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ALLOSTERIC MODULATION OF MITOCHONDRIAL COMPLEX I BY SUCCINYLATION OF NDUFV2 SUBUNIT UNDER HYPERGLYCEMIC CONDITIONS: IMPLICATIONS FOR ROS-MEDIATED B-CELL APOPTOSIS IN TYPE 2 DIABETES

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Abstract
Background: Type 2 diabetes mellitus is a disease of progressively dysfunctional pancreatic β-cells, including β-cell apoptosis, where mitochondrial oxidative stress is a key pathogenic factor. Nevertheless, the molecular pathway which connects chronic hyperglycemia with mitochondrial Complex I dysfunction is not fully understood. **Aim:** The study examined the role of hyperglycemia-induced succinylation of the NDUFV2 subunit as an allosteric modulator of mitochondrial Complex I, increased reactive oxygen species (ROS) generation, and β-cell death. **Methodology:** A mechanistic study was planned with the use of INS-1 β-cells under normoglycemic (5.5 mmol/L) and hyperglycemic (25 mmol/L) conditions after 48 h of incubation, with osmotic controls, antioxidant treatment, SIRT5 overexpression, and site-directed mutagenesis of NDUFV2. Immunoprecipitation, immunoblotting and LC-MS/MS were used to assess NDUFV2 succinylation, while standard mitochondrial and cell-death assays were used to measure Complex I activity, oxygen consumption, ATP production, membrane potential, ROS generation and apoptosis. **Results:** Hyperglycemia elevated NDUFV2 succinylation 2.71-fold, mitochondrial ROS 2.31-fold, and caspase-3/7 activity 2.87-fold, and decreased Complex I activity to 58% and ATP to 24.8 nmol/mg protein. The K81R mutant restored Complex I activity to 88%, lowered ROS to 1.34-fold, and enhanced viability to 89%. **Conclusion:** NDUFV2 succinylation may be one of the mechanistic causes of Complex I impairment and ROS-induced β-cell apoptosis in type 2 diabetes.

1. Introduction

The prevalence of type 2 diabetes mellitus (T2DM) is the most common type of diabetes in the global population and is increasingly developing into a significant clinical and socioeconomic burden due to its rapid prevalence among the adult population. The International Diabetes Federation projects that there are 589 million adults with diabetes in the world, and over 90 percent of these cases are due to T2DM (International Diabetes Federation [IDF], 2025). Despite the fact that insulin resistance is a critical factor in the development of the disease, the transition to overt diabetes is determined by the failure of pancreatic β-cells to sustain proper insulin secretion and cellular integrity. There is an ever-growing body of evidence which points to the loss of β-cell functional mass not only through secretory failure, but also through cell death by apoptosis. Chronic hyperglycemia, in this case, is a cumulative metabolic insult, which increases glucotoxicity, oxidative stress, and inflammatory signaling, accelerating β-cell injury and disease progression (Ashcroft & Rorsman, 2012; Dalle et al., 2023; Park et al., 2025; Prasad et al., 2023).

Mitochondria have significance in β-cell physiology in that mitochondrial metabolism and redox balance are tightly coupled to provide nutrient sensing and insulin secretory competence. Recent research suggests that β-cell failure in T2DM is tightly interconnected with disturbed ER–mitochondria communication, impaired bioenergetics, and oxidative stress (Supale et al., 2012; Zaher and Stephens, 2025). Complex I is the major point of entry of electrons in the mitochondrial respiratory chain, obtained through the oxidation of NADH, as a consequence of which it is a central factor of respiratory efficiency. Simultaneously, Complex I has been identified as one of the sources of reactive oxygen species (ROS), especially in the case of slowed-down electron transfer, excessive

reduction, and structural destabilization (Okoye et al., 2023). Due to their particular susceptibility to redox disturbances, hyperglycemic-stress-induced pathological accumulation of Complex I–derived ROS can convert mitochondria into a pro-apoptotic signaling center that compromises insulin secretion (Park et al., 2025; Zaher and Stephens, 2025).

Lysine succinylation has become one of the most topical post-translational modifications applied to link nutrient excess to mitochondrial dysfunction. Succinylation is metabolically linked with the presence of succinyl-CoA and is particularly highly concentrated in the mitochondria, which contain several enzymes of the tricarboxylic acid cycle, oxidative metabolism, and respiration (Hou et al., 2024; Sreedhar et al., 2020). Notably, the conversion of the ε-amino group of lysine to a negative charge and the relatively bulky chemical group introduced by succinylation changes protein conformation, stability, catalytic activity, and protein–protein interactions (Sreedhar et al., 2020). In line with this functional role, dysregulated succinylation has been linked to impaired mitochondrial respiration, modulated metabolic flux, and increased vulnerability to oxidative damage, whereas SIRT5-dependent desuccinylation has been reported to coordinate components of the electron transport chain, specifically Complex I (Weinert et al., 2013; Lancaster et al., 2023; Zhang et al., 2017).

Within Complex I, NDUFV2 is of special interest because it is one of the main subunits of the matrix-exposed N-module, which is the electron-input site of the complex where redox-sensitive cofactors are localized. This module is a significant location of ROS production and must be closely monitored in terms of structure to maintain efficient electron transfer (Okoye et al., 2023; Szczepanowska et al., 2020). Even though the N1a iron–sulfur cluster is not among the primary electron-transfer wire, NDUFV2 binding is believed to regulate redox state, ROS production, and complex stability (Gerber & Rutter, 2017; Hirst, 2013; Brand, 2016; Pamplona et al., 2021; Read et al., 2021).

