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Exosomal MicroRNA-142-3p: A Potential Contributor to the Beneficial Effects of Postprandial Exercise in Type 2 Diabetes

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ABSTRACT

Background: The study explored postprandial exercise's impact on exosomal miRNAs and novel protective biomarkers in men with Type 2 diabetes (T2D).

Methods: A total of 24 male participants, including 13 patients and 11 healthy individuals, were recruited. T2D patients underwent a randomized three-period crossover 1-hour postprandial intervention: high-intensity interval exercise (T2D-HIIE), moderate-intensity continuous exercise (T2D-MICE) with cycling and sedentary control (T2D-CON) for 30 min, while healthy participants served as controls (HP-CON) without exercise intervention. Plasma glucose and insulin levels were measured at pre-exercise and immediately post-exercise. Exosomes were extracted from plasma using differential ultra-centrifugation at 1.5 hours postprandial, which equates to immediately post-exercise for T2D patients. Four miRNAs (miR-142-3p, miR-144-3p, miR-126-3p, and miR-30d-5p) were tested to identify the differentially expressed miRNAs. The relationship between exosomal miRNAs and glucose and insulin levels was achieved by Pearson correlations.

Results: Both postprandial exercise interventions (HIIE and MICE) led to immediate reductions in glucose and insulin levels ($P < 0.05$), with HIIE showing a more pronounced decrease in glucose level compared to MICE ($P = 0.04$). Both HIIE and MICE induced a decrease in exosomal miR-142-3p ($p = 0.010$ and $p = 0.020$, respectively), and circulating miR-144-3p ($p = 0.040$ and $p = 0.033$, respectively), with no significant differences between HIIE and MICE. Additionally, only exosomal miR-142-3p expression positively related with glucose levels ($r = 0.624$, $p = 0.01$).

Conclusions: Exosomal miR-142-3p is reduced by postprandial exercise and positively associated with glucose regulation. We propose that exosomal miR-142-3p may serve as a potential contributor for exercise-induced benefits in middle-aged men with T2D.

Keywords: Type 2 Diabetes; microRNA; Exosome; Postprandial Exercise

Abbreviations: T2D: Type 2 Diabetes; MICE: Moderate Intensity Continuous Exercise; HIIE: High Intensity Interval Exercise; EVs: Extracellular Vesicle; CISS: China Institute of Sport Science; FBG: Fasting Blood Glucose; IPAQ: International Physical Activity Questionnaire; PARQ: Physical Activity Readiness Questionnaire; TEM: Transmission Electron Microscopy; NTA: Nanoparticle Tracking Analysis; T1D: Type 1 Diabetes; HIIT: High Intensity Interval

Introduction

Type 2 diabetes (T2D), characterized by hyperglycemia, is a prevalent metabalic disorder that poses a significant threat to human health worldwide. Exercise has long been recognized as an essential component in the management of T2D. Although individuals with T2D experience impaired insulin sensitivity, exercise-induced muscular contraction can facilitate glucose uptake independently of insulin [1]. In particular, postprandial exercise has been found to stimulate glucose uptake [2]. Among the different exercise recommendations for individuals with T2D, moderate intensity continuous exercise (MICE) is the most widely recommended. However, recent studies have shown that high intensity interval exercise (HIIE), which differs from MICE in terms of intensity and exercise pattern, is equally effective or even superior in terms of glycemic control [3-5]. This suggests that HIIE could hold potential as a promising intervention approach for individuals with T2D [6]. In the search for improved or even novel interventional strategies, researchers need to revert back to clarify the molecular mechanisms underlying T2D. While altered miRNAs have been documented to contribute to the positive effects of physical exercise [7,8], the involvement of exosomes, the regulation of exosomal miRNAs, and the function of miRNAs' corresponding target genes in T2D patients remain to be elucidated. Exosomes, with diameters ranging from 40 nm to 120 nm, are a type of extracellular vesicle (EVs) originating from various cells and can be detected in most biofluids [9,10].

