Tracking Sirtuin 3 in Diabetic Children: A Bioinformatics and Clinical Approach to Cellular Metabolism

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Abstract

***Background*: Sirtuin 3 (SIRT3) is the main mitochondrial deacetylase that modifies the biochemical reactions of diverse proteins within mitochondria through lysine deacetylation. This enzyme is essential for regulating mitochondrial respiratory functions, redox homeostasis, reactive oxygen species detoxification, and insulin response across various organs. SIRT3 deficiency has been linked to mitochondrial dysfunction with insufficient ATP production, which both contribute to aging and metabolic disorders such as type 2 diabetes mellitus (T2DM), insulin insensitivity, and cardiovascular complications. *Aim*: The present study aimed to assess serum SIRT3 levels in children with type 1 diabetes mellitus (T1DM). *Materials and Methods:* This study involved 90 children, divided into 45 children with T1DM and 45 healthy individuals as controls. A full medical history was taken, and biochemical tests such as fasting blood glucose (FBG), HbA1c, and lipid profile were performed. Serum levels of SIRT3 were also measured in both groups using the sandwich ELISA technique. A bioinformatic analysis was performed to explore SIRT3 structure, function, and diabetes-related networks. *Results:* Serum SIRT3 levels were markedly decreased in diabetic children, with significant differences between the two groups in FBG, HbA1c, triglycerides (TAG), and very low-density lipoprotein cholesterol (VLDL-C). Bioinformatics revealed that SIRT3 and its interactome are involved in diabetes-related pathways and processes. *Conclusion*: This research suggests that SIRT3 may be related to the pathophysiology of the disease, and that identifying the complex interplay between SIRT3 and mitochondrial function in DM could aid in developing more advanced treatment strategies.**

Keywords: Mitochondrial deacetylase, Redox homeostasis, Mitochondrial dysfunction

Introduction

Diabetes mellitus is a chronic, complex metabolic condition marked by hyperglycemia resulting from abnormalities in either insulin production, insulin action, or a combination of both [(1)]. As reported by the International Diabetes Federation (IDF), about 537 million adults suffered from DM in 2021 worldwide. By 2030, this worldwide estimate is predicted to rise to 643 million, and by 2045, it would reach 783 million. Egypt is considered the 9th country with the highest burden of DM worldwide, with an estimated 8 million affected adults and a prevalence rate of 15.2% at the beginning of 2020 [(2,3)]. In the MENA region, children and adolescents with T1DM have reached about 149,400 cases, and a total of 20,800 new cases were

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recognized every year, according to the IDF report of 2019 [4].

Type 1 diabetes is classified as an autoimmune condition where the immune system recognizes pancreatic β-cells through autoreactive T lymphocytes, causing destruction of β-cells and insulin deficiency. Many genetic and environmental factors also contribute to the etiology of T1DM [5].

Chronic hyperglycemia is the hallmark of diabetes mellitus pathogenesis, as it upregulates multiple metabolic pathways, such as the polyol pathway, protein kinase C, and the glycation pathway, resulting in NADH/NAD^+ redox imbalance, overproduction of reactive oxygen molecules, and mitochondrial dysfunction [6].

Oxidative stress and inflammatory mediators, which are both induced by hyperglycemia and metabolic dysregulation, are linked to β-cell failure and apoptosis, decreasing insulin secretion from these cells [6].

One of the vital characteristics of diabetes mellitus is the molecular imbalance of NADH/NAD^+ with an excess of NADH and a lack of NAD^+ caused by deranged glucose metabolism. In diabetes mellitus, oxidative stress induces DNA damage, which activates repair mechanisms mediated by the poly (ADP-ribose) polymerase, a NAD^+-dependent enzyme, resulting in NAD^+ depletion. As a result, sirtuin activity that is NAD^+-dependent is decreased [7].

SIRT3 functions as a significant mitochondrial deacetylase that removes acetyl moieties from target proteins, requiring NAD^+ as a catalytic cofactor, thereby regulating the biochemical reactions of many proteins within the mitochondria. It is classified as a class III histone deacetylase and plays a key role in mitochondrial respiratory functions, redox homeostasis, and insulin resistance [8].

SIRT3 has been shown to have a positive impact on the muscles of the skeleton, fatty tissues, and the pancreas through lowering inflammation, regulating oxidative stress, as well as improving mitochondrial defects that occur in diabetes [9]. SIRT3 depletion exposes beta cells to oxidative stress, which interferes with their activity and accelerates type 2 diabetes development [8].

Previous studies have not clearly demonstrated the relation between SIRT3 and T1DM pathogenesis. Thus, the goal of this study was to evaluate SIRT3 levels in T1DM for better understanding the pathophysiology of the disease and further improvement of its therapeutic modalities.

Materials and Methods

A total of 90 children, aged 2 to 10 years and of both sexes, participated in this study and were divided into two groups. The diabetic group included 45 children with T1DM, diagnosed based on the criteria of the American Diabetes Association (ADA) [10], who were enrolled from the Pediatric Endocrinology Clinic at Suez Canal University Hospital. All diabetic participants had a disease duration of six months or more. Children were excluded from the diabetic group if they had a chronic illness other than type 1 diabetes or another endocrine or autoimmune disease, were receiving long-term steroid therapy, or had acute conditions such as diabetic ketoacidosis. The control group included 45 age- and gender-matched healthy children. Control participants were included if they had no history of diabetes or other chronic illness with normal fasting blood glucose and HbA1c at the time of enrollment.

A full history was taken from their parents, including full name, age, gender, address, diabetes duration, any complications, inflammatory or endocrinal diseases other than diabetes, genetic predisposition to diabetes, and treatment type. Anthropometric data regarding weight (kg) and height (m), with body mass index calculated as BMI = kg/m^2, were also measured. All study participants underwent laboratory tests, including FBG, HbA1c, lipid profile, and serum SIRT3 levels.

Blood sample collection

A five ml venous blood sample was obtained from each child within the study groups: two ml in an EDTA tube labelled with the patient ID number was assigned to measure HbA1c and FBG, and three ml of blood were collected in plain tubes labelled with the patient ID, centrifuged, and serum was separated for assessing serum levels of SIRT3 and lipid profile.

