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Amelioration of fructose-induced hepatic lipid accumulation by vitamin D_3 supplementation and high-intensity interval training in male Sprague–Dawley rats

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Abstract

Background Intrahepatic lipid accumulation (IHL), a hallmark of metabolic disorders, is closely associated with de novo lipogenesis (DNL). Notably, fructose feeding increased the DNL. Lifestyle modifications resulting from dietary changes and increased physical activity/exercise can decrease the IHL content. We examined the effects of vitamin D_3 supplementation (VDS), high-intensity interval training (HIIT), and their combination on the transcription factors and enzymes of the DNL pathway in male Sprague–Dawley rats fed a high-fructose diet (HFrD).

Methods Forty male rats were assigned to 5 groups ($n=8$): CS (the control group had a standard diet); CF (the control group had HFrD (10% (w/v) fructose solution in tap water)); and FT (HFrD + HIIT: 10 bouts of 4 min of high-intensity running, corresponding to 85–90% of the maximal speed with 2 min active rest periods of 50% maximal speed, 5 days per week); FD (HFrD + intervention of intraperitoneal injection of 10000 IU/kg/week VDS); FTD (HFrD + HIIT + VDS) that were maintained for 12 weeks. ELISA, the GOD-POD assay, folch, western blotting, and oil red O staining were used to determine insulin, fasting blood glucose (FBG), hepatic triglyceride (TG) and cholesterol levels; SREBP1c, ChREBP-β, ACC1, FASN, p-ACC1, AMPK, p-AMPK, and PKA protein expression; and IHL content, respectively.

Results Both HIIT and VDS led to significant increases in the levels of PKA, AMPK, p-AMPK, and p-ACC1, as well as significant decreases in the levels of SREBP1c, ChREBP-β, ACC1, FASN, insulin, FBG, liver TG, liver cholesterol, and IHL. HIIT exhibited superior efficacy over VDS in reducing ChREBP-β, ACC1, insulin, FBG, liver TG and cholesterol, as well as increasing p-ACC1 and PKA. Notably, the combined intervention of HIIT and VDS yielded the most substantial improvements across all the parameters.

Conclusions HFrD causes IHL accumulation and the onset of diabetes, whereas VDS and HIIT, along with their combined effects, prevent the consequences of HFrD.

Keywords Intrahepatic lipid; SREBP1C, ChREBP-β, ACC1, FASN, High-intensity interval training

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Background

Intrahepatic lipid (IHL) accumulation results from an imbalance between lipid acquisition and elimination [1]. This lipid accumulation in hepatocytes is a key factor in the development of nonalcoholic fatty liver disease [2]. IHL is a significant aspect of the constellation of metabolic abnormalities linked to central obesity and insulin resistance [3]. Elevated IHL levels have been linked to impaired hepatic glucose metabolism [4] and are independently correlated with type II diabetes [5], components of metabolic syndrome, and atherosclerosis [6]. Additionally, IHL accumulation may directly contribute to the development of dyslipidemia associated with obesity [7].

IHL is positively associated with de novo lipogenesis (DNL) [8], which is the process of synthesizing fatty acid chains from acetyl-CoA subunits produced during glycolysis [9]. On the other hand, fructose acts as both a substrate and an inducer of hepatic DNL [10] and elevates the protein levels of all DNL enzymes during its conversion of fructose into triglycerides [11]. In hepatocytes, fructose bypasses the rate-controlling step of glucose metabolism and is converted into glyceraldehyde-3-phosphate [10]. Additionally, fructose promotes lipogenesis even in the context of insulin resistance, as it does not require insulin for its metabolism. It directly activates sterol regulatory-element binding protein 1c (SREBP1c), a key transcriptional regulator of DNL [11], and enhances the upregulation of carbohydrate-responsive element-binding protein (ChREBP), which governs the expression of lipogenic genes [12]. ChREBP-β expression in the liver is correlated with hepatic insulin resistance and IHL [13].

Lifestyle modifications have been shown to reduce the IHL content [14]. Moreover, nutritional interventions can correct the imbalances in lipid storage, disposal, and partitioning that contribute to liver fat accumulation [15]. Dietary supplements can also aid in improving hepatic lipid homeostasis [16]. Notably, vitamin D_3 may inhibit SREBP1c, thereby controlling DNL in hepatocytes and directly suppressing the activity of two major enzymes of DNL, acetyl-CoA carboxylase (ACC1), and fatty acid synthase (FASN), leading to reduced synthesis of free fatty acids [17]. Furthermore, engaging in exercise training alone has demonstrated effectiveness in lowering IHL levels [18]. Specifically, high-intensity interval training (HIIT) is more effective in preventing liver lipid accumulation by restoring the mRNA levels of genes involved in hepatic lipogenesis, such as SREBP1, ACC1 and FASN [19].

On the basis of this research background, it was hypothesized that a high-fructose diet (HFrD) increases hepatic de novo lipogenesis and leads to fat accumulation in the liver, whereas vitamin D_3 supplementation (VDS) and HIIT may prevent hepatic fat accumulation. Previous studies have investigated the effects of HIIT [19] and VDS [20] on de novo lipogenesis markers in rodents fed high-fat and standard diets. Additionally, the therapeutic effect of HIIT [21] on DNL markers in diabetic rats (induced by HFrD) has been examined. It is crucial to assess the preventive effects of VDS and HIIT, as well as their combined impact on DNL markers in healthy rats fed a high-fructose diet. Accordingly, the following research questions were addressed in the present study:

1. Does engaging in HIIT and taking VDS while simultaneously consuming a HFrD help mitigate the adverse effects of fructose?
2. Which training and supplement interventions are more effective in impacting DNL markers?
3. Can the combination of VDS and HIIT yield superior results in preventing IHL compared with the individual effects of each?

Methods

Animals

Forty male Sprague–Dawley rats, each 7 weeks old and weighing between 200 and 250 g, were obtained from the Pasteur Institute Animal Care Center in Karaj, Iran. These rats were then relocated to the University of Guilan's Faculty of Physical Education and Sports Sciences. Upon arrival, they were randomly assigned to groups of four per cage and kept on a 12-hour light/dark cycle in a low-stress and controlled environment (in air-conditioned rooms, 24±2 °C, 50% humidity, and low noise). The rats were fed a standard diet (3.77 kcal/g, 21.23% protein, 11.95% fat, 66.82% carbohydrate). The formulations followed the AIN-93 M recommendations for macro- and micronutrients [22]. The diet was produced by the Production and Research Complex Pasteur Institute of Iran. After one week of acclimatization, the animals were randomly (simple random sampling) allocated into 5 groups (n=8/group): a control group with a standard diet (CS), a control group with HFrD (CF), HFrD+HIIT (FT), HFrD+VDS (FD), and HFrD+HIIT+VDS (FTD). The rats in the CS group were provided plain water, whereas those in the fructose-fed groups received a 10% (w/v) fructose solution in their water, which was prepared fresh daily for 12 weeks. The rodent diet was isocaloric and contained high levels of fructose. All animals had unrestricted access to their respective drinks and food throughout the study period.

