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Modulation of Brown Adipose Tissue Activity by HIIT and Vitamin D_3 Supplementation in Type 2 Diabetes Rat Models

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Abstract

Background

Type 2 diabetes mellitus (T2DM) is a chronic disorder marked by hyperglycemia from insulin resistance and impaired secretion. Because adipose tissue regulates energy and glucose, activating brown adipose tissue (BAT) may offer therapeutic benefit. High-intensity exercise improves metabolic health, and low vitamin D is linked to worse insulin function and higher T2DM risk. Hence, the aim of the current study was to investigate the impact of high-intensity interval training (HIIT) and vitamin D_3 supplementation on BAT in T2DM rats.

Methods and Results

Forty male Wistar rats (6–8 weeks old, ~ 220 g) were used to induce T2DM via a high-fat diet (HFD) and Streptozotocin (STZ). Rats were randomized into five groups (n = 8 each): healthy control (HC), diabetic control (DC), diabetic HIIT (DT), diabetic vitamin D_3 (DV), and diabetic HIIT plus vitamin D_3 (DTV). Interventions lasted 8 weeks, with HIIT performed on a treadmill (5 days/week) and vitamin D_3 administered by weekly subcutaneous injection (10,000 IU/kg). Western blotting was used to assess protein changes related to the interventions. This study shows that eight weeks of HIIT combined with vitamin D_3 supplementation significantly enhanced the expression of FGF21, GPR120, PGC-1α, and UCP1 in BAT of T2DM rats. Vitamin D_3 alone elevated all four proteins, while HIIT alone increased only GPR120 and UCP1.

Conclusion

These results suggest vitamin D_3 as a potential means to enhance BAT activity in type 2 diabetes, highlighting its value as a non-pharmacological approach to improving metabolic control.

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic, multifactorial metabolic disorder marked by persistent hyperglycemia, arising from a combination of insulin resistance and impaired insulin secretion[1, 2]. Characterized by systemic pathology, this disease influences virtually every organ and is tightly linked to obesity and the metabolic syndrome[3]. A sustained increase in diabetes incidence has been observed worldwide in recent decades, highlighting its growing impact on global health systems[2]. As such, targeted and widely accessible treatments are key to mitigating complications and extending the life expectancy of patients with T2DM[4]. Among the organs involved in metabolic regulation, adipose tissue has gained attention as a central hub of inter-organ communication. Systemic glycemic control depends on such communication in response to nutritional and physiological cues, with adipose tissue serving as a key regulator of energy balance and glucose metabolism[5, 6]. Through UCP1-dependent

thermogenesis, brown adipose tissue (BAT) plays a specialized role in energy dissipation and heat generation[7]. Consequently, targeting BAT activation and white adipose tissue (WAT) browning represents a promising therapeutic strategy for restoring metabolic flexibility in the context of obesity and diabetes[6]. Importantly, by oxidizing glucose and fatty acids to generate heat, brown and beige adipocytes contribute significantly to systemic regulation of energy, glucose, and lipid metabolism[8]. In addition to intrinsic thermogenic activity, lifestyle factors such as exercise exert powerful effects on adipose tissue metabolism. Both aerobic endurance and resistance training improve metabolic health by lowering fat mass and increasing muscle mass, while high-intensity exercise, such as high-intensity interval training (HIIT), enhances these benefits by boosting energy expenditure and improving insulin sensitivity in various tissues[6]. Brown and beige adipose tissues are activated by Sympathetic nervous system (SNS) signaling, where norepinephrine binds to β-adrenergic receptors (β-AR), inducing Uncoupling protein 1 (UCP1) and Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) via the p38 MAPK pathway. Exercise upregulates β-AR, enhances Adipose triglyceride lipase (ATGL) and Hormone-sensitive lipase (HSL) activity, and promotes BAT thermogenesis and WAT browning[9].

Beyond physical activity, nutritional and hormonal factors also influence adipose tissue metabolism. Multiple investigations[10] have found associations between low vitamin D levels and alterations in insulin production, glucose homeostasis, and insulin sensitivity, all of which may increase the risk of T2DM[11]. As a fat-soluble vitamin, vitamin D can be obtained through the diet or synthesized in the skin from 7-dehydrocholesterol upon exposure to ultraviolet B radiation[12]. In addition to serving as a storage site for vitamin D, adipose tissue expresses the vitamin D receptor (VDR) and enzymes involved in its metabolism. Recent studies have demonstrated that vitamin D_3 is biologically active in adipocytes, where it interacts with various cellular components, including membrane-bound receptors, adaptor proteins, phosphatases, and nuclear coregulatory proteins[13].

Another important regulator linking adipose tissue function and systemic metabolism is fibroblast growth factor 21 (FGF21)[14]. By reducing blood glucose without relying on insulin, improving cholesterol balance, and increasing both adiponectin and markers of bone formation, FGF21 shows promise as a treatment for metabolic diseases such as diabetes and obesity[15]. Its secretion induces the expression of PGC-1α and UCP1, promoting mitochondrial biogenesis and enhancing thermogenesis in BAT[16]. The liver is typically the main producer of serum FGF21, though WAT, BAT, and skeletal muscle also release it. Interestingly, in response to cold, BAT has been shown to become the major source[15]. Closely related is G-protein coupled receptor 120 (GPR120), also known as Fatty acid receptor 4 (FFAR4), a G protein–coupled receptor that recognizes unsaturated long-chain fatty acids and their derivatives. It plays a key role in mediating the anti-inflammatory effects of polyunsaturated fatty acids and contributes to protection against obesity and T2DM. [17, 18]. GPR120 deficiency suppresses FGF21 gene expression in BAT and lowers circulating FGF21, whereas GPR120 activation induces its release; this reduction in knockout mice likely accounts for the blunted thermogenic and browning response[7, 18]. UCP1, a BAT-specific protein located in the inner mitochondrial membrane, enables heat production by uncoupling oxidative phosphorylation, causing protons to leak back into the mitochondrial