They modulate cell functions by transporting proteins, lipids, and nucleic acids [11]. Emerging evidence has suggested that exosomes play a role in a variety of pathological processes such as chronic inflammation, endocrine disorders and insulin resistance and regulate multiple biochemical signaling through specific transfer of various molecules including miRNAs [12]. Exosomal miRNAs can be shielded from degradation by enzymes while in circulation, thanks to exosome-mediated protection. Subsequently, they are delivered to target cells, where they modulate the expression of specific genes, serving as regulators of intercellular communication [13] to affect insulin secretion, adipose tissue metabolism, and the development of diabetes [14,15]. Indeed, the role of biomarkers of miRNAs carried by exosomes in the body fluids for diabetes and its related complications has been previously described [16-19], while the effects of exercise are yet to be fully clarified. It's worth noting that exercise acts as a stimulus, inducing cellular responses such as gene expression and exosome release [20-22]. Exosomes from diverse sources can mediate a number of favorable effects induced by exercise on important metabolic tissues through their carried cargoes. Some pioneering work [23,24] highlighted the distinctiveness of exosomal miRNA patterns, portraying them as a distinct factor capable of facilitating inter-tissue communication throughout the body in response to a single exercise bout.

Furthermore, recent reviews have offered an important insight into the changes of exosomal miRNAs, which are molecules of growing importance in exercise physiology and probably being involved in the innovative mechanisms behind the positive effects of exercise for patients with T2D [18,25]. Given that the vital role of comprehensive mechanistic insights in T2D prevention and treatment, the exploration of novel perspective players contributing to the positive impacts of exercise on T2D holds promise and urgency. Thus far however, the role of exosomal miRNAs induced by acute postprandial exercise in T2D and their impact on glucose regulation, as well as the underlying signal pathways, remain largely unclear. By means of intervention of HIIE and MICE in middle-aged men with T2D, this study aimed to identify the differentially expressed exosomal miRNA associated with T2D and their response to exercise stimulus. This research aims to explore the exosomal miRNA-related mechanisms underlying the beneficial impacts of postprandial exercise on T2D.

Methods

Study Design and Ethical Approval

This study was a randomized three-period crossover intervention conducted solely for T2D patients in the laboratory of China Institute of Sport Science (CISS). The sample size was determined using G*Power Version 3.1.9.7 (Franz Faul, University of Kiel, Germany, 2020) based on a calculated mean between-group difference of 2.5 ± 2.82 mmol/L in postprandial blood glucose, with a two-tailed effect size of 0.5, a significant level α of 0.05, and a power of 0.8. The required sample size was 12. Ethical approval was obtained from the CISS Ethics Board in accordance with the principles of the Declaration of Helsinki. All participants provided written consent prior to participation.

Participants

Recruitment took place from October 10, 2021, to October 30, 2021. We enrolled 24 male participants aged between 30 and 60 through advertisements and social networks. Out of these, 13 participants had T2D, while 11 were healthy volunteers, matched for age and BMI. T2D patients were eligible if they had been diagnosed for at least 6 months and had not engaged in structured physical activity for the past 6 months (less than 150 minutes per week). Exclusion criteria for T2D patients included T2D-related complications and contraindications to exercise. Healthy participants had to meet specific criteria, including fasting blood glucose (FBG) < 6.1 mmol/L, oral glucose tolerance test within two hours with blood glucose < 7.8 mmol/L, freedom from chronic diseases and injuries, and no family history of diabetes.

Procedures

Procedures of T2D were described in our previous study [4]. For the first visit, participants fasted for 12 hours and arrived at the CISS laboratory at 8 a.m. for fasting blood sampling, resting blood pressure measurement, and anthropometric assessment. They also completed a basic information questionnaire, the international physical activity

questionnaire (IPAQ) [26], the physical activity readiness questionnaire (PAR-Q) [27], and a list of contraindications for graded exercise test [27]. Healthy individuals had a second visit on a different day to conduct blood sampling 1.5 hours after breakfast. For T2D patients, a randomized crossover trial consisted of three experimental days: a control day (CON) without exercise, and two exercise days—HIIE and MICE, was conducted. The interventions were performed 1 hour after breakfast, including: (1) HIIE (7 × 1 min, 90% VO2max + 2 min, 30% VO2max) with cycling; (2) MICE (30 min, 50% VO2max) with cycling; or (3) a sedentary control (CON) for 30 min. A 10-day washout period separated each intervention. The energy expended in MICE and HIIE protocols was approximately equivalent. Random allocation was accomplished through sealed envelopes, each specifying an intervention type. The assessors, investigators, statistician and T2D patients were blinded to the grouping. However, exercise intervention implementer was not blinded. The study flow is shown in (Figure 1).