Vitamin D_3 supplementation

An intraperitoneal (IP) injection of 10,000 international units per kilogram (IU/kg) of VDS was administered to the FD and FTD groups of rodents [23]. The entire dose

was given all at once on a specific day each week for 12 weeks. The easy-to-master IP technique is quick and minimally stressful for animals [24]. VDS was manufactured by Aburaihan Pharmaceutical Company, Iran.

Exercise performance test

The maximum running speed of the rats was assessed via a five-line rodent treadmill (DSI-580; Danesh Salar Iranian Company, Tehran, Iran). The rats were first placed on the treadmill, set to a 0° incline, and given a 5-min warm-up at a speed of 6 m/min. The speed was subsequently increased by 2 m/min every 2 min until the rats reached exhaustion [25]. Exhaustion was defined as the point when the rats stayed on the shockers, which are meant to encourage running, for more than 10 s instead of continuing to run on the treadmill [26]. Running speed serves as a metric to adjust training intensity throughout the training program [27]. Performance tests were conducted at the start and end of the program for all groups. Additionally, for the training groups, these tests were also performed after 5 weeks of training to readjust the training intensity [25].

Exercise training protocol

The HIIT protocol consisted of 10 intervals of 4 min each at a high intensity, running at 85–90% of maximum speed, interspersed with 2-minute active rest periods at 50% of maximum speed. Over the 10-week period, the interval pace was progressively increased and maintained for the final two weeks. Each session included 5 min of low-intensity running (45–50% of maximum speed) for warm-up and cool-down. The rats underwent a 12-week training program involving treadmill running 5 days a week [28].

Anthropometric determinations

At the conclusion of the experiment, body length, measured from the nose to the anus, was assessed in anesthetized rats. Body weight (BW) was recorded weekly throughout the study period. Waist circumference is measured via the midline. These measurements were utilized to calculate the following anthropometric parameters:

1. The Lee index is calculated as the cube root of body weight (in grams) divided by nose-to-anus length (in centimeters) [29].
2. The specific rate of body mass gain (in grams per kilogram) is expressed as dM/M dt, where dM represents the gain in body weight during the time interval dt = t2−t1 and where M is the rat's body weight at time t1 [30].

Nutritional determinations

Daily food and liquid intake, determined as the difference between the amount provided and the remaining quantity after 24 h, were measured consistently at 09:00 to 10:00 h. From these measurements and considering caloric intake, the following nutritional parameters were computed [31]:

1. Energy intake (kJ/day): Calculated by multiplying the mean food consumption by the amount of dietary metabolizable energy.
2. Feed efficiency (%): This metric represents the percentage of energy intake (Kcal) utilized for body weight gain, calculated as the product of mean body weight gain (in grams) and 100, divided by the energy intake (Kcal).

Although the diets were uniform in energy macronutrient content and density, the total daily energy consumption was determined by summing the energy density of the diet and that of the high-fructose beverage.

Tissue and blood sample collection

The animals were subjected to a 12-h fast and then anesthetized with an intraperitoneal injection of ketamine (60 mg/kg) and xylazine (6 mg/kg) 48 h after their final training session to eliminate any immediate exercise effects. They were subsequently euthanized. Blood was drawn from the inferior vena cava, left to settle, and then centrifuged at 3,000×g for 15 min at 4 °C to separate the serum, which was stored at −80 °C for later biochemical tests. Liver samples were dissected at the same time and weighed, and the right lobes were fixed in 10% formalin for histological studies. Additional liver tissue was rapidly frozen in liquid nitrogen and stored at −80 °C for future biochemical analyses. The relative weight, which is the ratio of the weight of the organ to the body weight, was also calculated.

Biochemical analysis

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

Insulin resistance and pancreatic β-cell function

We utilized homeostasis model assessment to evaluate insulin resistance (HOMA-IR), which is recognized as a straightforward, cost-effective, and reliable surrogate marker of insulin resistance [32]. Additionally, we employed the HOMA of β-cell function (HOMA-β)

index, which is considered a reliable measure of β-cell function [33]. These indices were calculated via the following formulas:

1. HOMA-IR = (Fasting insulin μU/ml) × (Fasting glucose mmol/l)/22.5
2. HOMA- β = (20 × fasting insulin μU/ml) / (fasting glucose mmol/l – 3.5)

Hepatic triglyceride and cholesterol analysis

The hepatic lipids were extracted according to the method described by Folch et al. [34]. The hepatic triglyceride (TG) and cholesterol contents were quantified via commercial colorimetric assay kits (Pars azmun, Alborz, Iran). The results are presented as mg triglyceride or cholesterol per gram of liver.

Western blotting analysis

Liver tissues were processed via RIPA lysis buffer supplemented with PMSF to extract proteins. The mixture was subsequently centrifuged at 13,523 × g for 30 min at 4 °C, after which the supernatant was collected, and the protein content was measured via the BCA assay. To denature the proteins, the samples were combined with 5X loading buffer and boiled for 10 min. The denatured proteins were then separated on a 12% SDS–PAGE gel, which was run first at 70 V for 30 min and then at 120 V for 90 min. The separated proteins were transferred to PVDF membranes via a wet transfer method (270 mA for 90 min). The membranes were blocked with a 5% non-fat milk mixture for 2 h at room temperature, followed by an overnight incubation at 4 °C with primary antibodies (diluted 1:300) against sterol regulatory element binding protein 1c (SREBP1c) (2A4) (sc-13551), carbohydrate-responsive element-binding protein (ChREBPβ) (G-12) (sc-515922), acetyl-CoA carboxylase (ACCά) (D-5) (sc-137104), fatty acid synthase (FASN) (G-11) (sc-48357), phospho-acetyl-CoA carboxylase (p-ACCα) (F-2) (sc-271965), and protein kinase A (PKA) [20] (sc-136231), which were manufactured by Santa Cruz Biotechnology, Shanghai, China. AMP-activated protein kinase (AMPK) (ab131512) and phosphorylated AMPK (p-AMPK) (ab23875) were purchased from Abcam (Cambridge, UK). After three 10-minute washes with TBST buffer, the membranes were incubated with HRP-conjugated secondary antibodies (diluted 1:1,000) for 1 h at room temperature. The membranes were then washed three times for 10 min each and treated with a 1:1 mixture of BeyoECL Plus A and B solutions according to the ECL kit instructions. Finally, the PVDF membranes were visualized via an Invitrogen iBright Imaging System (FL1000, Thermo Fisher Scientific) [35].