matrix and generate heat instead of ATP[6]. Exercise increases UCP1 levels and promotes mitochondrial biogenesis, thereby enhancing the thermogenic capacity of brown fat and elevating the body's basal energy expenditure. Furthermore, PGC1-α, a key transcriptional coactivator, facilitates the activation of nuclear receptors such as Peroxisome proliferator-activated receptor gamma (PPAR-γ), driving both transcriptional and post-transcriptional upregulation of UCP1 in brown adipocytes[19].

Given these interconnections between exercise, vitamin D, and thermogenic regulators such as FGF21, GPR120, and UCP1, it is important to explore their combined influence on BAT activation in the diabetic state. The present investigation therefore examines the effects of an 8-week HIIT protocol in conjunction with vitamin D_3 supplementation on the expression of proteins implicated in BAT activation in a T2DM rat model. While previous research has independently explored the influence of exercise on these proteins within BAT or in other tissues, to our knowledge, this is the first study to evaluate the integrative effects of HIIT and vitamin D_3 supplementation on BAT activation, thereby addressing a critical gap in the current understanding of exercise–nutrient interactions in the context of T2DM.

Materials and Methods

Animal Experiments. Forty male Wistar rats with an average age of 6–8 weeks and a weight of 220 grams were obtained from the Pasteur Institute, Karaj, Iran, and transferred to the Animal Laboratory of the Faculty of Medicine, Kurdistan University of Medical Sciences. All procedures in this study followed the ARRIVE guidelines. The animals were housed at a constant temperature of 22.2°C under a 12-hour light/dark cycle, with ad libitum access to drinking water[20, 21]. After a one-week acclimatization period with standard chow, the rats were randomly assigned to two dietary groups: standard normal diet (ND) (containing 20% protein, 70% carbohydrate, 10% fat) and high fat diet (HFD)[21] (containing 20% protein, 35% carbohydrate, 45% fat) (Fig. 1).

Diabetes Induction Method. Body weight was recorded prior to grouping to ensure homogeneity between groups. Rats in the HFD group were fed the HFD for six weeks to induce insulin resistance, followed by intraperitoneal injection of STZ to induce T2DM. After the 6-week feeding period and a second weight measurement, a single intraperitoneal dose of STZ (35 mg/kg body weight) dissolved in 0.1 mol/L citrate buffer (pH 4.5) was administered following a 6-hour fast[22]. Concurrently, rats in the ND group received an intraperitoneal injection of sodium citrate–citric acid buffer solution as a control[23].

Grouping. Rats were randomly assigned to five groups (n = 8 each): healthy control (HC), diabetic control (DC), diabetes + HIIT (DT), diabetes + vitamin D_3 (DV), and diabetes + HIIT + vitamin D_3 (DTV). (Fig. 1).

Exercise Protocol. Rats involved in HIIT intervention (DT and DTV groups) underwent an 8-week HIIT protocol, five days per week, using a treadmill for animal models. Prior to training, a one-week familiarization phase involved walking and running at 10 to 12 m/min. on a level gradient for 15 min. Maximal running speed (Vmax) was assessed using the Bedford exhaustion test, which consisted of ten 3-minute intervals on a treadmill starting at 0.3 km/h and increasing by 0.3 km/h until exhaustion. The

last completed speed was recorded as the true exhaustion time (TTE) and Vmax[23]. This assessment was conducted pre-intervention and biweekly during the HIIT program. The HIIT protocol included 4 to 10 intervals of 2-minute running bursts at 85–95% of Vmax, with active recovery periods of 3 to 9 intervals of 1-minute running at 50% of Vmax. Each session began and ended with a 5-minute warm-up and cool-down at 40% of Vmax.

Vitamin D_3 Supplementation. Rats assigned to the vitamin D_3 supplementation groups (DV and DTV) received weekly subcutaneous injections of 10,000 IU/kg vitamin D_3 (Caspian Pharmaceutical Company, Rasht, Iran) for eight weeks. The dose withdrawn from a 300,000 IU/kg ampoule using a 1 mL insulin syringe, diluted in 5 mL of sesame oil to achieve a concentration suitable for injecting 0.1–0.5 mL per rat (based in body weight), and stored in amber 20 mL tubes at 4°C to protect it from light. Body weight of each rat was recorded with a digital scale prior to each injection to adjust dosing. Injections were administered into the loose skin of the flank region once per week, between 8:00 AM and 10:00 AM, on non-training days, to avoid interference with the exercise-induced metabolic responses (Fig. 1).

Anesthesia, Sampling, and Tissue Collection in Rats. Twenty-four hours after the final HIIT session and following an 8-h fast, the rats were anesthetized by subcutaneous injection of ketamine (75 mg/kg; Stroop, Belgium, batch no. 200178) and xylazine (10 mg/kg; Nixgen, USA, batch no. 200178). After induction, animals were left undisturbed for 5 min to minimize stress and anesthesia depth was confirmed by absence of reflexes and no response to stimuli. While deeply anesthetized, animals were euthanized by rapid decapitation using a guillotine. This method was chosen and performed by trained personnel to minimize suffering. Death was confirmed by absence of respiration and irreversible cessation of cardiac activity. Interscapular brown adipose tissue (iBAT) was excised on a dissecting board; a portion was fixed in 10% neutral buffered formalin for paraffin embedding and histology, and another portion was snap-frozen in liquid nitrogen and stored at − 80°C until analysis.

