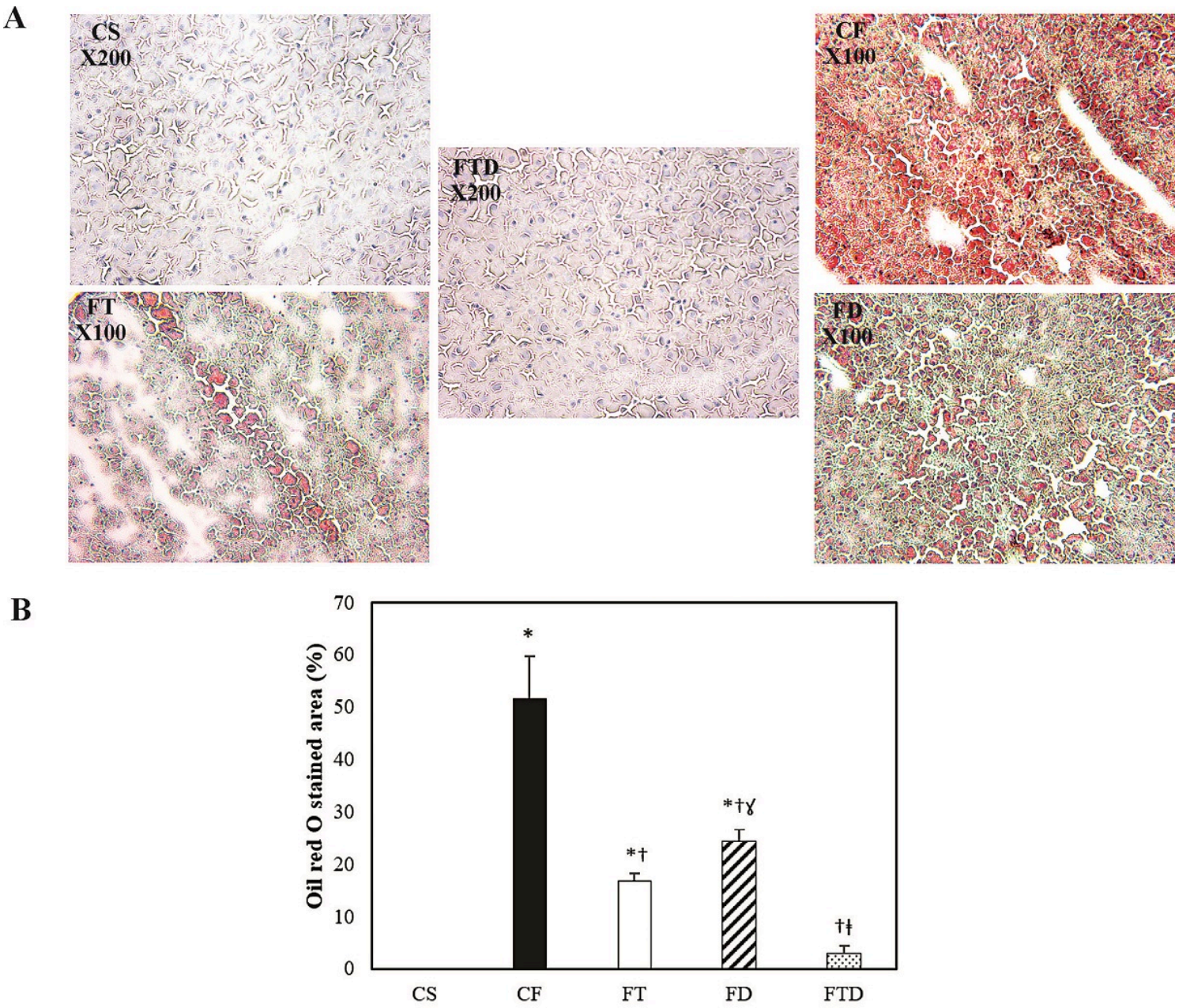


Figure 5

Liver analysis: (A) Oil red O staining shows as red, defining the lipid deposition area, and hematoxylin staining in blue, indicated the cell nucleus of lipids in the liver of rats. CS: Control group with a Standard Diet; CF: Control group with High-fructose Diet; FT: High-fructose Diet + HIIT intervention; FD: High-fructose Diet + Vitamin D_3 intervention; FTD: High-fructose Diet + HIIT + Vitamin D_3 interventions. (B) The percentage of oil red O stained area. Values are mean ± standard deviation. Means with different superscripts differ significantly, $P < 0.05$ ($n = 5$ in each group). The percentage of intrahepatic lipid was measured using Image J software. *: Significant difference with CS. †: Significant difference with CF. ɣ: Significant difference with FD and FT. ǂ: Significant difference with FT.

