scientific reports

Check for updates

OPEN

Vitamin D and exercise improve VEGF-B production and IGF-1 levels in diabetic rats: insights the role of miR-1 suppression

Fatemeh Mazaheri[1], Rastegar Hoseini[2]✉ & Ahmad Gharzi[3]

Background Type 2 Diabetes Mellitus (T2DM) is closely associated with the development of vascular damage in the heart. In this study, the researchers aimed to determine whether Aerobic Training (AT) and Vitamin D supplementation (Vit D) could alleviate heart complications and vascular damage caused by diabetes. The effects of an eight-week AT program and Vit D on the expression of miR-1, IGF-1 genes, and VEGF-B in the cardiomyocytes of rats with T2DM.

Methods This study was an experimental investigation. Fifty male Wistar rats were divided into 2 groups Non-Diabetic Obese Control (NC; $n=10$), and diabetic ($n=40$). The rats were then randomly divided into four groups: AT plus Vit D (AT + Vit D; n-=10), AT ($n=10$), Vit D (Vit D; $n=10$), and Control Diabetic (C; $n=10$). The exercise groups underwent treadmill training for 8 weeks at an aerobic intensity equal to 50–60% of their maximal oxygen uptake (VO2max), which corresponded to a speed of 15–25 m/min at a 0% incline, for 30–60 min per day, 5 days per week. The Vit D and AT + Vit D groups received 5,000 international units (IU) of Vitamin D (combined with sesame oil) per week via a single-dose injection. Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons among the groups. Paired data were analyzed using paired t-tests.

Results The results showed that BW, BMI, and FI significantly decreased in the AT + Vit D ($p=0.001$ for all variables), AT ($p=0.001$ for all variables), and Vit D ($p=0.001$ for all variables) groups compared to baseline. In contrast, BW, BMI, and FI increased in the C ($p=0.001$, $p=0.006$, $p=0.020$, respectively) and NC ($p=0.001$ for all variables) groups. Significant differences were observed between the groups in terms of visceral fat, insulin, glucose, and HOMA-IR ($p=0.001$ for all variables). Serum 25-hydroxyvitamin D levels varied significantly among the groups ($p=0.002$). The AT + Vit D group showed significantly increased VEGF-B ($p=0.001$ for both comparisons), upregulated IGF-1 ($p=0.001$ for both comparisons), and downregulated miR-1 ($p=0.001$ for both comparisons) compared to the AT and Vit D groups, respectively.

Conclusions AT and Vit D increased the expression of IGF-1 and VEGF-B in the heart of T2DM rats while decreasing the expression of miR-1. These effects were more pronounced when AT and Vit D were combined. The study concludes that the combination of AT and Vit D has cardio-protective effects in T2DM rats, counteracting abnormal angiogenesis induced by diabetes. These effects are mediated, at least in part, by the upregulation of IGF-1 and VEGF-B, and the downregulation of miR-1.

Keywords Exercise, Vitamin D, Type 2 diabetes Mellitus, Vascular damage

Abbreviations

AT	Aerobic Training
Vit D	Vitamin D
T2DM	Type 2 Diabetes Mellitus
VEGF-B	Vascular Endothelial Growth Factor B
miR-1	microRNA-1
IGF-1	Insulin-Like Growth Factor 1

[1]Department of Exercise Physiology, Faculty of Sport Sciences, Razi University, Kermanshah, Iran. [2]Department of Exercise Physiology, Faculty of Sport Sciences, Razi University, P.O.Box. 6714414971, Kermanshah, Iran. [3]Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran. ✉email: R.hoseini@razi.ac.ir

Background

Type 2 diabetes mellitus (T2DM) has been proven to correlate strongly with the increasing prevalence of features of vascular damage in the heart, kidneys, and eyes[1], causing several metabolic disorders starting from insulin resistance to chronic heart disease (CHD)[2]. Clinical trials have shown that diabetic patients are prone to cardiomyopathy, which is characterized by diastolic dysfunction, the development of cardiac hypertrophy, and cardiomyocyte fibrosis and apoptosis regardless of microvascular and macrovascular disorders[3]. In addition, adults with T2DM are more at risk of heart attacks and strokes[4]. However, its main pathophysiology needs to be elucidated, but perturbance of the molecular mechanism related to cardiac angiogenesis might be involved[5]. Vascular Endothelial Growth Factor B (VEGF-B) plays an important role in controlling the transfer of fatty acids in the heart and skeletal muscles[6]. Several studies have reported reduced production and activity of cardiac VEGF-B isoforms to be associated with angiogenesis disorder, reduction of myocardial perfusion, and ischemia[7,8]. Studies showed abnormal angiogenesis and vascular growth in diabetic models of animals[9] and humans[4]; since diabetic hyperglycemia disturbs the downstream signaling of VEGF (i.e. Akt-1 and eNOS)[10]. Additionally, Insulin-Like Growth Factor 1 (IGF-1) plays a critical role in the normal growth of tissues, and dysregulation of IGF-1 is associated with atherosclerosis, and cardiovascular disease[11]. Studies show a relationship between low levels of IGF-I with diabetes, high blood pressure, and higher triglyceride levels[12,13]. Furthermore, the IGF-1 expression level was shown to be directly controlled through MicroRNA-1 (miR-1)[14]. The effect of Aerobic training (AT) on angiogenesis, a crucial physiological process for improving oxygen and nutrient delivery to active tissues, remains a topic of ongoing debate[15]. While numerous studies have consistently shown that exercise induces angiogenesis through various mechanisms, including paracrine regulation, mechanical stimuli, and cellular mediators, some research suggests that the impact of exercise on angiogenesis may be more complex and context-dependent[16]. For instance, a recent review highlighted that the effects of exercise on angiogenesis-related factors, such as VEGF and PGC-1α, can vary significantly depending on factors like exercise intensity, duration, and type, as well as individual characteristics like age and sex[17,18]. Additionally, studies have shown that AT-induced myokines, such as TGF-β and irisin, can both promote and inhibit angiogenesis, depending on the specific cellular context and molecular pathways involved[19]. Also, AT ameliorates hypertriglyceridemia, insulin resistance, and heart tissue angiogenic signaling in rat models of diabetes[20–22]. However, the effect of AT on angiogenesis-inducing factors in the Cardiac muscle cells (cardiomyocytes) is still a debatable topic.

