Biochemistry and Biophysics Reports 40 (2024) 101882

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Comparative effects of metformin and varying intensities of exercise on miR-133a expression in diabetic rats: Insights from machine learning analysis

Elahe Alivaisi [a], Sabrieh Amini [a,*], Karimeh Haghani [b,**], Hori Ghaneialvar [c], Fatemeh Keshavarzi [a,***]

[a] Department of Biology, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran
[b] Department of Clinical Biochemistry, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran
[c] Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran

ARTICLE INFO

Keywords:
Diabetes
miR-133a
Metformin
HIIT
MCT
Machine learning

ABSTRACT

This study investigated the effects of metformin, high-intensity interval training (HIIT), and moderate-intensity continuous training (MCT) on miR-133a expression in a diabetic rat model. miR-133a, a microRNA associated with skeletal muscle insulin resistance, served as a key indicator of treatment efficacy. Diabetic rats exhibited elevated miR-133a levels compared to healthy controls. Both HIIT and MCT, alone and in combination with metformin, significantly reduced miR-133a expression. Importantly, the combination of HIIT and metformin demonstrated the most potent effect, reducing miR-133a levels more than other treatments. We used the CatBoost algorithm to develop a predictive model for miR-133a expression based on metabolic parameters. The model accurately predicted miR-133a levels using body weight, blood glucose, insulin levels, and cholesterol metrics. The findings suggest a potential clinical strategy combining metformin and exercise, with miR-133a potentially serving as a biomarker for personalized diabetes management.

1. Introduction

The World Health Organization (WHO) estimates that 347 million people worldwide suffer from diabetes [1], making it a global epidemic [2] that could double the number of deaths between 2005 and 2030 [3,4]. Diabetes mellitus (DM) is characterized by hyperglycemia and is a major threat to human health [5]. Long-term metabolic dysregulation in diabetes leads to microvascular, macrovascular, and neuropathic complications, highlighting the need for novel therapeutic strategies targeting glucose homeostasis [6].

Exercise is commonly used to manage blood glucose levels, delay or prevent complications, and reduce inflammation in diabetic patients [7–9]. The health benefits of exercise are influenced by the duration and intensity of the activity [10]. Regular exercise improves blood glucose levels in type 2 diabetes mellitus (T2DM) and reduces the risk of cardiovascular disease in individuals with prediabetes [11]. The Framingham study has shown that high-intensity interval training (HIIT) can enhance cardiac and metabolic health in diabetic patients [12]. Several studies suggest that combining moderate continuous training (MCT) with HIIT improves survival rates in T2DM patients [13]. Both HIIT and MCT have been found to lower blood sugar levels in diabetic patients, with HIIT showing greater effectiveness [14]. Strength training enhances insulin sensitivity primarily by increasing muscle mass, whereas aerobic exercises like HIIT and MCT improve insulin sensitivity by boosting skeletal muscle metabolic activity [15].

There is a growing interest in the molecular mechanisms underlying the effects of increased exercise and dietary changes in managing diabetes [16]. Lifestyle modifications are often combined with pharmacological interventions, such as the use of metformin, to help regulate blood glucose levels [17]. Metformin has been a widely used first-line treatment for T2DM for over six decades, although its exact molecular mechanism of action is still not fully elucidated [18]. However, studies

* Corresponding author. Department of Biology, Sanandaj Branch, Islamic Azad University, 6616935391, Sanandaj, Iran.
** Corresponding author.
*** Corresponding author. Department of Biology, Sanandaj Branch, Islamic Azad University, 6616935391, Sanandaj, Iran.
E-mail addresses: Amini.biology@gmail.com (S. Amini), haghani.bio@gmail.com (K. Haghani), Fatemeh.Keshavarzi@iau.ac.ir, F.keshavarzi@iausdj.ac.ir (F. Keshavarzi).

https://doi.org/10.1016/j.bbrep.2024.101882
Received 6 August 2024; Received in revised form 12 November 2024; Accepted 18 November 2024

have shown that this antihyperglycemic medication reduces hepatic glucose production and intestinal glucose absorption, and enhances insulin sensitivity by facilitating peripheral glucose uptake and utilization [19]. Importantly, unlike some other antidiabetic drugs, metformin does not typically induce hypoglycemia when used as a standalone therapy [20].

MicroRNAs (miRNAs or miRs) have emerged as potential novel biomarkers for T2DM due to their ability to modulate the expression of various genes involved in metabolism [21,22]. MicroRNAs are a class of small endogenous non-coding RNAs, approximately 22 nucleotides in length, that target and bind to the 3′-untranslated region (3′-UTR) of specific transcripts. This action leads to the downregulation of the expression of genes implicated in a range of cellular processes, including development, metabolism, differentiation, survival, proliferation, maturation, inflammation, angiogenesis, and immune responses [23–25]. Dysregulated expression of miRNAs can contribute to the pathogenesis of various diseases, including diabetes [23].

MiRNAs have been identified as significant players in the pathophysiology of diabetes and its associated complications, as they exert regulatory control over glucose metabolism and insulin activity in pivotal metabolic tissues such as the liver, adipose tissue, and skeletal muscle [24]. Skeletal muscle, in particular, plays a crucial role in governing whole-body energy metabolism and glycemic regulation, accounting for approximately 80 % of glucose uptake [25]. The impact of exercise on cellular homeostasis involves the modulation of miRNA expression in skeletal muscle [25]. Among these miRNAs are myomiRNAs, including miR-1, miR-133a, and miR-206, which are encoded by myosin genes and are specifically expressed in skeletal muscle during exercise and in the context of T2DM [26].

Prolonged aerobic exercise induces rapid fluctuations in plasma miR-133a, suggesting its role in exercise adaptation [27]. While the miR-133 family (miR-133a-1, miR-133a-2, and miR-133b) promotes myoblast proliferation and differentiation by suppressing serum response factor (SRF) in C2C12 cells [28], exercise upregulates SRF in human skeletal muscle [28]. Interestingly, skeletal muscle miR-133a levels decrease during exercise, returning to near-normal levels post-exercise [29].

In the context of T2DM, the expression of miR-133a is elevated in the skeletal muscles of affected individuals, suggesting a potential link to insulin resistance. Endurance exercise has been shown to reduce miR-133a levels, but research findings are somewhat contradictory. For instance, a 14-week endurance training regimen resulted in increased expression of both miR-1 and miR-133a in certain muscle types. Specifically, miR-133a gene expression decreased in finger flexor muscles but increased in soleus muscles following the same duration of endurance training [30]. Moreover, other studies have indicated that the induction of diabetes leads to decreased expression of miR-133a and miR-1, followed by an increase in their levels post swimming exercise. These observations indicate that the regulation of miRNAs is complex and may be influenced by various factors such as muscle type, exercise modality, and the metabolic state of the individual. Consequently, the impact of resistance training on miR-133a expression remains to be fully elucidated, prompting further investigation into the differential effects of various exercise types on microRNA regulation in the context of diabetes [31].