Western Blotting

Sample Preparation. Brown adipose tissue (500 mg) or cells (1×10^6) were homogenized in 500 µL PIPA buffer containing 50 mM Tris-HCl (pH 8.0), 3 mM EDTA, 80 mM NaCl, 25 mM sodium deoxycholate, 10 mM SDS, one protease inhibitor cocktail tablet (Roche), and 1% NP-40 (10 µL). Lysates were centrifuged at 4°C for 10 min at 12000 × g (Eppendorf 5415 R), and the supernatants were collected and stored at −20°C until use. Protein concentrations were determined using the Bradford assay (Bio-Rad, USA). Samples were mixed with Laemmli sample buffer (3X) and denatured at 100°C for 5–10 min.

SDS-PAGE and Transfer. Proteins (20 µg per lane, 12 µL) and a pre-stained protein marker (2 µL; Bio-Rad, USA) were loaded onto 12% SDS–polyacrylamide gels. Electrophoresis was performed at 120 V for ~ 45 min, until the dye front reached the bottom of the gel. Proteins were transferred to a PVDF membrane (Immun-Blot PVDF Membrane, Roll, 26 cm x 3.3 m, Cat. No1620177) using a wet transfer system (Trans-Blot® Turbo™ Transfer System, Cat. No. 1704150) in transfer buffer at 120 V for 90 min. Membranes were then processed for immunodetection.

Blocking and Antibody Incubation. Membranes were blocked with 2% nonfat dry milk in TBST for 75 min at room temperature to prevent nonspecific antibody binding. They were then incubated with primary antibodies overnight at 4°C: β-actin (sc-517582, 1:300), FGF21 (sc-81946, 1:300), PGC1α (66369-1-Ig, 1:300), UCP1 (sc-293418, 1:300), GPR120 (sc-390752, 1:300). After three washes with TBST (15 min each), membranes were incubated with secondary antibodies: donkey anti-goat IgG-HRP (sc-2020, 1:1000), m-IgGκ BP-HRP (sc-516102, 1:1000), mouse anti-rabbit IgG-HRP (sc-2357, 1:1000).

Detection and Stripping. Protein bands were visualized using enhanced chemiluminescence (ECL) reagents (Bio-Rad, USA). Equal volumes of Reagents A and B were mixed and applied to cover the PVDF membrane (~ 0.1 mL/cm²). The membrane was sealed in cellophane and exposed to X-ray film (Bio-Rad, USA) for 10–80 s. Films were developed using standard developer, fixer, and water washes.

For reprobing, membranes were stripped with stripping buffer at 37°C for 2–3 min, washed twice with TBST (5 min each), and reblocked before incubation with new primary antibodies. β-actin was used as a loading control to verify equal protein loading across samples.

Statistical Analyzes. Statistical analyses were performed using SPSS software, version 23 (SPSS, USA). Data are presented as mean ± standard deviation (SD) in tables and graphs. The Shapiro–Wilk test was applied to assess the normality of distribution. Group differences were evaluated using one-way analysis of variance (ANOVA), followed by the Bonferroni post hoc test when a significant F value was obtained. A p-value of $p < 0.05$ was considered statistically significant.

Results

Results from the one-way ANOVA confirmed significant between-group differences (Table 1) for FGF21 ($P < 0.0001$, F: 8.712), GPR120 ($P < 0.0001$, F: 46.634), PGC-1α: ($P < 0.0001$, F: 8.652), and UCP1: ($P < 0.0001$, F: 10.004).

Table 1
Descriptive statistics for the study measures.

Proteins	**Groups**			
(β-actin relative density fold of control)	**DC**	**DT**	**DV**	**DTV**
FGF21	0.58 ± 0.20	0.81 ± 0.07	0.99 ± 0.19*	0.94 ± 0.14*
GPR120	0.50 ± 0.05	0.66 ± 0.11*	0.78 ± 0.06*	0.90 ± 0.04*
PGC-1α	0.55 ± 0.09	0.76 ± 0.13	0.83 ± 0.22*	0.93 ± 0.15*
UCP1	0.56 ± 0.07	0.86 ± 0.08*	0.85 ± 0.24*	0.96 ± 0.12*

Note: DC: diabetic control, DT: diabetes + HIIT, DV: diabetes + vitamin D_3, DTV: diabetes + HIIT + vitamin D_3. *Significant difference versus the diabetic control group.

Effect of HIIT on BAT in T2DM rats: After posthoc analysis showed that 8 weeks of HIIT alone (DT) significantly increased UCP1 ($P = 0.005$) (Fig. 2) and GPR120 ($P = 0.005$) (Fig. 3) levels in diabetic rats compared to the diabetic control group. However, no significant changes were observed in PGC1-α (Fig. 4) and FGF21 (Fig. 5).

Effect of vitamin D_3 supplementation on BAT in T2DM rats: Posthoc analysis revealed that 8 weeks of only vitamin D3 supplementation (DV) significantly elevated GPR120 ($P < 0.0001$), FGF21 ($P < 0.0001$), PGC1-α ($P = 0.017$), and UCP1 ($P = 0.006$) levels in BAT compared to the diabetic control group.