Biochemical measurements

Assessment of lipid profile parameters**:** Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), TAG [11], FBG, and HbA1c, all done using Cobas C311, Roche Diagnostics, Mannheim, Germany [12]. Low-density lipoprotein cholesterol (LDL-C) was determined by the Friedwald equation [13]: (LDL-C) = TC - HDL-C - TAG/5. Very low-density lipoprotein cholesterol was estimated also by the Friedwald equation, where VLDL-C is calculated by dividing serum TAG levels by 5 (VLDL-C = TAG/5) [14].

Assessment of serum SIRT3 level was done using the SIRT3 ELISA kit based on the sandwich technique (Develop, Cat. No. DL-SIRT3-Hu, China) following the manufacturers' instructions [15].

In Silico Bioinformatics Analysis of SIRT3

The structural and functional analysis of the SIRT3 gene and its protein product was conducted in silico using a variety of bioinformatics resources. These included the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/gene/23410), GeneCards (www.genecards.org), Ensembl (www.ensembl.org), and the UniProt Knowledgebase (https://www.uniprot.org). Also, intracellular distribution was assessed by using the Compartments database (https://compartments.jensenlab.org).

In addition, interactive transcription factors and microRNAs associated with the SIRT3 protein were analyzed by Network Analyst version 3.0 (https://www.networkanalyst.ca) [16]. A pathway enrichment analysis was carried out via DIANAv.3 [17] and Enrichr (https://maayanlab.cloud/Enrichr/), g:Profiler [18] (https://biit.cs.ut.ee/gprofiler/gost), and visual representation was created by using Python's Seaborn and Matplotlib packages for data visualization. Pandas was used to process data [19]. Protein–protein interaction networks were evaluated with the STRING database version 11.5 (https://string-db.org).

Statistical Analysis Statistical analysis was performed by IBM SPSS Statistics version 26. Data were expressed as mean ± standard deviation. Statistical difference was evaluated by using a T-test for parametric independent samples and a Mann-Whitney test for nonparametric data. The Spearman test for nonparametric data, in addition to the Pearson test for parametric data, was used to test bivariate correlations. A multiple predictor

regression analysis was done. P < 0.05 was statistically significant.

Results

A. SIRT3 structural and functional analysis.

Sirtuin 3 (*SIRT3*; Gene ID: 23410, ENSG00000142082) is positioned at the 11p15.5 region of the short arm of chromosome 11, spanning positions 215,030 to 236,931 on the reverse strand (GRCh38.p14). The gene comprises seven exons and gives rise to 16 transcript variants, including 8 protein-coding isoforms. The remaining transcripts include non-coding variants resulting from alternative splicing, with one retained intron transcript, five subjects to nonsense-mediated decay, and two lacking defined coding sequences. Additionally, regulatory elements (both cis- and trans-acting) and transcriptional control factors are both illustrated in Fig. 1A.

The protein encoded by *SIRT3,* represented by UniProt entry Q9NTG7, has 399 amino acids (43.573 Da). SIRT3 protein is found in the nucleus, mitochondria, lysosome, cytosol, endoplasmic reticulum, peroxisome, extracellular space, plasma membrane, endosome, cytoskeleton, and Golgi apparatus (Fig. 1C). The protein's three-dimensional structure is depicted in Fig. 1B.

The distribution of SIRT3 mRNA expression across various human tissues has been analyzed using multiple transcriptomic datasets that utilize data from GTEx (RNA-seq), Illumina Body Map, and BioGPS (microarray). Results indicate a widespread SIRT3 expression across different tissues. Notably, higher expression levels were observed in organs with high metabolic demands, including the liver, kidney, and heart, as well as skeletal muscle. This pattern was consistently reported across all datasets. Additionally, SIRT3 expression was detected in reproductive tissues, particularly the testis, as well as in secretory organs like the thyroid and adrenal gland (Fig. 1D).

In contrast, the nervous system tissues, including the brain, cerebellum, and spinal cord, showed comparatively lower expression levels (Fig. 1D). These findings are in line with SIRT3's functions as a key mitochondrial deacetylase involved in regulating metabolism, oxidative stress, and energy balance, with elevated expression in metabolically active tissues.