Histological analysis

Liver samples were initially fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned into 6-μm thick slices. These sections were deparaffinized in xylene followed by rehydration. After rinsing with PBS, the tissue sections were stained with oil red O for 30 min and subsequently counterstained with hematoxylin for 1 min to assess lipid droplet formation in liver tissues [36]. The sections were also stained with hematoxylin and eosin (H&E) for examination of any structural abnormalities [37]. The stained sections were mounted and visualized via a fluorescence microscope (BX50, Olympus-Germany, AXIOM).

Data analysis

All the data are presented as the means±standard deviations. One-way analysis of variance (ANOVA) was conducted, followed by Tukey's post hoc test, and SPSS (version 23.0, IBM Corporation, Armonk, NY, USA) was used for analysis. The normality of the data was evaluated with the Shapiro–Wilk test, whereas the homogeneity of variance was assessed via the Levene test.

Results

Anthropometric determinations

The anthropometric indices of the five groups of rats (CS, CF, FT, FD and FTD) are presented in Table 1. The collected data revealed no significant differences in terms of body length, initial or final BW, body weight gain, waist circumference, or the Lee index among the groups. The consumption of fructose led to a significant increase in the liver and relative weight of the rats in the CF group compared with those in the CS group ($P<0.05$). In contrast, the liver and relative weights in the intervention groups were similar to those in the CS group ($P>0.05$). The results further revealed that the specific rate of body mass gain was significantly greater in the FT group than in the CS group ($P<0.05$).

Nutritional determinations

As shown in Table 2, food consumption significantly decreased in fructose-fed rats compared with that in the CS group ($P<0.05$). The results revealed no significant difference in water/fructose solution consumption between the fructose-fed rats and the rats fed a standard diet ($P>0.05$). Compared with those in the CS group, a significant reduction in energy intake in the CF and FTD groups and a significant increase in feed efficiency in the FTD group were observed ($P<0.05$).

Exercise performance test

Table 3 shows the changes in exercise performance during the two stages (baseline and final (after 12 weeks)) in the CS, CF and FD groups of rats and three stages

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]

The values are the means ± standard deviations

Means with different superscripts differ significantly, $P < 0.05$ ($n = 8$ per group)

CS: control group with standard diet; CF: control group with HFrD; FT: HFrD + HIIT; FD: HFrD + VDS; FTD: HFrD + HIIT + VDS

[a]: significant difference from CS, [b]: significant difference from CF

Table 2 Nutritional indices in 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/rat)	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]

The values are the means ± standard deviations

Means with different superscripts differ significantly, $P < 0.05$ ($n = 8$ per group)

CS: control group with standard diet; CF: control group with HFrD; FT: HFrD + HIIT; FD: HFrD + VDS; FTD: HFrD + HIIT + VDS

* Energy intake from food and fructose solution

[a]: significant difference from CS

Table 3 Exercise performance during the two stages (baseline and final (after 12 weeks)) in the CS, CF and FD groups of rats and three stages (baseline, middle and final) in the FD and FTD groups of rats

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]

The values are the means ± standard deviations

Means with different superscripts differ significantly, $P < 0.05$ ($n = 8$ per group)

CS: control group with standard diet; CF: control group with HFrD; FT: HFrD + HIIT; FD: HFrD + VDS; FTD: HFrD + HIIT + VDS

[a]: significant difference from FT and FTD

[b]: significant difference compared with Pretime

(baseline, middle and final) in the training groups. Twelve weeks of HIIT led to significant increases in maximal speed, running time and running distance ($P<0.05$).

Biochemical analysis, insulin resistance and pancreatic β-cell function

Compared with those in the CS group, there was a significant increase in FBG and a significant decrease in the HOMA-β index in fructose-fed rats. We also observed a significant increase in serum insulin levels and insulin resistance (HOMA-IR) in the CF, FT and FD groups compared with those in the CS group. Compared with the CF group, both the VDS and HIIT groups presented significant reductions in FBG, insulin levels and resistance. HIIT was significantly more effective than VDS in reducing FBG and insulin levels and resistance. Moreover, the combined effect of VDS and HIIT had a greater impact on decreasing FBG and insulin levels than the individual effects did. Another result of the research was a significant increase in the HOMA-β index in the FT and FTD groups compared with that in the CF group ($P<0.05$), without any significant difference between them ($P>0.05$) (Fig. 1).

Hepatic triglyceride and cholesterol analysis

As shown in Fig. 2, rats in the CF, FT and FD groups had significantly greater TG and cholesterol levels in the liver than those in the CS group did in response to 12 weeks of HFrD. In contrast, VDS and HIIT were able to decrease TG and cholesterol levels compared with those in the CF group, with the difference being that HIIT was more effective than VDS was ($P<0.05$). The results further revealed that, as a result of the interaction effect of VDS and HIIT, there was no significant difference in TG or cholesterol levels between the CS and FTD groups of rats ($P>0.05$).

Liver de novo lipogenesis markers

Compared with those in the CS group, a significant increase in the levels of DNL transcription factors, including SREBP1c and ChREBP-β, as well as a significant decrease in the levels of AMPK, p-AMPK, and PKA, was observed in the CF, FT, and FD groups. No significant difference in the ratio of p-AMPK to AMPK was detected among the research groups. VDS and HIIT led to a significant reduction in the protein levels of SREBP1C and ChREBP-β and a significant increase in the protein levels of PKA, AMPK, and p-AMPK compared with those in the CF group ($P<0.05$). There was no significant difference between VDS and HIIT in terms of their effects on changes in SREBP1C, AMPK, and p-AMPK levels ($P>0.05$). However, HIIT was significantly more effective than VDS in altering the levels of ChREBP-β and PKA ($P<0.05$). Additionally, the levels of all proteins in the FTD group were close to those observed in the CS group, with no significant differences between them ($P>0.05$) (Fig. 3).

Figure 4 shows a significant increase in ACC1 levels and a significant decrease in p-ACC levels (in the CF, FT, and FD groups of rats), as well as a significant increase in FASN levels (in fructose-fed rats), after 12 weeks of fructose feeding compared with those in the CS group. HIIT and VDS resulted in a significant reduction in ACC1 and FASN levels as well as a significant increase in p-ACC levels compared with those in the CF group. HIIT was significantly more effective than VDS in altering ACC1 and p-ACC1 levels ($P<0.05$). However, there was no significant difference between them in reducing FASN levels. Additionally, no significant difference in ACC1 or p-ACC1 levels was observed between the CS and FTD groups ($P>0.05$).