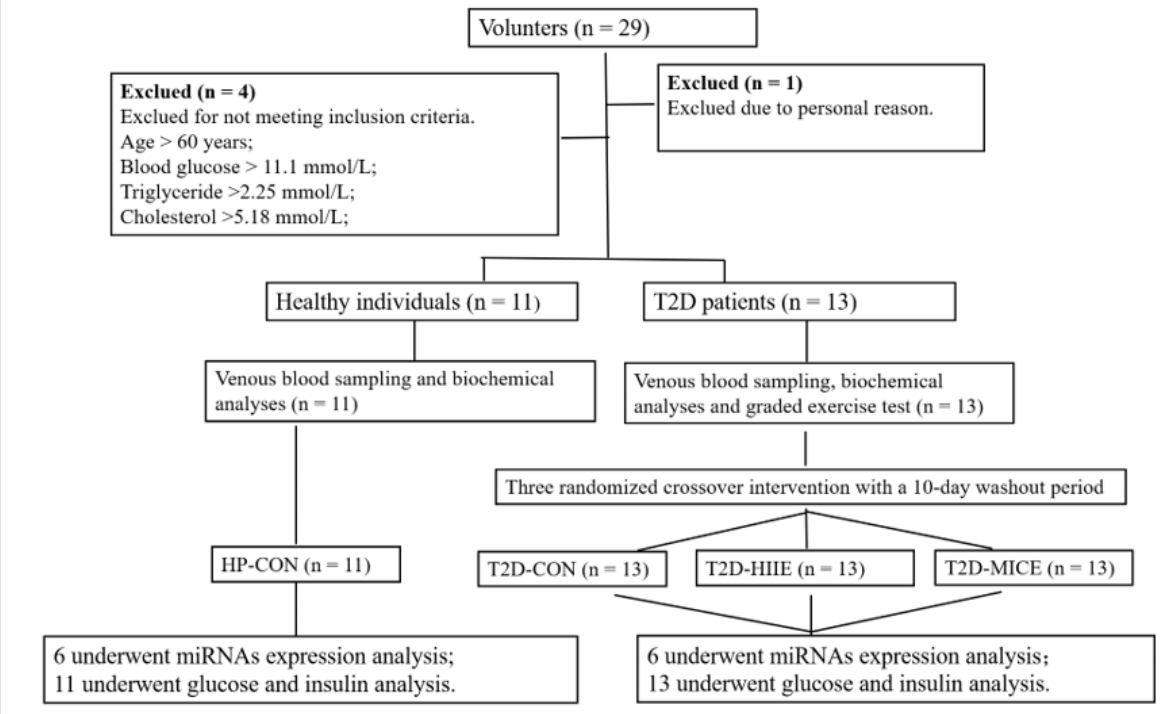

Figure 1: Study flow chart.

Throughout the study, participants were advised to maintain their regular daily routines, including diet, exercise, and medication. They consumed consistent standard meals (breakfast) and water intake on each intervention day, along with the same dinner before interventions. Alcohol, caffeine, and physical activity were avoided 48 hours prior to each intervention. We randomly selected 6 out of 13 diabetes patients and 6 out of 11 healthy individuals for exosome extraction. All the randomization were conducted via opaque, sealed envelopes.

Venous Blood Sampling and Biochemical Analyses

A trained professional collected blood samples from the antecubital vein. During the initial visit, following a 12-hour fast, we measured various biochemical markers. Throughout the three interventions for T2D patients, we assessed plasma glucose and insulin levels before and immediately after exercise. Exosomes were extracted from a randomly selected subset of 6 out of 13 T2D patients immediately after exercise. During the second visit of healthy individuals, we measured plasma glucose and insulin levels from 11 healthy individuals and extracted exosomes from a randomly selected subgroup of 6 individuals, also at1.5 hours after breakfast (immediately after exercise). All samples for biochemical analyses were duplicated, and the experimenters conducting the analyses were kept 'blinded' to the interventional conditions.

Graded Exercise Test

Graded exercise test was conducted using a stationary cycle ergometer (Ergoselect 100, Ergoline, Bitz, Germany) to measure the peak heart rate (HRpeak) and peak oxygen uptake (V̇O2peak), as detailed in our previous study [4].

Exosome Isolation from Plasma

Blood samples were initially centrifuged at 1900g for 10 minutes at 4°C, and the resulting supernatant was further centrifuged at 3000g for 15 minutes at the same temperature. The obtained plasma

was promptly frozen at -80°C. Exosome isolation involved a multi-step process of ultracentrifugation using a Beckman Optima L-100XP centrifuge. This process included initial centrifugation of the plasma at 2000 x g at 4°C for 30 minutes, followed by a subsequent centrifugation at 12000 x g at 4°C for 45 minutes. The supernatant was then passed through 0.45 µm disposable filter units and centrifuged at 110000 x g at 4°C for 70 minutes. Following resuspension in 10 ml of nuclease-free water, the exosomes underwent an additional centrifugation step at 110000 x g at 4°C for 70 minutes. Finally, the exosomes were suspended in 0.1 ml of nuclease-free water and stored at -80°C.