The effect of Vitamin D (Vit D) on angiogenesis is a complex and context-dependent process that involves various cellular and molecular signaling pathways. Several studies have reported both pro-angiogenic and anti-angiogenic effects of Vit D, depending on factors such as cell type, cellular condition, and the presence of specific chemokines[23,24]. In cells with excessive angiogenesis, such as those found in tumor vasculature or after vascular injury, Vit D has been shown to exert an anti-angiogenic effect by reducing the activation, proliferation, and migration of endothelial cells[24,25]. This effect is mediated through the Vit D receptor (VDR), which regulates the expression of genes involved in angiogenesis and inflammation[25]. Conversely, in the absence of vascular injury, Vit D has been found to promote angiogenesis by increasing the expression of hypoxia-inducible factor 1-α (HIF1-α) and stromal cell-derived factor 1 (SDF1), which stimulate vascular repair[26,27]. Also, there are contradictory results on the effect of Vit D supplementation attenuating diabetic complications in both humans[28] and rats[29], and the mechanisms of the cardio-protective effects of Vit D have not been fully elucidated[30]. The interplay between AT and Vit D is a complex and multifaceted relationship, involving various cellular and molecular signaling pathways. AT has been shown to modulate Vit D metabolism by increasing the production of 1,25-dihydroxyvitamin D, the active form of Vit D, through the upregulation of the enzyme 1α-hydroxylase in skeletal muscle and other tissues[31,32]. This enhanced Vit D activation can, in turn, promote the expression of genes involved in muscle growth, repair, and mitochondrial biogenesis, thereby improving AT capacity and performance[33]. Conversely, Vit D has been found to enhance the angiogenic response to AT, increasing the delivery of oxygen and nutrients to active muscles and facilitating recovery from AT-induced damage[34]. Thus, we hypothesized that AT and Vit D could attenuate diabetes-induced heart complications and vascular damage by altering the expressional level of angiogenesis-inducing factors. Therefore, we investigated the effects of eight-week AT and Vit D on miR-1, IGF-1 gene expressions, and VEGF-B in the cardiomyocyte of rats with T2DM.

Methods

Animals, diets, and diabetes induction

Fifty male Wistar rats were obtained at 4-5wk of age weighing 180 ± 10 gr from the Medical University (Kermanshah, Iran). Animals were housed in transparent polycarbonate cages in the rodent's care facility under a 12:12-h light-dark cycle at a controlled temperature of 25 ± 2 °C and 45–55% humidity with provided food and water. The ethical principles of working with laboratory animals were considered in the present study. After 2 wk of new environment acclimation, standard chow was replaced by a High-Fat Diet (HFD) containing a mixture of standard mouse food powder (365 mg/kg), mixed vitamins and minerals (60 mg/kg), yeast powder (1 mg/kg), sheep fat (310 mg/kg), DL-methionine (3 mg/kg), and chloride Sodium (1 mg/kg) in the form of pellets (purchased from Beh-Parvar Company) (45% of calories from fat; 35% of calories from carbohydrates, 20% of calories from protein). This diet has been previously reported to induce obesity in rats[35,36].

Fifty male Wistar rats were divided into 2 groups Non-Diabetic Obese Control (NC; $n = 10$), and diabetic ($n = 40$). The rats were then randomly divided into four groups: AT plus Vit D (AT + Vit D; n-=10), AT ($n = 10$), Vit D (Vit D; $n = 10$), and Control Diabetic (C; $n = 10$). (Fig. 1). To determine the sample size, based on previous research, a moderate effect size of 0.5, a power level of 0.8, and a significance level of 0.05, with an estimated standard deviation of 1.5, and a five-group design with an equal allocation ratio was used suggesting a total sample size of 50 rats.

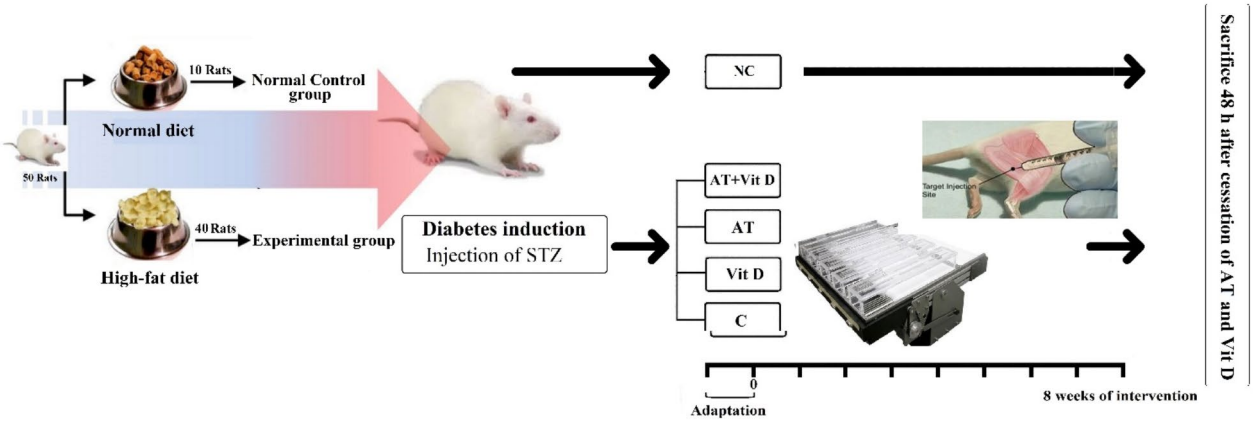

Fig. 1. Flow chart of the study.
AT + Vit D, Aerobic training + Vitamin D supplement, **AT**, Aerobic Training; **Vit D**, Vitamin D Supplement; **C**, Control; **NC**, Non-diabetes Control.

After increasing weight (more than 300 g), streptozotocin (60 mg/kg BW, dissolved in 0.1 M citrate buffer with pH = 4.5) and nicotinamide (15 min later, 110 mg/kg BW) were injected to induce diabetes. After 2 wk, blood samples were taken from the lateral tail vein to measure blood sugar; any values above 200 mg/l were considered diabetes[37]. No insulin treatments were given to animals during the study. The animals used in our study were obtained from the Kermanshah University of Medical Sciences. Additionally, we would like to mention that all necessary permissions and approvals were obtained before conducting the study. Also, the research protocol followed the tenets of the Iranian Convention for the protection of vertebrate animals used in experimental and other scientific contexts and was approved by the ethics committee of Razi University of Kermanshah, Kermanshah, Iran (IR.RAZI.REC.1401.011).

Protocols

To investigate the effects of AT on cardiomyocyte angiogenesis-inducing factors, diabetic rats were allocated to the following four groups: an AT + Vit D supplementation group (AT + Vit D; $n = 10$), an AT group ($n = 10$), a Vit D supplementation group (Vit D; $n = 10$), and a control diabetic group (C).

Protocol 1 Aerobic Training. After 1 week of acclimation to the treadmill (5 min, at 8–10 m/min, 5 days a week), the exercised groups underwent treadmill exercise for 8 wk at an aerobic intensity equal to 50–60% of VO2max (15–25 m/min, 0°, for 30–60 min/day, 5 days/week)[38]. Ten minutes of warm-up and cool-down were performed before and after the AT at 5 m/min (Fig. 1).

Protocol 2 Vitamin D supplementation. Supplement administration was performed during the last 8 wk of the intervention, and Vit D and AT + Vit D groups received 5000 international units (IU) of Vit D (combined with sesame oil) per week by single-dose injection. AT and C were injected with the same amounts of sesame oil. The serum concentration of Vit D was measured by a Vit D rat kit (enzyme-linked immunosorbent assay (ELISA); immune diagnostics system Ltd, Boldon, UK; with an intraassay coefficient of variation = 1.63%, and sensitivity of method = 1.33 mg/dL))[39] (Fig. 1).