Histological analysis

As shown in Fig. 2, rats in the CF, FT and FD groups presented significantly greater lipid accumulation in the liver than did those in the CS group in response to 12 weeks of HFrD. In contrast, VDS and HIIT were able to decrease liver lipid deposition in the rats compared with the CF group, with the difference being that HIIT was more effective than VDS was ($P<0.05$). The results further revealed that, as a result of the interaction effect of VDS and HIIT, there was no significant difference in IHL between the CS and FTD groups of rats ($P>0.05$). The results obtained from H&E staining revealed normal liver structure in both the CS and FTD groups. However, liver sections from the CF group rats presented a loss of liver structure, whereas those from the FD and FT groups presented improvements in hepatic structure (Fig. 5).

Discussion

With respect to the first research question mentioned in the introduction, 12 weeks of fructose consumption significantly increased the expression of DNL transcription factors and enzymes and IHL accumulation independent of BW, waist circumference and the Lee index. In contrast, VDS and HIIT were able to help mitigate the adverse effects of fructose. Despite reduced food consumption and energy intake in the FTD group, body weight gain was greater than that in the FD group, raising questions about the metabolic effects of HIIT. Therefore, examining this issue requires further research.

Fructose is more lipogenic than glucose [9]. Fructose-activated ChREBP and peroxisome proliferator-activated receptor gamma (PPARγ) coactivator-1β (PGC-1β) are believed to act as transcriptional coactivators for SREBP-1c, leading to increased expression of enzymes essential for DNL [38]. This process limits fatty acid β-oxidation by producing malonyl coenzyme A, which inhibits carnitine

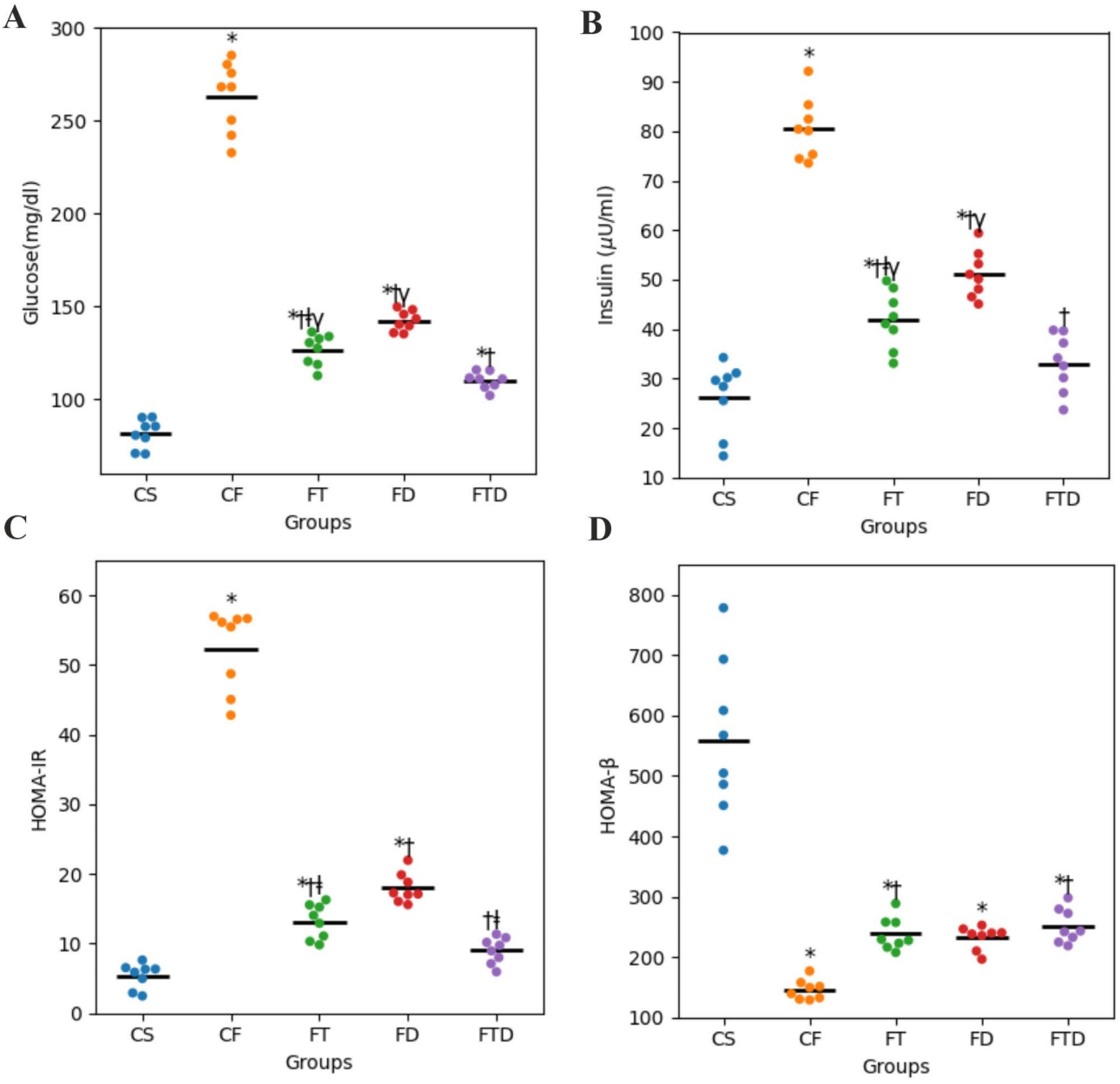

Fig. 1 Changes in glucose, insulin, and insulin resistance (HOMA-IR) and the HOMA of β-cell function in five groups of rats. CS: control group with standard diet; CF: control group with HFrD; FT: HFrD + HIIT; FD: HFrD + VDS; FTD: HFrD + HIIT + VDS. The values are the means ± standard deviations. Means with different superscripts differ significantly, $P < 0.05$. *: significant difference compared with CS. †: significant difference from CF. Y: significant difference from FD. Y: significant difference from FTD

palmitoyltransferase-1 (CPT-1), preventing fatty acyl derivatives from entering the mitochondrial matrix for β-oxidation [39]. Additionally, fructose has been shown to lower the levels of mitochondrial fatty acid oxidative enzymes by downregulating PPARα [40]. This preference for lipogenesis over fatty acid oxidation may contribute to insulin resistance via mechanisms involving diacylglycerol accumulation and protein kinase C epsilon type ϵ (PKCϵ) activation [41]. Furthermore, insulin and glucose activate SREBP-1c and ChREBP, respectively, which transcriptionally activate genes involved in DNL [42]. Insulin further promotes the transcription of genes necessary for fatty acid synthesis [43], thereby establishing a positive feedback loop where insulin resistance enhances hepatic DNL and where hepatic DNL exacerbates insulin resistance [44]. Continuous hepatic glucose production and release in the fed state, despite reduced insulin sensitivity, is thought to result in chronic hyperglycemia and hyperinsulinemia, impairing muscle insulin sensitivity and promoting the development of type 2 diabetes