Characterization of Isolated Exosomes

To assess exosome purity, we employed transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and Western blotting. For TEM, exosomes were fixed, stained, and visualized with an HT-7800 TEM (Hitachi, Tokyo, Japan) at 80 kV. NTA measured particle size and concentration using a Flow NanoAnalyzer (N30E, China). Western blotting targeted exosomal proteins (CD63, CD81, TSG101, Calnexin) with specific primary antibodies and secondary antibodies for detection.

Isolation of RNA from Exosomes

Exosomal RNA was Isolated using the miRNeasy Serum/Plasma Advanced Kit (217204, TIANGEN, Germany) following the instructions. RNA quality and quantity were assessed using NanoDrop (Thermo Fisher Scientific, USA) and Agilent 2100 bioanalyzer (Thermo Fisher Scientific, MA, USA), respectively.

Quantitative Real-Time PCR

RNA was converted into cDNA through reverse transcription using the miRCURY LNA RT Kit (339340, TIANGEN, Germany). Real-time PCR for miR-142-3p, miR-144-3p, miR-126-3p and miR-30d-5p (miRCURY LNA miRNA Probe Assay, 339350, TIANGEN, Germany) was carried out using the miRCURY LNA Probe PCR Kit (339371, TIANGEN, Germany) on the CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA), with miR-26a used to normalize the qPCR data. PCR reaction began with an initial denaturation at 95°C for 2 min, then 40 cycles of 95°C for 5 s, 56°C for 30 s. Relative fold-changes were calculated as using the 2-ΔΔCq method [28]. Samples exhibiting a qRT-PCR result of Ct < 40 were deemed positive [29].

Statistical Analysis

Statistical analysis was performed with SPSS 19.0 (IBM Corp., Armonk, NY, USA). Data normality was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Non-normally distributed data were transformed using logarithm or square root methods. Glucose and insulin values among the three interventions were compared using repeated measures two-way ANOVA for intervention and time interaction. Differentially expressed miRNAs were analyzed using one-way ANOVA. Statistical significance was set at $P < 0.05$. The relationship between exosomal miRNAs and glucose and insulin levels was achieved by Pearson correlations. Data are expressed as means and 95%CI.

Results

Participant' characteristics are showed in (Table 1). All participants were male. Blood samples from 11 healthy individuals and 13 patients with T2D were obtained.

Table 1: Participants' characteristics and pharmacological regimen (n =24). Mean with 95%CI.

Characteristics	Healthy Individuals (n = 11)	Type 2 Diabetes (n = 13)	P-Value
Age (years)	43.7 (39, 56)	47.0 (34, 57)	$P = 0.233^{\#}$
Diabetes duration (years)		3.7 (2, 6)	
Body mass (kg)	69.3 (62.5, 83.8)	70.8 (58.5, 84.3)	$P = 0.660^{\&}$
BMI (kg/m^2)	25.6 (22.2, 27.7)	26.0 (22.3, 28.0)	$P = 0.581^{\&}$
Glycemic control			
Fasting glucose (mmol/L)	4.2 (3.1, 4.8)	6.7 (3.3, 11.0)	$P < 0.001^{\#}$
HbA1c (%)	5.2 (4.5, 5.4)	7.1 (5.5, 9.9)	$P < 0.001^{\&}$
FFA (mmol/L)	0.5 (0.3, 0.8)	0.6 (0.2, 1.4)	$P = 0.828^{\&}$
Total cholesterol (mmol/L)	4.7 (4.0, 5.1)	5.3 (3.5, 7.7)	$P = 0.306^{\&}$
Triglycerides (mmol/L)	1.5 (1.0, 2.1)	2.0 (0.8, 4.5)	$P = 0.998^{\&}$
HDL-cholesterol (mmol/L)*	1.3 (1.0, 2.0)	1.2 (0.7, 1.5)	$P = 0.445^{\#}$
LDL-cholesterol (mmol/L)*	2.8 (2.2, 3.5)	3.0 (1.5, 5.0)	$P = 0.467^{\#}$
Fitness variables			
Maximal oxygen consumption (VO_{2max}) (ml/min/kg)	30.0 (17.9, 40.1)	25.0 (14.1, 34.9)	$P = 0.060^{\#}$
Maximal heart rate (HR_{max})(bpm)	177.6 (152.5, 202.9)	152 (104.5, 171.0)	$P = 0.004^{\#}$
Oral hypoglycemic agents		n = 10 (76.9%)	
Metformin only		n = 7 (53.8%)	

Metformin + acarbose		n =1 (7.7%)
Metformin + repaglinide		n =1 (7.7%)
Metformin + gliptin		n =1 (7.7%)
No medication used		n =3 (23.1%)

Note: *HDL-cholesterol, high-density lipoprotein cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol; &Comparison by Mann-Whitney U test.

