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Correlation between circulating microRNAs and vascular biomarkers in type 2 diabetes based upon physical activity: a biochemical analytic study

Hadeel A. Al-Rawaf[1,2], Sami A. Gabr[2], Talal Alghadir[3], Faisal Alghadir[3], Amir Iqbal[2*] and Ahmad H. Alghadir[2]

Abstract

Background This research investigated how physical activity (PA) might impact the expression of several microRNAs, specifically miR-126, miR-146a, miR-34a, miR-124a, miR-155, and miR-221, in the blood of elderly individuals with type 2 diabetes (T2D). Additionally, the study examined the relationship between these microRNAs and markers of vascular endothelial dysfunction, including vascular endothelial growth factor (VEGF), apolipoprotein A-I (apoA-I), and apolipoprotein B (apoB), to assess their potential in the prevention, early detection, and treatment of diabetes.

Methods This correlational observational study involved 100 male participants, aged between 18 and 65 years, all of whom had been living with type 2 diabetes (T2D) for over six years. The participants were divided into three groups: inactive, moderate, and active, depending on their level of physical activity (PA). Real-time PCR and immunoassays were employed to measure the expression of selected miRNAs, as well as VEGF, apoA-I, apoB, and diabetic management indicators. PA levels were determined using ACTi graph GT1M accelerometer (model WAM 7164; Fort Walton Beach, FL) and energy expenditure was measured in the form of metabolic equivalent (MET) by indirect calorimetry method.

Results The expression levels of miR-146a, miR-34a, and miR-124a were significantly higher in patients with higher physical activity, while no such increase was observed for the other miRNAs in less active participants. Additionally, PA-active individuals showed a more pronounced decrease in fasting blood sugar (FBS), insulin resistance (IR), fasting insulin (FINS), HOMA-IR, HbA1c (%), and levels of VEGF, apoAI, apoB, and the apoB/apoA-I ratio. The alteration in miRNA expression was positively associated with physical activity, VEGF, apoAI, apoB, the apoB/apoA-I ratio, and diabetes-related metrics, while being inversely related to BMI.

Conclusions In diabetic patients with higher physical activity levels, circulating miR-146a, miR-34a, and miR-124a showed elevated expression, accompanied by a notable decrease in vascular biomarkers, including apoAI, apoB, and the apoB/apoA-I ratio. The findings revealed a strong correlation between these vascular biomarkers and the

*Correspondence:
Amir Iqbal
physioamir@gmail.com; ajamaluddin@ksu.edu.sa

Full list of author information is available at the end of the article

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physiological responses of miR-146a, miR-34a, and miR-124a, though larger studies are required to validate these results further.

Trial registration Not applicable.

Keywords Diabetes mellitus, Fasting blood sugar, Vascular biomarkers, Physical activity, Circulating mRNAs expressions, Insulin resistance

Introduction

Type 2 diabetes (T2D) was estimated to affect more than 150 million people worldwide. In addition, it accounts for about 80% of the cases globally [1]. Insulin secretion from target tissues and pancreatic β-cells is affected by adverse genetic background and environmental factors [2–3]. Severe complications affecting both microvascular and macrovascular systems, linked to factors such as hypertension, high cholesterol levels, poor blood sugar management, and the length of time a person has had diabetes [4–5], contribute to the development and worsening of diabetic retinopathy (DR). In diabetic patients, DR is characterized by microaneurysm with hemorrhage, neovascularization, and finally produces poor vision loss [6–7].

The advancement of diabetic retinopathy was attributed to the increased inflammatory process and vascular endothelium dysfunction with a significant increase of cytokines, chemokines, and adhesion molecules in diabetic patients [8–9]. In addition, several vascular endothelial markers, especially VEGF [10], apolipoprotein AI (apoAI), and apolipoprotein B (apoB) linked to retinopathy in diabetic patients [11–13]. ApoAI and apoB provide a more accurate representation of physiological changes linked to diabetic retinopathy compared to standard lipid measurements [14]. ApoAI is more indicative of lipid buildup in peripheral tissues [15], while apoB has been detected in the retinas of individuals with diabetic retinopathy [16]. Numerous genes associated with type 2 diabetes (T2D) play a key role in managing the balance between insulin production from β-cells and insulin resistance [17–20]. Although gene expression regulation during T2D progression holds promise for improving prevention, early diagnosis, and treatment, further research is needed to establish these genes as reliable biomarkers for predicting and diagnosing diabetes [8, 17, 18].

MicroRNAs are stable, single-stranded RNA molecules made up of 21–23 nucleotides that have been found to control gene expression at the post-transcriptional stage [21–22]. It was reported that miRNAs were protected from endogenous RNase activity. They could be easily determined as novel, non-invasive biomarkers specifically expressed in serum/plasma of various types of diseases [23–28]. Moreover, in chronic diabetes, dysregulation of miRNA is attributed to serious physiological abnormalities which lead to the pathogenicity and progression of DR [29–32].