Artificial intelligence (AI) is revolutionizing healthcare, particularly in managing chronic conditions like diabetes and researching molecular markers such as microRNAs (miRNAs). AI significantly enhances prediction, diagnosis, and treatment strategies in these areas. AI's impact on diabetes management is profound, improving patient outcomes through early detection, precise monitoring, and tailored treatments. Applications range from risk prediction using large datasets (genetic, clinical, lifestyle) to predict diabetes onset and identify high-risk individuals for proactive screening; to automated insulin delivery systems using continuous glucose monitoring (CGM) data for optimized glycemic control; and to AI models predicting diabetic complications (retinopathy, nephropathy, neuropathy, cardiovascular events) enabling early diagnosis and intervention. Furthermore, AI personalizes treatment plans by considering individual factors and provides patient support tools and educational platforms for enhanced self-management [31–34].

Similarly, AI significantly advances miRNA research. AI algorithms can identify and classify miRNAs from large datasets, predict their target genes and regulated pathways, identify miRNAs as biomarkers for diagnosis, prognosis, and treatment response, and integrate miRNA data with clinical and genomic information for a holistic understanding of disease mechanisms supporting precision medicine approaches. This research highlights the synergistic effects of metformin and exercise training on miR-133a expression in diabetic rats and uses machine learning (specifically, the CatBoost algorithm) to develop a predictive model for miR-133a expression. This model identifies key metabolic predictors that could serve as biomarkers for personalized diabetes management. The combined power of AI in diabetes management and miRNA research promises significant advancements in diagnosis, treatment, and personalized medicine.

2. Materials and methods

2.1. Animals

The experiments adhered to the NIH Guide for the Care and Use of Laboratory Animals (IR.MEDILAM.REC.1399.097). Sprague-Dawley rats (240 ± 20 g) were randomly assigned to one of nine groups (n = 10 rats/group).

1. Control group (CG): Distilled water
2. Control group + MCT intervention (CMTG)
3. Control group + HIIT intervention (CHTG)
4. Diabetic control group (CDG): Distilled water
5. Diabetic group + metformin (DMG)
6. Diabetic group + MCT intervention (DMTG)
7. Diabetic group + HIIT intervention (DHTG)
8. Diabetic group + metformin + MCT intervention (DMMTG)
9. Diabetic group + metformin + HIIT intervention (DMHTG)

Rats were housed under standard laboratory conditions (21 ± 2 °C, 60 ± 5 % humidity, 12-h light/dark cycle) with ad libitum access to standard rodent chow and water. An 8-day treadmill acclimation period preceded the main exercise protocols.

2.2. Induction of diabetes

Diabetes was induced in overnight-fasted rats via intraperitoneal injection of streptozotocin (STZ, 60 mg/kg in normal saline), followed 15 min later by nicotinamide (200 mg/kg in normal saline) [16]. Blood glucose levels were measured seven days later; animals with plasma glucose >300 mg/dL were considered diabetic [32].

2.3. Metformin administration

Rats in groups DMG, DMMTG, and DMHTG received metformin (200 mg/kg BW) by oral gavage daily for 8 weeks [33]. All other groups received distilled water. It is important to note that this study investigated the effects of metformin within the context of pre-existing diabetes. A separate control group of non-diabetic rats receiving metformin was not included.

2.4. Training protocol

The MCT regimen involved a 6-min warm-up at 40–50 % of VO2max (maximal oxygen consumption) at the start of the running protocol, followed by 40–60 min of exercise at 65%–75 % of VO2max, and concluded with a 6-min cool-down at 40–50 % of VO2max. The HIIT

protocol consisted of a 6-min warm-up phase at 50–60 % of VO2max at the beginning of the running protocol, followed by 3 intervals of 4 min each at an intensity of 90–100 % of VO2max. This was then succeeded by a 6-min cool-down phase at 50–60 % of VO2max. **This was performed three days a week for 15 min per session, for a total of 14 weeks.**

2.5. Measurement of biochemical factors

The rats' body weights were measured 24 h post their final training session. The animals were anesthetized with ketamine (30–50 mg/kg, i.p) and xylazine (3–5 mg/kg, i.p). Blood samples were collected from their hearts for the analysis of glucose, triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and insulin levels. The serum samples were divided into aliquots and stored at −20 °C.

Anticoagulant EDTA tubes were used to transfer the blood specimens for measuring HbA1c. Insulin concentrations in the serum were determined through the enzyme-linked immunosorbent assay (ELISA) method using the ELISA plate reader ELx800TM from BioTek, Winooski, VT, USA, following the manufacturer's guidelines (DEVELOP, Canada).

Winooski, VT, USA, following the manufacturer's guidelines (DEVELOP, Canada).

Fasting blood glucose (FBG), TG, TC, HDL, and LDL serum concentrations were enzymatically assessed using commercial kits from Pars Azemoon, Tehran, Iran, and analyzed with an autoanalyzer from Hitachi, Tokyo, Japan, according to the manufacturer's instructions.

2.6. Skeletal muscle removal

The skeletal muscles of the thigh were carefully excised post blood sample collection to ensure minimal degradation. Following the removal, the tissues were promptly placed in a cryogenic container and immediately stored at −80 °C to preserve RNA integrity for subsequent extraction.

2.7. Total RNA extraction and real-time PCR

Total RNA (mRNA and microRNA) was extracted from skeletal muscles using TRIzol reagent from Biotechnology-Iran following the manufacturer's instructions. The concentration, purity, and quality of the total RNA were assessed using a NanoDrop 1000 spectrophotometer from Thermo Fisher Scientific, and sample integrity was confirmed through gel electrophoresis.

MicroRNA was transcribed into cDNA and quantified with a QuantiMir RT kit from Bonyakhteh as per the manufacturer's guidelines. Real-time PCR, employing SYBR Green reagent from Biotechnology-Iran, was utilized to measure the gene expression levels of miR-133a, with Snord-47 serving as an internal normalization control for RNA. Duplicate runs of all samples were conducted simultaneously, including negative controls without cDNA. The 2-ΔΔct method was applied to determine the relative quantitative levels of miR-133a.

2.8. Statistical analysis

Data analysis was performed using SPSS software (version 16.0). Normality of all parameters was assessed with a one-sample Kolmogorov-Smirnov test. Differences within each group were analyzed with paired independent Student t-tests. Post-hoc least significant difference tests and one-way analysis of variance (ANOVA) were employed to compare differences between groups. Statistical significance was set at $p < 0.05$.

2.9. Machine learning model development and evaluation

A dataset comprising the measured metabolic parameters (body weight, blood glucose, insulin levels, HOMA-IR, HbA1c, total cholesterol, HDL, LDL, and triglycerides) and the corresponding miR-133a expression levels was used to train and evaluate ML models. We compared the performance of multiple ML models, including Linear Regression, Ridge Regression, Lasso Regression, Random Forest, Gradient Boosting, Support Vector Regression, K-Nearest Neighbors, Decision Tree, XGBoost, CatBoost, and LightGBM. Model performance was assessed based on Mean Squared Error (MSE) and R-squared (R^2) metrics.

2.9.1. Model evaluation metrics

To assess the performance of the models, we utilized the following metrics.

- Mean Squared Error (MSE): This metric measures the average of the squared differences between actual and predicted values. The formula for calculating MSE is:

$$\mathrm{MSE} = \frac{1}{n}\sum_{i=1}^{n}(Y_i - \widehat{Y}_i)^2 \tag{1}$$

where yi*yi* represents the actual values, ŷiŷi the predicted values, and n*n* the number of data points. Lower MSE values signify better model performance, indicating smaller discrepancies between actual and predicted values.