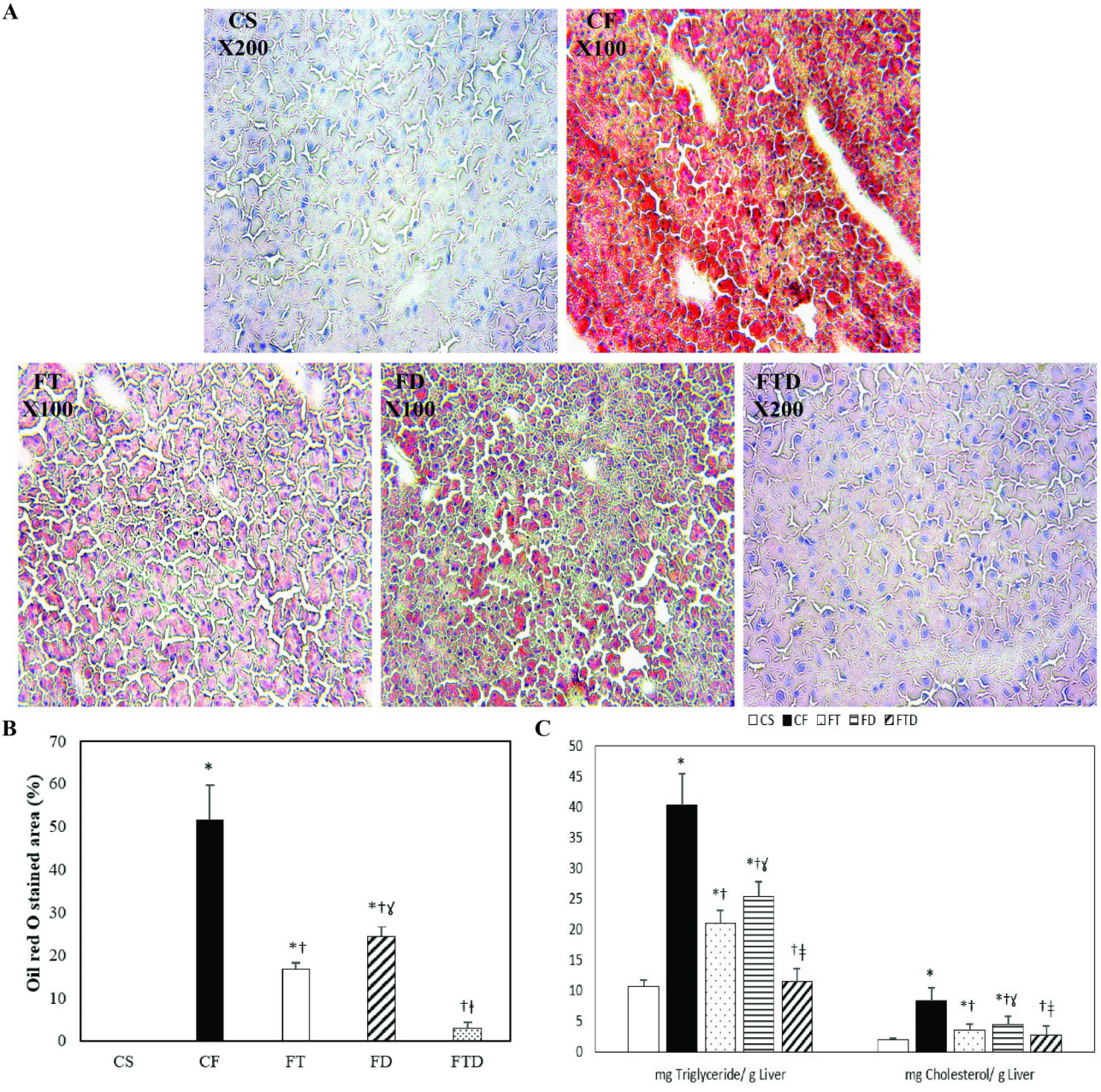

Fig. 2 Liver analysis: (**A**) Oil red O staining is shown in red, indicating the lipid deposition area, and hematoxylin staining in blue indicates the cell nucleus of lipids in the livers of the rats. CS: control group with standard diet; CF: control group with HFrD; FT: HFrD + HIIT; FD: HFrD + VDS; FTD: HFrD + HIIT + VDS. (**B**) The percentage of Oil Red O-stained area. (**C**) TG and cholesterol levels in the liver. The values are the means ± standard deviations. Means with different superscripts differ significantly, $P < 0.05$. The percentage of intrahepatic lipids was measured via ImageJ software. *: significant difference compared with CS. †: significant difference from CF. Y: significant difference from FD and FT. Y: significant difference from FT

mellitus [45]. In our study, after 12 weeks of fructose consumption, the FBG in the CF group was >13.8 mmol/L, which indicated the incidence of type 2 diabetes in the rats [46]. In this regard, in the CF group, compared with those in the CS group, the levels of insulin, hepatic TG and cholesterol, HOMA-IR, and IHL increased, and the HOMA-β decreased, which could be considered a confirmation of the adverse effects of fructose consumption.

In relation to the second research question, HIIT is more effective than VDS in inhibiting hepatic DNL markers.

There are several mechanisms involved in decreasing the expression of genes involved in the DNL pathway via HIIT: 1. AMPK: HIIT activates AMPK in skeletal muscle, liver, and adipose tissue [26], leading to reduced expression of lipogenic genes, decreased fatty acid synthesis, and decreased hepatic fat content [47]. Exercise