Recently, microRNAs (miRNAs) have been identified as being associated with T2D, with a strong correlation observed between glucose levels and the blood concentrations of specific miRNAs, including miR-126, miR-146a, miR-155, and miR-221 [33–39].

Moreover, miRNAs as small, non-coding RNA molecules that play a crucial role in regulating gene expression at the post-transcriptional level. The data previously reported miRNAs to play an integral role of various pathophysiological processes, including insulin resistance, β-cell dysfunction, and chronic inflammation. For instance, miR-126 is known to be downregulated in T2DM, contributing to endothelial dysfunction and impaired angiogenesis. In addition, at molecular mechanisms, especially at the epigenetic level, microRNAs (miRNAs) are significantly associated with the regulation of the defect in both insulin signaling and insulin resistance [40, 41].

Type 2 diabetes (T2D) is strongly associated with physical inactivity, poor eating habits, and worsened physical functioning, which, in combination with genetic predisposition, contribute to the chronic metabolic syndrome linked to the disease [42–46]. On the other hand, physical exercise (PE) showed to modulate the expression of miRNAs, which in turn could induce a beneficial modification in signaling pathways in T2DM, increasing insulin sensitivity, reducing insulin resistance, in addition, to being an excellent non-pharmacological strategy to combat T2DM [47–53]. PE protocols like aerobic and resistance training sessions which prescribed based on scientific variables, such as volume, frequency, intensity, and duration, significantly were reported to enhance insulin sensitivity and cardiovascular functioning, hyperglycemia, and reducing the risk for developing T2DM [54–55].

In molecular level, homeostasis in the circulating and cellular miRNA profile within cells have shown to be influenced by exercise and physical activity of mild or chronic intensity. In both healthy and diabetic individuals, the results previously reported that the expression of different sets of miRNAs was significantly modulated with different PE of resistance and aerobic training types. The change of miRNAs profile was correlated with body

fat loss and with changes in fatty acid metabolism in normal and diabetic patients [56–60].

The rationale for studying the relationship between miRNAs and physical activity in diabetes lies in the potential to develop targeted therapeutic strategies. By understanding how exercise influences miRNA expression, it may be possible to design interventions that mimic or enhance these effects, leading to improved glycemic control and reduced complications associated with T2DM. it was reported previously that, both regular physical exercise and improved physical fitness could beneficially regulate skeletal muscle miRNAs in T2DM patients, potentially affecting glycemic control [61].

Thus, the objective of this review is to analyze the expression pattern of miRNAs in T2DM and compare with the expression pattern induced by PE, analyzing the signaling pathways associated with these miRNAs in the pathophysiological processes in T2DM.

Despite this, few studies have explored the connection between microRNAs, physical activity, and vascular biomarkers in T2D patients. Understanding these interactions could offer novel insights into preventing and managing vascular complications, potentially leading to more personalized and effective therapeutic strategies [62]. Given the global burden of T2D and the growing emphasis on individualized approaches in clinical practice, this study aims to fill critical knowledge gaps and improve long-term patient outcomes.

The primary objective of this research was to determine the correlation between circulating microRNAs (miR-126, miR-146a, miR-34a, miR-124a, miR-155, and miR-221) and vascular biomarkers (VEGF, apoA-I, and apoB) in type 2 diabetes (T2D) patients based on their levels of physical activity (PA).

The secondary objective was to assess how physical activity affects diabetic management parameters such as fasting blood sugar (FBS), insulin resistance (IR), fasting insulin (FINS), HOMA-IR, and HbA1c (%), and their relationship with the expression of selected microRNAs. The study hypothesized that the higher levels of physical activity in T2D patients will be associated with increased expression of certain microRNAs (miR-146a, miR-34a, and miR-124a) and a reduction in vascular biomarkers (VEGF, apoA-I, apoB, and the apoB/apoA-I ratio), which in turn may improve metabolic control and reduce vascular endothelial dysfunction.

By integrating physical activity as a modifiable factor with molecular biomarkers, this research will provide a framework for more precise, individualized care in type 2 diabetes, enhancing both prevention and treatment strategies aimed at reducing long-term vascular complications and improving overall patient outcomes.

Materials and methods

Study design

The study was based on a correlational observational design.

Participants

One hundred male participants, aged 18 to 65, who had been diagnosed with type 2 diabetes (T2D) for over six years according to the American Diabetes Association guidelines [63], were included in this study. The participants had metformin as prescribed drug for type 2 diabetes. Metformin is generally considered to have a neutral to positive effect on exercise performance, especially for aerobic activities like walking or cycling. In addition, it was suggesting that metformin may not impair performance during moderate-intensity exercise; however, it could potentially reduce the performance in high-intensity activities due to its effects on mitochondrial function and lactate clearance [64–65]. Data collection occurred between October 2016 and May 2017.Patients with obesity (BMI: ≥ 25), which may interfere with the data of lipoprotein markers and lipid accumulation in peripheral tissues, type1 diabetes, anemia, smokers, with heart diseases, with chronic diabetic complications such as nephropathy, neuropathy, retinopathy, chronic liver disease, hypothyroidism, and drugs (diuretics, oral contraceptives) were excluded from this study. All participants were subjected to standard anthropometric measurements to estimate BMI, WHR, and WC according to the World Health Organization [66].