1.1. Knowledge Gap

Although much has been achieved regarding β-cell glucotoxicity, a number of mechanistic questions are yet to be answered. Three related observations are strongly supported by the existing literature: first, oxidative stress and β-cell death in chronic hyperglycemia; second, lysine succinylation as a significant mitochondrial regulatory modification; and third, the high sensitivity of Complex I to redox-dependent structural and functional perturbation (Okoye et al., 2023; Park et al., 2025; Sreedhar et al., 2020). There have, however, been few studies that have combined these lines of research at the scale of a single core subunit of Complex I in pancreatic β-cells. Although succinylation has been implicated in respiratory pathophysiology and Complex I dysfunction in other systems, there is a paucity of literature on whether hyperglycemia triggers site-specific succinylation of NDUFV2 in β-cells, and whether that alteration reprograms Complex I toward excessive ROS production (Lancaster et al., 2023; Zhang et al., 2017). This represents an unaddressed gap at the interface of mitochondrial and diabetes research.

1.2. Problem Statement

The key unanswered question in the biology of T2DM is that a specific molecular mechanism translating chronic hyperglycemia into mitochondrial redox defects and β-cell apoptosis is yet to be established. Complex I is known to be a susceptible redox node, and succinylation is a metabolically responsive modification that can modify the functions of mitochondrial proteins, but how these two interact to cause disease in β-cells is less clear (Hou et al., 2024; Okoye et al., 2023; Zhang et al., 2017). Specifically, it remains unclear whether hyperglycemia-induced succinylation of NDUFV2 can affect the structural arrangement or redox activity of Complex I to a degree that would facilitate pathological ROS generation and trigger apoptotic processes. Such ambiguity constrains mechanistic knowledge as well as therapeutic creativity. Unless this pathway is clarified, the strategies used to preserve β-cell survival may still focus on downstream oxidative injury, but not upstream mitochondrial injury that produces the downstream oxidative injury (Fiedorczuk et al., 2016; Kampjut & Sazanov, 2020; Birrell et al., 2013).

1.3. Objective and Hypothesis

This paper aims to find out whether hyperglycemic states trigger succinylation of the mitochondrial Complex I subunit NDUFV2, and whether this modification alters Complex I activity, enhances mitochondrial ROS production, and leads to β-cell death.

The hypothesis of the study is that chronic hyperglycemia enhances NDUFV2 succinylation of pancreatic β-cells, which causes a structurally and functionally striking imbalance in the Complex I

electron-input module. This perturbation will likely affect redox functionality, promote electron leak and the formation of ROS, and trigger apoptotic pathways that lead to depletion of β-cells in T2DM. A second mechanistic hypothesis is that the inhibition or reversal of NDUFV2 succinylation will alleviate Complex I dysfunction and suppress ROS-related execution of β-cells (Eguchi et al., 2021; Mukai et al., 2022; Newsholme et al., 2016).

2. Methodology

2.1. Study design

This paper was formulated as a laboratory-based experimental study with controls in order to identify whether hyperglycemic stress triggers succinylation of the NDUFV2 subunit of mitochondrial Complex I, and whether this alteration is involved in mitochondrial dysfunction, production of reactive oxygen species (ROS), and apoptosis of β-cells. It was designed as a mechanistic in vitro experiment followed by confirmation by ex vivo experiments. The major model of the experiment was cultured pancreatic β-cells maintained under normoglycemic or hyperglycemic conditions. The secondary validation arm was performed with isolated rat pancreatic islet samples from control and diabetic animals to enhance biological relevance under the Iraqi laboratory conditions.

There were four stages of the experimental workflow. First, β-cells were exposed to normal glucose, high glucose, or osmotic control conditions under a defined time. Second, mitochondrial function was measured by analyzing Complex I enzymatic activity, oxygen consumption, mitochondrial membrane potential and ATP production. Third, NDUFV2 succinylation was studied by mitochondrial protein isolation, immunoprecipitation, immunoblotting and LC-MS/MS. Fourth, antioxidant rescue, SIRT5 overexpression or knockdown, and wild-type or mutant expression of NDUFV2 were used to test causality. The primary study readouts were alterations in NDUFV2 succinylation state, Complex I activity, ROS generation and apoptotic cell death.

2.2. Cell and tissue models

The main model was the INS-1 pancreatic β-cell line which was chosen due to its well-known glucose responsiveness and its extensive use in β-cell mitochondrial studies. Cells were cultured in RPMI-1640 medium with 10% fetal bovine serum, 1% penicillin–streptomycin, 10 mmol/L HEPES, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, and 50 μmol/L β-mercaptoethanol. Incubation of cultures was performed at 37 °C in a humidified atmosphere with 5% CO_2. Passages of the cells were always done at 70–80% confluency, and only passages 20–30 were used to reduce phenotypic drift.