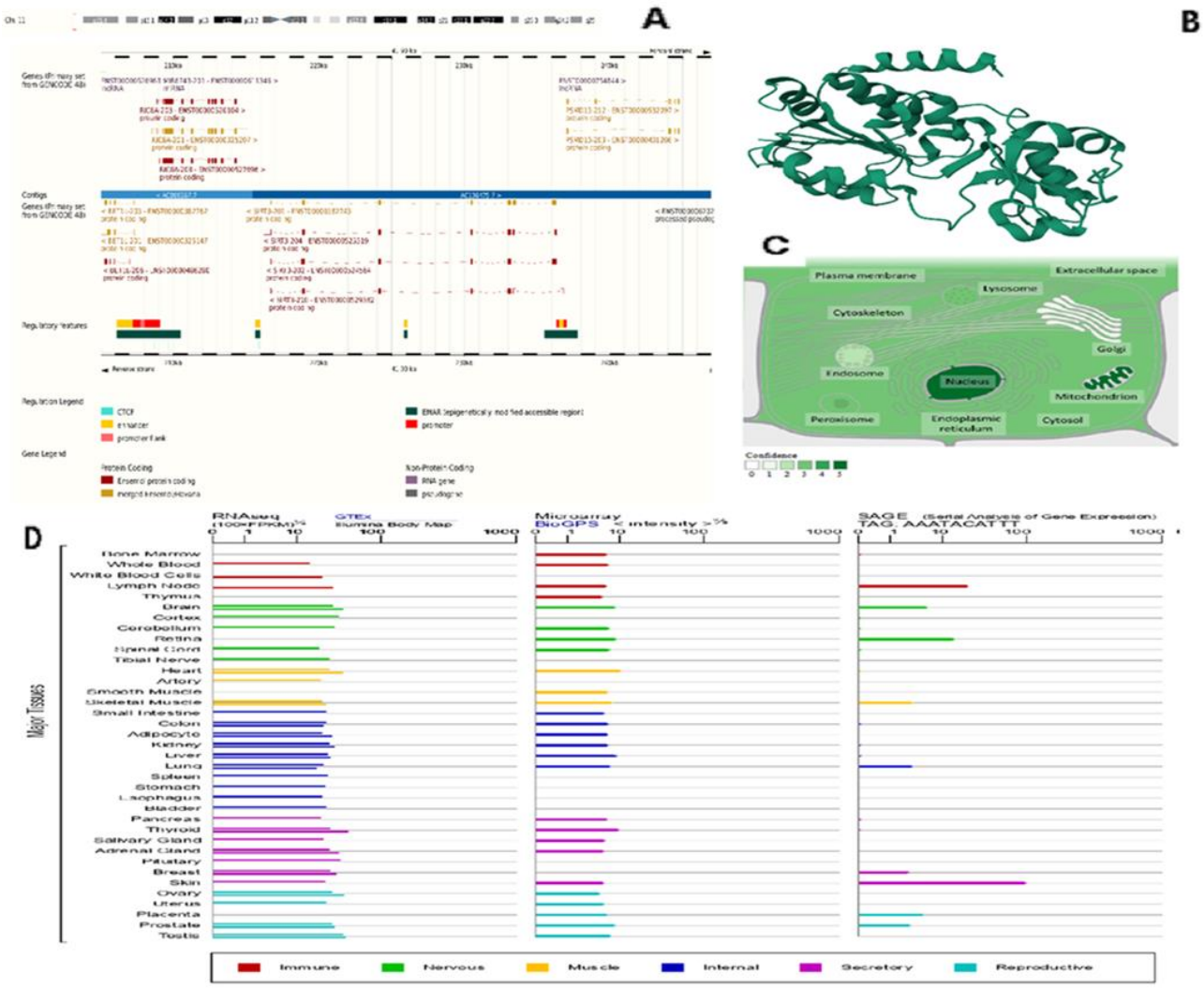


Figure 1. SIRT3 structural and functional analysis. **(A)** The chromosomal location and the related transcripts; **(B)** the predicted 3D structure of SIRT3; **(C)** the localization of the SIRT3. The color degree is related to protein abundance and arranged according to degree of confidence from highest to smallest. They arise from database notes, sequence-based predictions along with computerized text mining of biomedical literature, and **(D)** mRNA levels in healthy human body tissues from GTEx, Illumina, BioGPS, and SAGE.

B. Protein-protein interactions of SIRT3

Protein-protein interactions (PPI) of our target SIRT3 via the STRING database (Fig. 2A) reveal high evidence of interactions with proteins such as PPARGC1A, NAMPT, NMNAT1/2, and FOXO3, indicating possible regulatory or functional relationships. The network also includes H3 histone variations (H3-3B, H3C13, and H3-5), which may indicate a chromatin or transcriptional regulatory relationship. The gene co-expression map generated using the STRING database (Fig. 2B) reveals strong co-expression among proteins such as SIRT3, PPARGC1A, and NMNATs, suggesting their involvement in related biological processes like mitochondrial metabolism and NAD^+ biosynthesis. This co-expression matrix helps assess functional relevance and biological coherence within the protein network.