Assessment of physical activity (PA)

Physical activity was estimated by using ACTi graph GT1M accelerometer (model WAM 7164; Fort Walton Beach, FL) as mentioned previously [67–68]. Validated reliable accelerometers showed to provide objective and detailed information on various aspects of physical activity [69–70].

The ActiGraph GT1M accelerometer (model WAM 7164) is a commonly used device for objectively measuring physical activity. The GT1M has been extensively validated in controlled laboratory and free-living conditions. Studies have shown strong correlations between GT1M output and energy expenditure measured by indirect calorimetry method, with correlation coefficients typically ranging between 0.7 and 0.9. In addition, the device has shown excellent test-retest reliability, with infraclass correlation coefficients (ICCs) generally above 0.90. The strength of the used devise showed that it can detect small movements and distinguish between different intensities of physical activity (e.g., sedentary, moderate, vigorous). Moreover, it is reliable for tracking changes in activity over time, making it suitable for intervention

studies and to ensure the accuracy and reliability of the data.

In this study, the diabetic participants performed walking as a form of moderate intensity aerobic activity for 30 min /3 times per week, in addition to that, the intensity and frequency of PA were achieved per week, and energy expenditure was measured in the form of metabolic equivalent (MET) of all participants [71–72].

Indirect calorimetry method for measuring energy expenditure

Indirect calorimetry is a method of measuring energy expenditure based on the respiratory exchange of gases, particularly oxygen (O2) and carbon dioxide (CO2), during both rest and activity. This method assumes that energy expenditure is proportional to oxygen consumption and carbon dioxide production. This method is used to estimate the resting metabolic rate (RMR), basal metabolic rate (BMR), and energy expenditure during physical activity. It is often used in controlled settings such as clinical trials, exercise physiology studies, or nutritional assessments. For each patient, the mean VCO2 measured by the mechanical ventilator (Hamilton-S1, Hamilton Medical AG, Bonaduz, Switzerland) during the 10-min measurement of the metabolic monitor was recorded. Because VO2 is not measured by the mechanical ventilator, an adjusted version of Weir's equation was used to estimate ventilator derived energy expenditure:

Subjects were considered physically active when participating in endurance activities during a year and had monitored wear at least 10 h a day for over 3 days per week. Moreover, subjects with fewer accelerometer counts (≤ 100 counts/min) or not engaged in any sport training were characterized by a sedentary lifestyle. The participants with type 2 diabetes were classified according to physical activity levels into three groups; mild PA group (MET minutes/week of ≤ 500, $n = 40$), moderate PA (MET minutes/week of 500–2,500, $n = 25$), and physically active group (≥ 2,500 MET minutes/week, $n = 35$).

Assessment of glucose control

A colorimetric assay was performed to estimate blood glucose for each participant using a QuantiChrom Glucose bioassay kit (DIGL-100, BioAssay Systems, Hayward, CA, USA). In addition, HbA1c and insulin serum levels were estimated using a commercial kit (Bio-Rad, Richmond, CA, USA) for HbA1c and an immune assay ELISA kit (human insulin ELISA kit, KAQ1251, Invitrogen Corporation, Camarillo, CA, USA) for insulin respectively. All kits used provide reliable results accurate measurements, whereas the study protocol strictly following a critical role of regular calibration of patient sample used with reference standards.

Isolation of miRNAs and RT–PCR

For each subject, total RNA was extracted from serum samples using the TRIzol LS reagent (Invitrogen, Carlsbad, CA), and subjected to RT–PCR analysis. In addition to that, TRIzol LS Reagent showed to be accurate and reliable for RNA isolation and RT-PCR Analysis also is highly accurate analysis for gene expression quantification, with proper primer design, RNA quality, and control implementation [73–74]. Thus, combining these methods provides robust and reproducible results, provided attention is given to sample quality, protocol adherence, and control inclusion.

In this regard, ready-made solution containing the primers and probes for human miR–126, miR-146a, miR-34a, miR-124a, miR-155, and miR-221 (Applied Biosystems, Foster City, CA) and real-time RT–PCR was estimated using an ABI 7300 system (Applied Biosystems) [39].

In this study, these miRNAs were selected according to their association with altered physiological states such as aging, PA, and diabetes, and also due to their important functions as intercellular communicators between endothelial cells and endothelial apoptotic bodies, smooth muscle cells, and cardiomyocytes [44, 46, 75–84].

RNU43 was used as an endogenous reference control, and all PCR cycles were performed according to the manufacturer's instructions as previously described [22], whereas the relative quantification of miRNAs was performed by the $2 - \Delta Ct$ method. To avoid errors and exactly determine the cycle threshold mean values for each sample including amplified miRNAs and endogenous control, all reactions were run in duplicate.