- R-squared (R^2): This metric indicates how well the model's predictions match the actual data. An R^2 value closer to 1 suggests a better fit. The R^2 is calculated using the formula:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(Y_i - \widehat{Y}_i)^2}{\sum_{i=1}^{n}(Y_i - \overline{Y})^2} \tag{2}$$

where $\bar{y}\bar{y}$ is the mean of the actual values, and it reflects how closely the predicted values approximate the actual data.

2.9.2. Model performance summary

The performance of each model on the test dataset is summarized in Table 1. To provide a visual representation of the model performance, a 3D scatter plot is presented in Fig. 1.

2.9.3. Analysis and best model selection

Based on the results outlined in Table 1, the CatBoost model emerges as the best-performing model with the lowest MSE (0.0003) and the highest R-squared value (0.8894). This indicates that CatBoost provides the closest predictions to the actual values and captures the variance in the data effectively.

Other models, such as XGBoost, also demonstrate strong performance with positive R^2 values, suggesting a good fit. Conversely, traditional models like Linear Regression and Ridge Regression exhibit poorer performance, as indicated by their negative R^2 values, implying

Table 1
Performance of each model.

Model	MSE	R-squared
Linear Regression	**0.0338**	**−12.5251**
Ridge Regression	**0.0241**	**−8.6554**
Lasso Regression	**0.0166**	**−5.6294**
Random Forest	**0.0050**	**−1.0114**
Support Vector Regression	**0.0234**	**−8.3768**
K-Nearest Neighbors	**0.0170**	**−5.8000**
Decision Tree	**0.0050**	**−1.0000**
XGBoost	**0.0022**	**0.1017**
CatBoost	**0.0003**	**0.8894**
LightGBM	**0.0515**	**−19.6122**

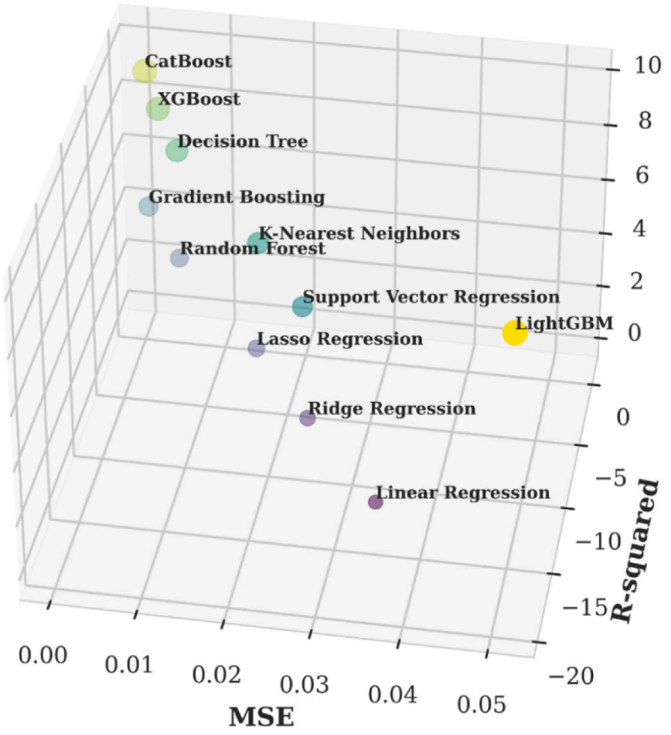

Fig. 1. 3D Scatter Plot of Model Performance. The plot visualizes the Mean Squared Error (MSE) and R-squared values for each model, with the z-axis representing the model indices.

that they are less suitable for this particular dataset.

3. Results

The biochemical characteristics of rats in the nine experimental groups (Table 2) revealed that initial body weights did not differ among the groups. As a result, a significant decrease in body weight was observed in the CDG group compared to all other diabetic treatment groups, with the metformin-treated group (DMG) showing the most significant weight improvement.

Blood glucose levels were notably lower in the DMHTG group compared to CDG, while insulin levels were significantly reduced in both DMHTG and DMMTG compared to CDG. The Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) index showed no significant differences among diabetic treated groups but significantly differed between these groups and CDG. HbA1c levels were significantly lower in DM and DM training groups than CDG. The body weight of rats in the healthy control group (CG) was 250 g, while the body weight of rats in the diabetic control group (DCG) decreased to 200 g. The body weight of rats in the treated diabetic groups that consumed metformin alone (DMG) and in combination with both type of exercise (DMMTG and DMHTG) increased compared to the diabetic control group (DCG).

Cholesterol levels were lower in all treated groups, particularly DMHTG, than in CDG. HDL levels increased in CDG but decreased in all diabetic treated groups. LDL levels were consistently low in diabetic treated groups. TG levels were significantly lower in DMHTG and DMMTG compared to CDG.

3.1. The expression levels of miR-133a in skeletal muscles

In skeletal muscles, the expression levels of miR-133a showed a significant increase in CDG compared to CG rats ($p < 0.001$) (Fig. 2). Treated diabetic rats exhibited decreased expression levels of miR-133a, with the lowest levels seen in DMMTG and DMHTG. While there were non-significant decreases in miR-133a expression in DMHTG and DMMTG groups, DMG showed lower expression of miR-133a compared to DMTG and DHTG ($p < 0.001$). Additionally, there was a non-significant decline in miR-133a expression in DMMTG compared to DMG ($p > 0.001$). The expression level of miR-133a in DMTG was higher than in DHTG, although this difference was not significant ($p > 0.001$).

3.2. Feature importance analysis

Understanding the importance of each feature in the predictive model is crucial for interpreting the model's behavior and making informed decisions. The CatBoost algorithm provides a mechanism to evaluate the importance of each feature used in the model. Fig. 3 illustrates the feature importance based on the tuned CatBoost model.

3.2.1. Key insights

1. Body Weight (g):This feature emerged as the most significant predictor of miR-133a expression, with an importance score of 27.273. This highlights the strong relationship between body weight and miR-133a expression levels.
2. Blood Glucose (mg/dL): The second most important feature, with an importance score of 14.008. This indicates that blood glucose levels play a substantial role in predicting miR-133a expression.
3. Insulin (μIU/mL): This feature also showed high importance (11.684), suggesting that insulin levels are a key factor in the model's predictions.

Table 2
Clinical and biochemical variables in diabetic and healthy rats.