Blood sampling

To exclude the acute effects of exercise rats were euthanized 48 h after the final training session. After 8 h of fasting, rats were anesthetized by injecting a combination of ketamine (70 mg/Kg BW) and xylazine (3–5 mg/Kg BW). After ensuring full anesthesia, blood samples were taken by exposing the vena cava following the dissection of the abdominal cavity. The supernatant, designated as serum, was used to measure glucose, insulin, and serum 25(OH)D concentration after centrifuging the blood samples for 10 min at 4,000 g. Then the supernatant was collected to measure glucose, insulin, and serum VEGF-B (Pars Azmoon Kit, Iran). Serum 25(OH)D concentration was measured by rat 25(OH)D enzyme-linked immunosorbent assay (ELISA) kit (Immunodiagnostics system Ltd, Boldon, UK). The intraassay coefficient of variation and sensitivity of the method was 1.63% and 1.33 mg/dL, respectively. Also, insulin resistance status was calculated using the homeostasis model assessment for insulin resistance (HOMA-IR) (17). After euthanization and decapitation, cardiac muscle cells were quickly dissected and homogenized as recommended by the manufacturer using a gentleMACS™ Octo Dissociator system, M tubes, and the RNA_02 program in 2 ml of TRI Reagent® buffer. The homogenate was snap-frozen in liquid nitrogen and then stored at -80 °C for future analysis.

Genes	Primers	Size (bp)	Annealing temperature (C)
IGF-1	F: TCGCATCTCTTCTATCTGGCCCTGT R: GCAGTACATCTCCAGCCTCCTCAGA	226	60
Mir-1	F: GCTATGGAATGTAAAGAAGTATGTAT R: CTCAACTGGTGTCGTGGAGTC	208	59

Table 1. The Forward and reverse primer sequences.

Variables	AT + Vit D	AT	Vit D	C	NC	P-Value [a]
Body Weight (g)						
Before	308.40 ± 2.67	310.20 ± 2.85	308.10 ± 2.72	307.30 ± 1.76	209.10 ± 3.90	
After	275.90 ± 3.57	288.90 ± 3.54	295.60 ± 4.42	312.10 ± 2.84	211.70 ± 3.91	
P†	0.001*	0.001*	0.001*	0.001*	0.001*	
Δ	-32.50 ± 2.01 [A]	-21.30 ± 1.70 [B]	-12.50 ± 3.13 [C]	4.80 ± 1.61 [D]	2.60 ± 0.69 [E]	0.001 ¥
BMI (kg/m^2)						
Before	0.76 ± 0.033	0.80 ± 0.019	0.77 ± 0.020	0.78 ± 0.030	0.53 ± 0.014	
After	0.58 ± 0.035	0.69 ± 0.036	0.70 ± 0.022	0.88 ± 0.041	0.55 ± 0.011	
P†	0.001*	0.001*	0.001*	0.006*	0.001*	
Δ	-0.17 ± 0.025 [A]	-0.11 ± 0.030[B]	-0.07 ± 0.014 [C]	0.09 ± 0.039 [D]	0.02 ± 0.006[E]	0.001 ¥
FI (g/d)						
Before	15.41 ± 0.025	15.43 ± 0.033	15.36 ± 0.026	15.47 ± 0.045	14.89 ± 0.022	
After	15.19 ± 0.054	15.26 ± 0.079	15.20 ± 0.064	15.63 ± 0.193	15.26 ± 0.135	
P†	0.001*	0.001*	0.001*	0.020*	0.001*	
Δ	-0.22 ± 0.058 [A]	-0.17 ± 0.054 [B]	-0.15 ± 0.58 [C]	0.16 ± 0.18[D]	0.37 ± 0.148 [E]	0.001 ¥

Table 2. Comparison of mean ± SD of body weight, BMI, and FI before and after intervention. **AT + Vit D**, Aerobic training + Vitamin D supplement, **AT**, Aerobic Training; **Vit D**, Vitamin D Supplement; **C**, Control; **NC**, Non-diabetes Control; **BMI**: Body Mass Index; **FI**: Food Intake. Data analysis was done by the analysis of one-way analysis of variance test followed by post hoc Tukey's test; **P†**: Statistical analysis was done by paired sample t-test; *: Significantly different in comparison pre- and post-within the groups; **P-Value[a]**: Statistical analysis was done by one-way analysis test; **¥**: Significantly different comparing Δ between groups. The mean values followed by different letters (A, B, C, D, and E) are significantly different at the 0.05 level ($p < 0.05$). The values followed by the same letter are not significantly different. Dissimilar letters represent a significant difference between the groups.

RNA extraction/real-time PCR

The homogenized samples were first incubated for 5 min at 15 to 30 °C and 0.2 ml of chloroform per 1 ml of TRIZOL Reagent was then added. After vortexing tubes for 15 s and incubating at 15–30 °C for 2 min, the samples were centrifuged at 12,000xg for 10 min at 2–8 °C. Then the upper layer was mixed into 0.5 ml of isopropyl alcohol per 1 ml of TRIZOL Reagent and centrifuged at 12,000xg for 10 min at 2–8 °C. After removing the supernatant, the RNA was washed with 75% ethanol and centrifuged at 7,500xg for 5 min at 2 to 8 °C. RNA was eluted in 30 µl of RNAse-free water and incubated for 10 min at 55 °C. Reverse transcription into cDNA was done using 1 µg of total RNA and a Prime Script RT reagent kit (Sinaclon, Iran). TB Green Premix Ex-Taq II (TaKaRa, Dalian, China) was used for quantitative RT-PCR. The expression levels of target genes were quantified by the comparative $2-\Delta\Delta ct$ method. The Sinaclon kit, Iran was used to measure IGF-1 and miR-1 gene expressions (sensitivity of 0.021, and 0.06, resp). All primers for the RT-PCR were designed using the Applied Biosystems Primer Express software V 2.0 as shown in Table 1.

Statistical analysis

All statistical analyses were performed using the SPSS statistical software (version 21; SPSS Inc., Chicago, IL, USA) was used at a significant level of $P < 0.05$. The Shapiro–Wilk test was used for evaluating the normality of distribution. Data were analyzed using one-way ANOVA followed by a Tukey test for multiple comparisons among the groups. Paired data were analyzed using paired t-tests. $P \leq 0.05$ was considered to indicate statistical significance. Data were analyzed by SPSS (version 26, IBM).

Results

Mean Body Weight (BW), Body Mass Index (BMI), and food intake (FI) are shown in Table 2. There was a significant difference between groups in the mean BW, BMI, and FI after 8 weeks of intervention; BW, BMI, and FI reduced significantly in the AT + Vit D, AT, and Vit D groups at the end of the study compared with the beginning. Mean BW, BMI, and FI increased significantly in the C and NC groups at the end of the study compared to the beginning. The results of one-way ANOVA showed significant differences in the Δ of BW, BMI,

and FI between the groups. The results of Tukey's post hoc test show that BW, BMI, and FI were significantly lower in AT + Vit D, AT, Vit D, and C compared to NC. The results indicate significant differences in BW, BMI, and FI in AT + Vit D, AT, and Vit D compared to C. Significant differences in the BW, BMI, and FI were also observed between AT + Vit D with AT, and Vit D. Additionally, there were significant differences in BW, BMI, and FI in AT compared to Vit D (Table 2).

As Table 3 shows, there was a significant difference between groups in visceral fat, insulin, glucose, and HOMA-IR ($p = 0.001$ for all variables). The results of Tukey's post hoc test show that visceral fat, insulin, glucose, and HOMA-IR were significantly lower in AT + Vit D, AT, Vit D, and C compared to NC ($p = 0.001$ for all variables). The results indicate significant differences in visceral fat, insulin, glucose, and HOMA-IR in AT + Vit D ($p = 0.001$; $p = 0.002$; $p = 0.001$), AT ($p = 0.004$; $p = 0.010$; $p = 0.007$), and Vit D ($p = 0.006$; $p = 0.0014$; $p = 0.016$) compared to C. Significant differences in the visceral fat, insulin, glucose, and HOMA-IR were observed between AT + Vit D with AT, and Vit D. The results indicate significant differences in visceral fat, insulin, glucose, and HOMA-IR in AT compared to Vit D (Table 3).