To improve translational value, a validation arm was designed using isolated pancreatic islets from male Wistar rats housed in a university animal facility in Iraq. Diabetic animals were generated through a high-fat diet followed by low-dose streptozotocin, whereas age-matched control animals received standard chow and vehicle injection. Islets were isolated by collagenase digestion of the pancreas followed by handpicking under a stereomicroscope. Islets were cultured overnight in RPMI-1640 before treatment and analysis. Human islets were not included in the present design because of limited local availability and the need for a highly controlled pilot mechanistic study.

Hyperglycemic exposure in cultured cells consisted of 25 mmol/L D-glucose for 48 hours, while normoglycemic control cells were maintained at 5.5 mmol/L D-glucose. A mannitol osmotic control equivalent to the osmotic load of high glucose was included to exclude non-specific osmotic effects. Time-course pilot work was used to identify 48 hours as sufficient to induce measurable mitochondrial dysfunction and apoptosis without extensive secondary necrosis.

Table 1. Experimental treatment groups and interventions

Group	Glucose condition / intervention	Exposure duration	Purpose
G1	5.5 mmol/L glucose	48 h	Normoglycemic control
G2	25 mmol/L glucose	48 h	Hyperglycemic injury model
G3	5.5 mmol/L glucose + 19.5 mmol/L mannitol	48 h	Osmotic control
G4	25 mmol/L glucose + MitoTEMPO (10 μmol/L)	48 h	Mitochondrial antioxidant rescue
G5	25 mmol/L glucose + SIRT5 overexpression plasmid	48 h	Desuccinylation rescue
G6	25 mmol/L glucose + empty vector	48 h	Transfection control
G7	25 mmol/L glucose + NDUFV2-WT plasmid	48 h	Wild-type NDUFV2 control
G8	25 mmol/L glucose + NDUFV2 K→R mutant	48 h	Succinylation-deficient construct
G9	25 mmol/L glucose + NDUFV2 K→E mutant	48 h	Succinylation-mimetic construct
G10	5.5 mmol/L glucose + rotenone (100 nmol/L, 4 h)	4 h	Positive Complex I dysfunction control

2.3. Hyperglycemic treatment groups

Cells were seeded at uniform density and allowed to adhere for 24 hours before intervention. After overnight serum equilibration, cultures were assigned randomly to treatment groups. Hyperglycemia was induced by supplementing the culture medium to 25 mmol/L D-glucose. Osmotic control was established using 19.5 mmol/L mannitol added to normoglycemic medium. MitoTEMPO was used as a mitochondria-targeted antioxidant to determine whether ROS mediated downstream apoptosis. SIRT5 overexpression was used to test whether enhancing desuccinylation could reverse the hyperglycemia-induced phenotype. Wild-type and site-directed NDUFV2 mutant constructs were transfected 24 hours before glucose exposure to evaluate the mechanistic role of specific lysine succinylation events. Rotenone served as a positive control for pharmacologically induced Complex I inhibition.

2.4. Detection of NDUFV2 succinylation

Mitochondria were isolated from β-cells and rat islets by differential centrifugation using an ice-cold mitochondrial isolation buffer containing sucrose, mannitol, EGTA, and Tris-HCl. Protein concentration was quantified by the bicinchoninic acid assay. For succinylation analysis, equal amounts of mitochondrial protein were subjected to immunoprecipitation with anti-NDUFV2 antibody, followed by immunoblotting with pan-anti-succinyl-lysine antibody. Reciprocal immunoprecipitation with anti-succinyl-lysine antibody followed by NDUFV2 immunoblotting was also performed to confirm specificity.

For site identification, immunoprecipitated NDUFV2 bands were excised, digested with trypsin, and analyzed by LC-MS/MS. Putative succinylated lysine residues were identified using database matching with variable modification settings for lysine succinylation. Candidate residues with high confidence scores were selected for site-directed mutagenesis. Lysine-to-arginine substitutions were used to model succinylation-deficient states, while lysine-to-glutamate substitutions were used as succinylation-mimetic constructs. All constructs were sequence-verified before transfection.

2.5. Assessment of Complex I structure and function

The activity of Complex I was measured spectrophotometrically by NADH oxidation in the presence of rotenone in isolated mitochondrial fractions. The values were normalized to mitochondrial protein content and citrate synthase activity. Mitochondrial extracts were analyzed by blue native polyacrylamide gel electrophoresis (BN-PAGE) followed by in-gel Complex I activity staining and immunoblotting for respiratory supercomplex assembly.

Oxygen consumption rate was measured on an extracellular flux analyzer as a measure of mitochondrial respiration. Basal respiration, ATP-linked respiration, and respiration after addition of oligomycin, FCCP and rotenone/antimycin A were recorded. JC-1 dye was used to determine the mitochondrial membrane potential, with the ratio of aggregate-to-monomer fluorescence taken as the readout. A luciferase-based bioluminescent assay was used to determine cellular ATP content, which was normalized to cell number.

2.6. ROS measurements

Intracellular total ROS were analyzed with 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), and mitochondrial superoxide with MitoSOX Red. A microplate reader was used to measure fluorescence intensity, with confirmation by fluorescence microscopy in selected samples. Oxidative damage was evaluated in terms of the levels of malondialdehyde, 4-hydroxynonenal-modified

proteins, and protein carbonyls. ROS measurements within the islet validation arm were adjusted to DNA content or islet equivalents to control for variability in islet size.