Variables Groups	Weight (g)	Glucose (mg/dl)	Insulin (μIU/mL)	Insulin resistance	HbA1C (mmol/l)	LDL (mg/dl)	HDL (mg/dl)	TC (mg/dl)	TG (mg/dl)
CG	250 ± 9	105.2 ± 20	3.6 ± 0.1	0.93 ± 0.10	4.1 ± 0.06	26.2 ± 0.65	27.0 ± 0.12	60.4 ± 2	106.01 ± 9
CMTG	240 ± 5	95.01 ± 15	3.12 ± 0.06	0.73 ± 0.09	4.0 ± 0.08	24.9 ± 0.3	25.5 ± 0.13	58.01 ± 9	95.02 ± 10
CHTG	231 ± 6	95.0 ± 10	3 ± 0.08	0.70 ± 0.06	3.8 ± 0.05	23.5 ± 0.48	22.0 ± 0.26	56.21 ± 4	89.1 ± 4
CDG	200 ± 4#	400.01 ± 22	5.58 ± 0.1	5.51 ± 0.02	7.0 ± 0.15	34.1 ± 0.3	36.7 ± 0.34#	82.1 ± 8	166.3 ± 5
DMG	230 ± 6#*	152.05 ± 9	4.02 ± 0.22	1.50 ± 0.06*	5.7 ± 0.06*	28.4 ± 0.24	29.5 ± 0.14	70.2 ± 4	114.4 ± 10*
DMTG	215 ± 7	175.1 ± 10	4.56 ± 0.19	1.97 ± 0.03*	6.4 ± 0.05	33.2 ± 0.2	29.5 ± 0.1	65.7 ± 10	141.2 ± 7
DHTG	212 ± 3	170.5 ± 17	4.38 ± 0.16	1.84 ± 0.01*	6.1 ± 0.1	31.5 ± 0.29	27.0 ± 0.15*	62.3 ± 10*	138.1 ± 4
DMMTG	225 ± 5*	140.3 ± 9*	3.6 ± 0.07*	1.24 ± 0.08*	5.5 ± 0.08*	28.4 ± 0.1*	26.0 ± 0.2*	63.1 ± 5*	109.3 ± 10*
DMHTG	222 ± 4*	128.5 ± 7*	3.6 ± 0.1*	1.14 ± 0.10*	5.3 ± 0.09*	25.6 ± 0.2*	25.0 ± 0.1*	62.2 ± 4*	106.4 ± 3*
p-value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

CG: Control group, CDG: diabetic control group, CMTG: control performing MCT group, CHTG: control performing HIIT group, DMG: diabetic receive metformin group, DMTG: diabetic performing MCT group, DHTG: diabetic performing HIIT group, DMMTG: diabetic receiving metformin and performing MCT group, DMHTG: diabetic receiving metformin and performing HIIT group. LDL: low-density lipoprotein, HDL: High-density lipoprotein, triglyceride (TG), total cholesterol (TC), Glycosylated Hemoglobin (HbA1C), Data are means ± SEM (n = 10). *Significant difference with diabetic (C) ($p<0.05$). # Significant difference with healthy group ($p<0.05$).

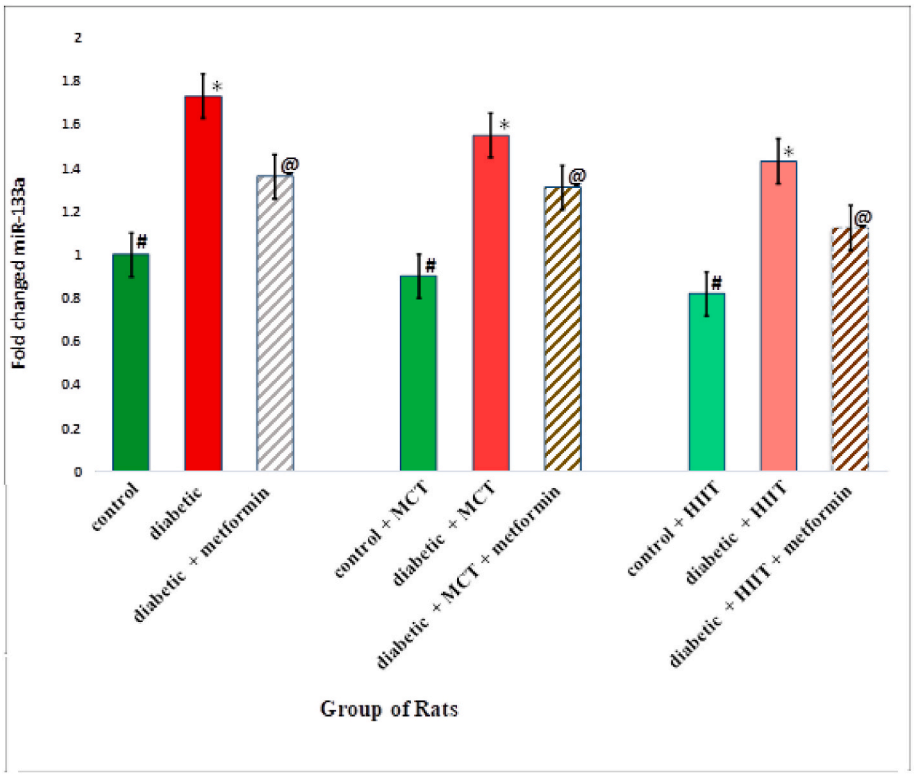

Fig. 2. Fold change in the expression levels of miR-133a in the skeletal muscles of healthy and diabetic rats (n = 10). The groups compared are indicated as follows: healthy rats (#), diabetic rats (*), and additional comparisons (@). #: Represents the expression levels in healthy rats,*: Represents the expression levels in diabetic rats, @: Indicates comparisons among different experimental groups.

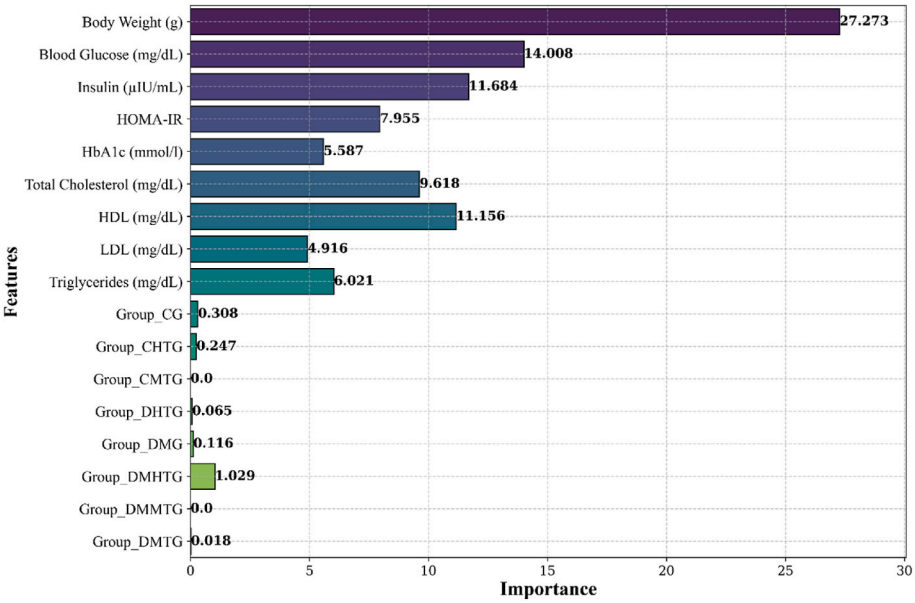

Fig. 3. Feature Importance in Predicting miR-133a Expression Levels Using CatBoost Regression Model.

4. HDL (mg/dL) and Total Cholesterol (mg/dL):** Both features had importance scores of 11.156 and 9.618, respectively, indicating their significant influence on the prediction of miR-133a expression.
5. HOMA-IR: With an importance score of 7.955, this feature was also identified as a critical predictor.
6. Triglycerides (mg/dL) and LDL (mg/dL): These features had importance scores of 6.021 and 4.916, respectively, contributing notably to the model.
7. HbA1c (mmol/l): This feature had a moderate importance score of 5.587.
8. Group Variables: Among the categorical group variables, Group_DMHTG showed the highest importance (1.029), while other group variables had lower importance scores, indicating their relatively smaller impact on the prediction.