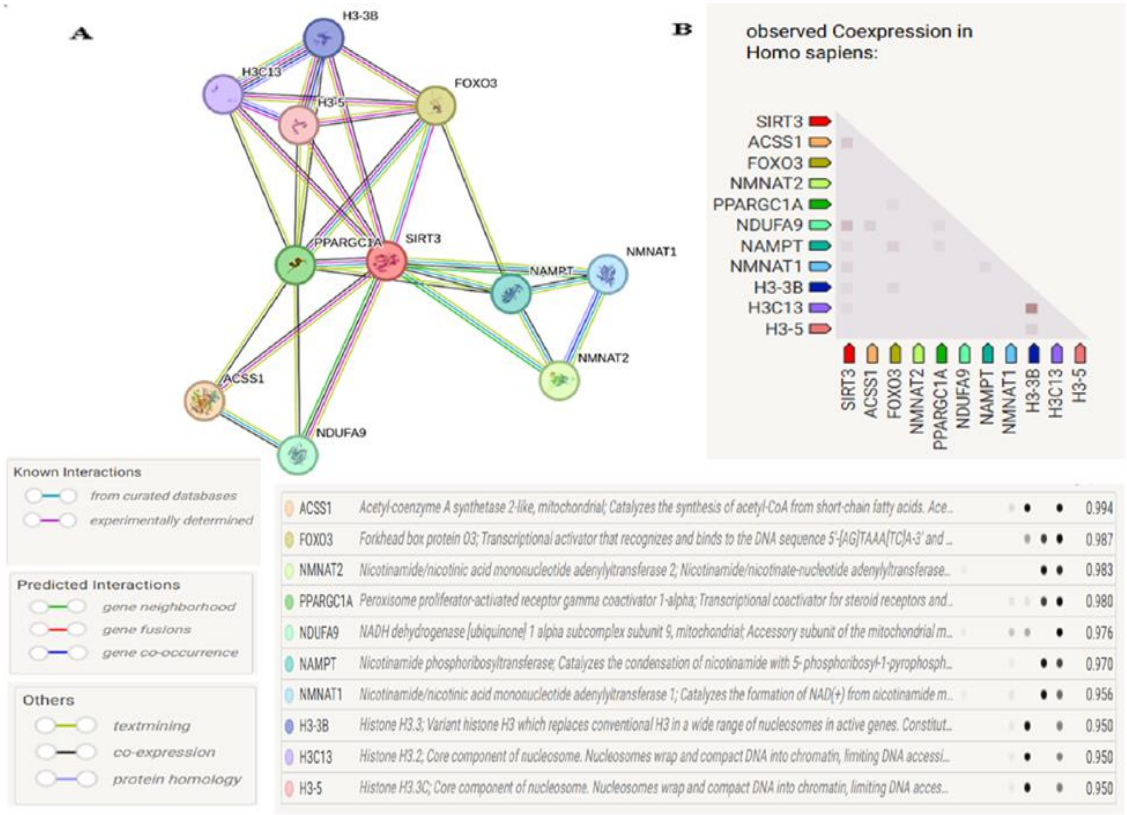


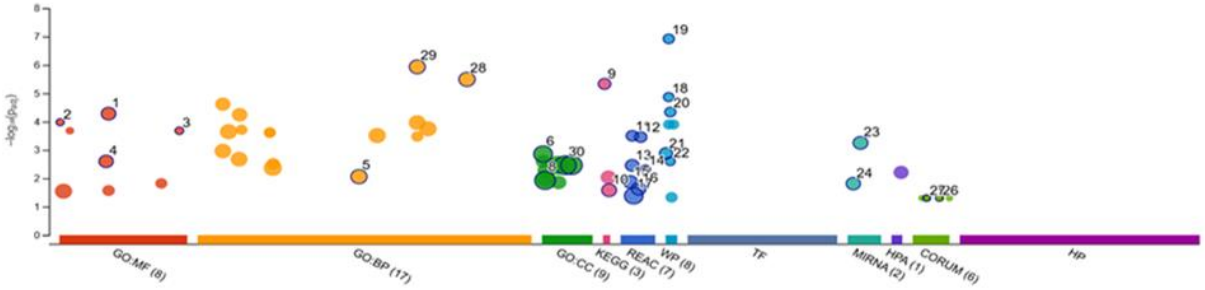
Figure 2. (A) Protein-protein interaction (PPI) between the examined proteins via the STRING database, where nodes indicate proteins and edges denote the relation between them. The resultant networks reveal a statistically significant enrichment, indicating strong associations among the proteins (p-value < 1.0 × 10−16) with a confidence score of 0.700. (**B**) A gene co-expression matrix for target protein & interacting proteins created by using STRING db.

C. SIRT3 and interacting protein involvement with the relevant pathways using network topology analysis:

Our target protein and interacting network were functionally enriched in multiple different pathway verifications via KEEG, Reactome, and wiki databases, revealing pathways such as energy metabolism, systemic lupus, programmed cell death, FOXO-mediated transcription of oxidative stress, and metabolism of water-soluble vitamins. Figure 3 shows additional evidence revealed from the respective gene ontology enrichment, which shows the involvement of these proteins in various biological processes, cellular compartments, and molecular functions. Our target protein and interacting network were functionally enriched in multiple biological processes and molecular functions related to the NAD synthesizing process (GO:0009435), nucleotide synthesizing process (GO:0009165), rhythmic process (GO:0048511), circadian rhythm (GO:0007623), chromatin remodeling (GO:0006338), protein/peptidyl-lysine deacetylation (GO:0006476 & GO:0034983), aerobic respiration (GO:0009060), and positive

regulation of insulin secretion (GO:0032024) (Fig. 3).

SIRT3 was enriched in various pathways for energy metabolism (WP1541), NAD+ metabolism/NAD metabolism, sirtuins and aging (WP3630 & WP3644), aerobic respiration and respiratory electron transport (Id: R-HSA-70268.10), cellular responses to stress (Id: R-HSA-2262752.13), the citric acid cycle (Id: R-HSA-71403.5), the FOXO signaling pathway (Id: R-HSA-9614085.3) controlling the transcriptional activation of genes linked to oxidative stress, metabolic regulation, and neuronal activity (Id: R-HAS-9615017.2), mitochondrial biogenesis (Id: R-HSA-1605416.1), the mitochondrial unfolded protein response (Id: R-HAS-9841251.1), and transcriptional activation of mitochondrial biogenesis (Id: R-HSA-2151201.4).



ID	Source	Term ID	Term Name	p_{adj} (query_1)
1	GO:MF	GO:0031490	chromatin DNA binding	5.222×10^{-5}
2	GO:MF	GO:0000309	nicotinamide-nucleotide adenylyltransferase activity	1.043×10^{-4}
3	GO:MF	GO:0140768	protein ADP-ribosyltransferase-substrate adaptor ...	2.086×10^{-4}
4	GO:MF	GO:0030527	structural constituent of chromatin	2.510×10^{-3}
5	GO:BP	GO:0048511	rhythmic process	8.686×10^{-3}
6	GO:CC	GO:0000785	chromatin	1.385×10^{-3}
7	GO:CC	GO:0070013	intracellular organelle lumen	3.431×10^{-3}
8	GO:CC	GO:0005654	nucleoplasm	1.178×10^{-2}
9	KEGG	KEGG:00760	Nicotinate and nicotinamide metabolism	4.646×10^{-6}
10	KEGG	KEGG:05322	Systemic lupus erythematosus	2.606×10^{-2}
11	REAC	REAC:R-HSA-96...	FOXO-mediated transcription of oxidative stress, ...	3.151×10^{-4}
12	REAC	REAC:R-HSA-19...	Nicotinate metabolism	3.487×10^{-4}
13	REAC	REAC:R-HSA-96...	FOXO-mediated transcription	3.490×10^{-3}
14	REAC	REAC:R-HSA-96...	Regulation of FOXO transcriptional activity by acet...	4.808×10^{-3}
15	REAC	REAC:R-HSA-96...	Diseases of programmed cell death	1.325×10^{-2}
16	REAC	REAC:R-HSA-19...	Metabolism of water-soluble vitamins and cofactors	2.309×10^{-2}
17	REAC	REAC:R-HSA-21...	Generic Transcription Pathway	4.127×10^{-2}
18	WP	WP:WP3630	NAD metabolism sirtuins and aging	1.355×10^{-5}
19	WP	WP:WP3644	NAD metabolism	1.198×10^{-2}
20	WP	WP:WP4210	Tryptophan catabolism leading to NAD production	4.584×10^{-5}
21	WP	WP:WP1541	Energy metabolism	1.301×10^{-3}
22	WP	WP:WP4191	Caloric restriction and aging	2.509×10^{-3}
23	MIRNA	MIRNA:hsa-miR...	hsa-miR-4328	5.581×10^{-4}
24	MIRNA	MIRNA:hsa-miR...	hsa-miR-223-3p	1.532×10^{-2}
25	CORUM	CORUM:2678	FOXO3-TP53 complex, oxidative stress stimulated	4.964×10^{-2}
26	CORUM	CORUM:6402	NMNAT2-HSP90 complex	4.964×10^{-2}
27	CORUM	CORUM:2593	FOXO3-SIRT1 complex, oxidative stress stimulated	4.964×10^{-2}
28	GO:BP	GO:1901293	nucleoside phosphate biosynthetic process	3.220×10^{-6}
29	GO:BP	GO:0072522	purine-containing compound biosynthetic process	1.165×10^{-6}
30	GO:CC	GO:0043233	organelle lumen	3.431×10^{-3}

Figure 3. GO terms and pathways enriched for proteins interacting with SIRT3. The figure was created via g:Profiler (https://biit.cs.ut.ee/gprofiler/gost). The enrichment evaluation was done by using g:Profiler (version e112_eg59_p19_25aa4782) with the Bonferroni correction test with a significance threshold of 0.05 (Kolberg, Raudvere et al. 2023).