Effect of combined HIIT and vitamin D_3 supplementation on BAT in T2DM rats: After 8 weeks of combined HIIT and vitamin D_3 supplementation, significant increases were observed in GPR120 ($P < 0.0001$), FGF21 ($P = 0.002$), PGC1-α ($P = 0.001$), and UCP1 ($P < 0.0001$) levels in BAT compared to the diabetic control group.

Discussion

This study was designed based on the hypothesis that eight weeks of HIIT combined with vitamin D_3 supplementation would stimulate BAT activity in rats with T2DM. Recent studies have increasingly focused on the biological pathways that regulate energy balance to counteract the negative effects of insulin resistance and metabolic diseases associated with obesity and overeating[6]. Accordingly, our aim was to explore how exercise and nutritional strategies together may activate metabolic pathways in BAT, ultimately improving insulin sensitivity.

The present study demonstrated that the combination of HIIT and vitamin D_3 supplementation did not elicit synergistic effects beyond those achieved with vitamin D_3 alone, indicating that vitamin D may play a predominant role in driving the observed metabolic improvements. Both vitamin D_3 supplementation alone and its combination with HIIT significantly elevated the levels of FGF21, GPR120, UCP1, and PGC-1α proteins in the brown adipose tissue of diabetic rats compared with diabetic controls. These findings emphasize the significance of vitamin D–associated signaling pathways as promising therapeutic targets and suggest that coordinated nutritional interventions may yield benefits in the management of T2DM.

HIIT is a relatively recent training method compared to endurance or resistance protocols and has gained attention due to its efficiency. It involves short bursts of intense effort followed by rest, resulting in a higher metabolic effect compared to other common training protocols [21, 24]. Because it requires less time, HIIT may be especially motivating for patients with low exercise adherence. Most previous studies on BAT activation have focused on endurance exercise[11, 15, 25], making the current study a novel contribution. Xiong et al. (2020) compared HIIT and moderate-intensity continuous training (MICT) in obese rats and found that both increased serum FGF21 and reduced diet-induced weight gain, but HIIT had a stronger effect on weight loss. In their study, MICT significantly elevated BAT FGF21 levels, while HIIT did not[26]. Consistent with these findings, our study showed that HIIT alone did not significantly raise FGF21 levels in BAT; however, combining it with vitamin D_3 supplementation did.

Similarly, Kartinah et al. (2024) reported that six weeks of HIIT improved glucose control in diabetic rats by increasing plasma and muscle FGF21 and GLUT4 expression through AMPK pathway activation[27]. Exercise promotes FGF21 expression and activation through several pathways, including elevated norepinephrine and cAMP levels[28]. In addition, factors such as energy and ER stress, mitochondrial dysfunction, cold exposure, short-chain fatty acids, low protein, and thyroid hormones can further stimulate the production, activation, and secretion of FGF21[29, 30]. FGF21 activation begins with binding to FGFRs and the co-receptor β-klotho (KLB), initiating downstream signaling[14, 29].

The role of exercise in regulating GPR120 has been less explored. GPR120 is essential for mediating the anti-inflammatory effects of polyunsaturated fatty acids and provides protection against obesity and diabetes[18]. GPR120 deficiency disrupts BAT FGF21 expression and reduces circulating FGF21 levels[7, 17, 18], showing their close interaction. In this study, HIIT, both alone and in combination with vitamin D_3, significantly increased GPR120 expression in BAT, further supporting its role in enhancing metabolism and anti-inflammatory responses in diabetes.

We also observed that HIIT elevated UCP1 expression, a key thermogenic protein in BAT, although PGC1α levels only increased with vitamin D_3 alone or when paired with HIIT. PGC1α is a major regulator of UCP1 expression and mitochondrial biogenesis[31, 32], the observed upregulation indicates that vitamin D_3 was the main driver of BAT activation, as combining it with HIIT did not produce additional benefits. Additionally, the SNS is known to regulate BAT by releasing norepinephrine, which activates the cAMP-PKA pathway[33, 34], leading to lipolysis and UCP1 expression[19, 35]. FGF21 can also independently stimulate PGC1α and UCP1, further promoting BAT thermogenesis and white fat browning[16]. Our observed increase in FGF21 with vitamin D_3 supplementation may therefore represent a key driver of the elevated PGC1α and UCP1 levels.

An intriguing outcome of our study is that while HIIT produced only modest enhancements in BAT activity, as evidenced by increases limited to UCP-1 and GPR120 proteins, vitamin D_3 supplementation alone elicited upregulation across all four target proteins, comparable to the combined intervention. These results indicate that vitamin D_3 may serve as the principal factor underlying the observed improvements in BAT function. Vitamin D_3 is a fat-soluble nutrient crucial for energy metabolism[12]. Its deficiency has been linked to impaired glucose control, reduced insulin sensitivity, and higher diabetes risk[11]. Khaledi et al. (2023) found that aerobic training combined with vitamin D_3 supplementation increased AMPK, PGC1α, and UCP1 expression in diabetic rat adipose tissue[11]. While our study demonstrated that vitamin D_3 supplementation drove significant increases in FGF21, GPR120, PGC1α, and UCP1 expression in BAT, prior research indicates the therapeutic value of integrating exercise and dietary interventions in managing metabolic disorders such as type 2 diabetes.