The results show significant differences between groups in serum 25-hydroxyvitamin D; with the highest level in NC and the lowest in the C group. Based on the results, the mean serum 25-hydroxyvitamin D was significantly higher in the AT + Vit D, AT, and Vit D than in the C group ($P < 0.05$ for all three variables). In addition, a significant difference was observed in serum 25-hydroxyvitamin D between the AT + Vit D group and other groups ($P < 0.05$ for all three variables). A significant difference in serum 25-hydroxyvitamin D was observed between AT compared to Vit D (Fig. 2A). The results of VEGF-B, IGF-1, and miR-1 are shown in Figures (Fig. 2B, C, and D). The results of one-way ANOVA showed a significant difference in VEGF-B, IGF-1, and miR-1 gene expression between all groups. Furthermore, AT + Vit D, AT, and Vit D increased VEGF-B, upregulated IGF-1, and downregulated miR-1 compared to the C. Based on the results, AT + Vit D significantly increased VEGF-B ($p = 0.001$; $p = 0.001$), upregulated IGF-1 ($p = 0.001$; $p = 0.001$), and downregulated miR-1 ($p = 0.001$; $p = 0.001$) compared to AT and Vit D, respectively. Also, AT induced more significant upregulations in the VEGF-B, and IGF-1 gene expression than Vit D. In addition, significant differences were observed in VEGF-B, and IGF-1 and miR-1gene expression between the C and NC ($P < 0.05$ for all three variables).

Discussion

AT and Vit D have been reported to ameliorate insulin resistance and reduce vascular complications in diabetic rats[40,41]. However, the effects of AT and Vit D on angiogenesis-inducing factors have not been well characterized in the cardiomyocytes. Therefore, the present study investigated the effects of eight-week AT and Vit D on the angiogenesis-inducing factors in the cardiomyocytes of rats with T2DM, specifically focusing on cardiac miR-1, IGF-1, and VEGF-B. T2DM disturbed the normal responses of angiogenesis-inducing factors in the cardiomyocytes of rats, and lipid accumulation, which is a novel finding of the present study. AT decreased the important heart adaptation regulator microRNA, miR-1 increased the expression of target angiogenesis-inducing factors (IGF-1 and VEGF-B), and restored T2DM-induced abnormalities in angiogenesis in the cardiomyocytes of diabetic rats. These findings indicate that AT has cardio-protective effects, ameliorating defects in cardiac angiogenesis in diabetic subjects. Angiogenic disorders in myocardial tissue have been reported during the progression of T2DM[42]. Therapy for restoring normal angiogenesis (thereby improving IGF-1 and VEGF-B) is believed to protect cardiac function against CHD progression in T2DM models[43]. Angiogenesis impairment plays a pathogenic role and deteriorates cardiac function which contributes to later heart complications and related morbidity in diabetic models[44]. Consistent with the present data, previous studies have reported that T2DM perturbed angiogenesis in different tissues and increased complications in these individuals[42,45]. Insulin-related disorders might decrease IGF-I production by reducing growth hormone (GH) receptor expression and causing GH resistance[46]. AT has been shown to have cardio-protective effects in various models of metabolic disease[47]. AT might reduce the risks of cardiovascular injuries in diabetic patients by improving the maximum oxygen consumption (VO_{2max}) and cardiac output[48,49]. Also, AT helps to improve glycosylated hemoglobin (HbA1c) and lipid profile metabolism[50]. Based on the results of studies, AT not only leads to weight loss and blood sugar control by reducing the number of fat cells through beta-oxidation in fat tissue but also improves the function of the left ventricle by increasing the functional capacity[51,52]. It has been shown that the effects of AT on VEGF-B gene expression and protein abundance might be associated with insulin resistance[53]. Based

Variables	AT + Vit D	AT	Vit D	C	NC	*P*-value
Visceral fat (g)	9.53 ± 0.028^{A}	10.10 ± 0.049^{B}	10.49 ± 0.051^{C}	11.40 ± 0.032^{D}	9.01 ± 0.045^{E}	0.001 ¥
Insulin (µU/ml)	2.66 ± 0.025^{A}	2.85 ± 0.025^{B}	2.99 ± 0.039^{C}	3.31 ± 0.021^{D}	1.20 ± 0.030^{E}	0.001 ¥
Glucose (mmol/L)	18.99 ± 0.028^{A}	19.76 ± 0.022^{B}	23.44 ± 0.034^{C}	29.57 ± 0.023^{D}	8.27 ± 0.023^{E}	0.001 ¥
HOMA-IR	0.12 ± 0.001^{A}	0.13 ± 0.001^{B}	0.17 ± 0.002^{C}	0.24 ± 0.001^{D}	0.024 ± 0.001^{E}	0.001 ¥

Table 3. Comparison of mean ± SD of visceral fat, insulin, glucose, and HOMA-IR after the intervention among the groups. **AT + Vit D**, Aerobic training + Vitamin D supplement, **AT**, Aerobic Training; **Vit D**, Vitamin D Supplement; **C**, Control; **NC**, Non-diabetes Control. Data analysis was done by the analysis of one-way analysis of variance test followed by post hoc Tukey's test; **P-Value[a]**: Statistical analysis was done by one-way analysis test; **¥**: Significantly different comparing Δ between groups. The mean values followed by different letters (A, B, C, D, and E) are significantly different at the 0.05 level ($p < 0.05$). The values followed by the same letter are not significantly different. Dissimilar letters represent a significant difference between the groups.