D. Different microRNAs, transcription factor interaction with SIRT3, and functional enrichment:

As SIRT3 is implicated in various pathways and biological processes, Network Analyst version processes recognize the different

microRNAs and the transcriptional factors that have a potential interaction with SIRT3. Figure 4A illustrates the list of miRNAs that interact with SIRT3. Figure 4B shows the revealed miRNAs were potentially enriched in various pathways and processes. KEGG pathways (e.g., *pathways in cancer*, *oxytocin signaling*) and GO categories (e.g., *organelle*, *ion binding*) were ranked by p-value, indicating their relevance in the context of the microRNA data input. SIRT3 seems to be involved with the control of enzymes involved in amino acid metabolism, particularly lysine breakdown. Changes to this route can have an impact on energy generation and metabolism. The oxytocin signaling pathway is typically connected with social behaviors, but it also regulates glucose utilization as well as insulin sensitivity. SIRT3's modulation of mitochondrial activity may have an indirect influence on this pathway; in addition, its role in cancer pathways emphasizes its relevance in cellular metabolism and stress response.

The KEGG pathway enrichment study of TFs interacting with SIRT3 identifies multiple pathways involved with diabetes [20], including insulin signaling [21], the NF-κB signaling, glycolysis [22], gluconeogenesis, and oxidative phosphorylation. These pathways are critical to the initiation and advancement of diabetes; in addition, their control by SIRT3 and other TFs highlights the possibility of targeting these interactions for therapeutic purposes (Fig. 5 A, B).

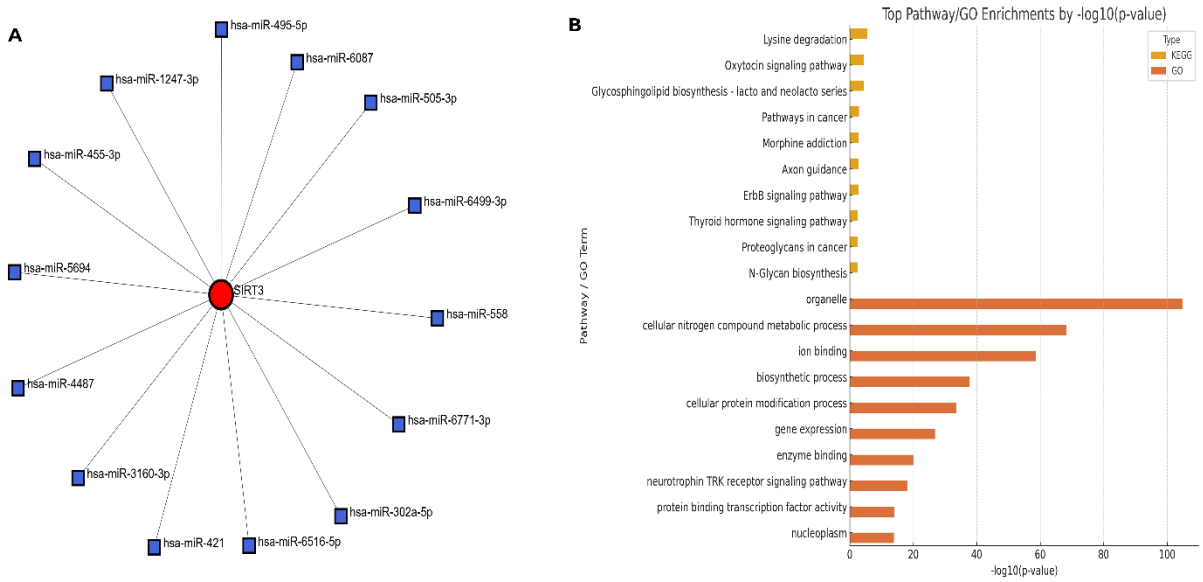


Figure 4. miRNA interacts with SIRT3, and the bar plot illustrates the top 10 enriched KEGG pathways and GO biological categories associated with microRNA targets, based on statistical significance (log_{10}(p-value)).

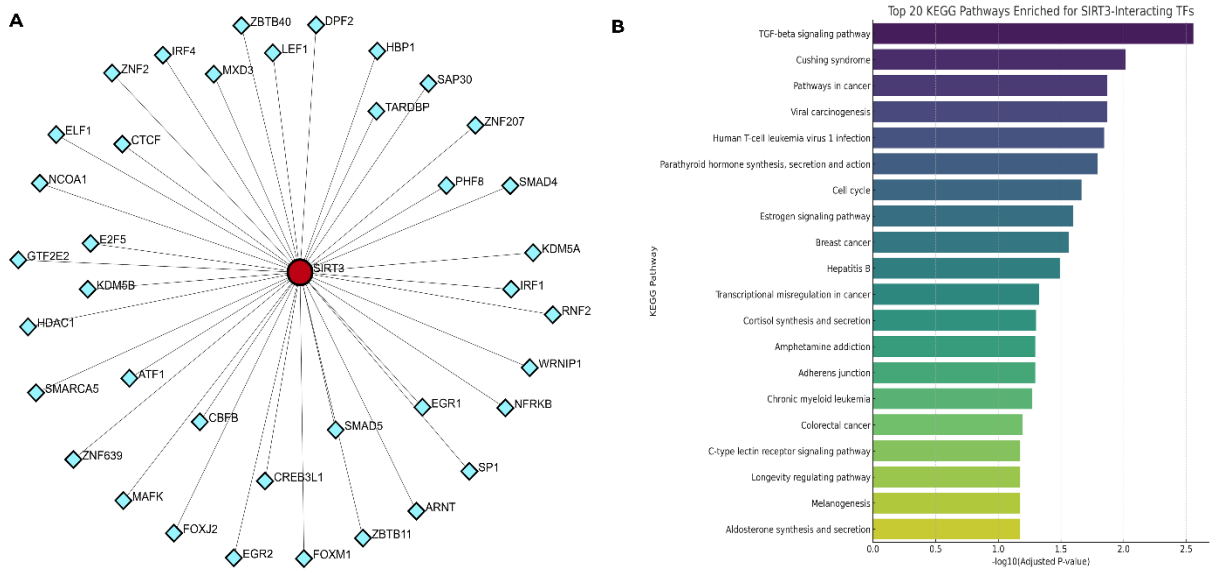


Figure 5. Transcription factors interact with SIRT3, and the bar plot illustrates the top 20 enriched KEGG pathways associated with transcription factor targets, based on statistical significance ($-\log_{10}$(p-value)).