#Comparison by Independent 2-sample t test.

Glucose and Insulin Responses

T2D patients exhibited good tolerance to both HIIE and MICE interventions. The responses of glucose and insulin following HIIE and MICE are presented in (Table 2). Both interventions led to immediate reductions in glucose and insulin levels ($P < 0.05$), with HIIE showing a more pronounced decrease in glucose level compared to MICE ($P = 0.04$).

Table 2: Plasma glucose (mmol/L) and insulin (μU/mL) values in T2D men. Mean with 95%CI.

Time	Group	Plasma glucose (mmol/L)	Plasma insulin(μU/mL)
Pre-exercise	CON	9.79 (4.3, 23.65)	35.40 (15.6, 95.6)
	HIIE	9.91 (5.8, 20.0)	36.07 (14.2, 89.7)
	MICE	9.55 (5.2, 18.0)	35.91 (16.1, 102.6)
Post-exercise	CON	9.26 (3.8, 23.1)	35.34 (14.9, 107.9)
	HIIE	6.60 (3.3, 15.5)$^{**@\#\#}$	14.37 (5.3, 20.2)$^{*\#\#}$
	MICE	7.55 (3.9, 20.0)$^{**\#\#}$	17.40 (4.4, 27.3)$^{*\#\#}$

Note: *P <0.05; **P<0.01; compared to CON; @compared to MICE post-exercise. #compared to pre-exercise

Isolation and Characterization of EVs

The identification of plasma exosomes was illustrated in (Figure 2).

Validation of miRNAs by qRT-PCR

Four significantly differentially expressed miRNAs (miR-142-3p, miR-144-3p, miR-126-3p, and miR-30d-5p), identified through sequencing (data not disclosed), were found to be upregulated in T2D patients compared to normal participants. However, these miRNAs were downregulated in T2D patients after the CON phase compared to immediately post-HIIE or post-MICE phases. These miRNAs are potentially associated with energy metabolism and are commonly expressed in T2D patients. To validate these findings, qRT-PCR was conducted in a sample of 6 T2D patients and 6 healthy participants. Additionally, the plasma levels of these four miRNAs were also analyzed. We found that exosomal miR-142-3p showed remarkably higher expression in T2D patients in comparison to healthy participants ($p = 0.021$) and decreased following HIIE and MICE ($p = 0.010$ and $p = 0.020$, respectively; (Figure 3A)), suggesting its potential role in exercise-induced changes. However, miR-144-3p and miR-126-3p did not show significant alterations in expression levels ($p > 0.05$) after exercise. Furthermore, miR-30d-5p was not detected in any exosomal group, indicating its absence or low expression in the collected samples. Regarding the expression of these 4 miRNAs in plasma, miR-144-3p was significantly higher in T2D patients compared to healthy controls ($p = 0.047$) and decreased following both HIIE and MICE ($p = 0.040$, $p = 0.033$, respectively; (Figure 3B)). Correlation analysis with glucose and insulin concentrations showed that only miR-142-3p exhibited a positive correlation with glucose concentrations ($r = 0.635$, $p = 0.008$).