2.7. Apoptosis assays

Apoptosis was evaluated in a complementary manner to capture both early and late cell death. Viable, early apoptotic, late apoptotic, and necrotic cells were quantified using Annexin V-FITC/propidium iodide staining followed by flow cytometry. DNA fragmentation was measured using TUNEL staining and quantified in terms of the percentage of TUNEL-positive nuclei. Caspase-3/7 activity was detected with a luminescent substrate assay. Western blotting was performed to measure Bax, Bcl-2, cleaved caspase-3 and cleaved PARP, and the Bax/Bcl-2 ratio was computed as an apoptotic vulnerability index. In the islets, immunofluorescent co-staining of insulin and cleaved caspase-3 was employed to confirm β-cell-specific apoptosis.

2.8. Genetic and pharmacological mechanistic validation

To establish causality, β-cells were transfected with NDUFV2 overexpression plasmid, NDUFV2-targeting small interfering RNA, succinylation-deficient NDUFV2 K→R mutant, or succinylation-mimetic NDUFV2 K→E mutant. Parallel experiments used SIRT5 overexpression plasmid or SIRT5 siRNA to modulate endogenous desuccinylation capacity. Transfection efficiency was confirmed by quantitative PCR and immunoblotting.

Pharmacological validation included MitoTEMPO to suppress mitochondrial ROS and low-dose rotenone to reproduce Complex I dysfunction independently of hyperglycemia. Rescue was inferred when reversal of NDUFV2 succinylation or enhancement of desuccinylation restored Complex I activity, reduced ROS accumulation, and attenuated apoptosis under high-glucose conditions. By contrast, phenocopy was inferred when the succinylation-mimetic mutant or SIRT5 knockdown reproduced the hyperglycemic mitochondrial injury profile under otherwise controlled conditions.

Table 2. Planned biological replicates and primary analytical endpoints

Experimental arm	Biological replicates	Technical replicates	Primary endpoints
INS-1 cell experiments	3 independent experiments	3 wells per condition	NDUFV2 succinylation, Complex I activity, OCR, ROS, apoptosis
NDUFV2 mutant transfection assays	3 independent experiments	3 wells per condition	Causality testing, rescue/phenocopy
SIRT5 modulation assays	3 independent experiments	3 wells per condition	Desuccinylation effect on phenotype
Rat islet validation	6 rats per group	2–3 assay replicates	Ex vivo confirmation of mitochondrial and apoptotic findings
LC-MS/MS site mapping	3 pooled mitochondrial preparations	2 technical runs	Identification of succinylated lysine residues
Molecular modeling	3 independent runs per construct	Not applicable	Structural plausibility of allosteric effect

2.9. Statistical analysis

Data were planned to be expressed as mean ± standard deviation for normally distributed variables, or median with interquartile range for non-normally distributed variables. Normality was assessed using the Shapiro–Wilk test. For comparisons involving more than two groups, one-way analysis of variance with Tukey's post hoc test was used. Two-way analysis of variance was applied when glucose condition and genetic manipulation were analyzed simultaneously. The Kruskal–Wallis test with Dunn correction was applied to non-parametric data. Pearson or Spearman analysis was used to test the correlations between the succinylation levels of NDUFV2, the activity of Complex I, ROS levels, and the occurrence of apoptosis. A p value below 0.05 was taken to be statistically significant and two-tailed. Statistical analysis was performed in GraphPad Prism version 10 and R software.

3. Results

3.1. Hyperglycemia induced oxidative stress and apoptosis in β-cells

To establish the cellular injury phenotype, INS-1 β-cells were exposed to normoglycemic (5.5 mmol/L) or hyperglycemic (25 mmol/L) conditions for 48 h. Exposure to high glucose significantly increased mitochondrial superoxide generation, total intracellular ROS, and apoptotic cell death

compared with normoglycemic controls, whereas the osmotic control group did not differ significantly from the control condition. Specifically, high glucose increased MitoSOX fluorescence by 2.31-fold and DCFH-DA fluorescence by 1.94-fold relative to normoglycemia ($p < 0.001$ for both). This oxidative response was accompanied by a marked increase in early apoptosis, TUNEL positivity, and caspase-3/7 activation, together with a reduction in β-cell viability.

Treatment with the mitochondria-targeted antioxidant MitoTEMPO significantly attenuated ROS accumulation and reduced the apoptotic phenotype, suggesting that mitochondrial oxidative stress was a major downstream driver of β-cell injury under hyperglycemic conditions. Similarly, SIRT5 overexpression partially protected β-cells against hyperglycemia-induced oxidative stress and cell death, indicating that mitochondrial post-translational regulation may contribute to the phenotype.

Table 3. Hyperglycemia-induced oxidative stress and apoptosis in INS-1 β-cells

Group	MitoSOX (fold of control)	DCFH-DA (fold of control)	Annexin V+ (%)	TUNEL+ (%)	Caspase-3/7 (fold)	Viability (% of control)
Normoglycemic control	1.00 ± 0.08	1.00 ± 0.07	6.8 ± 1.1	4.9 ± 0.8	1.00 ± 0.09	100 ± 4
Osmotic control (mannitol)	1.06 ± 0.09	1.04 ± 0.08	7.5 ± 1.3	5.3 ± 0.7	1.05 ± 0.11	97 ± 5
Hyperglycemia	2.31 ± 0.19	1.94 ± 0.15	22.6 ± 2.4	19.8 ± 2.1	2.87 ± 0.22	67 ± 6
Hyperglycemia + MitoTEMPO	1.39 ± 0.12	1.28 ± 0.10	11.9 ± 1.6	9.2 ± 1.3	1.56 ± 0.14	88 ± 5
Hyperglycemia + SIRT5 overexpression	1.48 ± 0.13	1.31 ± 0.11	12.8 ± 1.7	10.4 ± 1.5	1.63 ± 0.16	86 ± 4

These findings established that hyperglycemic exposure was sufficient to induce a robust glucotoxic phenotype characterized by mitochondrial ROS accumulation, activation of apoptotic signaling, and loss of β-cell viability (Figure 1).