Assessment of vascular endothelial biomarkers

Spectrophotometric analyses were performed to estimate VEGF, apoAI, and apoB by using ELISA immunoassay kits (Cusabio Biotech, Newark, NJ) for VEGF, and (Duplex ELISA kit, Cat. N. STA-361, Cell Biolabs, Inc., San Diego, USA) for apoAI, and apoB respectively. ELISA procedures were performed according to the manufacturer's instructions and the concentrations of VEGF, apoAI, and apoB were measured at 450 nm. All kits used are highly specific and sensitive for their respective targets, ensuring accurate measurements. In these analyses, ELISA assays generally have excellent reproducibility whereas, the use of monoclonal antibodies ensures minimal cross-reactivity and a pre-coated plate with a clear instruction provided by manufacturers facilitate consistent execution.

Ethical considerations

The Ethics Sub-Committee of King Saud University, Kingdom of Saudi Arabia, reviewed and approved the study protocol under file ID: RRC-2015-048. The study

Table 1 Demographics characteristics of patients with type 2 diabetes (T2D) based up on physical activity

Parameters	Mild –PA (≤ 500 METs min/ week)	Moderate –PA (500–2500 METs-min/week)	Physically active (≥ 2500 METs-min/week)
Number	40	25	35
Age (years) #	49.1 ± 3.4	49.6 ± 4.7	49.8 ± 5.3
BMI(kg/m2) #	24.7 ± 2.8	23.8 ± 6.1**	21.9 ± 3.9**
Waist (cm) #	85.1 ± 13.2	76.1 ± 10.4**	68.1 ± 16.2**
Hips (cm) #	87.2 ± 21.3	86.9 ± 15.7**	84.5 ± 18.3**
WHR#	0.98 ± 0.75	0.87 ± 0.49**	0.81 ± 0.36**
Systolic BP (mmHg)	115.1 ± 2.1	110.5 ± 3.7	112 ± 4.1
Diastolic BP (mmHg)	74.2 ± 6.5	74.5 ± 6.1	74.8 ± 5.4
VO_2 max (ml/kg min) # #	31.8 ± 6.5	34.7 ± 7.1*	38.4 ± 3.1*
Disease duration (years)	6.7 ± 2.5	9.1 ± 4.5	9.8 ± 3.6

Notes: All values represent mean ± standard deviation. #Student's t-test; ##Mann–Whitney U-test; *$P < 0.05$; **$P < 0.01$. **Abbreviations**: BMI: body mass index, WHR: waist to hip ratio, VO2 max: maximal oxygen consumption, PA: physical activity

Table 2 Changes in glucose control traits and vascular endothelial biomarkers in type 2 diabetic patients with different PA (M ± SD)

Parameters	Mild –PA (≤ 500 METs min/ week)	Moderate –PA (500–2500 METs-min/week)	Physically active (≥ 2500 METs-min/week)
FBS (mg/dl)	215 ± 12.1	168 ± 21.3**	136.4 ± 16.3**
HbA1c (%)	9.5 ± 2.1	7.9 ± 0.85**	6.7 ± 0.58**
FINS (mUI/ml)	18.7 ± 3.4	14.2 ± 3.1**	12.2 ± 2.6**
HOMA-IR	5.5 ± 1.4	4.3 ± 1.7**	2.8 ± 1.2**
VEGF (pg/ml)	89.7 ± 3.4	76.2 ± 1.76**	62.7 ± 2.3**
ApoAI (mg/dL)	1.8 ± 0.52	1.5 ± 0.38**	1.2 ± 0.46**
ApoB (mg/dL)	1.2 ± 0.26	0.98 ± 0.31**	0.75 ± 0.24**
ApoB /apoA-I ratio	0.67 ± 0.31	0.65 ± 0.33*	0.62 ± 0.25*

Notes: All values represent mean ± standard deviation. *$P < 0.05$; **$P < 0.01$. A Student's t-test or the Mann-Whitney U test was used for comparing diabetic participants with different PA-scorers

Abbreviations: HOMA: homeostatic model assessment, IR: insulin resistance, VEGF: Vascular endothelial growth factor (vascular molecule), ApoA-I: apolipoprotein A-I, ApoB: apolipoprotein B, FINS: fasting serum insulin, HbA1c: Glycated hemoglobinA1c, PA: physical activity

followed the ethical guidelines of the Declaration of Helsinki (2013). All participants were assigned written informed consent before data collection. Blood was collected from all participants and serum samples were obtained following centrifugation for 1 min at the rate of 1400 rpm. Samples were given a coded study identification number, and were shipped frozen at 20° C for analysis.

Sample estimation

A computer software G*Power (v.3.1.9.4) was sued to estimate the sample size of this study. A total sample size of 100 participants was required to achieve a statistically significant difference at an α level of 0.05 and a power of 0.95 between the groups.