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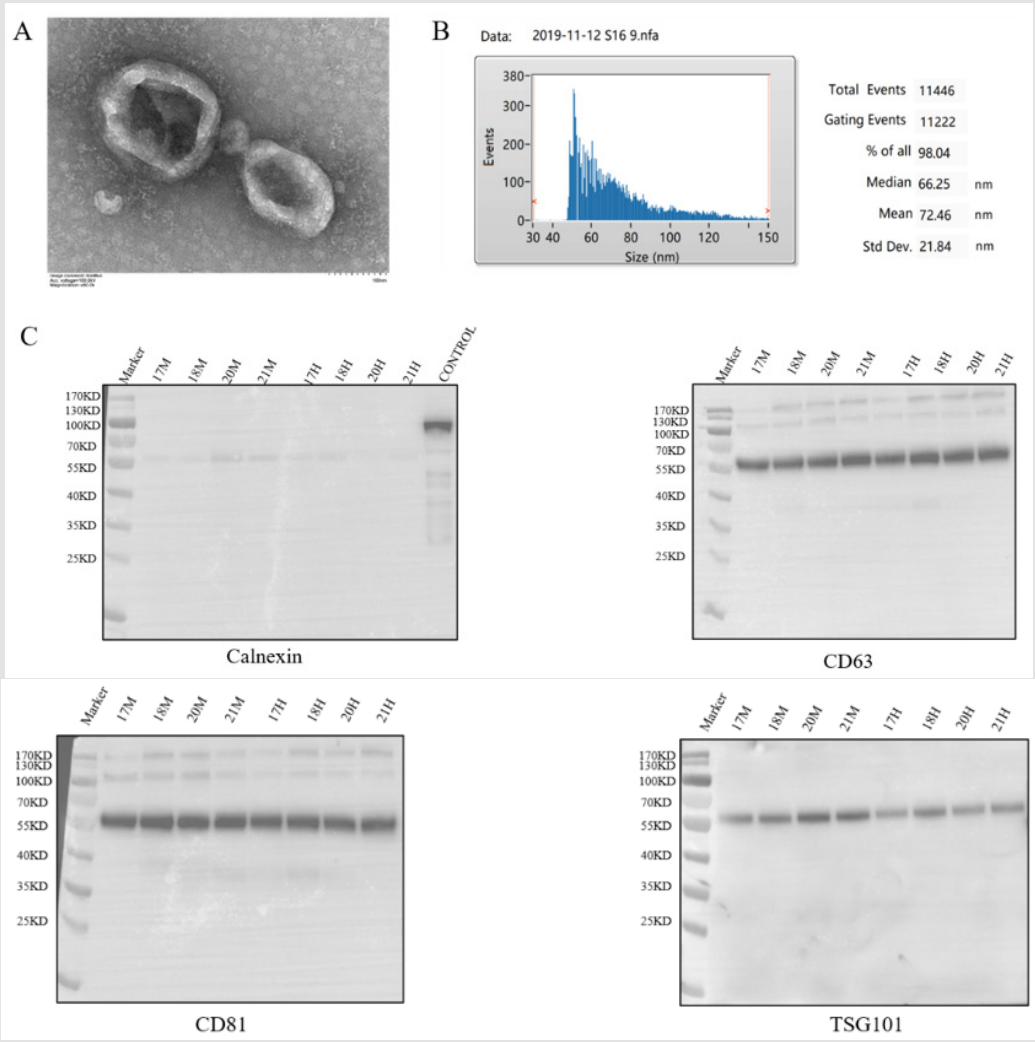

Figure 2: Identification of plasma exosomes.
A. Transmission electron microscopy (scale bar 100 nm).
B. Analysis of exosomes isolated from plasma by Nanoparticle Tracking Analysis (size range: 50 nm to 150 nm; concentration: 1.0 E+8 ~ 1.0 E+10 particles/mL).
C. Confirmation of the exosomes markers with Western blotting indicated the presence of CD63, CD81 and TSG101, but the absence of calnexin in exosomes. 17M-21M: 4 T2D patients following MICE; 17H-21H: 4 T2D patients following HIIE; Numbers 17 to 21 are the identification numbers for each patient.