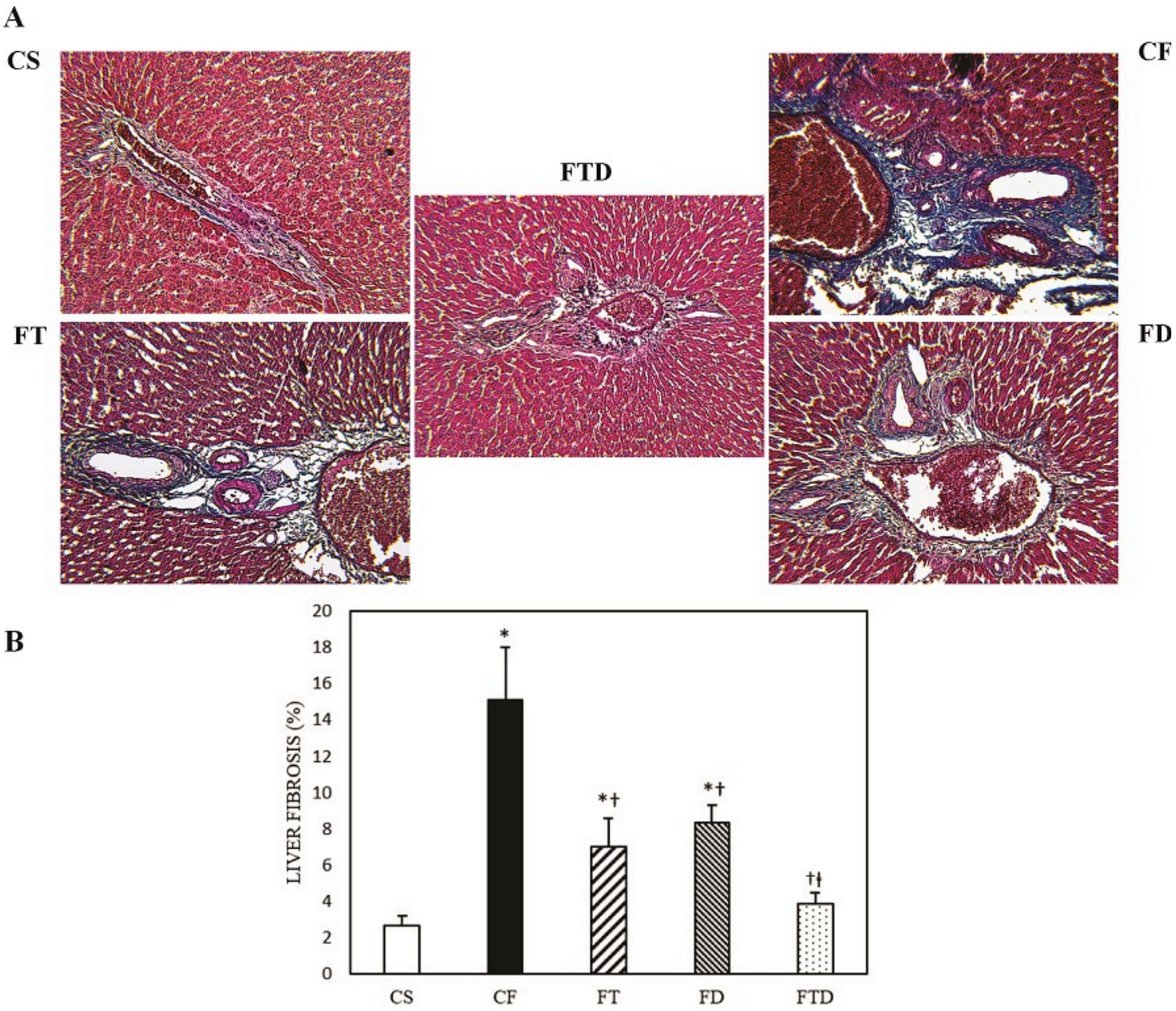


Figure 6

Liver Analysis: (A) Masson's trichrome staining showing collagen deposition in rat liver tissue. the fibrotic area is stained blue. Magnification, ×100. CS: Control group with a Standard Diet; CF: Control group with a High-fructose Diet; FT: High-fructose Diet + HIIT intervention; FD: High-fructose Diet + Vitamin D_3 intervention; FTD: High-fructose Diet + HIIT + Vitamin D_3 interventions. (B) Quantification of fibrotic areas. Values are presented as mean ± standard deviation. Means with different superscripts are significantly different ($P < 0.05$) (n = 5 in each group). Liver fibrosis percentage was measured using Image J software. *: Significant difference with CS. †: Significant difference with CF. ‡: Significant difference with FD and FT.