Statistical analyses

The Shapiro–Wilk test was conducted to assess the normality of data distribution, and the data were logarithmically transformed for further statistical analysis. Differences between the study groups were evaluated using both Student's t-test and ANOVA, followed by Bonferroni's multiple comparison post-hoc analysis. Micro-RNA levels across the different groups were compared using univariate analysis, adjusted by a general linear model. To estimate associations between MicroRNA levels and vascular endothelial parameters (VEGF, apoA-I, and apoB), multiple stepwise regression analyses and Pearson's correlation analysis were performed. A p-value of < 0.05 was considered statistically significant. All analyses were conducted using SPSS software (version 13.0, SPSS Inc., Chicago, IL, USA).

Results

A total of 100 male subjects with T2D participated in this study. Table 1 shows the clinical and demographic properties of the patients based on physical activity scores.

Only 60% of the patients were physically active. In moderate to physically active patients, there was a significant decrease ($P = 0.01$) in BMI, waist, hip, and WHR, and an increase ($P = 0.05$) in VO2 max as a marker of fitness score was reported in comparison with those of mild activity. In addition, a significant improvement ($P = 0.01$) in diabetic control traits was observed in patients with higher physically active scores (Table 2).

In this study, in order to study the role of physical activity in the reduction of diabetic complications, VEGF, ApoAI, ApoB, and ApoB /apoA-I ratio was measured

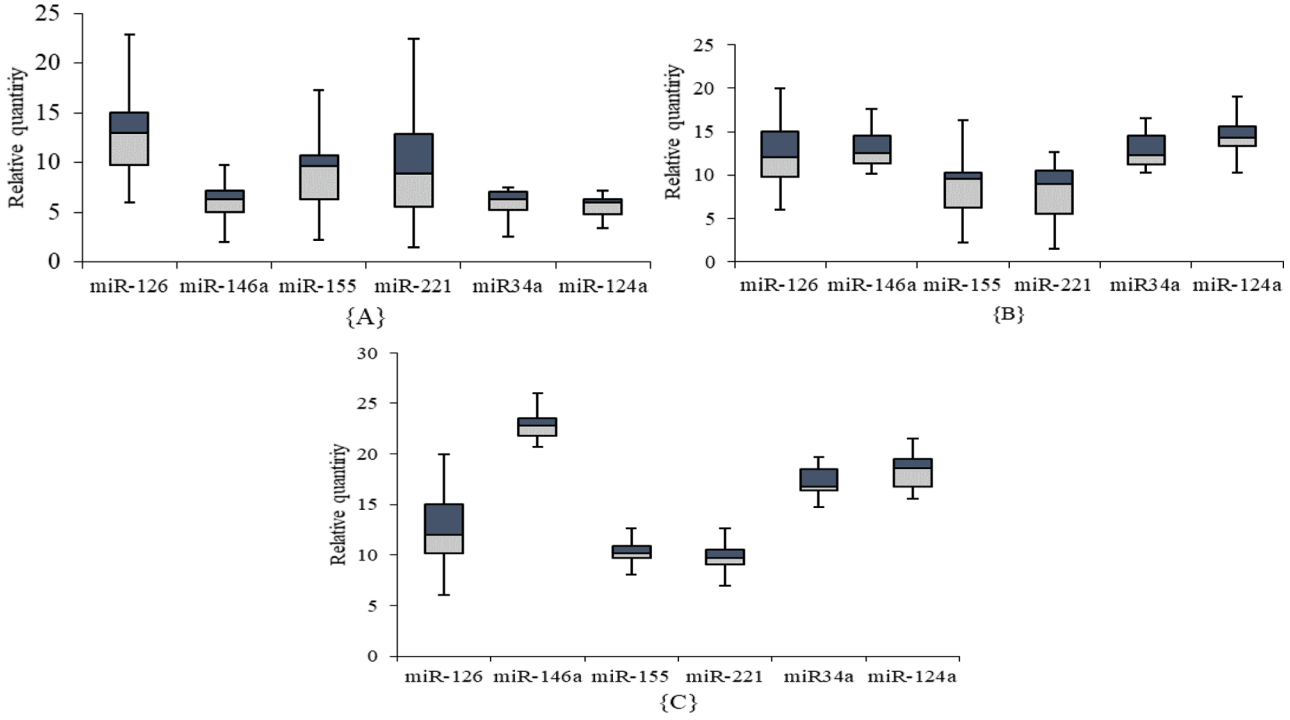

Fig. 1 The magnitude of miRNAs levels expression in type 2 diabetic patients with different physical activity scores presented with corresponding SD. Type 2 diabetic patients with mild physical activity (≤ 500 METs min/ week) (**A**), with moderate physical activity (500–2500 METs-min/week) (**B**), and physically active (≥ 2500 METs-min/week) (**C**)