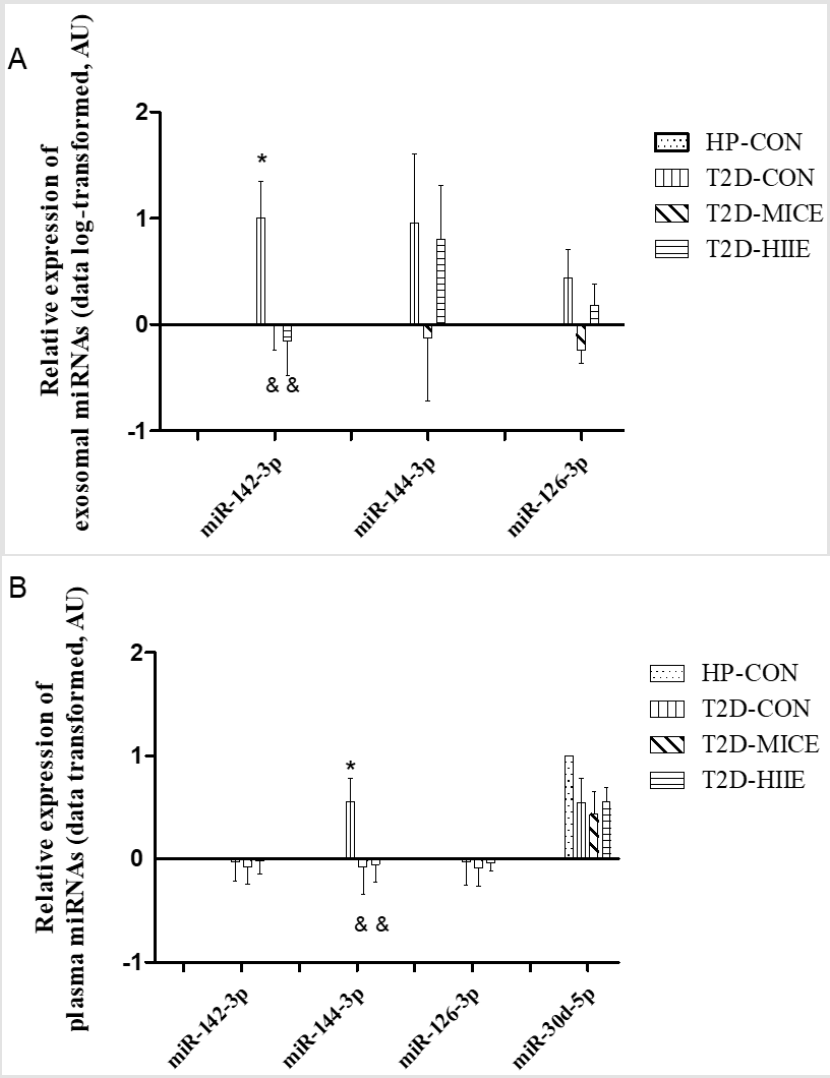

Figure 3: Validation of 4 selected miRNAs in another cohort. Expression levels of 4 miRNAs (miR-142-3p, miR-144-3p, miR-126-3p and miR-30d-5p) in the plasma exosomes

A. And plasma

B. Were determined by qRT-PCR. The relative gene expression of the target miRNAs was normalized to miR-26a.The data of miR-142-3p, miR-144-3p, miR-126-3p were log-transformed, miR-30d-5p was square root-transformed. *P < 0.05, **P < 0.01.

Discussion

We found that miR-142-3p was significantly upregulated in T2D patients but downregulated after one bout of HIIE/MICE, which correlated with the positive glucose response to exercises. While a larger sample size would strengthen our findings, this report suggests that exosomal miR-142-3p could contribute to the positive impacts of postprandial exercise on T2D. Exercise is considered a significant intervention for enhancing postprandial glucose levels. A single bout of moderate or high-intensity interval exercise following breakfast has the potential to mitigate postprandial hyperglycemia, aligning with our findings. However, the exact cellular and molecular mechanisms behind this exercise-induced improvement require further investigation. Studies report that acute exercise may result in the release of exosomes or small extracellular vesicles and the changes of various miRNA profiles inside the exosomes [20,21,23,30,31]. Exercise, acting as a stressor, triggers molecular responses such as gene expression and exosome release in various cell types. MiRNAs transported by exosomes are subject to modification, uncovering biological mechanisms behind exercise effects. However, the intricate regulation of exosomal miRNAs post-exercise in T2D remains incompletely elucidated. In our study, we selected four differentially expressed miRNAs

(miR-142-3p, miR-144-3p, miR-126-3p, and miR-30d-5p) based on sequencing conducted in our previous study (data not disclosed) and tested them using qRT-PCR.

MiR-142-3p and miR-144-3p showed elevated levels in T2D patients but returned to normal expression after postprandial exercise, with exosomal miR-142-3p exhibiting a positive correlation with plasma glucose levels. Previous studies have also reported increased plasma levels of miR-142-3p and miR-144-3p in T2DM patients, suggesting their potential as biomarkers for T2D [32-36]. Guay, et al [37] found that T lymphocyte-specific exosomal miR-142-3p/-5p could induce chemokine expression and apoptosis in pancreatic β cells in type 1 diabetes (T1D), and inhibiting miR-142-3p/-5p in β cells led to improved insulin levels, reduced inflammation, and prevented T1D development. Additionally, miRNA-144-3p may regulate glucose metabolism and energy homeostasis by directly targeting insulin receptor substrate 1, an important component of the insulin signaling pathway, and glucose transporter 1 [38]. Physical exercise, however, can influence the miRNA levels in T2DM patients by inducing a rapid change of the release of exosomes and alterations in circulating miRNA levels and exosomal miRNA levels [8,23,39]. In our study, exosomal miRNA-142-3p showed a decrease in response to exercise, indicating postprandial exercise-induced changes in specific miRNA-enriched exosomes. This unique exosomal miRNA response may contribute to the improvements in insulin sensitivity and glycemic control observed after postprandial exercise [39].