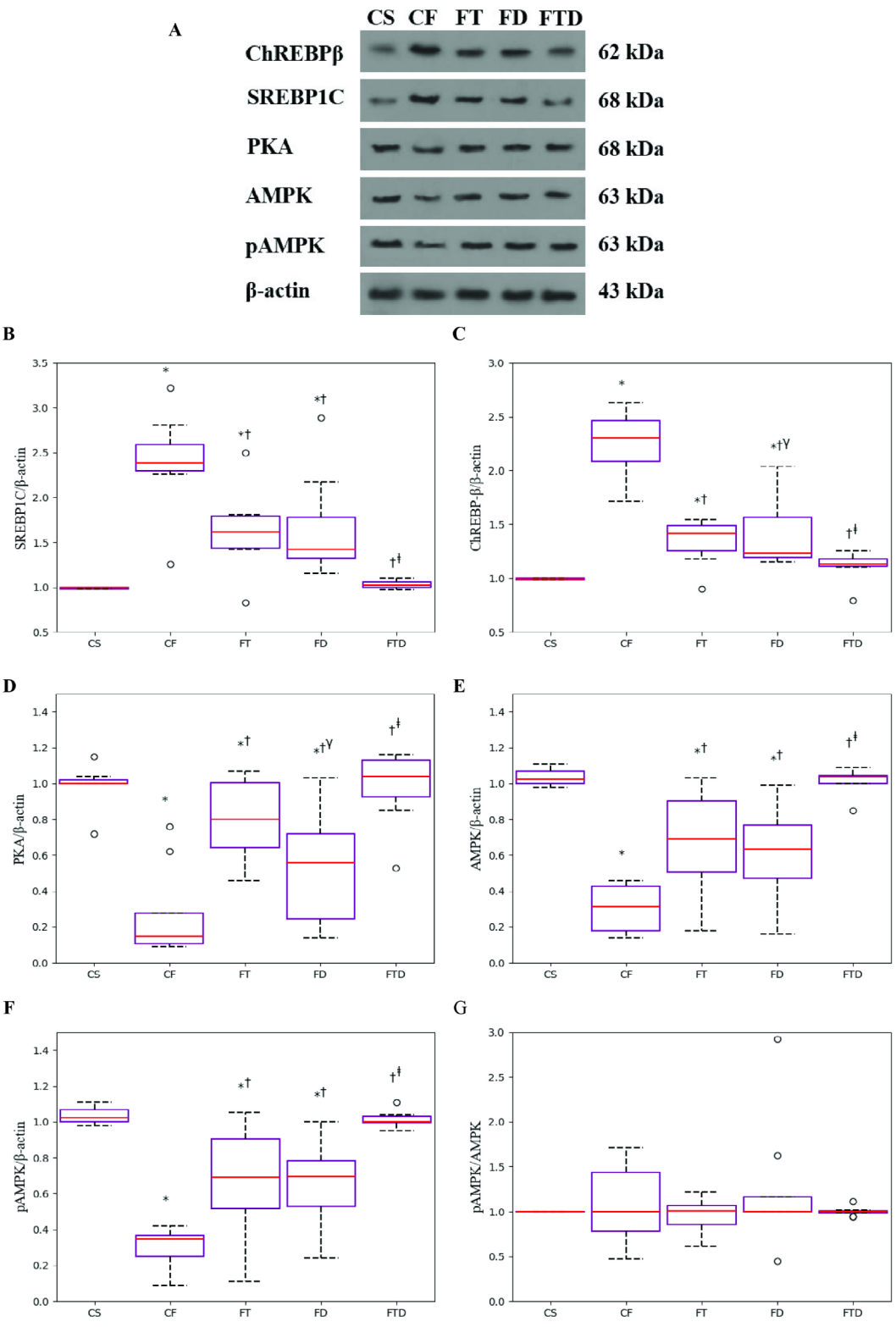

Fig. 3 (**A**) Western blot analysis of SREBP1C, ChREBP-β, PKA, AMPK, p-AMPK, and β-actin protein expression. (**B**, **C**, **D**, **E**, **F**, and **G**) Box and whisker plots of SREBP1C, ChREBP-β, PKA, AMPK, and p-AMPK relative to β-actin and p-AMPK/AMPK. CS: control group with standard diet; CF: control group with HFrD; FT: HFrD + HIIT; FD: HFrD + VDS; FTD: HFrD + HIIT + VDS. The middle line in the box represents the median, the upper and lower areas of the center box indicate the 75th and 25th percentiles, respectively, and the vertical bars indicate the standard deviation. Means with different superscripts differ significantly, $P < 0.05$. *: significant difference compared with CS. †: significant difference from CF. Y: significant difference from FD and FT. **Ɣ**: significant difference from FT