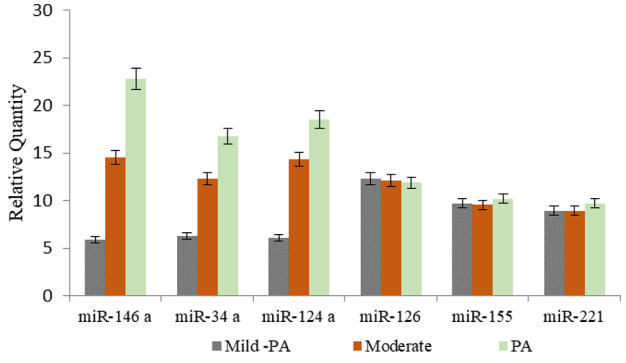

Fig. 2 change in miRNAs expression in diabetic patients (T2D) with different physical activity scores. miRNAs expression levels of miR-146-a, miR-34-a, and miR-124-a were significantly increased in physically active patients compared with those of mild PA ($P = 0.001$, $R = 0.145$)

as markers of vascular endothelial changes related to chronic diabetic complications (Table 2). In moderate to physically active patients, there was a significant decrease ($P = 0.01$) in VEGF, ApoAI, ApoB, and ApoB /apoA-I ratio ($p = 0.05$) compared to patients with lower PA or characterized by a sedentary lifestyle.

Circulating miRNAs as molecular markers of risk related to type 2 diabetes (T2D) were measured in patients with different PA scores. Figure 1 compares the relative quantities of the expressed miRNAs; miR–126, miR-146a, miR-34a, miR-124a, miR-155, and miR-221 according to the physical activity status of each diabetic patient. In moderate and physically active diabetic patients exhibiting a significant increase ($P = 0.001$, $R = 0.145$) in the expression levels of miR-146a, miR-34a, miR-124a compared with those with mild PA (Fig. 1A, B and C) and Fig. (2). However, little or no change ($p = 0.123$, $R = 0.543$) was reported in the expression levels of miR–126, miR-155, and miR-221 in diabetic patients with various PA scores (Fig. 2).

Correlation coefficient analysis showed that diabetic control parameters; FBS, HbA1c (%), FINS, and HOMA-IR correlated negatively with the expression levels of miR-146a, miR-34a, miR-124a, miR–126, miR-155, and miR-221. However, physical activity correlated positively with miR-146a, miR-34a, and miR-124a and negatively with miR–126, miR-155, and miR-221 (Table 3).

Moreover, the expression of circulating miRNAs correlated positively with VEGF, ApoAI, ApoB, and ApoB / apoA-I ratio and negatively with obesity-related factor BMI (Table 4).

Discussion

Chronic complications of type 2 diabetes are associated with the patient's lifestyle such as aggravated status of physical functioning, physical inactivity, and poor eating habits [42–43]. In addition; genetic predisposition adds an extra potential impact on diabetic status [44–46].

Table 3 Correlation coefficients of significantly expressed circulating miRNAs with parameters of diabetic control, and physical activity of patients with type 2 diabetes (T2D)

Parameters	miR-146 a	miR-34 a	miR-124 a	miR-155	miR-221	miR-126
FBS	0.254**	0.124**	0.145**	0.235*	0.231*	0.425 *
HbA1c (%)	0.123**	0.356**	0.235**	0.361*	0.254*	0.961*
FINS	0.112**	0.568**	0.365**	0.234*	0.235*	0.358*
HOMA-IR	0.356**	0.135**	0.235**	0.234*	0.123*	0.235*
PA	0.235**	0.384**	0.368**	0.165*	0.118*	0.235*

HOMA: homeostatic model assessment, IR: insulin resistance, FINS: fasting serum insulin, HbA1c: Glycated hemoglobinA1c, PA: physical activity. *$P<0.01$; **$P<0.001$

Table 4 Correlation coefficients of significantly expressed circulating miRNAs with vascular endothelial biomarkers and BMI of patients with type 2 diabetes (T2D)

Parameters	miR-146 a	miR-34 a	miR-124 a	miR-155	miR-221	miR-126
VEGF	0.325**	0.204**	0.545**	0.615*	0.538*	0.515 *
Apo AI	0.543**	0.156**	0.635**	0.251*	0.434*	0.761*
Apo B	0.322**	0.268**	0.445**	0.154*	0.197*	0.424*
Apo B /Apo A-I ratio	0.426**	0.345**	0.465**	0.184*	0.213*	0.346*
BMI	-0.515**	-0.534**	-0.218**	-0.465*	-0.295*	-0.415*

BMI: body mass index, VEGF: Vascular endothelial growth factor (vascular molecule), ApoA-I: apolipoprotein A-I, ApoB: apolipoprotein B. *$P<0.01$; **$P<0.001$

The main findings obtained in this pilot study were a greater reduction in the levels of diabetic control factors such as FBS, IR, FINS, HOMA-IR, and HbA1c (%) in T2D patients with moderate and active physical scores. The extant diabetic control was significantly observed in physically active patients, which were paralleled with a significant reduction in serum levels of VEGF, ApoAI, ApoB, and ApoB /apoA-I ratio as markers of vascular endothelial changes during diabetes. Our results confirmed previously reported studies which showed that physical activity significantly helps in improving or adding a potentially positive effect on glycemic control by enhancing the sensitivity of tissues towards insulin uptake and also a reduction in body weight which greatly exerts more glucose uptake by muscles and provides with more regulation in insulin-regulated GLUT4 [85–86]. Moreover, non-drug-based strategies showed that changes in lifestyle such as diet adequacy and adoption of regular exercise practices potentially provide good results in a reduction of glycemic levels and related serious complications [87–88]. Prevention and control of T2DM are significantly dependent on the potential positive effects of physical activity, which exerts minor and major physiological effects following participation in exercise programs [89–90].