Signaling pathway – Our findings demonstrated that vitamin D_3 supplementation, either alone or combined with HIIT, significantly increased FGF21 expression in BAT. This aligns well with known FGF21 signaling mechanisms. The RAF/RAS-MAPK pathway[14, 28, 30] is one of the most extensively investigated signaling routes in brown adipose tissue and other tissues (Fig. 6). FGF21 activates this

pathway by binding to its receptor and KLB, which triggers FGFR dimerization and autophosphorylation of tyrosine residues[14, 30]. This event enables the recruitment and activation of the adaptor protein FRS2α, which subsequently relays FGF signals into the MAPK cascade through additional adaptor molecules. Within this pathway, Extracellular signal-regulated kinases 1 and 2 (ERK1/2) play a pivotal role, rapidly initiating the transcription of early-response genes[14, 29]. Notably, inhibition of ERK signaling diminishes the capacity of FGF21 to promptly stimulate glucose uptake in adipocytes[14]. GPR120 triggers several signaling pathways that influence metabolic activity in brown adipocytes. It interacts with Gαq/11 proteins, leading to intracellular calcium release and activation of phosphorylation events within the ERK1/2 cascade. The rise in intracellular Ca^{2+} promotes mitochondrial depolarization and fission, thereby enhancing cellular respiration. In addition, GPR120 activation increases the expression of FGF21 and specific microRNAs, such as miR-30b, which subsequently stimulate the expression of genes associated with brown adipocyte function[7].

Vitamin D exerts most of its effects through the vitamin D receptor (VDR), which regulates hundreds of genes by binding to specific DNA response elements[12, 36]. When bound to its ligand, VDR migrates into the nucleus and forms a heterocomplex with the retinoid X receptor (RXR), where it regulates gene transcription by attaching to vitamin D response elements on target genes. In addition, vitamin D influences gene expression through interactions with other nuclear receptors and transcription factors, such as nuclear factor kappa B (NF-κB), SP1, and STAT5. The active form, $1,25(OH)_2D_3$–VDR, controls the expression of hundreds of genes in a cell- and tissue-specific manner, thereby affecting metabolic regulation, immune function, and cellular homeostasis[13, 37]. Beyond its genomic actions, VDR also mediates rapid non-genomic signaling at the plasma membrane. By interacting with proteins such as phospholipase A2 and protein kinases, it initiates the production of secondary messengers including calcium and cAMP. These cascades contribute to both immediate cellular responses and the longer-term regulation of gene expression[36, 37].

Limitations

This study has several limitations. First, findings derived from a rat model may not be directly generalizable to humans. Second, only a limited set of pathway-related proteins was analyzed, without assessment of upstream or downstream signaling components. Third, the absence of gene expression analyses restricts the mechanistic insight of the work. Although the combined intervention produced numerically greater effects, statistical significance was not achieved, likely reflecting limited statistical power. Additionally, measurements were obtained at a single time point, which may not capture temporal or dynamic responses. Finally, vitamin D metabolite levels and VDR activation were not evaluated, and no dose–response analysis was performed.

Conclusion

The present study shows that eight weeks of combined HIIT and vitamin D_3 supplementation produced marked increases in FGF21, GPR120, PGC-1α, and UCP1 expression in BAT of type 2 diabetic male rats.

Importantly, vitamin D_3 supplementation alone significantly elevated all four proteins, whereas HIIT alone increased only GPR120 and UCP1. These findings indicate that vitamin D_3 is the primary driver of the observed effects. Given the central roles of FGF21, GPR120, PGC-1α, and UCP1 in mitochondrial biogenesis, thermogenesis, and glucose homeostasis, vitamin D_3 may represent a promising non-pharmacological strategy to enhance BAT activity and improve metabolic control in type 2 diabetes. Further mechanistic work with higher powered studies, and clinical studies are warranted to confirm translatability and optimize intervention parameters.

Abbreviations

BAT: Brown adipose tissue

FGF21: Fibroblast growth factor 21

GPR120: G-protein coupled receptor 120

HIIT: High-intensity interval training

KLB: β-klotho

MAPK: Mitogen-activated protein kinase

PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

STZ: Streptozotocin

T2DM: Type 2 diabetes mellitus

UCP1: Uncoupling protein 1

VDR: Vitamin D receptor

Vmax: Maximal running speed

Declarations

Ethics approval and consent to participate. All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of the University of Kurdistan (Approval ID: IR.UOK.REC.1404.063) and were conducted in accordance with the ethical standards for the care and use of laboratory animals. All efforts were made to minimize animal suffering and reduce the number of animals used.

Consent for publication. Not applicable.

Availability of data and materials. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests. The authors declare that they have no competing interests.

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Ethics approval. All experimental procedures involving animals were approved by Research Ethics Committees of University of Kurdistan (Approval No. IR.UOK.REC.1404.063) and conducted in accordance with relevant guidelines and regulations.

Consent to participate. Not applicable.

Clinical trial number. Not applicable.