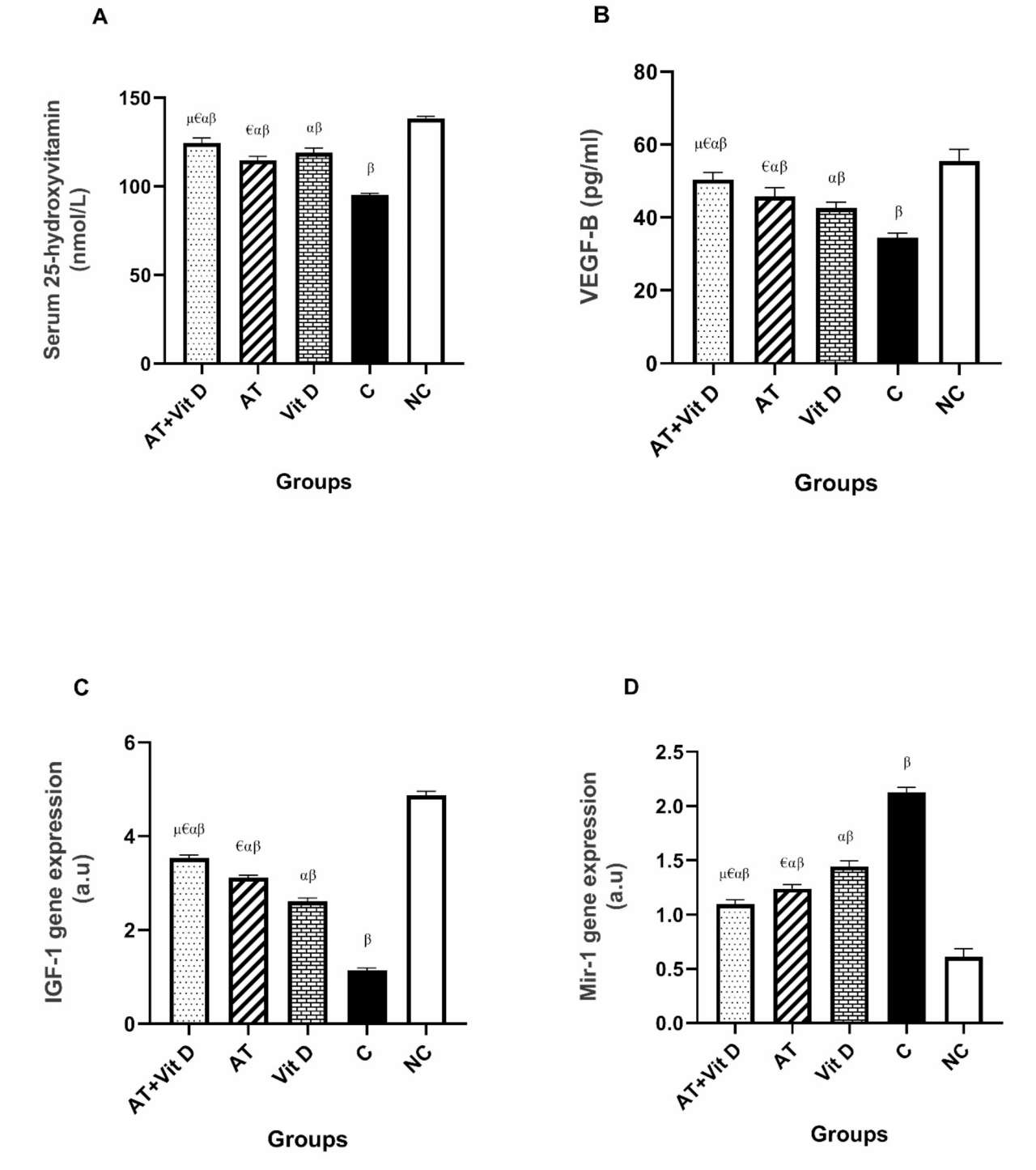

Fig. 2. Effect of aerobic training and vitamin D supplementation on 25-hydroxyvitamin D, VEGF-B, IGF-1, and Mir-1 in type-2 diabetic rats.
AT + Vit D, Aerobic training + Vitamin D supplement, **AT**, Aerobic Training; **Vit D**, Vitamin D Supplement; **C**, Control; **NC**, Non-diabetes Control.
VEGF-B: Vascular Endothelial Growth Factor B; **IGF-1**: Insulin-like growth factor 1; **Mir-1**: MicroRNA-1.
Results from analysis of one-way analysis of variance (ANOVA), post-hoc Tukey's test. μ: Significantly different compared to AT. ϵ: Significantly different compared to Vit D. α: Significantly different compared to C. β: Significantly different compared to NC.

on these studies, AT counteracts the effects of T2DM-induced cardiac complications, specifically defected cardiac angiogenesis[54,55]. To elucidate the mechanisms, the present study revealed that AT reduces miR-1 and increases IGF-1 and VEGF-B expression in the cardiomyocytes of rats with T2DM. Hoier et al. (2020), showed that AT increases cardiac muscle metabolism creates oxygen deficiency conditions, and stimulates hypoxia-inducible factor 1-alpha (HIF-1) that increases VEGF-B and improves cardiac angiogenesis, in turn[56]. IGF-1 is an anti-apoptosis factor that exerts a protective effect towards apoptosis in rat cardiomyocytes by decreasing the cytotoxic effects of glucose. There are contradictory results on the IGF-I levels following AT that are limited to serum investigations. Birzniece et al. (2019) reported a significant increase in IGF-I following AT[57]; while Moazami et al. (2018) reported a significant decrease in the serum levels of IGF-I in middle-aged women after a six-month AT[58]. Studies have reported IGF-I as one of the targets of miR-1[59,60]. The miR-1 plays a major role in cardiomyocyte apoptosis and ultimately cardiomyopathy by downregulating IGF-1 at the translational level[61]. In addition, miR-1 has been implicated in diabetes-induced cardiomyocyte apoptosis[62], which is overexpressed in the hearts of diabetic patients[63], and miR-1 overexpression inhibits the anti-apoptotic action of IGF-1[64]. Consistent with the present data, Delfan et al. (2020) reported that AT increases the IGF-1 expression gene by reducing the overexpression of miR-1 and thus can be an effective intervention to reduce the complications of diabetes cardiopathy[65]. The present study further indicates that Vit D increased IGF-1 and VEGF-B and decreased miR-1 after 8 weeks. Studies report contradictory results on the effect of Vit D supplementation on cardiac biomarkers[66,67]. Kord-Varkaneh et al. (2020) reported no significant alteration in IGF-1 following Vit D supplementation[68]. Also, Latino and African-American people with pre-diabetes and hypovitaminosis D showed no significant changes in IGF-1 following Vit D supplementation[69]. However, variations in Vit D measurement techniques, confounding factors affecting 25-hydroxyvitamin D levels, lack of consensus on optimal thresholds, and differences between observational and interventional studies all contribute to the contradictory findings surrounding Vit D and health. While Consistent with our findings, there is evidence that Vit D supplementation modulates circulating IGF1 concentrations[68,69]. Moreover, several studies have showed positive association between IGF1 and 25(OH)D in healthy and unhealthy subjects[70,71]. The possible mechanism involved in increasing IGF-1 and VEGF-B expression in cardiomyocytes following Vit D supplementation might indicate activation of the IGF-1 and VEGF-B promoter that promotes a further proangiogenic response and vascularization[72]. According to our data, increased IGF-1 and VEGF-B expressions in the cardiomyocytes were also multiplied when combining Vit D with AT. These changes suggest that combined AT and Vit D ameliorated the T2DM-induced defect in cardiac angiogenesis. In this model, separate Vit D was less effective at ameliorating the features of T2DM-induced angiogenesis disorders in T2DM rats than combined AT and Vit D, suggesting that combining AT and Vit D might be an essential mediator of both basal angiogenic responses and the effects of AT on this.

The interplay between AT and Vit D in the regulation of angiogenesis, the process of new blood vessel formation, involves complex cellular and molecular signaling pathways. Emerging evidence suggests that the combination of these two factors can have a synergistic effect on promoting or inhibiting angiogenesis, depending on the physiological context.

Exercise has been shown to modulate Vit D metabolism by increasing the production of the active form, 1,25-dihydroxyvitamin D (1,25(OH)2D), through the upregulation of the enzyme 1α-hydroxylase in skeletal muscle and other tissues[73]. This enhanced Vit D activation can, in turn, influence the expression of genes involved in angiogenesis. For instance, 1,25(OH)2D has been reported to upregulate the expression of vascular endothelial growth factor (VEGF) and stromal cell-derived factor 1 (SDF-1), which are potent pro-angiogenic factors[66,67]. This can lead to increased endothelial cell proliferation, migration, and the formation of new blood vessels, thereby facilitating the delivery of oxygen and nutrients to active muscles during exercise.