The feature importance analysis underscores the significance of body

weight, blood glucose, insulin levels, and cholesterol-related metrics in predicting miR-133a expression. This information is valuable for understanding the underlying factors influencing miR-133a levels and for potentially guiding further biological research or clinical interventions.

3.3. Visualizing model predictions vs. actual values

To assess the performance of our CatBoost regression model, we visualized the relationship between the actual and predicted values for miR-133a expression levels. The plot in Fig. 4 provides a scatter plot of the actual versus predicted values, with additional annotations highlighting the differences between these values. We created a combined dataset that includes both the training and testing data predictions. The difference between the actual and predicted values was calculated for each data point, and these differences were annotated on the scatter plot. The scatter plot in Fig. 4 reveals the accuracy of the CatBoost regression model by illustrating how closely the predicted values align with the actual values. The red dashed line serves as a benchmark for perfect predictions. Data points that lie closer to this line indicate higher prediction accuracy. The annotations showing the differences provide insight into the magnitude of prediction errors, which helps in identifying areas where the model may require further tuning or improvement.

In Fig. 4, each data point represents the relationship between actual and predicted miR-133a expression levels across different experimental groups in the study. The x-axis displays the actual miR-133a expression levels obtained through real-time PCR measurements, while the y-axis shows the predicted values generated by the CatBoost machine learning model based on metabolic parameters. Each point on the scatter plot corresponds to an individual observation (rat), where the proximity of the point to the red dashed line indicates the accuracy of the prediction. Points closer to the line signify a minimal difference between actual and predicted values, reflecting higher model accuracy.

4. Discussion

The purpose of this research was to explore the influence of two different exercise regimens (HIIT and MCT) as well as metformin, either independently or in combination, on the expression of miR-133a in rats with diabetes and those without. Moreover, we investigated the effects of exercise training, metformin, and their combination on body weight, various biochemical parameters, and lipid profiles across all the animal groups.

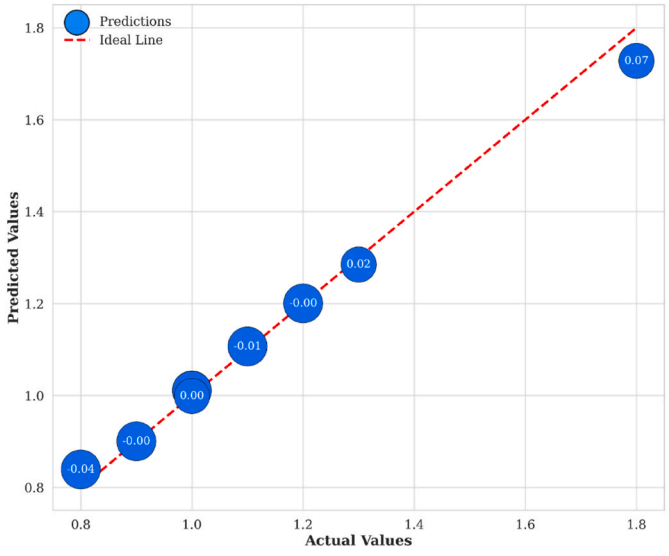

Fig. 4. Model Performance: Actual vs. Predicted values.

Our results revealed a notable increase in miR-133a expression in the skeletal muscles of the CDG group in comparison to the CG group. In summary, miR-133 is implicated in the pathophysiology of diabetes through various mechanisms. Specifically, research has demonstrated that miR-133 in human skeletal muscle is regulated by sterol regulatory element-binding protein 1c (SREBP-1) and myocyte enhancer factor 2C (MEF2C), which are associated with impaired insulin response in patients with T2D [35]. Notably, SREBP-1 is activated by insulin, which inhibits MEF2C, leading to the downregulation of miR-133 [36,37]. Additionally, studies on insulin-resistant mice revealed that miR-133 is dysregulated in skeletal muscle due to altered insulin-like growth factor 1 (IGF-1) signaling, which includes validated targets of miR-133 [38]. This microRNA has been shown to downregulate IGF-1 receptor (IGF-1R) expression, impacting the signaling pathways involved in skeletal myogenesis, hence positioning miR-133 as a potential therapeutic target for muscle disorders [37]. Importantly, low levels of IGF-1 are correlated with insulin resistance, contributing to the development of impaired fasting glucose, impaired glucose tolerance, and T2D [39,40]. Furthermore, overexpression of miR-133 in cardiomyocytes has been linked to a reduction in insulin-sensitive glucose transporter (GLUT4) expression, thereby diminishing insulin-stimulated glucose uptake through the targeting of KLF15, a key transcription factor regulating GLUT4 [41]. Additionally, several targets of miR-133, including IGF-1, SLC7A8, SLC46A1, SLC2A12, and CD47, have been predicted [39]. Collectively, these findings underscore the significance of miR-133 in glucose metabolism and insulin signaling, suggesting its potential role in the management of diabetes and related muscle disorders.

The levels of miR-133a were significantly reduced in the groups that underwent exercise training and/or received metformin, as opposed to the CDG group. Particularly, diabetic rats engaged in MCT and HIIT exercises along with metformin administration exhibited a substantial decrease in miR-133a expression when compared to the other groups receiving diabetic treatments. Additionally, Drigny et al. have substantiated that both long-term HIIT and MCT training can bring about similar effects on ventricular repolarization indices, with HIIT showing a more pronounced effect in enhancing certain cardio-metabolic risk factors [40].

Adaptive responses to HIIT compared to traditional exercise require less time commitment [42]. The efficacy of HIIT over MCT in T2DM patients may stem from its rapid metabolic impact due to maximal energy utilization [43]. While MCT proves as effective as HIIT over an extended period for enhancing glucose metabolism, recent research indicates that the myomiRs miR-1, miR-133a/b, and miR-206 exhibit variations in response to endurance exercise and training [44]. Studies reveal an initial rise in miR-1 and miR-133a levels pre-training, which return to baseline post-training cessation. However, contrary to some findings, miR-133a expression in T2DM patients' skeletal muscles is reportedly reduced. Endurance exercise has been linked to increased miR-133a expression, while endurance training may downregulate miR-192 expression. Notably, resistance and endurance training in muscle tissue did not significantly impact miR-133a levels in some investigations. Circulating miRNAs remained stable after a combined exercise regimen in healthy men, whereas swimming training notably increased miR-133a and miR-21 gene expression [45].

The regulation of miR-133a expression in skeletal muscles through exercise training is intricately linked to the intensity, duration, and type of exercise [44]. While the role of specific miRNAs in metabolic regulation is known, only a limited number have been studied in diabetic patients' skeletal muscles. Limited research exists on the effects of HIIT and MCT, alone or combined with metformin, on miR-133a expression in diabetic rats' skeletal muscles. The observed reduction in miR-133a expression in diabetic rat muscles with metformin treatment suggests its potential in regulating glucose levels.