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Figures

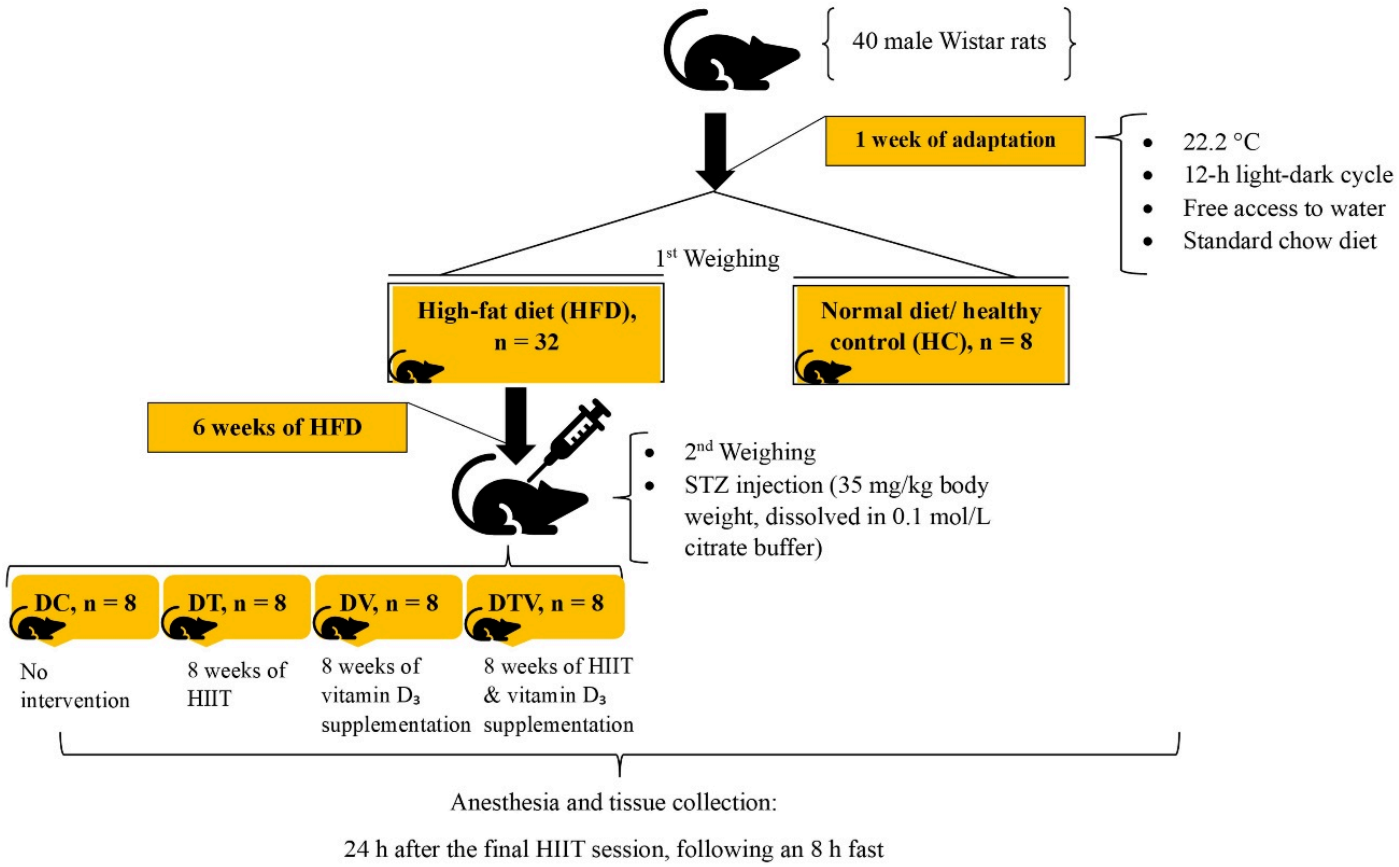

Figure 1

Forty male Wistar rats were acclimated (1 week, 22.2 °C, 12 h light/dark) then randomized to normal diet (HC, n=8) or high-fat diet (HFD, n=32). After 6 weeks HFD, STZ induced T2DM and animals were assigned to five groups (n=8 each): HC, diabetic control (DC), diabetic HIIT (DT), diabetic vitamin D_3 (DV), and diabetic HIIT plus vitamin D_3 (DTV).

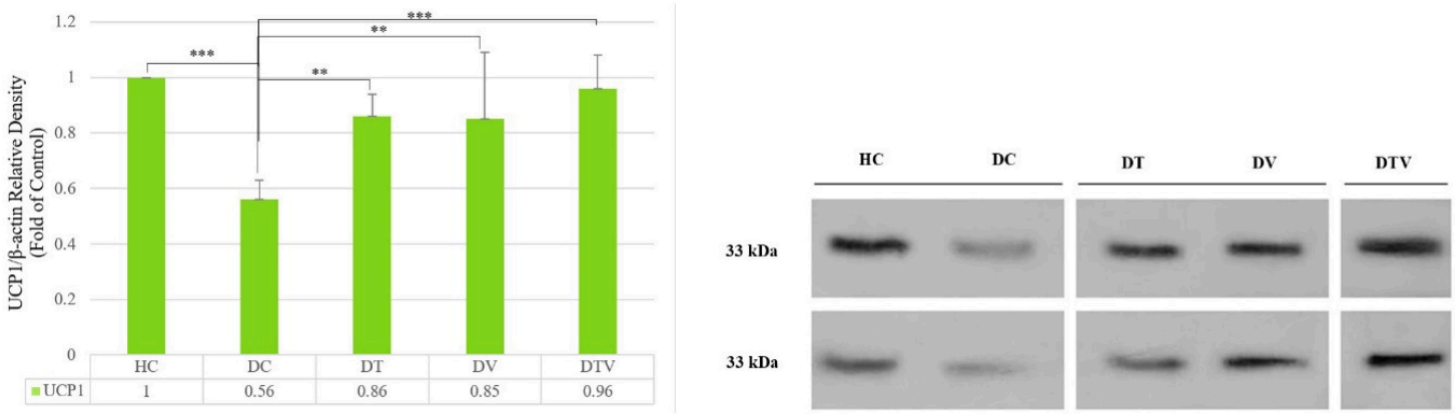

Figure 2

The effect of 8 weeks of HIIT and Vitamin D_3 supplementation on UCP1 levels in the brown adipose tissue of T2DM rats. HC: Healthy Control. DC: Diabetic Control. DT: HIIT+T2DM. DV: Vitamin D_3+T2DM. DTV: HIIT+Vitamin D_3+T2DM. **P < 0.01, ***P < 0.001. Brightness/contrast were adjusted uniformly to improve visibility; no individual bands were digitally altered.