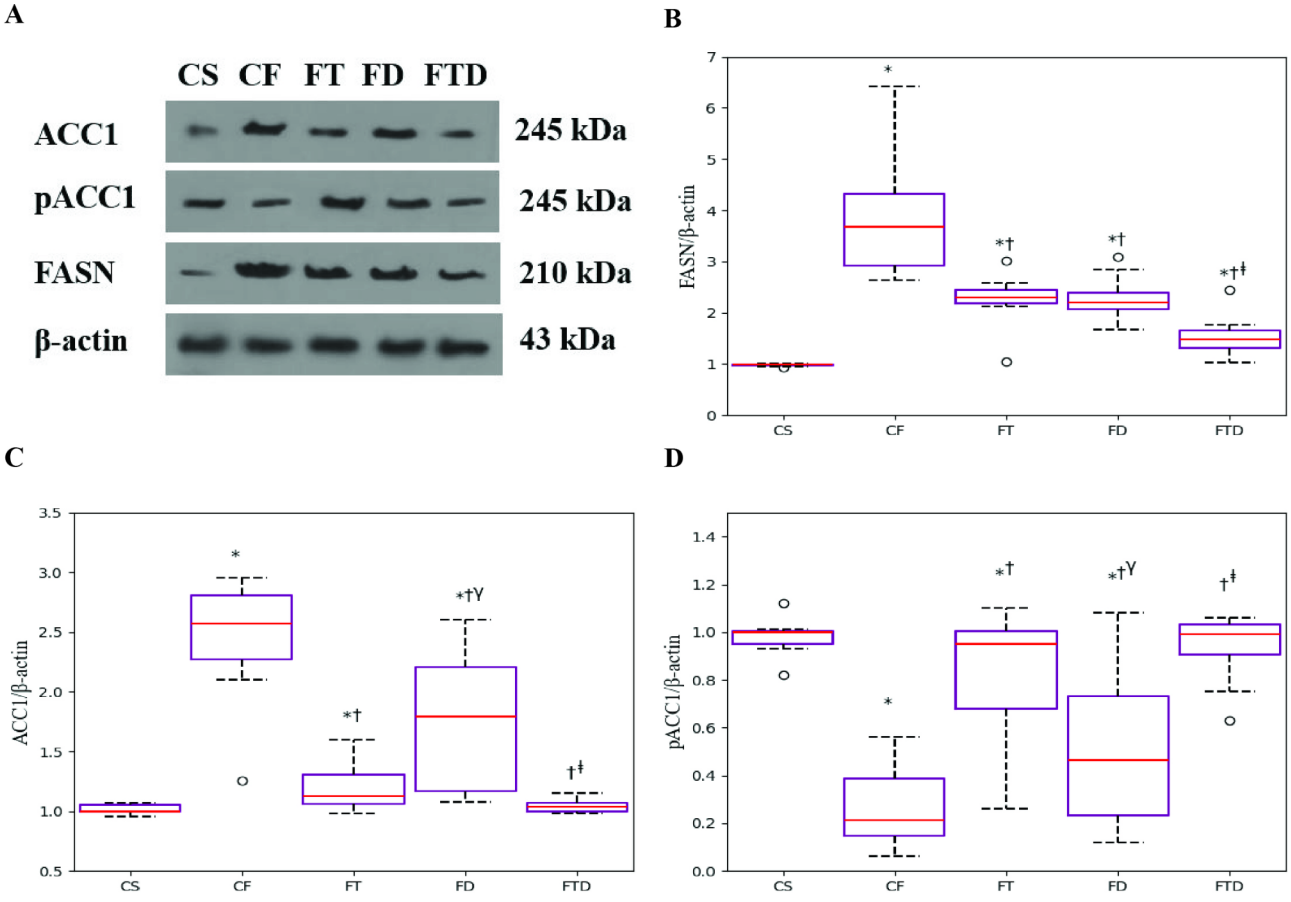

Fig. 4 (**A**) Western blot analysis of the protein expression of ACC1, FASN, p-ACC1, and β-actin. (**B**, **C**, and **D**) Box and whisker plots of ACC1, FASN, and p-ACC1 relative to β-actin. CS: control group with standard diet; CF: control group with HFrD; FT: HFrD + HIIT; FD: HFrD + VDS; FTD: HFrD + HIIT + VDS. The middle line in the box represents the median, the upper and lower areas of the center box indicate the 75th and 25th percentiles, respectively, and the vertical bars indicate standard errors. Means with different superscripts differ significantly, $P < 0.05$. *: significant difference compared with CS. †: significant difference from CF. Y: significant difference from FD and FT. Ɣ: significant difference from FT

influences the activation of G protein-coupled receptors (GPCRs). This activation leads to the production of the second messenger, 3',5'-adenosine monophosphate (cyclic AMP or cAMP), through the cyclization of ATP by adenylyl cyclase. Once cAMP is produced, it activates PKA [48], which in turn stimulates the phosphorylation (activation) of AMPK [49]. AMPK shifts liver lipid metabolism away from fatty acid synthesis by phosphorylating and thereby inhibiting key enzymes such as ACC, FASN, SREBP-1 [50], , and ChREBP [51], ultimately reducing the expression of these lipogenic enzymes. In addition to phosphorylating AMPK, protein kinase A directly phosphorylates SREBP1c and suppresses its transcriptional activity [52]. Furthermore, PKA directly phosphorylates liver X receptor alpha (LXRalpha), a dominant activator of SREBP-1c [53] and ChREBP [54] expression. The target genes of SREBP-1 include ACC1 and FASN. Therefore, PKA activation attenuates SREBP-1c and ChREBP activity and SREBP-1-mediated lipogenesis [55]. In this study, after 12 weeks of HIIT, no significant difference was observed in the p-AMPK/AMPK ratio, but there was a significant increase in the expression levels of PKA, AMPK, and p-AMPK. 2. PPAR-gamma (PPARγ): While PPARγ normally promotes DNL and lipid droplet deposition in hepatocytes [56], HIIT significantly inhibits PPARγ gene expression in the liver [46, 57]. 3. miR-122: This microRNA, the most abundant in hepatocytes, has an antilipogenic role by suppressing the expression of lipogenic genes such as SREBP-1c. HIIT has been shown to increase miR-122 expression in rats [21]. 4. Insulin sensitivity: HIIT improves insulin sensitivity, reduces FBG levels, and decreases insulin resistance [26]. As shown in the present study, after 12 weeks of HIIT, the levels of FBG, insulin, HOMA-IR, TG and cholesterol and the IHL content decreased, which may be evidence of reduced activity in the DNL pathway as a result of HIIT.

HIIT enhances fatty acid oxidation in the liver. ACC is crucial for regulating this process, as it catalyzes the conversion of acetyl-CoA to malonyl-CoA [58]. When AMPK phosphorylates ACC, it inhibits its activity,

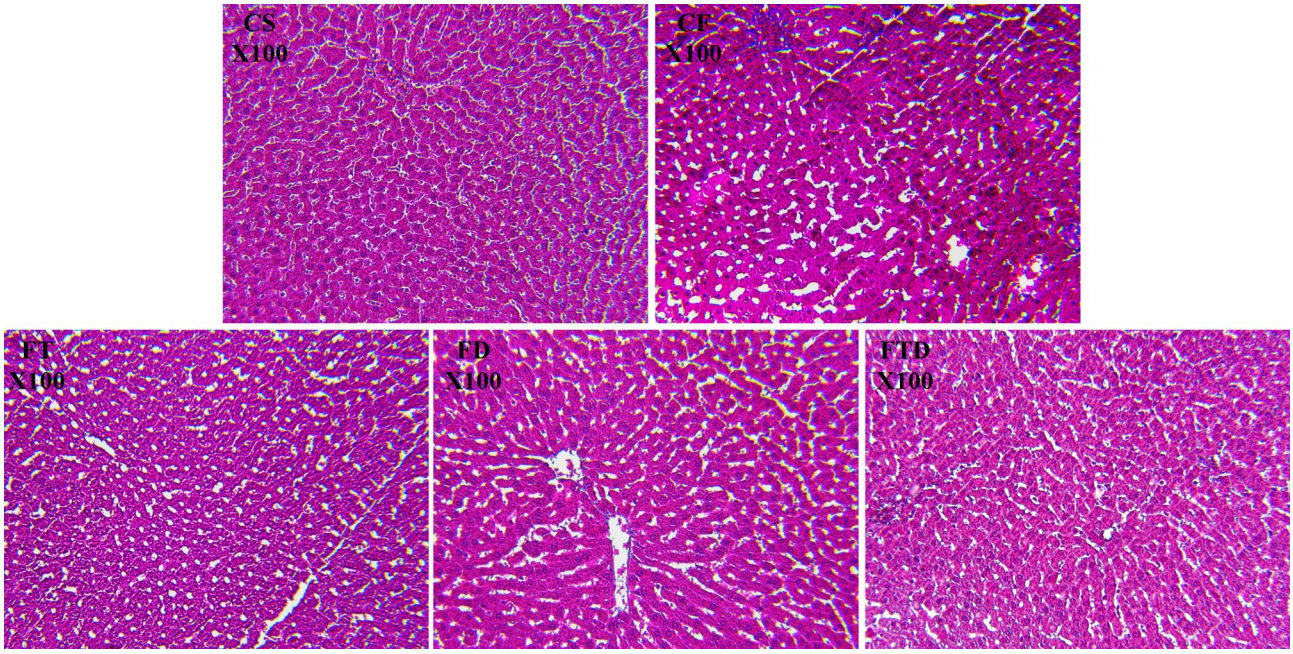

Fig. 5 Liver analysis: H&E staining of the livers of the rats. CS: control group with standard diet; CF: control group with HFrD; FT: HFrD + HIIT; FD: HFrD + VDS; FTD: HFrD + HIIT + VDS