Interestingly, the greater reduction immediately after HIIE during the postprandial period was not consistently reflected in the change of exosomal miRNA-142-3p levels, as no significant distinction was observed between HIIE and MICE at the immediate postprandial time point. A larger sample size is needed to confirm this finding. MiR-126 is mainly expressed in endothelial cells and regulates endothelial cell function, angiogenesis, and vascular integrity [40,41]. Previous studies have suggested that plasma miR-126 hold promise as an early predictive biomarker for T2D in susceptible individuals [29]. In our study, however, no difference was found between T2D and healthy but sedentary individuals, which is consistent with previous findings [42]. We also failed to observe any difference in miR-126-3p levels between pre-exercise and post-exercise in T2D patients. Previous study suggested that 126-3p could be elevated after a 4-week regimen high intensity interval training (HIIT) intervention [43]. In more depth, studies contrasting acute and long-term HIIT failed to reveal immediate impacts on miR-126 levels, while an elevation in resting miR-126 levels was observed after regular HIIT, indicating an adaptation to regular physical activity [44]. Of note, circulating miR-126 levels seem to be readily elevated in trained elite athletes [45]. Therefore, future studies should investigate the effects of regular exercise on the exosomal miRNA profile in T2D to gain further insight into the underlying mechanisms of physical adaptation.

Regarding miR30d-5p, it may be involved in glucose metabolism, insulin signaling, blood coagulation, platelet activation, and inflammation. Dysregulation of miR-30d has been shown in circulating blood and urinary exosomes [46,47]. However, the present study failed to detect the expression of miR-30d-5p in circulating plasma exosomes. This discrepancy may be influenced by factors such as the duration of the disease, the presence of diabetic complications, or the age of the participants, and requires further investigation. Taken together, two of the four measured miRNAs exhibited alterations in plasma and exosome-enriched plasma following HIIE or MICE in T2D patients. This highlights the potential impact of exercise-induced mechanisms on miRNAs. However, further research is required to comprehensively elucidate their roles. Meanwhile, several limitations should be acknowledged. Firstly, it was an exploratory study with a relatively small cohort of T2D patients and healthy participants. However, these preliminary insights into the exercise-induced effects of exosomal miRNAs in T2D patients can guide the sample size calculation for future large-scale studies. Secondly, medication discontinuation or consistency in diabetic patients was not controlled in this study. Nevertheless, the absence of notable disparities in pre-exercise (1 hour postprandial) glucose and insulin values among exercise interventions implies the viability of comparing post-exercise blood glucose and insulin levels.

Future research will aim to control patients' medication status to minimize the potential impact of medications on exosomes. Additionally, there are some questions yet to be explored, such as, where are these exosomal miRNAs released? Are they skeletal muscle-derived, adipose tissue-derived or stem cells-derived? It is likely that the exosomes released by distinct cells may possess varying miRNA profiles. Further experimental investigations are needed to clarify the origin of these miRNAs. Furthermore, the short laboratory duration of this study limited the observations to the immediate post-exercise period. Monitoring exosomal miRNA kinetics throughout the day could result in more informative results and will be considered in our future studies. Finally, due to the scarcity of exosome samples, we were unable to compare the exosomal miRNA expression at the pre-exercise state. Further studies will investigate the therapeutic function of miR142-3p and other miRNAs to understand the physiological mechanisms underlying exercise's beneficial effects on T2D outcomes. Despite its limitations, our results reinforce the role of postprandial exercise induced exosomal miRNA in the regulation of glucose, as well as possibly provide new target for the intervention of T2D.

Conclusion

Exosomal miR-142-3p is reduced by postprandial exercises and positively associated with glucose regulation. We propose that exosomal miR-142-3p may serve as a potential contributor for exercise-induced benefits in middle-aged men with T2D.

Conflicts of Interest

The authors confirm no financial conflicts of interest related to the manuscript's content.

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Author's Contributions

Xinwen Cui and Peng Zhao significantly contributed to the study design. Xinwen Cui, Rongxin Zhu, Xingya Yang, and Yirui Wang were involved in conducting the experiments. Peng Zhao conducted data analysis and interpretation. Xinwen Cui drafted the manuscript. All authors, including Rongxin Zhu, Xingya Yang, Yirui Wang, and Yimin Zhang, critically reviewed and revised the manuscript. The final version was approved by all authors.

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Ethical Approval and Consent to Participate

This study was approved by the Ethics Committee of the China Institute of Sport Science (CISSLA-20210909). All participants provided written informed consent prior to the enrollment.

Consent for Publication

Not applicable.

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