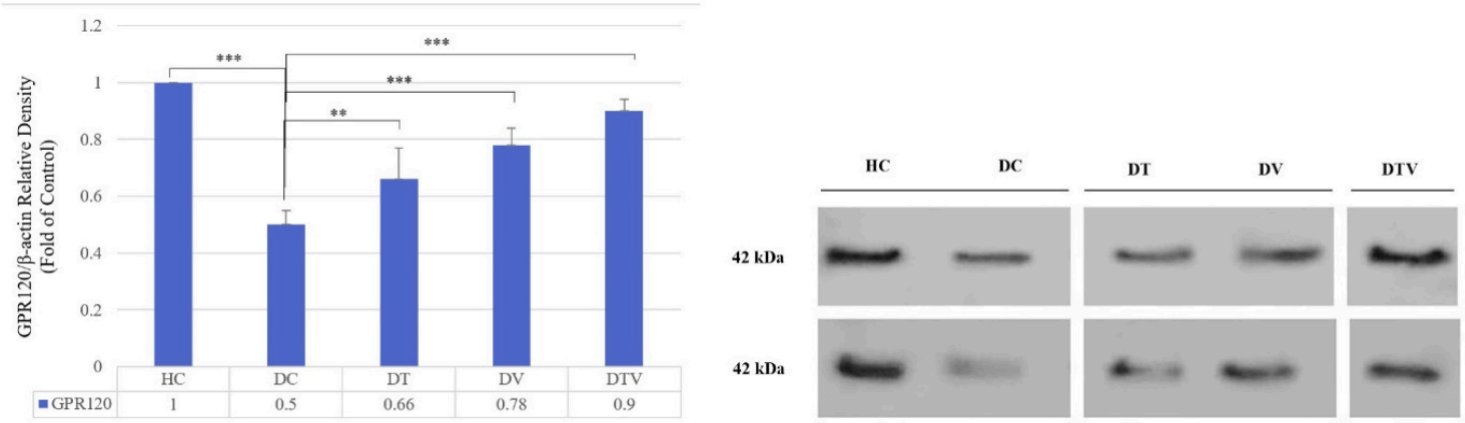

Figure 3

The effect of 8 weeks of HIIT and Vitamin D_3 supplementation on GPR120 levels in the brown adipose tissue of T2DM rats. **P < 0.01, ***P < 0.001. Brightness/contrast were adjusted uniformly to improve visibility; no individual bands were digitally altered.

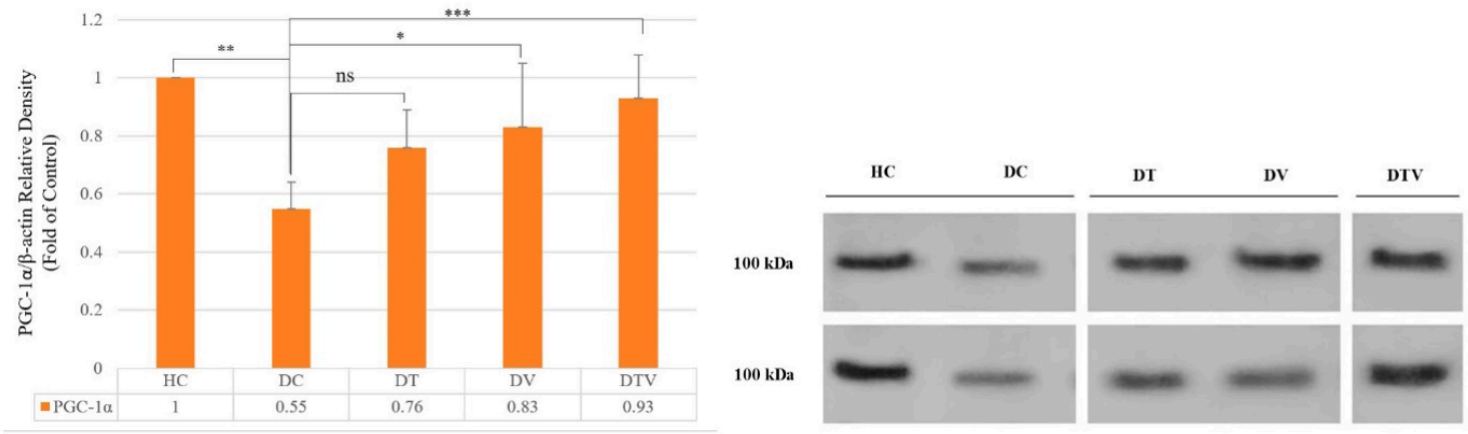

Figure 4

The effect of 8 weeks of HIIT and Vitamin D_3 supplementation on PGC-1α levels in the brown adipose tissue of T2DM rats. *P < 0.05, **P < 0.01, ***P < 0.001, ns: P > 0.05. Brightness/contrast were adjusted uniformly to improve visibility; no individual bands were digitally altered.