Physical activity was shown to improve vascular endothelial function in adults [91–92], and in children [68–69], via nullifying the loss of vasodilatory function which occurs with aging [93]. Moreover, potential positive effects of physical activity on reducing chronic complications associated with diabetes were previously approved, such as the progression of carotid IMT in healthy adults [94], coronary heart disease, or hypertension [95–96].

Recently, it was concluded that a moderate increase in physical activity and an improving lifestyle efficiently prevent the progression of atherosclerotic vascular changes in healthy adolescents [97]. Supporting our data, interventions based upon enhancing physical activity performance have shown to be the most realistic option to prevent type-2 diabetes, especially in obese patients with impaired glucose tolerance [98], whereas exercise was shown as an effective antioxidant and anti-atherogenic therapy [99]. The beneficial effects of increased physical activity, such as reduction in blood pressure and prevention of atherogenesis, are mainly controlled by changes in the biology of vascular tissues [100–104]. Moreover, vascular and angiogenic factors such as VEGF, ApoAI, and ApoB are regulated following exercise-induced endothelial adaptation [88, 105–108].

Circulating microRNAs were shown as new ways of biological markers to explain the physiological changes accompanying physical activity [109–110]. They are found in the entire cell nucleus and circulate in the blood and other tissue fluids in nucleated bodies such as leukocytes in the systemic circulation as small vesicles (exosomes) [111–113]. These characteristics provide miRNAs with a resistant property against endogenous RNases, which makes them appropriate to be considered as potential non-invasive biomarkers of health and disease status [114].

In this study, we are trying to find a probable link between the physical activity of diabetic patients and the expression of a set of miRs and its correlation with both diabetic control traits and markers of vascular endothelial changes. Our results refer to consistent changes in serum levels of miRs according to the physical activity status of T2D patients. There was a significant increase

in the levels of miR-146a, miR-34a, and miR-124a with little or no change in the expression levels of miR-155, miR-221, and miR-126 observed in moderate or physically active T2D patients compared with those of sedentary lifestyle or participating in activities with lower PA. miRNAs associated with several diseases, especially T2DM [33–34]. Serum concentrations of miR-126, miR-155, and miR-146a were found in inversely proportional situations with glucose levels, IR, and glucose tolerance rate [35–36]. Moreover, matched with our results, other studies have suggested a reduction in the concentrations of miR-146a, miR-34a, miR-124a, and miR-155 in physically active T2DM patients. This may be related to the proposed higher proinflammatory state associated with diabetes [37–39]. In addition, a significant correlation was previously obtained between glucose levels and blood concentration of miR-126, miR-146a, miR-155, and miR-221 in diabetic patients, respectively [35–40].

Similarly, the reduction in miRNAs concentration in the circulation of diabetic patients negatively correlated with systemic glucose, insulin resistance, and inflammatory cytokines, and this may be related to the regulation of immune mediators [47, 115]. Thus, the reduction or impaired expression of miR-146a, miR-34a, and miR-124a in the serum of patients with mild PA may be related to oxidative stress and proinflammatory pathways which significantly trigger or worsen the uncontrolled glycemic state [116].

In moderate or physically active patients of our study, the increase in the expression rates of miR-146a, mIR-34a, and miR124a may be related to increases in the levels of antioxidant enzymes that counteract free radical oxidative stress and sugar metabolism in muscle tissues and a decrease in adiposity tissues provide a suitable climate for micro-RNAs expressions. Thus, the estimation of circulating miR-146a, mIR-34a, and miR124a may correspond to a potential physiological change that is beneficial as a biomarker to measure the functioning of glucose metabolism in the body [45, 117–125]. Moreover, our findings showed that the expression of microRNAs closely correlated with VEGF, ApoAI, ApoB, and ApoB / apoA-I ratio as markers of vascular endothelial changes that occurred during diabetes. Circulating miRNAs estimated in this study correlated positively with VEGF, ApoAI, ApoB, and ApoB /apoA-I ratio and negatively with obesity-related factor BMI.

In endothelial cells, most of the miRNAs were downregulated [126], whereas miR-221 has been implicated in the inhibition of the formation and migration of endothelial cell tubes [127], and miR-126 was shown to modulate angiogenesis in a positive way. The deletion of the miR-126 expression gene leads to vascular leakage, hemorrhage, and early death in mice via negative signaling pathways [128]. In addition, the expression of miR-146a, miR-34a, miR-124a, miR-155, miR-221, and miR-126 appears to increase the response of endothelial cells to vascular promoters such as VEGF which promotes angiogenesis in vivo and in vitro [129–130]. Several studies reported that all angiogenesis-related miRNAs have potential angiogenic effects, such as miR-126, miR-155 of miR-146a, miR-34a, and miR-124a, while miR-221 has been found to be antiangiogenic [131–132]. Moreover, the change in the expression of microRNAs estimated in this study linked with lipid profile, especially cholesterol metabolism and severe complications in patients with diabetes. MiR-122 was shown to have a direct role in cholesterol metabolism [123–124], and the change in miR-146a levels was affected by an increment in the plasma concentrations of proinflammatory cytokines [125].