E. Baseline characteristics of patient and control groups

A total of 90 children participated in this study, divided equally into a control group (n=45) and a diabetic group (n=45). The demographic and anthropometric characteristics of the study population were listed in table 1. There was no statistically significant difference in gender distribution between both groups, with 42.2% males and 57.8% females in the control group, compared to 51.1% males and 48.9% females in the diabetic group (p=0.399). The mean age of the control group was 6.77 ± 1.83 years, compared to 7.43 ± 1.62 years in the diabetic patients, revealing no statistically significant difference (p=0.09). Regarding the anthropometric data, a significant difference in height was observed, with diabetic children being taller (123.78 ± 11.94 cm) than controls (117.56 ± 13.05 cm, p=0.02). The mean weight was higher in the diabetic group (27.07 ± 7.97 kg) than in the control group (23.88 ± 5.74 kg), but this difference was not statistically significant, with (p=0.09). Similarly, the diabetic children showed higher values of body mass index (17.39±2.82 kg/m²) than the controls (17.12 ± 2.11 kg/m²), with no significant difference (p=0.9).

Table (1): Baseline features of patient and control groups.

Groups / Parameters	Control N=45	Diabetic patients N=45	P-value
Gender Male n (%) Female n (%)	 (42.2%) (57.8%)	 (51.1%) (48.9%)	0.399
Age (years)	6.77 ± 1.83	7.43 ± 1.62	0.09
Height (cm)	117.56 ± 13.05	123.78 ± 11.94	0.02*
Weight (kg)	23.88 ± 5.74	27.07 ± 7.97	0.09
Body mass index (kg/m²)	17.12 ± 2.11	17.39±2.82	0.9

*Data are reported as mean ± standard deviation (SD). Nonparametric data were analyzed by the Mann-Whitney test, parametric data with the student t-test, and the chi-square test for categorical variables. * P < 0.05 is considered significant*

F. Biochemical lab investigations in patient and control groups

Diabetic children showed statistically significant higher FBG (192.24 ± 82.17 mg/dl) and HbA1c values (9.64 ± 1.74 %) compared to controls (100.76 ± 12.68 mg/dl and 4.79 ± 0.30 %, respectively, with p < 0.001), denoting poor glycemic control. Similarly, they had a statistically significant elevation in TAG (102.56 ± 22.36 mg/dl) and VLDL-C levels (20.51 ± 4.47 mg/dl) compared to controls (71.91 ± 28.2 mg/dl and 14.38 ± 5.64 mg/dl, respectively, with p < 0.001). However, no significant differences in TC (p=0.19), HDL-C (p=0.43), LDL-C (p=0.06), non-HDL-C (p=0.36), LDL-C/HDL-C risk ratio (p=0.24), or TC/HDL-C risk ratio (p=0.89) were identified. Regarding serum SIRT3 level, there was a statistically significant difference in both groups, with the level being lower in the diabetic group (12.36 ± 4.85 ng/ml) compared to controls (16.87 ± 3.02 ng/ml; p < 0.001) (table 2)

G. Correlation of SIRT3 levels with biochemical parameters and duration of diabetes in diabetic children and controls

In the control group, SIRT3 levels showed non-significant weak negative correlations with FBG (r=-0.27, p=0.07), HbA1c (r=-0.24, p=0.11), and HDL-C (r=-0.2, p= 0.18); non-significant very weak negative correlations with LDL-C (r=-0.04, p=0.8) and TC (r=-0.14, p=0.37); and non-significant very weak positive correlations with non-HDL-C (r=0.04, p=0.81), LDL-C/HDL-C risk ratio (r=0.1, p=0.5), and TC/HDL-C risk ratio (r=0.12, p=0.44). There was negligible correlation of SIRT3 with TAG and VLDL-C (r < 0.001, p=1).

In the diabetic group, SIRT3 levels exhibited non-significant very weak positive correlations with FBG (r=0.02, p=0.92), HbA1c (r=0.12, p=0.43), LDL-C/HDL-C risk ratio (r=0.11, p=0.48), TC/HDL-C risk ratio (r=0.004, p= 0.98), TAG, and VLDL-C (r=0.09, p=0.58); non-significant very weak negative correlations with TC (r=-0.09, p=0.57), LDL-C (r=-0.04, p=0.82), and non-HDL-C (r=-0.02, p=0.9); and non-significant weak negative correlation with HDL-C (r=-0.2, p=0.18). In addition, the correlation between SIRT3 and the duration of diabetes in diabetic children was statistically non-significant with a p-value of 0.28 (table 3).

Table (2): Comparison between the diabetic group and the control group regarding biochemical data			
Groups / Parameters	Control N=45	Diabetic patients N=45	P-value
FBG (mg/dl)	100.76 ± 12.68	192.24 ± 82.17	< 0.001***
HbA1c (%)	4.79 ± 0.30	9.64 ± 1.74	< 0.001***
TC (mg/dl)	191.87 ± 14.18	186.24 ± 41.51	0.19
TAG (mg/dl)	71.91 ± 28.2	102.56 ± 22.36	< 0.001***
HDL-C (mg/dl)	63.96 ± 7.75	63.69 ± 13.98	0.43
LDL-C (mg/dl)	113.51±15.03	102 ± 36.88	0.06
VLDL-C (mg/dl)	14.38 ± 5.64	20.51 ± 4.47	< 0.001***
Non HDL-C (mg/dl)	127.91 ± 12.3	122.56 ± 36.48	0.36
LDL-C/HDL-C risk ratio	1.8±0.3	1.66±0.71	0.24
TC/HDL-C risk ratio	3.02 ± 0.29	3.14 ± 0.80	0.89
SIRT 3 (ng/ml)	16.87 ± 3.02	12.36 ± 4.85	< 0.001***
*Data are reported in the form of mean ± SD. The Mann-Whitney test was used for analyzing nonparametric data, while the student t-test was used for parametric data. * Significant when p-value < 0.05.*			

Table (3): Correlation of SIRT3 levels with biochemical parameters and duration of diabetes in diabetic children and controls .		
Groups / Parameters	SIRT3 in diabetic group	
	R	*P*
DM duration (years)	-0.16	0.28
*Correlations were performed by Spearman's correlation analysis. * Significant when p < 0.05.*		

H. Correlation of SIRT3 levels with biochemical parameters in the whole study group

In the whole group (both diabetics and controls), correlation of SIRT3 levels with the biochemical parameters revealed that there were statistically significant moderate negative correlations between SIRT3 and FBG (r=-0.37, p < 0.001) & HbA1c (r=-0.44, p <0.001) and statistically significant weak negative correlations between SIRT3 levels and TAG (r=-0.23, p=0.03) & VLDL-C (r=-0.23, p=0.03). No significant correlations existed between SIRT3 and TC (p=0.67), HDL-C (p=0.2), LDL-C (p=0.56), non-HDL-C (p=0.66), LDL-C/HDL-C risk ratio (p=0.14), or TC/HDL-C risk ratio (p=0.94) (table 4).