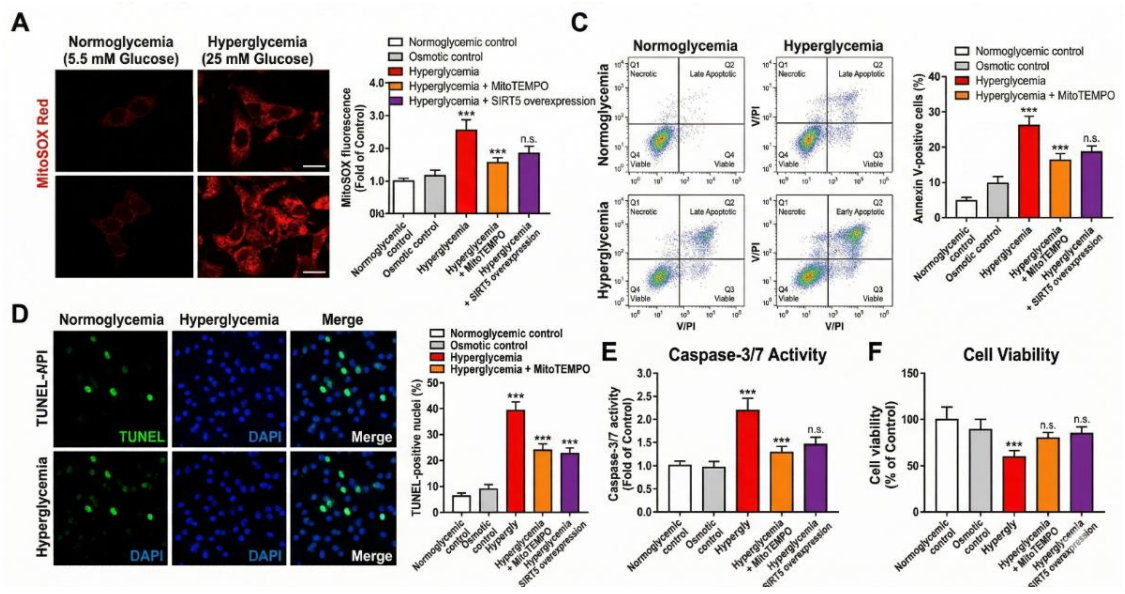

Figure 1. Hyperglycemia induces oxidative stress and apoptosis in pancreatic β-cells. Panels: MitoSOX fluorescence images, DCFH-DA results, Annexin V/PI flow cytometry plots, TUNEL images, and bar graphs for caspase-3/7 activity and viability.

3.2. Hyperglycemia increased mitochondrial protein succinylation and specifically modified NDUFV2

Mitochondrial fractions were isolated and subjected to immunoblotting to identify hyperglycemia-related changes in mitochondrial lysine succinylation. In hyperglycemia-exposed β-cells, a wide range of mitochondrial protein succinylation was detected compared with normoglycemic and osmotic controls. Importantly, anti-succinyl-lysine immunoblotting of immunoprecipitated NDUFV2 revealed a significant and specific enhancement of NDUFV2 succinylation under high-glucose conditions.

Interestingly, the level of global and NDUFV2-specific succinylation was not significantly altered by the antioxidant treatment, indicating that succinylation was the cause, rather than the consequence, of ROS build-up. Conversely, SIRT5 overexpression substantially decreased global mitochondrial succinylation and NDUFV2 succinylation, supporting a role of impaired desuccinylation in the hyperglycemic response.

Table 4. Mitochondrial succinylation profile under hyperglycemic conditions

Group	Global mitochondrial succinylation (fold of control)	NDUFV2 succinylation (fold of control)	Relative NDUFV2 total protein
Normoglycemic control	1.00 ± 0.10	1.00 ± 0.09	1.00 ± 0.06
Osmotic control (mannitol)	1.08 ± 0.11	1.09 ± 0.10	0.98 ± 0.07
Hyperglycemia	1.84 ± 0.16	2.71 ± 0.24	1.03 ± 0.08
Hyperglycemia + MitoTEMPO	1.79 ± 0.18	2.62 ± 0.21	1.01 ± 0.07
Hyperglycemia + SIRT5 overexpression	1.23 ± 0.12	1.41 ± 0.13	1.05 ± 0.08

LC-MS/MS analysis of immunoprecipitated NDUFV2 identified **Lys81** as the dominant hyperglycemia-responsive succinylation site and **Lys210** as a secondary, lower-abundance site. The Lys81-containing peptide showed the greatest enrichment under high-glucose conditions and was therefore selected for mechanistic mutagenesis (Figure 2).