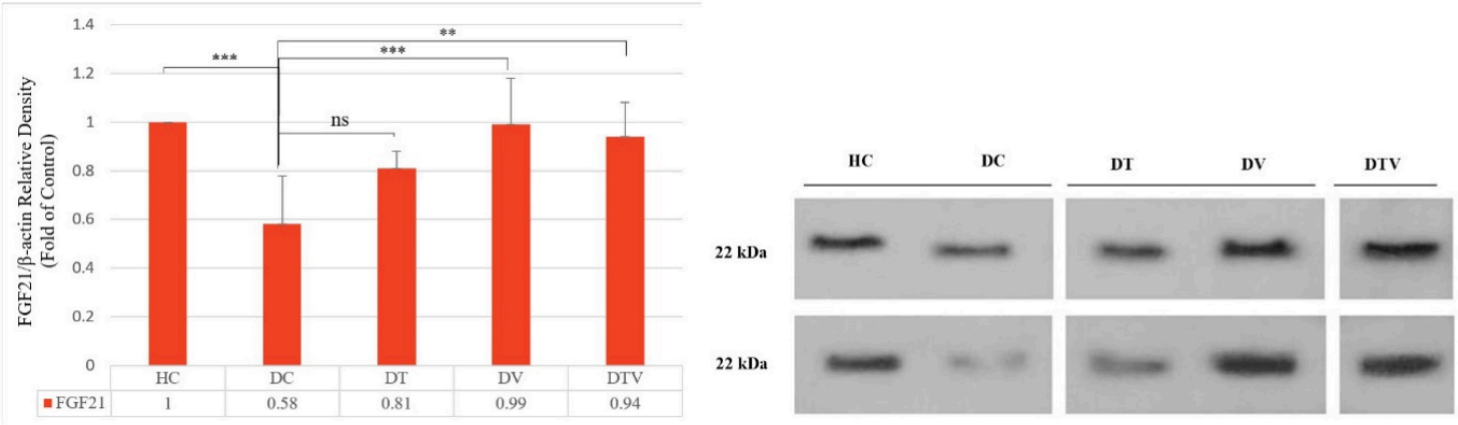

Figure 5

The effect of 8 weeks of HIIT and Vitamin D_3 supplementation on FGF21 levels in the brown adipose tissue of T2DM rats. *P < 0.05, **P < 0.01, ***P < 0.001, ns: P > 0.05. Brightness/contrast were adjusted uniformly to improve visibility; no individual bands were digitally altered.

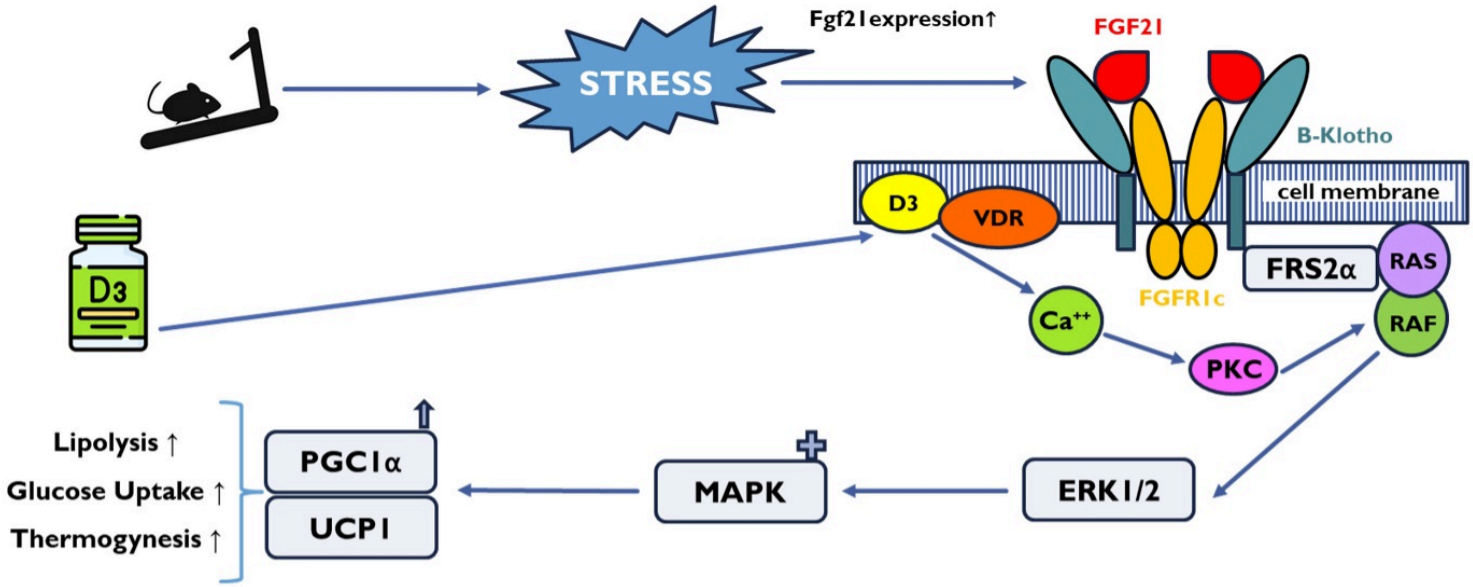

Figure 6

FGF21 binds to its receptor and cofactor KLB, inducing FGFR dimerization and autophosphorylation, which recruits FRS2α to activate the RAS/RAF-MAPK signaling cascade. FGF21: fibroblast growth factor 21, FGFR1c: Fibroblast growth factor receptor 1, VDR: vitamin D receptor, C^{++}: calcium, PKC: protein kinase C, FRS2α: fibroblast growth factor receptor substrate 2 alpha, RAS: a Small GTPase, ERK1/2: extracellular signal-regulated kinases 1 and 2, MAPK: mitogen-activated protein kinase, PGC-1α: peroxisome proliferator-activated receptor gamma coactivator 1-alpha, UCP1: uncoupling protein 1.

Supplementary Files

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