Finally, our findings may provide prospects for studies to modulate the expression of miR-miR-146a, mIR-34a, and miR124a as a possible mechanism to improve insulin sensitivity, and to regulate vascular endothelial complications related to uncontrolled glycemic state [77, 81, 126, 127]. Serum miRNA expression profiles were reported to serve as fingerprints for disease detection, whereas it is significantly associated with the expression of vascular angiogenesis parameters such as VEGF [123–128]. Thus, based on the physical activity of the patients, the data of our study suggest possible new metabolic pathways to study the correlation between vascular biomarkers and the expression of miRNAs, and to provide a new challenge towards chronic biological complications resulting from glucose intolerance in older adults. This may need more investigations with large-sized samples to confirm the results proposed in this study.

Strength and limitations of the study

Although limiting sample size, the strength of our research work is that combining the status of physical activity of diabetic patients with molecular changes in cellular miRNAs, and an improvement in vascular biomarkers, including apoAI, apoB, and the apoB/apoA-I ratio. The findings revealed a strong correlation between these vascular biomarkers and the physiological responses of miR-146a, miR-34a, and miR-124a.

Our study had several limitations. This study examined the correlation between physical activity and the role of microRNAs (miRNAs) in enhancing diabetes management among 100 diabetic males. While the findings provide valuable insights, several limitations warrant discussion. The small sample size limits the generalizability of the results, as it may not capture the full spectrum of variability in miRNA expression and physical activity responses. Furthermore, the exclusive inclusion of males introduces gender bias, neglecting potential sex-specific differences in miRNA regulation and metabolic

responses to exercise. Additionally, the study did not account for potential confounding variables, such as age, comorbidities, dietary habits, and medication use, which could influence miRNA expression and diabetes outcomes.

Finally, a low sample size of the current research work leads that our results can be interpreted as preliminary findings and, we still need more studies to understand the potential association and establishment of physical activity status, molecular changes in miRNA expression, diabetes outcomes, and vascular biomarkers in improving diabetes and related vascular biomarkers among patients with type 2 diabetes.

Future directions

Future research should aim to address these limitations by including larger, more diverse cohorts that encompass both genders and varied demographic backgrounds. Longitudinal studies with comprehensive data collection on confounding factors are essential to establish causal relationships and strengthen the findings. Furthermore, exploring the interaction between specific exercise regimens and miRNA expression across different diabetes phenotypes could yield tailored interventions. This expanded approach would enhance the translational potential of miRNA-targeted therapies in diabetes care.

Conclusion

In physically active diabetic patients, circulating miR-146a, mIR-34a, and miR124a were highly expressed in association with a significant enhancement in the levels of vascular biomarkers; apoAI, apoB, and apoB /apoA-I ratio. In addition, there is a significant correlation between vascular biomarkers and the predictable physiological responses of miR-146a, miR-34a, and miR124a. The study results imply that improving one's physical fitness is helpful for the regulation of the expression of cellular miRNAs in T2DM patients. Further research based on larger samples of both genders is recommended for identifying whether or not changes in the miRNA profile can affect the clinical situation of T2DM patients and the translational potential of new miRNA-targeted therapies in diabetes.

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Author contributions
H.A.R. S.A.G. T.A. F.A. A.H.A. and A.I. proposed the study's research ideas, conception, and design. H.A.R. T.A. F.A. and S.A.G. completed practical work and collected the data. A.H.A., S.A.G. and A.I. analysed and interpreted the data. H.A.R. S.A.G. T.A. F.A. and A.I. prepared the manuscript's initial drafts. H.A.R. A.H.A. S.A.G. and A.I. critically reviewed the manuscript's intellectual content. All authors reviewed, understood, and approved the manuscript's final version to be submitted or published.

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Data availability
The analyzed data used to support the findings of this study are included within the article.

Declarations

Ethics statement and consent to participate
The Ethics Sub-Committee of King Saud University, Kingdom of Saudi Arabia, reviewed and approved the study protocol under file number ID: RRC-2015-048. The study followed the ethical guidelines of the Declaration of Helsinki (2013). All participants were assigned written informed consent before data collection.

Consent to publish
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh 11433, Saudi Arabia
[2]Rehabilitation Research Chair, Department of Rehabilitation Sciences, College of Applied Medical Sciences, King Saud University, P.O. Box 10219, Riyadh 11433, Saudi Arabia
[3]College of Medicine, King Saud University, Riyadh 11433, Saudi Arabia

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