Table (4): Correlation of SIRT3 levels with biochemical parameters in the whole study group.		
Groups / Parameters	SIRT3 in the whole Group	
	R	P-value
FBG (mg/dl)	-0.37	< 0.001***
HbA1c (%)	-0.44	< 0.001***
TC (mg/dl)	-0.05	0.67
TAG (mg/dl)	-0.23	0.03*
HDL-C (mg/dl)	-0.14	0.2
LDL-C (mg/dl)	0.062	0.56
VLDL-C (mg/dl)	-0.23	0.03*
Non HDL-C (mg/dl)	0.05	0.66
LDL-C/HDL-C risk ratio	0.16	0.14
TC/HDL-C risk ratio	0.01	0.94
*Correlations were performed using Spearman's correlation analysis. * Significant when p < 0.05*		

I. Multiple linear regression analysis for predicting SIRT3 level

A multiple regression analysis was performed to assess the independent effects of HbA1c and VLDL-C on serum SIRT3 level. Serum SIRT3 level served as the dependent variable, while HbA1c and VLDL-C were involved as predictors. In this model, HbA1c was identified as a significant predictor of serum SIRT3 level independently of VLDL-C (B=-0.44, p=0.001), with a 95% confidence interval ranging from -0.68 to -0.19, indicating a significant inverse association. This means that, independent of VLDL-C, serum SIRT3 decreases by 0.44 for each one-unit increase in HbA1c. However, VLDL-C was not a significant predictor (B=0.001, p=0.93, with 95% CI ranging from -0.01 to 0.01), suggesting no significant relationship with serum SIRT3 levels (table 5).

Table (5): Multiple regression analysis showing significant associations with SIRT3.					
Model	Variables	B	Significance	95%CI for B	
				Lower bound	Upper bound
1	HbA1c	-0.44	0.001**	-0.68	-0.19
	VLDL-C	0.001	0.93	-0.01	0.01
** P < 0.05 is considered significant.*					

Discussion

Diabetes mellitus represents a chronic metabolic illness that is marked by high blood glucose levels due to either a complete or partial lack of insulin or impaired insulin sensitivity. Type 1 diabetes is an autoimmune disorder where insulin secretion is deficient, induced through T lymphocyte-related death of pancreatic beta cells. In contrast, T2DM is characterized by insulin resistance associated with pancreatic β-cell dysfunction [23].

Chronic inflammatory states and oxidative damage are well-established contributors to the pathophysiological mechanisms underlying diabetes mellitus. Hyperglycemia promotes reactive oxygen molecules production by a complex interplay of various factors, including

polyol pathway stimulation, accumulation of advanced glycation end-products (AGEs), increased flux through the hexosamine pathway, and triggering protein kinase C (PKC) signaling. These processes lead to beta cell malfunction coupled with apoptotic processes, impairing insulin secretion. Moreover, oxidative stress further contributes to diminished insulin signaling efficacy by impairing glucose uptake in target tissues [24].

Mitochondria are majorly specialized in energy production, maintaining redox equilibrium, cellular communication, and regulating longevity; thus, mitochondrial dysfunctions could contribute to the pathogenesis of numerous diseases, including diabetes. Protein acetylation, a frequent post-translational modification of many target proteins, is a significant element in controlling mitochondrial activity and is partly regulated by sirtuins [6].

SIRT3, a known mitochondrial deacetylase requiring NAD^+, is classified as a class III HDAC. SIRT3 insufficiency has been reported to be linked to age-associated metabolic disorders, particularly heart problems, insulin insensitivity, T2DM, and hepatic steatosis. In the pathogenesis of diabetes, SIRT3 is thought to have a preventive function, contributing to multiple stress responses. SIRT3 plays a necessary role in decreasing inflammation and oxidative damage while also enhancing mitochondrial health in muscles of the skeletal system, fatty tissues, and the pancreas. Deficiency of SIRT3 has been linked to loss of β cell structure and function through inhibition of genetic markers that are responsible for insulin release [9].

To contextualize our experimental findings, we conducted a complete in silico investigation of SIRT3 with a variety of bioinformatics tools and curated databases. Which revealed that SIRT3 interacts with several mitochondrial and metabolic regulator proteins, including PPARGC1A, FOXO3, NMNATs, and NAMPT. These partners play a crucial role in various biological processes, including oxidative metabolism, NAD^+ biosynthesis, mitochondrial biogenesis, aerobic respiration, positive insulin secretion regulation, and circadian rhythm regulation. Furthermore, SIRT3 and its interactome have been connected to various signaling pathways, including insulin signaling, energy metabolism, oxidative phosphorylation, and FOXO-mediated signaling, elucidating its central role in stress response, metabolic homeostasis, and insulin sensitivity [25,26].

Several miRNAs revealed through the in-silico analysis, such as miR-495-3p, miR-302a-5p, miR-1247-5p, miR-3160-3p, and miR-4487, are predicted to control SIRT3 and have recently been implicated in diabetes pathogenesis, including diabetic cardiomyopathy, gestational diabetes, and early-onset diabetes. These findings corroborate the biological significance of our bioinformatics-identified SIRT3-targeting miRNAs in the context of diabetes [27].

Furthermore, enrichment analysis of microRNA and transcription factor (TF) demonstrated that they are engaged in diabetes-related pathways such as glycolysis/gluconeogenesis, NF-κB signaling, oxidative stress, and oxytocin signaling. These signaling pathways, in addition to oxytocin signaling, which is reported to promote glucose absorption and insulin sensitivity in addition to its

traditional activities, have been demonstrated to play an important role in the pathogenesis of diabetes. These findings place SIRT3 at the crossroads of metabolic regulation, oxidative defense, and insulin responsiveness processes critical to the development and progression of T1DM [28].