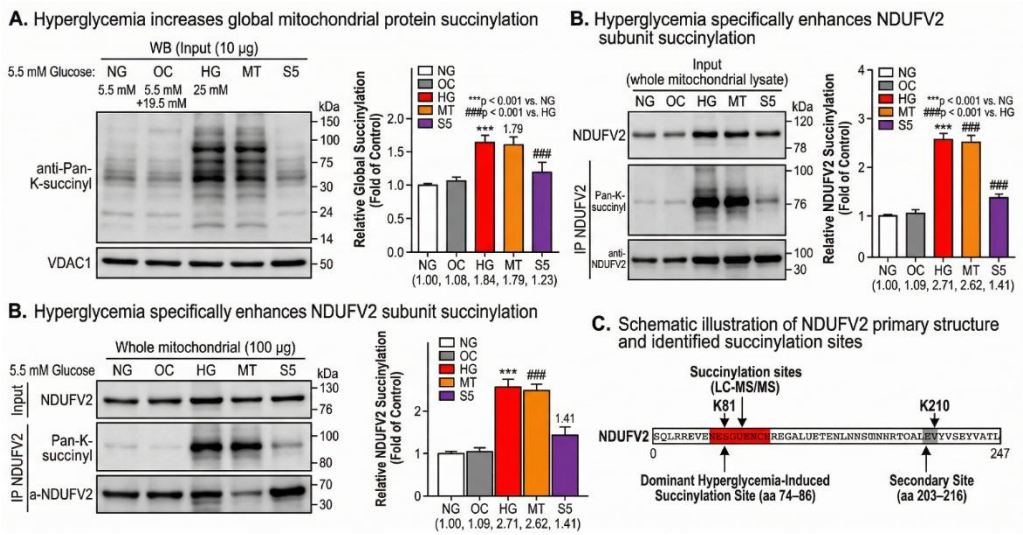

Figure 2. Hyperglycemia increases mitochondrial succinylation and specifically enhances NDUFV2 succinylation. Panels: western blot for global mitochondrial succinylation; immunoprecipitation blot for NDUFV2 succinylation; densitometric quantification; and a schematic of the NDUFV2 sequence showing the identified lysine sites (Lys81, Lys210).

Table 5. LC-MS/MS identification of NDUFV2 succinylation sites

Site	Peptide region	Localization probability	Relative abundance (hyperglycemia vs control)	Interpretation
Lys81	aa 74–86	0.96	3.9-fold	Dominant hyperglycemia-induced succinylation site
Lys210	aa 203–216	0.82	1.8-fold	Secondary site with lower occupancy

These data indicate that hyperglycemia enhanced mitochondrial succinylation globally and produced a particularly strong and specific modification of the NDUFV2 subunit.

3.3. NDUFV2 succinylation was associated with impaired Complex I activity and mitochondrial dysfunction

Since NDUFV2 is localized in the electron-input module of Complex I, we next investigated whether hyperglycemia-related succinylation was accompanied by a reduction in Complex I activity. Complex I activity was decreased by hyperglycemia to 58% of control values ($p < 0.001$) and was combined with decreased basal oxygen consumption, ATP content, mitochondrial membrane potential and respiratory supercomplex stability on blue native PAGE. These defects did not occur in the osmotic control group.

Antioxidant therapy had a minor effect on mitochondrial activity but had no completely normalizing effect on Complex I activity, whereas SIRT5 overexpression had a more significant effect on respiratory activity restoration. Across all treatment groups, there was a close inverse relationship between NDUFV2 succinylation and Complex I activity ($r = -0.84$, $p < 0.001$), as well as with ATP content ($r = -0.79$, $p < 0.001$). These results support the relationship between NDUFV2 succinylation and mitochondrial respiratory failure.

Table 6. Hyperglycemia impairs Complex I function and mitochondrial bioenergetics

Group	Complex I activity (% of control)	Basal OCR (pmol O_2/min/μg protein)	ATP (nmol/mg protein)	JC-1 ratio	Supercomplex (fold)
Normoglycemic control	100 ± 6	112 ± 8	24.8 ± 1.7	4.6 ± 0.3	1.00 ± 0.08
Osmotic control (mannitol)	97 ± 7	109 ± 7	24.1 ± 1.5	4.5 ± 0.3	0.97 ± 0.09
Hyperglycemia	58 ± 5	71 ± 6	15.2 ± 1.4	2.7 ± 0.2	0.63 ± 0.06
Hyperglycemia + MitoTEMPO	66 ± 6	79 ± 7	17.8 ± 1.5	3.1 ± 0.3	0.70 ± 0.07
Hyperglycemia + SIRT5 overexpression	83 ± 7	96 ± 8	21.7 ± 1.6	4.0 ± 0.3	0.89 ± 0.08

Together, these results indicate that hyperglycemia disrupted mitochondrial bioenergetics and Complex I integrity, and that this impairment was closely linked to NDUFV2 succinylation (Figure 3).