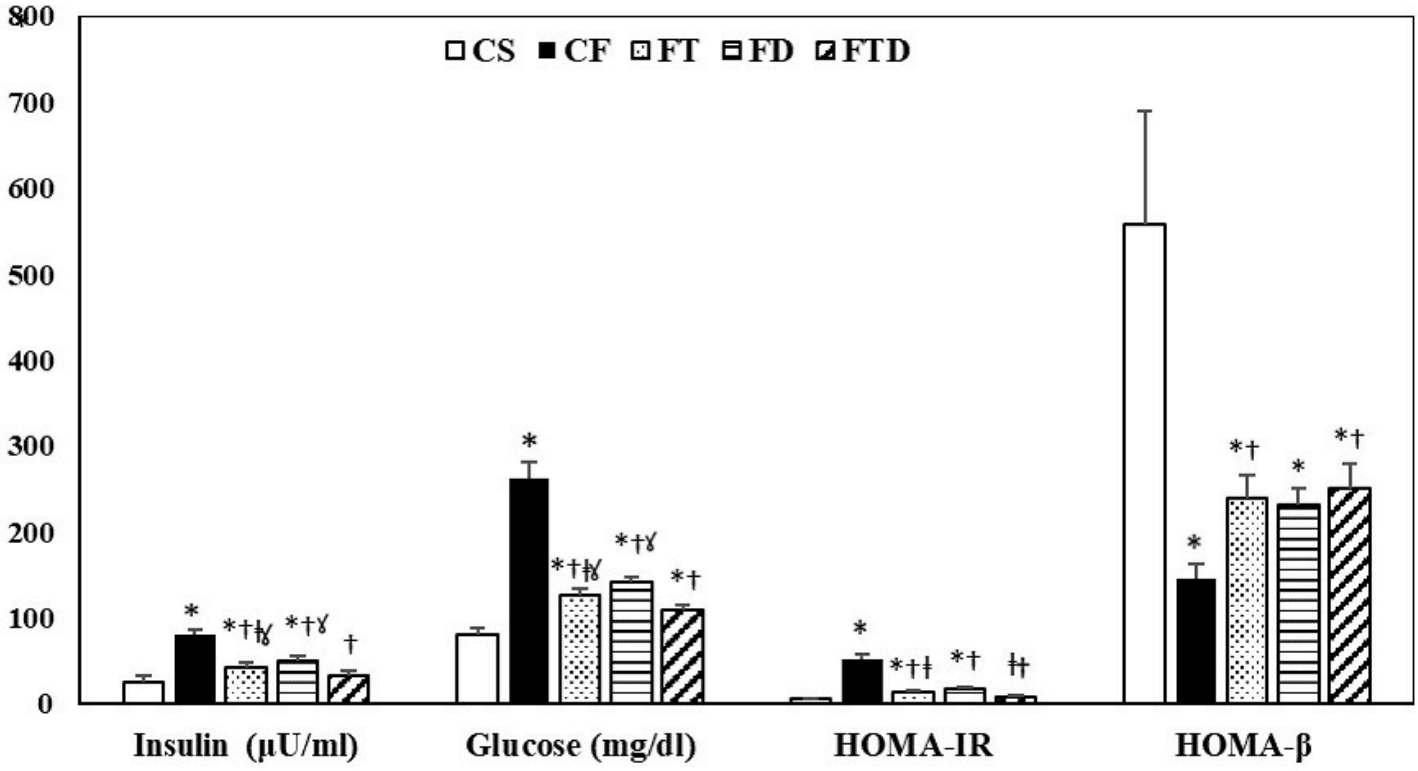


Figure 7

Changes in glucose, insulin, insulin resistance (HOMA-IR) and HOMA of β-cell function in five groups of rats. CS: Control group with Standard Diet; CF: Control group with a High-fructose Diet; FT: High-fructose Diet + HIIT intervention; FD: High-fructose Diet + Vitamin D_3 intervention; FTD: High-fructose Diet + HIIT + Vitamin D_3 interventions. Value are mean ± standard deviation of the mean. Means with different superscripts differ significantly, $P < 0.05$ ($n = 8$ in each group). *: Significant difference with CS. †: Significant difference with CF. ‡: Significant difference with FD. ¥: Significant difference with FTD.