leading to reduced intracellular malonyl-CoA levels. This reduction alleviates the inhibition of CPT1, resulting in increased fatty acid oxidation [59]. In this context, the present study revealed that 12 weeks of HIIT led to a decrease in ACC1 levels but an increase in p-ACC1 levels. The key factors for fatty acid oxidation are CPT1α, PPARα, and acyl-CoA oxidase 1 (ACOX1). HIIT increases the expression of these genes in the livers of mice [60]. Furthermore, exercise studies in rodents have demonstrated improvements in mitochondrial respiration and elevated levels of cytochrome C, suggesting that exercise not only enhances β-oxidation but also increases downstream oxidative phosphorylation [50].

Recent studies have indicated an inverse relationship between liver lipid levels and vitamin D_3 status, showing that as the liver lipid content increases, vitamin D_3 levels significantly decrease [61]. A cross-sectional study involving 6,567 healthy Korean men by Rhee et al. revealed that participants with higher serum 25-hydroxyvitamin D3 (25(OH)D3) levels had a significantly lower risk of developing nonalcoholic fatty liver disease than those with lower 25(OH)D3 levels, regardless of obesity or metabolic syndrome [62]. In Maia-Ceciliano's study, the mice were divided into different groups, with high-dose (10,000 IU/kg) and low-dose (1,000 IU/kg) vitamin D_3 and no vitamin D_3 supplementation. Research has shown that FASN and SREBP1c are significantly reduced by VD3 at a dose of 10,000 IU/kg [23]. In another animal study, the administration of low-dose (1 μg/kg), middle-dose (2.5 μg/kg) and high-dose (5 μg/kg) 1,25-dihydroxyvitamin D3, a biologically active form of vitamin D (1,25(OH)2D3), attenuated hepatic steatosis in a dose-dependent manner and downregulated the mRNA expression of SREBP-1c and its target genes, ACC and FAS [63]. In another investigation, diabetic mice treated with vitamin D_3 (at doses of 300 ng/kg or 600 ng/kg). As a result, low-dose treatment significantly reduced levels of FAS and SREBP1c. High-dose treatment led to a marked reduction in both FAS levels and triglyceride content [64]. Thus, investigating whether the effect of vitamin D_3 on liver fat is dose dependent requires further studies.

In association with the mechanisms involved in the protective effect of vitamin D against HFrD through a reduction in the protein levels of the DNL pathway, the following findings have been suggested: (1) Enhancement of the AMPK pathway: Vitamin D activates PKA [65] and AMPK, which are involved in the attenuation of lipogenic gene expression [66]. In this study, after 12 weeks of vitamin D_3 consumption, no significant difference was observed in the p-AMPK/AMPK ratio, but the protein expression of PKA, AMPK, and p-AMPK increased. (2) Inhibition of PPARγ expression: Vitamin D inhibits PPARγ expression at the molecular level through a vitamin D receptor (VDR)-dependent mechanism [67]. (3) Regulation of Insig proteins: Insulin-induced gene-1 (Insig-1) and its homolog Insig-2, which encode endoplasmic reticulum proteins that block the proteolytic activation of SREBP, thereby reducing insulin-stimulated lipogenesis. 1,25-(OH)2D3 may upregulate Insig-2 [68].

(4) Improvement in glucose tolerance and insulin resistance: Vitamin D positively impacts glucose tolerance and insulin resistance by increasing insulin sensitivity [69] and stimulating the expression of insulin receptors, thus improving insulin responsiveness for glucose transport [68]. The present study also revealed a decrease in the levels of FBG, insulin, HOMA-IR, hepatic TG and cholesterol, and IHL content, which indicates a reduction in DNL pathway activity due to the consumption of VDS.

In response to the third question of the study, the combined effect of the two interventions was more effective than their individual effects in reducing IHL induced by HFrD through the DNL pathway. Considering the independent mechanisms of VDS and HIIT in reducing the expression of the proteins involved in the DNL pathway, the combination of these two interventions may have a greater effect on decreasing the levels of these proteins, as demonstrated in our study.

Study strengths and limitations

This research is the first to explore the effects of HIIT, vitamin D_3 supplementation, and their combination on preventing hepatic fat accumulation due to fructose consumption. The findings offer valuable insights into how these interventions can help prevent diseases associated with increased liver fat. Furthermore, the results have practical applications. Nonetheless, the study has some limitations, including the absence of individual assessments of maximum speed in rats during the exercise performance test and the failure to analyze all proteins involved in the hepatic de novo lipogenesis pathway.

Conclusions

Overall, HFrD leads to increased IHL content and diabetes in rats, whereas VDS and HIIT likely prevent the increase in IHL accumulation and the onset of diabetes, probably by reducing the levels of transcription factors and enzymes involved in the DNL pathway. The combined effect of these two interventions is more effective.

Abbreviations

HIIT High	Intensity interval training
FBG	Fasting blood glucose
IHL	Intrahepatic lipid accumulation
DNL	De Novo Lipogenesis
VDS	Vitamin D< Subscript>3</Subscript> supplementation
HFrD	High-fructose diet
SREBP1c	Sterol regulatory-element binding protein 1c
ChREBP	Carbohydrate-Responsive Element-Binding Protein
ACC1	Acetyl-CoA carboxylase
FASN	Fatty acid synthase
p-ACC	Phospho-acetyl-CoA carboxylase
AMPK	AMP-activated protein kinase
p-AMPK	Phosphorylated AMPK
PKA	Protein kinase A
IP	Intraperitoneal Injection
BW	Body weight
GOD-POD	Glucose oxidase-peroxidase
HOMA-β	HOMA of β-cell function
ANOVA	One-way Analysis of Variance
PGC-1β	Peroxisome proliferator-activated receptor gamma coactivator-1β
CPT-1	Carnitine palmitoyltransferase-1
PKCϵ	Protein kinase C epsilon type
PPARγ	PPAR-gamma
VDR	Vitamin D Receptor
Insig-1	Insulin-induced gene-1
GPCRs	G protein-coupled receptors
cAMP	3′, 5′-Adenosine monophosphate
25(OH)D3	25-hydroxyvitamin D3
ACOX1	Acyl-CoA oxidase 1
LXRalpha	Liver X receptor alpha

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Author 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.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

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

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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