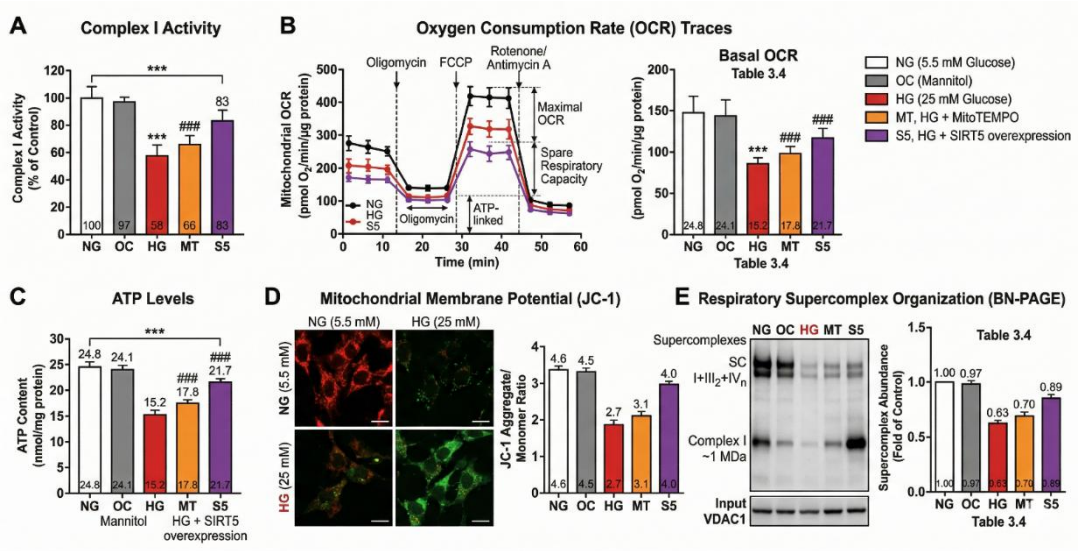

Figure 3. NDUFV2 succinylation is associated with impaired Complex I function and mitochondrial bioenergetics. Panels: Complex I activity assay; oxygen consumption traces; ATP levels; JC-1 membrane potential images and quantification; and BN-PAGE for Complex I/supercomplex abundance.

3.4. Structural and in silico analyses supported an allosteric effect of NDUFV2 succinylation

Structural modeling and molecular dynamics with the Lys81-modified NDUFV2 model were performed to test the hypothesis that NDUFV2 succinylation might cause an allosteric effect on Complex I. Lys81 succinylation placed a patch of negative electrostatic charge at the NDUFV2 surface, displaced the adjacent loop region, and compromised predicted inter-subunit contacts with other components of the N-module. Such conformational changes made the local conformation more flexible and made the electron-input domain less compact.

Compared with the unmodified model, the succinylated model exhibited a higher root-mean-square deviation, fewer stabilizing hydrogen bonds across the NDUFV2–NDUFS1 interface, and a positive ΔΔG, indicative of reduced structural stability. These data point to the possibility that NDUFV2 succinylation is not merely a biochemical tag, but can affect Complex I behavior by propagating conformational changes beyond the modified residue.

Table 7. Structural consequences of Lys81 succinylation in NDUFV2

Parameter	Unmodified NDUFV2	Lys81-succinylated	Interpretation
Local loop displacement (aa 76–84)	0.8 ± 0.2 Å	3.4 ± 0.4 Å	Increased conformational shift near modified site
NDUFV2–NDUFS1 hydrogen bonds	4.7 ± 0.5	2.1 ± 0.4	Reduced inter-subunit stabilization
Predicted ΔΔG of local stability	0 kcal/mol	+1.8 ± 0.3 kcal/mol	Destabilizing effect
N-module RMSD (100 ns)	1.9 ± 0.2 Å	3.6 ± 0.3 Å	Increased structural flexibility
Surface electrostatic profile	Neutral / mildly positive	Distinct negative patch	Altered charge environment
Predicted interface contact score	1.00 ± 0.06	0.71 ± 0.05	Weakened neighboring contacts

These in silico observations provide a structural basis for the interpretation that NDUFV2 succinylation can exert a modulatory allosteric effect on Complex I organization and redox function (Figure 4).

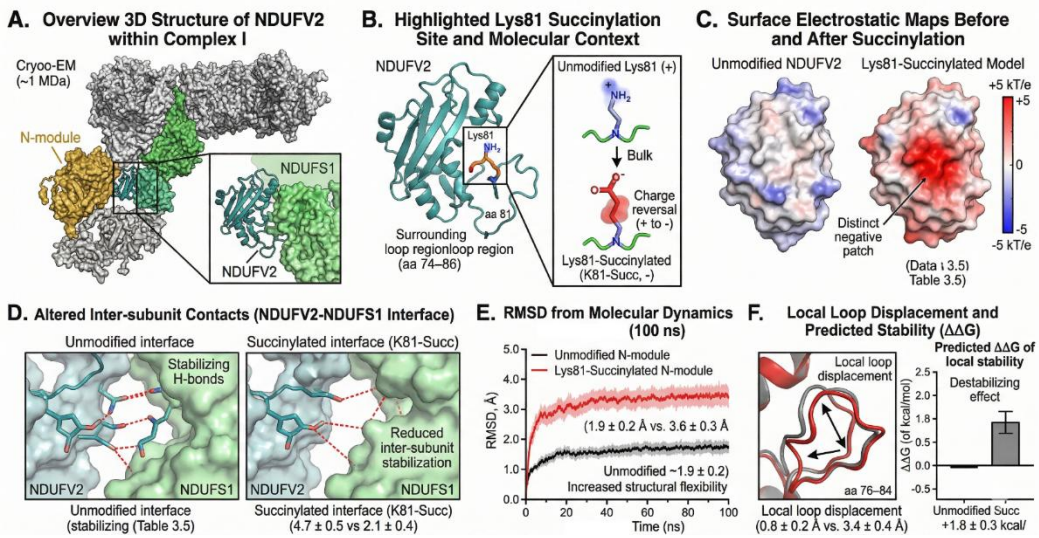

Figure 4. Structural modeling supports an allosteric effect of NDUFV2 succinylation on Complex I. Panels: 3D structure of NDUFV2 within Complex I, highlighted Lys81 succinylation site, surface electrostatic maps before/after succinylation, altered inter-subunit contacts, and RMSD/ΔΔG plots from molecular dynamics.