The current research suggests that a combination of metformin and different types of exercise training can lead to decreased insulin and

glucose levels in diabetic rats [46]. Various studies offer both supporting and contradictory evidence to this conclusion. Reductions in insulin and serum glucose levels following exercise may be attributed to factors such as increased glucose transporter proteins (GLUT4), reduced free fatty acid secretion, enhanced muscle glucose uptake, and improved glucose availability for muscles [47]. In the diabetic group receiving metformin, blood glucose levels decreased compared to the control group. Metformin is known to lower blood glucose levels by inhibiting hepatic glucose production and enhancing insulin sensitivity [48]. A decrease in insulin and glucose levels typically signifies improved insulin sensitivity. In this study, the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) values in the treated diabetic group decreased compared to the control group, indicating lower insulin resistance and higher insulin sensitivity [49].

HbA1C levels in diabetic rats and those undergoing MCT and HIIT training decreased compared to the control group [50]. While some studies have reported various effects or no changes in HbA1C levels post-exercise, our findings align with significant reductions [51]. Metformin-treated diabetic rats showed unexpected decreases in average weight and blood glucose levels, possibly attributed to enhanced fat burning and reduced fat mass in the HIIT group compared to MCT. Notably, HDL, LDL, total cholesterol (TC), and triglyceride (TG) levels were lower in treated diabetic rats compared to the control group. These changes in lipid profile could be linked to increased lipoprotein lipase activity, which promotes fat metabolism, and reduced triglyceride lipase activity [52].

HDL levels increased unexpectedly in the control diabetic group compared to the healthy control group, likely due to its role in removing and transferring excess cholesterol in the diabetic group. Cholesterol levels in the diabetic group also significantly increased, possibly due to HDL's removal action, leading to higher HDL levels. These findings are in line with a study by Eatemady-Boroujeni et al. creating a consistent pattern of results [53]. The synergistic effect observed with HIIT combined with metformin highlights the potential for multimodal therapeutic strategies in diabetes management. This effect might be attributed to the combined actions of HIIT, which increases glucose uptake and utilization in skeletal muscle, and metformin, which enhances insulin sensitivity and reduces hepatic glucose production. The decreased miR-133a levels, in turn, may contribute to the improved metabolic response.

Nie Y.(2022) reported that six weeks of endurance exercise training increased the transcriptional level of miR-133a and stimulated mitochondrial biogenesis in wild-type mice, but failed to improve mitochondrial function in miR-133a–deficient mice. He mentioned an increase in the potential target of miR-133a, the IGF-1 receptor, along with hyperactivation of Akt signaling in miR-133a-deficient mice, which was consistent with lower transcription of mitochondrial biogenesis regulators. These findings indicate an essential role of miR-133a in skeletal muscle mitochondrial biogenesis, exercise tolerance, and response to exercise training [54].

In this study, the expression level of mir-133a decreased with exercise but increased in diabetic control rats. It was determined that the effect of HIIT exercise was greater than MCT in reducing miR-133a expression, although this difference was not significant. These discrepancies may be due to variation in the severity, duration, age and gender of the research samples. The results indicated that skeletal muscle miR133a is upregulated in diabetic control rats, and through exercise, its expression is downregulated. Therefore, we hypothesize that mir-133a may serve as a biomarker for T2DM. Additionally, microRNAs could be used for early diagnosis, and monitoring the progression and severity of T2DM, given their pancreatic specific expression and stability in various body fluids and muscles (57).

4.1. Implications for biological research and clinical interventions

The identification of key predictors such as body weight, blood glucose, insulin levels, and cholesterol metrics suggests important considerations for biological research and clinical practice, warranting further investigation.

4.1.1. Biological research

Mechanistic Studies: The observed association between body weight and miR-133a expression indicates a potential link between adiposity and miRNA regulation. Further research is needed to confirm these findings explore the molecular mechanisms that may underlie these associations.

Metabolic Pathways: By examining the relationships between blood glucose, insulin levels, and miR-133a expression, future studies could investigate how these metabolic pathways interact. This could enhance our understanding of metabolic regulation linked to miRNA profiles.

Lipid Metabolism: The connection between HDL, total cholesterol, and triglycerides in miR-133a expression suggests that lipid metabolism may influence this regulation. Further research could investigate how change in lipid homeostasis impact miRNA expression, potentially identifying areas for therapeutic exploration.

4.1.2. Clinical interventions

Biomarker Development: Changes in miR-133a levels may be investigated as indicators in patients with abnormal body weight or blood glucose levels. More research is essential.

Personalized Medicine: The insights from studies like this one could inform more individualized treatment strategies based on a patient's metabolic profile. This might help prioritize interventions in at-risk populations based on miR-133a expression patterns.

Therapeutic Targeting: The new findings from future studies, may provide initial ideas for therapeutic avenues aimed at modulating miR-133a expression.

4.1.3. Future directions

Further validation in larger and more diverse cohorts is essential to confirm our findings. Also, Future research should include longitudinal studies to track changes in miR-133a expression over time in relation to the identified key predictors. This would help establish causal relationships and temporal dynamics. Integrating miRNA data with other omics datasets (e.g., genomics, proteomics, metabolomics) could provide a more comprehensive understanding of the regulatory networks influencing miR-133a

expression.Additionally, experimental studies exploring the effects of specific interventions (e.g., dietary changes, pharmacological treatments) on miR-133a expression and its key predictors could provide actionable insights for clinical practice.

Elevated miR-133a expression is observed in the skeletal muscle of diabetic rats. Combining exercise training (especially HIIT) with metformin shows promising synergistic effects in reducing miR-133a levels and managing diabetes. Both MCT and HIIT training, even without metformin, are effective in lowering miR-133a levels in diabetic rats, indicating that intensive exercise alone can positively impact miR-133a levels and regulate biochemical factors in diabetic animals.

5. Conclusion

This study indicated the notable potential of combined interventions, including exercise (HIIT and MCT) a long with metformin, in reducing miR-133a expression. The synergistic effect of these interventions is especially, noticeable when HIIT is combined with metformin. Additionally, the use of machine learning techniques, specifically CatBoost, in predicting miR-133a expression, yielded promising results. Therefore, it seems that miR-133a may be useable as a biomarker for monitoring intervention effectiveness and predicting disease progression. Further research should focus on exploring the underlying mechanisms of these synergistic effects and translating these findings into actionable clinical

interventions.

CRediT authorship contribution statement

Elahe Alivaisi: Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sabrieh Amini:** Visualization, Validation, Supervision, Project administration, Investigation, Data curation, Conceptualization. **Karimeh Haghani:** Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hori Ghaneialvar:** Visualization, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Fatemeh Keshavarzi:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Investigation, Data curation, Conceptualization.

Ethics approval

This research was ethically approved by the Research & Ethics Committee of the Ilam university of Medical Science, Iran(IR.MEDILAM.REC.1399.097). Also, all protocols involving human subjects comply with the requirements of the Declaration of Helsinki of the Iranian Ministry of Health and Medical Education(33).

Funding/support

None.

All data generated or analyzed during this study are included in this published article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

None.

Data availability

The data that has been used is confidential.

References

[1] J.B. Buse, D.J. Wexler, A. Tsapas, P. Rossing, G. Mingrone, C. Mathieu, D. A. Dalessio, M.J. Davies, 2019 update to: management of hyperglycemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD), Diabetes Care 43 (2020) 487–493.