Conversely, in the presence of excessive or pathological angiogenesis, such as in the context of tumor growth or vascular injury, Vit D has been shown to exert anti-angiogenic effects[33]. This is mediated through the Vit D receptor (VDR), which can regulate the expression of genes involved in the inhibition of endothelial cell activation, proliferation, and migration[74]. Additionally, Vit D has been found to downregulate the expression of pro-angiogenic microRNAs, such as miR-1, further contributing to its anti-angiogenic actions[23,24].

The interplay between AT and Vit D in the modulation of angiogenesis is further highlighted by their combined effects on the immune system and inflammation. Exercise can stimulate the production of anti-inflammatory cytokines, while Vit D has been reported to suppress pro-inflammatory pathways, such as those mediated by nuclear factor-κB (NF-κB)[34,75]. This synergistic anti-inflammatory effect may help to maintain a balanced angiogenic response and promote vascular health[34].

In summary, the interaction between AT and Vit D in the regulation of angiogenesis involves a complex interplay of cellular and molecular signaling pathways. The combination of these two factors can have either pro-angiogenic or anti-angiogenic effects, depending on the physiological context, highlighting the importance of considering both AT and Vit D status in the optimization of vascular function and overall health. In summary, both AT and Vit D increased cardiac expression of IGF-1 and VEGF-B in T2DM rats, whereas decreased miR-1, these responses were greater when combining AT and Vit D.

The provided study highlights several limitations of a study on the effects of AT and Vit D on angiogenesis-inducing factors in rats with type-2 diabetes mellitus (T2DM). Firstly, the study primarily focuses on animal models of T2DM, which may restrict the applicability of the findings to human populations. To validate the observed effects, human studies are necessary. Secondly, the study's characterization of angiogenesis-inducing factors is limited to AT and Vit D, without comprehensively investigating other potential factors involved in angiogenesis. This narrow focus may hinder a comprehensive understanding of the overall angiogenic response in the context of T2DM. Thirdly, while the study reports the effects of AT and Vit D on miR-1, IGF-1, and VEGF-B expression in the cardiomyocytes, it lacks a detailed mechanistic explanation of how these interventions modulate angiogenesis. Further investigation is required to elucidate the underlying molecular pathways via histological,

immunohistochemical (IHC), and Western blot (WB) analyses to explore the activity of mir-1 and other relevant molecular markers. Lastly, the study only investigates the effects of eight weeks of AT and Vit D supplementation, without exploring the long-term effects and sustainability of these interventions on angiogenesis and cardiac function. Longer follow-up periods would provide a more comprehensive understanding of the potential benefits. Overall, these limitations highlight the need for further research to overcome these constraints and enhance the understanding of the interventions' effects on angiogenesis in the context of T2DM.

Conclusions

We conclude that combined AT and Vit D have cardio-protective effects in T2DM rats and counteracts diabetes-induced abnormal angiogenesis, which is mediated, at least in part, by upregulation of IGF-1 and VEGF-B and down-regulation of miR-1.

Data availability

The results of the current study are available from the corresponding author on reasonable request.

Received: 23 December 2023; Accepted: 25 November 2024
Published online: 08 January 2025

References

1. Faselis, C. et al. Microvascular complications of type 2 diabetes mellitus. *Curr. Vasc. Pharmacol.* **18** (2), 117–124 (2020).
2. Nesti, L., Pugliese, N. R., Sciuto, P. & Natali, A. Type 2 diabetes and reduced exercise tolerance: a review of the literature through an integrated physiology approach. *Cardiovasc. Diabetol.* **19** (1), 134 (2020).
3. Tan, Y. et al. Mechanisms of diabetic cardiomyopathy and potential therapeutic strategies: preclinical and clinical evidence. *Nat. Reviews Cardiol.* **17** (9), 585–607 (2020).
4. Ma, C-X. et al. Cardiovascular disease in type 2 diabetes mellitus: progress toward personalized management. *Cardiovasc. Diabetol.* **21** (1), 1–15 (2022).
5. Hoseini, R., Rahim, H. A. & Ahmed, J. K. Decreased inflammatory gene expression accompanies the improvement of liver enzyme and lipid profile following aerobic training and vitamin D supplementation in T2DM patients. *BMC Endocr. Disorders*. **22** (1), 245 (2022).
6. Li, L-J., Ma, J., Li, S-B., Chen, X. & Zhang, J. Vascular endothelial growth factor B inhibits lipid accumulation in C2C12 myotubes incubated with fatty acids. *Growth Factors*. **37** (1–2), 76–84 (2019).
7. Bahmani, E., Hoseini, R. & Amiri, E. Home-based aerobic training and vitamin D improve neurotrophins and inflammatory biomarkers in MS patients. *Multiple Scler. Relat. Disorders*. **60**, 103693 (2022).
8. Räsänen, M. et al. VEGF-B promotes endocardium-derived coronary vessel development and cardiac regeneration. *Circulation* **143** (1), 65–77 (2021).
9. Khaledi, K., Hoseini, R. & Gharzi, A. Effects of aerobic training and vitamin D supplementation on glycemic indices and adipose tissue gene expression in type 2 diabetic rats. *Sci. Rep.* **13** (1), 10218 (2023).
10. Jiao, W. et al. Distinct downstream signaling and the roles of VEGF and PlGF in high glucose-mediated injuries of human retinal endothelial cells in culture. *Sci. Rep.* **9** (1), 1–13 (2019).
11. Higashi, Y. et al. Endothelial deficiency of insulin-like growth factor-1 receptor reduces endothelial barrier function and promotes atherosclerosis in apoe-deficient mice. *Am. J. Physiol. Heart Circ. Physiol.* **319** (4), H730–H743 (2020).
12. Ohshima, H. et al. Association between growth hormone and hypertension in a general population. *Hypertens. Res.* **43** (12), 1430–1436 (2020).
13. Ricotti, R. et al. The relationship between cortisol and IGF-I influences metabolic alteration in pediatric overweight and obesity. *Eur. J. Endocrinol.* **182** (3), 255–264 (2020).
14. Jung, H. J. & Suh, Y. Regulation of IGF – 1 signaling by microRNAs. *Front. Genet.* **5**, 472 (2014).
15. Arabzadeh, E., Samadian, Z., Tofighi, A. & Tolouei Azar, J. Alteration of follistatin-like 1, neuron-derived neurotrophic factor, and vascular endothelial growth factor in diabetic cardiac muscle after moderate-intensity aerobic exercise with insulin. *Sport Sci. Health*. **16** (3), 491–499 (2020).
16. Riahi, S., Mohammadi, M. T., Sobhani, V. & Ababzadeh, S. Chronic aerobic exercise decreases lectin-like low density lipoprotein (lox-1) receptor expression in heart of diabetic rat. *Iran. Biomed. J.* **20** (1), 26 (2016).
17. Ross, M., Kargl, C. K., Ferguson, R., Gavin, T. P. & Hellsten, Y. Exercise-induced skeletal muscle angiogenesis: impact of age, sex, angiocrines and cellular mediators. *Eur. J. Appl. Physiol.* **123** (7), 1415–1432 (2023).
18. Li, S. et al. The effect of blood flow restriction exercise on angiogenesis-related factors in skeletal muscle among healthy adults: a systematic review and meta-analysis. *Front. Physiol.* **13**, 814965 (2022).
19. Qi, C., Song, X., Wang, H., Yan, Y. & Liu, B. The role of exercise-induced myokines in promoting angiogenesis. *Front. Physiol.* **13**, 981577 (2022).
20. Dastah, S., Tofighi, A., Azar, J. T. & Alivand, M. Aerobic exercise leads to upregulation of Mir-126 and angiogenic signaling in the heart tissue of diabetic rats. *Gene Rep.* **21**, 100914 (2020).
21. Yu, Q., Xia, Z., Liong, E. C. & Tipoe, G. L. Chronic aerobic exercise improves insulin sensitivity and modulates Nrf2 and NF–κB/IκBα pathways in the skeletal muscle of rats fed with a high fat diet. *Mol. Med. Rep.* **20** (6), 4963–4972 (2019).
22. Wang, G. Aerobic exercise ameliorates myocardial ischemia/reperfusion injury and thrombosis of diabetic rats via activation of AMPK/Sirt1/PGC-1α pathway. *Gen. Physiol. Biophys.* **41** (4), 319–328 (2022).
23. Dovnik, A. & Fokter Dovnik, N. Vitamin D and ovarian cancer: systematic review of the literature with a focus on molecular mechanisms. *Cells* **9** (2), 335 (2020).
24. Chan, H-N. et al. Vitamin D and ocular diseases: a systematic review. *Int. J. Mol. Sci.* **23** (8), 4226 (2022).
25. Song, Y-S., Jamali, N., Sorenson, C. M. & Sheibani, N. Vitamin D receptor expression limits the angiogenic and inflammatory properties of Retinal endothelial cells. *Cells* **12** (2), 335 (2023).
26. Shanmugavadivu, A., Balagangadharan, K. & Selvamurugan, N. Angiogenic and osteogenic effects of flavonoids in bone regeneration. *Biotechnol. Bioeng.* **119** (9), 2313–2330 (2022).
27. Looi, C-K. et al. Therapeutic challenges and current immunomodulatory strategies in targeting the immunosuppressive pancreatic tumor microenvironment. *J. Experimental Clin. Cancer Res.* **38**, 1–23 (2019).
28. Hu, Z. et al. Effects of long-term vitamin D supplementation on metabolic profile in middle-aged and elderly patients with type 2 diabetes. *J. Steroid Biochem. Mol. Biol.* **225**, 106198 (2023).
29. Mitrašinović-Brulić, M. et al. Vitamin D3 attenuates oxidative stress and regulates glucose level and leukocyte count in a semi-chronic streptozotocin-induced diabetes model. *J. Diabetes Metabolic Disorders*. **20** (1), 771–779 (2021).