Although SIRT3's association with T2DM is well-documented in both animal models and humans, no definitive results exist for T1DM. Thus, we decided to conduct this study, which aimed to assess SIRT3 in children with T1DM. Our study was a descriptive cross-sectional study that included 90 children aged 2-10 years. They were divided into two main groups: 45 diabetic children and 45 controls that were age and gender-matched. Diabetic children were diagnosed using ADA criteria. Laboratory tests, including FBG, HBA1C, and lipid profile, were performed on both groups.

Our results showed that diabetic and control groups had statistically significant differences in fasting blood glucose and HbA1c levels. These results are matched to a case-control study conducted on 40 diabetic and 40 control children and adolescents under 18 years in Port Said City [29].

Regarding our results about serum triglycerides and VLDL-C, there was a significant increase in diabetic children compared to the control group. These results were similar to Muhsen et al., 2022, who conducted a case-control study that involved 150 T1DM patients with a wide age range and 90 healthy controls and showed similar comparative results in the control and diabetic groups [30].

Regarding SIRT3, our results showed a significant variation in serum SIRT3 level in diabetics compared to the control group. This finding was supported by Al-Khaldi and Sultan (2019), who revealed a significant decrease in SIRT3 in peripheral blood samples from the diabetic group compared to the healthy control group when they studied the impact of diabetes on SIRT3 expression levels in patients with T1DM and T2DM. He explained his results as the observed reduction of SIRT3 may be due to oxidative stress and increased reactive oxygen molecules concentration in mitochondria as a result of diabetes-linked hyperglycemia [31].

Our finding was supported by an experimental research carried out by Locatelli et al. (2020), who observed a reduction in SIRT3 mRNA level in renal tissue of diabetic rodent models of the *BTBR ob/ob* strain compared to wild-type mice. This was linked to a decrease in its deacetylase function to superoxide dismutase 2 (SOD2) and high reactive oxygen molecules concentration, indicating the key role of SIRT3 in the pathogenesis of T2DM [32].

Fu et al. (2022) justified his results that polyol pathway stimulation and PARP pathway enhancement, with complex I dysfunction, which occurred in T2DM rats, lead to NADH/NAD^+ redox imbalance. With the depletion of NAD^+, SIRT3 activity is decreased [33].

Also, our results were similar to Song et al. 2021, who found that SIRT3 mRNA levels were lower in the cardiac tissue of the diabetic male C57BL/6 mice developed by streptozotocin (STZ) compared to controls. Focusing on the actual function of SIRT3 in diabetic cardiomyopathy, studied groups were further divided into male 129S1/SvImJ wild-type and SIRT3-deficient mice, aged eight weeks for both diabetic and control groups [34].

Song et al. (2021) found that SIRT3 deficiency was linked to cardiac dysfunction, increased serum lactate dehydrogenase, low ATP concentration in the cardiac tissue, and promoted reactive oxygen molecules generation. SIRT3 depletion has been associated with high levels of necroptosis-linked proteins in the cardiac tissue of mice suffering from diabetic cardiomyopathy, for example, receptor-interacting protein kinase 1 (RIPK1), RIPK3 as well as cleaved caspase 3, and inflammation-linked proteins, for example, NLRP3, caspase 1 p20 as well as interleukin-1β. Compared with the wild-type diabetic mouse models, all these findings were more profound in the SIRT3-deficient group, suggesting the importance of SIRT3 in diabetes development [34].

In 2020, Mao et al. investigated the function of SIRT3 in diabetic retinopathy in an STZ-induced diabetic rat model. This study included four different groups: control, diabetic, diabetic+scrambled adenovirus, and diabetic+Sirt3 overexpression group. He observed that the mRNA and protein concentration of SIRT3 in the diabetic group was decreased dramatically compared with the control group, mediating the pathological changes of retinal tissue. Those effects were ameliorated through overexpression of SIRT3, which upregulates the expression of autophagy-linked proteins while decreasing the expression of angiogenesis-linked genes [35].

Also, our results showed non-significant correlations between SIRT3 and other biochemical parameters in each single group, which may be explained by small sample size. However, when analyzing the correlation between SIRT3 and other parameters in the whole sample, there was a significant moderate negative correlation between SIRT3 and FBG & HbA1c and a significant weak negative correlation between SIRT3 and TAG & VLDL-C. HbA1c was also found to have a significant effect on serum SIRT3 level in the regression model.

Consistent with our results, Hua et al., 2020 showed a closely related finding regarding the effect of dihydromyricetin (DHY) as a SIRT3 activator on diabetes-induced endothelial impairment. He found a decline in SIRT3 expression within the thoracic aortic tissue of male C57BL/6 diabetic mouse models, which is related to increased HbA1c and FBS levels that were restored after DHY treatment. Hua et al. (2020) assessed the role of SIRT3 on the endothelium-mediated relaxation of thoracic aortic tissue. Wild-type 129S1/SvImJ and SIRT3-deficient diabetic mice were also used in the study, which revealed that the protective effects of DHY against diabetic vascular endothelial impairment were mediated by SIRT3. DHY attenuated superoxide level and reactive oxygen molecules production but increased total antioxidant capacity and glutathione redox ratio with SOD2 action in the thoracic aortic tissue of wild-type diabetic mouse models, which was abolished in SIRT3-diabetic mice [36].

Paramesha et al. (2021) further evaluated the impact of SIRT3 on cardiac tissue insulin insensitivity. They reported that SIRT3 activation enhances cellular glucose entry in palmitate-linked insulin-intolerant cardiomyoblast (H9c2) cells [37].

At last, we can conclude that SIRT3 is critically involved in diabetes mellitus pathophysiology, suggesting that SIRT3 activation may be a successful strategy in the treatment of the disease and its consequences.

Conclusion

In conclusion, our findings emphasize SIRT3's crucial role in diabetes pathogenesis. SIRT3 acts as a fundamental mitochondrial regulator, reducing inflammation and oxidative damage while promoting mitochondrial function. Our combined clinical and bioinformatic data argue that pharmacological SIRT3 stimulation could be a promising treatment strategy for managing diabetes and preventing complications.

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