[2] S.-X. Liu, F. Zheng, K.-L. Xie, M.-R. Xie, L.-J. Jiang, Y. Cai, Exercise reduces insulin resistance in T2DM via mediating the lncRNA MALAT1/microRNA-382-3p/resistin axis, Mol. Ther. Nucleic Acids 18 (2019) 34–44.

[3] C.-Y. Wang, D.L. Neil, P. Home, 2020 vision–An overview of prospects for diabetes management and prevention in the next decade, Diabetes Res. Clin. Pract. 143 (2018) 101–112.

[4] H. Chen, H.-Y. Lan, D.H. Roukos, W.C. Cho, Others Application of microRNAs in diabetes mellitus, J. Endocrinol. 222 (2014) R1–R10.

[5] A.B. Olokoba, O.A. Obateru, L.B. Olokoba, T2DM: a review of current trends, Oman Med. J. 27 (2012) 269.

[6] S. Vasu, K. Kumano, C.M. Darden, I. Rahman, M.C. Lawrence, B. Naziruddin, MicroRNA signatures as future biomarkers for diagnosis of diabetes states, Cells 8 (2019) 1533.

[7] S.H. Seyedizadeh, S. Cheragh-Birjandi, M.R. Hamedi Nia, The effects of combined exercise training (resistance-aerobic) on serum kinesin and physical function in type 2 diabetes patients with diabetic peripheral neuropathy (randomized controlled trials), J. Diabetes Res. 2020 (2020) 6978128.

[8] F. Magkos, M.F. Hjorth, A. Astrup, Diet and exercise in the prevention and treatment of T2DM, Nat. Rev. Endocrinol. 16 (2020) 545–555.

[9] S. Amanat, S. Ghahri, A. Dianatinasab, M. Fararouei, M. Dianatinasab, Exercise and type 2 diabetes, Physical Exercise for Human Health (2020) 91–105.

[10] B. Schwaab, M. Windmöller, I.R. König, M. Schütt, Evaluation of aerobic exercise intensity in patients with coronary artery disease and T2DM, J. Clin. Med. 9 (2020) 2773.

[11] D.Y. Seo, J.R. Ko, J.E. Jang, T.N. Kim, J.B. Youm, H.-B. Kwak, J.H. Bae, A.H. Kim, K.S. Ko, B.D. Rhee, And others Exercise as a potential therapeutic target for diabetic cardiomyopathy: insight into the underlying mechanisms, Int. J. Mol. Sci. 20 (2019) 6284.

[12] J.K.S. Lew, J.T. Pearson, D.O. Schwenke, R. Katare, Exercise mediated protection of diabetic heart through modulation of microRNA mediated molecular pathways, Cardiovasc. Diabetol. 16 (2017) 1–20.

[13] D. E. da Silva, A.J. Grande, L. Roever, G. Tse, T. Liu, G. Biondi-Zoccai, J.M. Farias, de High-intensity interval training in patients with T2DM: a systematic review, Curr. Atherosclerosis Rep. 21 (2019) 1–10.

[14] R. Mendes, N. Sousa, J.L. Themudo-Barata, V.M. Reis, High-intensity interval training versus moderate-intensity continuous training in middle-aged and older patients with type 2 diabetes: a randomized controlled crossover trial of the acute effects of treadmill walking on glycemic control, Int. J. Environ. Res. Publ. Health 16 (2019) 4163.

[15] S.P. Tokmakidis, C.E. Zois, K.A. Volaklis, K. Kotsa, A.-M. Touvra, The effects of a combined strength and aerobic exercise program on glucose control and insulin action in women with type 2 diabetes, Eur. J. Appl. Physiol. 92 (2004) 437–442.

[16] H. Oghbaei, N.A. Asl, F. Sheikhzadeh, M.R. Alipour, And others the effect of regular moderate exercise on miRNA-192 expression changes in kidney of streptozotocin-induced diabetic male rats, Adv. Pharmaceut. Bull. 5 (2015) 127.

[17] Z. Lv, Y. Guo, Metformin and its benefits for various diseases, Front. Endocrinol. 11 (2020) 490991.

[18] J. Zhou, S. Massey, D. Story, L. Li, Metformin: an old drug with new applications, Int. J. Mol. Sci. 19 (2018) 2863.

[19] M. Li, X. Li, H. Zhang, Y. Lu, Molecular mechanisms of metformin for diabetes and cancer treatment, Front. Physiol. 9 (2018) 354854.

[20] F. Paneni, T.F. Lüscher, Cardiovascular protection in the treatment of type 2 diabetes: a review of clinical trial results across drug classes, Am. J. Cardiol. 120 (2017) S17–S27.

[21] J. Pordzik, D. Jakubik, J. Jarosz-Popek, Z. Wicik, C. Eyileten, S. De Rosa, C. Indolfi, J.M. Siller-Matula, P. Czajka, M. Postula, Significance of circulating microRNAs in diabetes mellitus type 2 and platelet reactivity: bioinformatic analysis and review, Cardiovasc. Diabetol. 18 (2019) 1–19.

[22] F. Barutta, S. Bellini, R. Mastrocola, G. Bruno, G. Gruden, MicroRNA and microvascular complications of diabetes, International journal of endocrinology 2018 (2018) 6890501.

[23] J.V. Esteves, F.J. Enguita, U.F. Machado, MicroRNAs-mediated regulation of skeletal muscle GLUT4 expression and translocation in insulin resistance, J. Diabetes Res. 2017 (2017) 7267910.

[24] L. Nigi, G.E. Grieco, G. Ventriglia, N. Brusco, F. Mancarella, C. Formichi, F. Dotta, G. Sebastiani, MicroRNAs as regulators of insulin signaling: research updates and potential therapeutic perspectives in type 2 diabetes, Int. J. Mol. Sci. 19 (2018) 3705.

[25] D. Dahlmans, A. Houzelle, J.A. Jörgensen, E. Phielix, L. Lindeboom, M.K. C. Hesselink, P. Schrauwen, J. Hoeks, Evaluation of muscle microRNA expression in relation to human peripheral insulin sensitivity: a cross-sectional study in metabolically distinct subject groups, Front. Physiol. 8 (2017) 711.

[26] C.P.C. Gomes, G.P. Oliveira-Jr, B. Madrid, J.A. Almeida, O.L. Franco, R.W. Pereira, Circulating miR-1, miR-133a, and miR-206 levels are increased after a half-marathon run, Biomarkers 19 (2014) 585–589.

[27] F. Pedregosa, G. Varoquaux, A. Gramfort, V. Michel, B. Thirion, O. Grisel, M. Blondel, P. Prettenhofer, R. Weiss, V. Dubourg, And others Scikit-learn: machine learning in Python, J. Mach. Learn. Res. 12 (2011) 2825–2830.

[28] G.-Q. Chen, W.-J. Lian, G.-M. Wang, S. Wang, Y.-Q. Yang, Z.-W. Zhao, Altered microRNA expression in skeletal muscle results from high-fat diet-induced insulin resistance in mice, Mol. Med. Rep. 5 (2012) 1362–1368.

[29] A.P. Russell, B. Lèger, Age-related Changes in the Molecular Regulation of Skeletal Muscle Mass. *Sarcopenia–Age-Related Muscle Wasting and Weakness: Mechanisms and Treatments*, 2011, pp. 207–221.