30. Garg, S. & Mallik, D. Role of Vitamin D as an adjuvant to oral hypoglycemic drugs in treatment of Type-2 Diabetic patients. *Asian J. Med. Sci.* **11** (2), 25–29 (2020).
31. Young, K., Beggs, M. R., Grimbly, C. & Alexander, R. T. Regulation of 1 and 24 hydroxylation of vitamin D metabolites in the proximal tubule. *Experimental Biology Med.* **247** (13), 1103–1111 (2022).
32. Caballero-García, A., Córdova-Martínez, A., Vicente-Salar, N., Roche, E. & Pérez-Valdecantos, D. Vitamin D, its role in recovery after muscular damage following exercise. *Nutrients* **13** (7), 2336 (2021).
33. Latham, C. M. et al. Vitamin D promotes skeletal muscle regeneration and mitochondrial health. *Front. Physiol.* **12**, 660498 (2021).
34. Hoseini, R., Rahim, H. A. & Ahmed, J. K. Concurrent alteration in inflammatory biomarker gene expression and oxidative stress: how aerobic training and vitamin D improve T2DM. *BMC Complement. Med. Ther.* **22** (1), 165 (2022).
35. Mostafavian, M., Abdi, A., Mehrabani, J. & Barari, A. Effect of eight weeks of aerobic progressive training with capsaicin on changes in PGC-1α and UPC-1 expression in visceral adipose tissue of obese rats with diet. *Complement. Med. J.* **10** (2), 106–117 (2020).
36. Mohammadi, K. & Khajehlandi, A. The effect of a period of swimming training with different temperatures on the expression of β3-AR and ERK2 genes in the visceral adipose tissue of streptozotocin-induced diabetic rats. *Feyz J. Kashan Univ. Med. Sci.* **25** (6), 1303–1312 (2022).
37. Khalili, A., Nekooeian, A. A. & Khosravi, M. B. Oleuropein improves glucose tolerance and lipid profile in rats with simultaneous renovascular hypertension and type 2 diabetes. *J. Asian Nat. Prod. Res.* **19** (10), 1011–1021 (2017).
38. Rocha-Rodrigues, S. et al. Effects of physical exercise on myokines expression and brown adipose-like phenotype modulation in rats fed a high-fat diet. *Life Sci.* **165**, 100–108 (2016).
39. Hoseini, R., Damirchi, A. & Babaei, P. Vitamin D increases PPARγ expression and promotes beneficial effects of physical activity in metabolic syndrome. *Nutrition* **36**, 54–59 (2017).
40. McDonald, M. W. et al. Aerobic exercise training improves insulin-induced vasorelaxation in a vessel-specific manner in rats with insulin-treated experimental diabetes. *Diabetes Vascular Disease Res.* **16** (1), 77–86 (2019).
41. Wang, W., Zhang, J., Wang, H., Wang, X. & Liu, S. Vitamin D deficiency enhances insulin resistance by promoting inflammation in type 2 diabetes. *Int. J. Clin. Exp. Pathol.* **12** (5), 1859 (2019).
42. Fadini, G. P., Albiero, M., Bonora, B. M. & Avogaro, A. Angiogenic abnormalities in diabetes mellitus: mechanistic and clinical aspects. *J. Clin. Endocrinol. Metabolism*. **104** (11), 5431–5444 (2019).
43. Rai, V., Moellmer, R. & Agrawal, D. K. Role of fibroblast plasticity and heterogeneity in modulating angiogenesis and healing in the diabetic foot ulcer. *Mol. Biol. Rep.* **50** (2), 1913–1929 (2023).
44. Dudley, A. C. & Griffioen, A. W. Pathological angiogenesis: mechanisms and therapeutic strategies. *Angiogenesis* **26** (3), 313–347 (2023).
45. Al-Kharashi, A. S. Role of oxidative stress, inflammation, hypoxia and angiogenesis in the development of diabetic retinopathy. *Saudi J. Ophthalmol.* **32** (4), 318–323 (2018).
46. Dichtel, L. E., Cordoba-Chacon, J. & Kineman, R. D. Growth hormone and insulin-like growth factor 1 regulation of nonalcoholic fatty liver disease. *J. Clin. Endocrinol. Metabolism*. **107** (7), 1812–1824 (2022).
47. Gao, J. et al. Exercise Training-Induced MicroRNA alterations with Protective effects in Cardiovascular diseases. *Rev. Cardiovasc. Med.* **24** (9), 251 (2023).
48. McDonald, M. W. et al. Exercise training induced cardioprotection with moderate hyperglycemia versus sedentary intensive glycemic control in type 1 diabetic rats. *Journal of Diabetes Research* 2018. (2018).
49. Seo, D. Y. et al. Exercise as a potential therapeutic target for diabetic cardiomyopathy: insight into the underlying mechanisms. *Int. J. Mol. Sci.* **20** (24), 6284 (2019).
50. Taghizadeh, M., Kargarfard, M., Braune, S., Jung, F. & Naderi, M. Long-term aerobic exercise training in type two diabetic patients alters the expression of miRNA-223 and its corresponding target, the P2RY12 receptor, attenuating platelet function. *Clin. Hemorheol. Microcirc.* **80** (2), 107–116 (2022).
51. Volterrani, M. et al. Effects of concurrent, within-session, aerobic and resistance exercise training on functional capacity and muscle performance in elderly male patients with chronic heart failure. *J. Clin. Med.* **12** (3), 750 (2023).
52. Goes-Santos, B. R. et al. Physical capacity increase in patients with heart failure is associated with improvement in muscle sympathetic nerve activity. *Int. J. Cardiol.* **378**, 48–54 (2023).
53. Zapata-Bustos, R. et al. Altered transcription factor expression responses to exercise in insulin resistance. *Front. Physiol.* **12**, 649461 (2021).
54. Wang, B. et al. Exercise ameliorating myocardial injury in type 2 diabetic rats by inhibiting excessive mitochondrial fission involving increased irisin expression and AMP-activated protein kinase phosphorylation. *J. Diabetes*. **16** (1), e13475 (2024).
55. Bhati, P., Hussain, M. E., Deepak, K., Masood, S. & Anand, P. Progressive resistance training ameliorates deteriorating cardiac autonomic dysfunction, subclinical inflammation and endothelial dysfunction in type 2 diabetes mellitus: a randomized control trial. *Diabetes Metabolic Syndrome: Clin. Res. Reviews*. **17** (5), 102778 (2023).
56. Hoier, B. et al. Early time course of change in angiogenic proteins in human skeletal muscle and vascular cells with endurance training. *Scand. J. Med. Sci. Sports*. **30** (7), 1117–1131 (2020).
57. Birzniece, V. Exercise and the growth hormone–insulin-like growth factor axis. *Curr. Opin. Endocr. Metabolic Res.* **9**, 1–7 (2019).
58. Moazami, M. & Askari, B. Effect of six months of Aerobic Exercise on serum levels of insulin, growth hormone and insulin-like Growth hormone 1 in sedentary obese women. *Med. Lab. J.* **12**(4). (2018).
59. Obradovic, M. et al. Effects of IGF-1 on the cardiovascular system. *Curr. Pharm. Design*. **25** (35), 3715–3725 (2019).
60. Liang, J. et al. MicroRNA profiling of different exercise interventions for alleviating skeletal muscle atrophy in naturally aging rats. *J. Cachexia Sarcopenia Muscle*. **14** (1), 356–368 (2023).
61. Lock, M. C. et al. The role of miRNA regulation in fetal cardiomyocytes, cardiac maturation and the risk of heart disease in adults. *J. Physiol.* **596** (23), 5625–5640 (2018).
62. Cheng, Y. et al. Inhibition of long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1 attenuates high glucose-induced cardiomyocyte apoptosis via regulation of miR-181a-5p. *Exp. Anim.* **69** (1), 34–44 (2020).
63. Kura, B., Kalocayova, B., Devaux, Y. & Bartekova, M. Potential clinical implications of miR-1 and miR-21 in heart disease and cardioprotection. *Int. J. Mol. Sci.* **21** (3), 700 (2020).
64. Ye, L-L., Cheng, Z-G., Cheng, X-E. & Huang, Y-L. Propofol regulates miR-1-3p/IGF1 axis to inhibit the proliferation and accelerates apoptosis of colorectal cancer cells. *Toxicol. Res.* **10** (4), 696–705 (2021).
65. Delfan, M. et al. High intensity interval training improves diabetic cardiomyopathy via miR-1 dependent suppression of cardiomyocyte apoptosis in diabetic rats. *J. Diabetes Metabolic Disorders*. **19** (1), 145–152 (2020).
66. Wu, Z. et al. Effect of monthly vitamin D supplementation on cardiac biomarkers: a post-hoc analysis of a randomized controlled trial. *J. Steroid Biochem. Mol. Biol.* **220**, 106093 (2022).
67. Limonte, C. P. et al. Effects of long-term vitamin D and n-3 fatty acid supplementation on inflammatory and cardiac biomarkers in patients with type 2 diabetes: secondary analyses from a randomised controlled trial. *Diabetologia* **64** (2), 437–447 (2021).
68. Kord-Varkaneh, H. et al. The influence of vitamin D supplementation on IGF-1 levels in humans: a systematic review and meta-analysis. *Ageing Res. Rev.* **57**, 100996 (2020).
69. Meshkini, F., Abdollahi, S., Clark, C. C. & Soltani, S. The effect of vitamin D supplementation on insulin-like growth factor-1: a systematic review and meta-analysis of randomized controlled trials. *Complement. Ther. Med.* **50**, 102300 (2020).

70. Contreras-Bolívar, V., García-Fontana, B., García-Fontana, C. & Muñoz-Torres, M. Mechanisms involved in the relationship between vitamin D and insulin resistance: impact on clinical practice. *Nutrients* **13** (10), 3491 (2021).
71. Chen, Y-C. et al. Association between metabolic body composition status and vitamin D deficiency: a cross-sectional study. *Front. Nutr.* **9**, 940183 (2022).
72. Chen, L. et al. Vitamin D3 induces mesenchymal-to-endothelial transition and promotes a proangiogenic niche through IGF-1 signaling. *Iscience* **24** (4), 102272 (2021).
73. Dzik, K. P. & Kaczor, J. J. Mechanisms of vitamin D on skeletal muscle function: oxidative stress, energy metabolism and anabolic state. *Eur. J. Appl. Physiol.* **119**, 825–839 (2019).
74. Pál, É., Ungvári, Z., Benyó, Z. & Várbíró, S. Role of vitamin D deficiency in the pathogenesis of cardiovascular and cerebrovascular diseases. *Nutrients* **15** (2), 334 (2023).
75. Saati-Zarei, A., Damirchi, A., Tousi, S. M. T. R. & Babaei, P. Myocardial angiogenesis induced by concurrent vitamin D supplementation and aerobic-resistance training is mediated by inhibiting miRNA-15a, and miRNA-146a and upregulating VEGF/PI3K/eNOS signaling pathway. *Pflügers Archiv-European J. Physiol.* **475** (4), 541–555 (2023).

Acknowledgements
The authors would like to thank the participants for their cooperation.

Author contributions
R.H. designed the study and analyzed the data. F.M. and A.G.H. conducted the experiments and contributed to data analysis. R.H. wrote the manuscript with input from F.M. and A.G.H. All authors were involved in the interpretation of data, critically revised the manuscript, and approved the final version for submission.

Funding
The authors declared that the research did not receive any financial grants.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Razi University of Kermanshah and was registered in the Iranian Clinical Trial Registration Center (code: IRCT20220512054832N1 on 09/06/2022). All procedures described in this study were performed following the guidelines of the Ethics Committee of the Razi University of Kermanshah (IR.RAZI.REC.1401.011). The authors confirm that this study is reported following ARRIVE guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to R.H.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.