[30] P.K. Davidsen, I.J. Gallagher, J.W. Hartman, M.A. Tarnopolsky, F. Dela, J. W. Helge, J.A. Timmons, S.M. Phillips, High responders to resistance exercise training demonstrate differential regulation of skeletal muscle microRNA expression, J. Appl. Physiol. (2011).

[31] M.F. Najafabadi, S. Keshavarz, S.A. Hosseini, E. Eftekhari, Interactive effects of swimming training and cinnamon supplementation on changing cardiac miR-133a and miR-21 in the streptozotocin-induced diabetic rats, Journal of Nutrition, Fasting \& Health 11 (2023).

[32] H. Dinari Ghozhdi, A. Heidarianpour, M. Keshvari, H. Tavassoli, Exercise training and de-training effects on serum leptin and TNF-α in high fat induced diabetic rats, Diabetology \& Metabolic Syndrome 13 (2021) 57.

[33] M. Al Zaabi, B.H. Ali, Y. Al Suleimani, S.A. Adham, H. Ali, P. Manoj, M. Ashique, A. Nemmar, The effect of metformin in diabetic and non-diabetic rats with experimentally-induced chronic kidney disease, Biomolecules 11 (2021) 814.

[34] L. Prokhorenkova, G. Gusev, A. Vorobev, A.V. Dorogush, A. Gulin, CatBoost: unbiased boosting with categorical features, Advances in neural information processing systems (2018) 31.

[35] A. Granjon, M.-P. Gustin, J. Rieusset, E. Lefai, E. Meugnier, I. Güller, C. Cerutti, C. Paultre, E. Disse, R. Rabasa-Lhoret, And others the microRNA signature in response to insulin reveals its implication in the transcriptional action of insulin in human skeletal muscle and the role of a sterol regulatory element–binding protein-1c/myocyte enhancer factor 2C pathway, Diabetes 58 (2009) 2555–2564.

[36] L. Elia, R. Contu, M. Quintavalle, F. Varrone, C. Chimenti, M.A. Russo, V. Cimino, L. De Marinis, A. Frustaci, D. Catalucci, And others Reciprocal regulation of microRNA-1 and IGF-1 signal transduction cascade in cardiac and skeletal muscle in physiological and pathological conditions, Circulation 120 (2009) 2377.
[37] M.-B. Huang, H. Xu, S.-J. Xie, H. Zhou, L.-H. Qu, Insulin-like growth factor-1 receptor is regulated by microRNA-133 during skeletal myogenesis, PloS one 6 (2011) e29173.
[38] F. de T. Frias, M. De Mendonca, A.R. Martins, A.F. Gindro, B. Cogliati, R. Curi, A. C. Rodrigues, MyomiRs as markers of insulin resistance and decreased myogenesis in skeletal muscle of diet-induced obese mice, Frontiers in endocrinology 7 (2016) 76.
[39] G. Al-Kafaji, H.A. Al-Muhtaresh, A.H. Salem, Expression and clinical significance of miR-1 and miR-133 in pre-diabetes, Biomedical reports 14 (2021) 33.
[40] M. Roozbayani, M. Peeri, H. Agha-Alinejad, M.A. Azarbayjani, Type of aerobic training effect on cardiac muscles MIR29A and collagen I gene expression in diabetic male rats, Iranian Journal of Diabetes and Obesity 8 (2016) 183–190.
[41] T. Horie, K. Ono, H. Nishi, Y. Iwanaga, K. Nagao, M. Kinoshita, Y. Kuwabara, R. Takanabe, K. Hasegawa, T. Kita, And others MicroRNA-133 regulates the expression of GLUT4 by targeting KLF15 and is involved in metabolic control in cardiac myocytes, Biochemical and biophysical research communications 389 (2009) 315–320.
[42] J.P. Little, J.B. Gillen, M.E. Percival, A. Safdar, M.A. Tarnopolsky, Z. Punthakee, M. E. Jung, M.J. Gibala, Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes, Journal of applied physiology 111 (2011) 1554–1560.
[43] J. Liu, L. Zhu, P. Li, N. Li, Y. Xu, Effectiveness of high-intensity interval training on glycemic control and cardiorespiratory fitness in patients with type 2 diabetes: a systematic review and meta-analysis, Aging clinical and experimental research 31 (2019) 575–593.
[44] H. Oghbaei, N.A. Asl, F. Sheikhzadeh, Can regular moderate exercise lead to changes in miRNA-146a and its adapter proteins in the kidney of streptozotocin-induced diabetic male rats? Endocrine Regulations 51 (2017) 145–152.
[45] C.A. Meza, M. Amador, A.J. McAinch, K. Begum, S. Roy, S. Bajpeyi, Eight weeks of combined exercise training do not alter circulating microRNAs-29a,-133a,-133b, and-155 in young, healthy men, European Journal of Applied Physiology 122 (2022) 921–933.
[46] A.P. Russell, S. Lamon, H. Boon, S. Wada, I. Güller, E.L. Brown, A.V. Chibalin, J. R. Zierath, R.J. Snow, N. Stepto, Others Regulation of miRNAs in human skeletal muscle following acute endurance exercise and short-term endurance training, The Journal of physiology 591 (2013) 4637–4653.
[47] P.L. Evans, S.L. McMillin, L.A. Weyrauch, C.A. Witczak, Regulation of skeletal muscle glucose transport and glucose metabolism by exercise training, Nutrients 11 (2019) 2432.
[48] \Ibrahim Halil Demirsoy, D.Y. Ertural, \cSenay Balci, Ü. Ç\ink\ir, K. Sezer, L. Tamer, N. Aras, Profiles of circulating miRNAs following metformin treatment in patients with type 2 diabetes, Journal of medical biochemistry 37 (2018) 499.
[49] A. Ngayimbesha, J.B. Bizimana, M.S. Gakima, Effect of eight weeks of exercise training on lipid profile and insulin sensitivity in obese person, Int J Sports Exerc Med 5 (2019) 119–125.
[50] A. Maiorana, G. O'Driscoll, C. Goodman, R. Taylor, D. Green, Combined aerobic and resistance exercise improves glycemic control and fitness in type 2 diabetes, Diabetes research and clinical practice 56 (2002) 115–123.
[51] M.L.M.P. Jorge, V. N. de Oliveira, N.M. Resende, L.F. Paraiso, A. Calixto, A.L. D. Diniz, E.S. Resende, E.R. Ropelle, J.B. Carvalheira, F.S. Espindola, And others the effects of aerobic, resistance, and combined exercise on metabolic control, inflammatory markers, adipocytokines, and muscle insulin signaling in patients with T2DM, Metabolism 60 (2011) 1244–1252.
[52] A.J. Scheen, Pathophysiology of type 2 diabetes, Acta Clinica Belgica 58 (2003) 335–341.
[53] A. Eatemady-Boroujeni, M. Kargarfard, H. Mojtahedi, R. Rouzbehani, H. Dastbarhagh, Comparison of the effects of 8-weeks aerobic training and resistance training on lipid profile in patients with diabetes type 2, Journal of Isfahan Medical School 32 (2014) 524–533.
[54] R. Li, Y. Nie, Letter by Li and nie regarding article, "inflammatory glycoprotein 130 signaling links changes in microtubules and junctophilin-2 to altered mitochondrial metabolism and right ventricular contractility", Circ Heart Fail 15 (8) (2022 Aug) e009531.