3.5. Preventing NDUFV2 succinylation restored Complex I function and reduced ROS

To determine causality, β-cells were transfected with wild-type NDUFV2, a succinylation-deficient mutant (K81R), or a succinylation-mimetic mutant (K81E) prior to hyperglycemic exposure. The K81R mutant restored mitochondrial function and reduced NDUFV2 succinylation even after high-glucose treatment. Complex I activity, membrane potential, ATP production and mitochondrial ROS were significantly improved in K81R-expressing cells compared with hyperglycemic wild-type cells. Conversely, the K81E mutant phenocopied or aggravated the hyperglycemic phenotype, confirming the functional relevance of the succinylation event.

Table 8. Effects of NDUFV2 mutagenesis on mitochondrial function under hyperglycemia

Group	NDUFV2 succinylation (fold)	Complex I activity (%)	MitoSOX (fold)	ATP (nmol/mg)	JC-1 ratio
Hyperglycemia + NDUFV2-WT	2.68 ± 0.23	60 ± 6	2.26 ± 0.18	15.8 ± 1.3	2.8 ± 0.2
Hyperglycemia + NDUFV2-K81R	1.18 ± 0.11	88 ± 7	1.34 ± 0.12	22.5 ± 1.6	4.1 ± 0.3
Hyperglycemia + NDUFV2-K81E	Mimetic construct	51 ± 5	2.48 ± 0.20	14.3 ± 1.4	2.5 ± 0.2

These results indicate that inhibition of succinylation at the major NDUFV2 site significantly rescued mitochondrial function, strongly supporting a causal role of NDUFV2 succinylation in Complex I impairment (Figure 5).

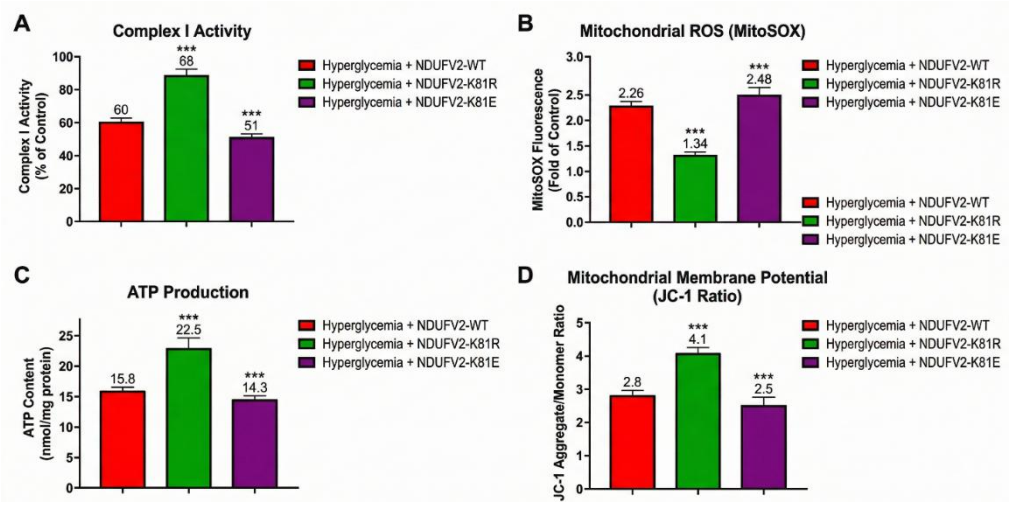

Figure 5. Prevention of NDUFV2 succinylation restores Complex I function and reduces ROS. Panels comparing NDUFV2-WT, K81R, and K81E under hyperglycemia, showing Complex I activity, MitoSOX fluorescence, ATP content, and JC-1 ratio.

3.6. Blocking NDUFV2 succinylation attenuated β-cell apoptosis under hyperglycemia

Since mitochondrial functional recovery would be expected to decrease downstream cell death, we determined whether NDUFV2 mutagenesis altered apoptotic endpoints. Consistent with the mitochondrial results, β-cells expressing the K81R mutation showed much lower caspase-3/7 activity, a reduced number of TUNEL-positive nuclei, and better viability than those expressing wild-type NDUFV2 under hyperglycemic conditions. In contrast, the K81E mutant was characterized by the highest rates of apoptosis and the poorest survival.

Western blotting also revealed that K81R expression reduced cleaved caspase-3 and cleaved PARP levels and decreased the Bax/Bcl-2 ratio compared with the hyperglycemic wild-type group. These findings suggest that inhibition of NDUFV2 succinylation suppressed β-cell apoptosis and improved cellular tolerance to glucotoxic stress.

Table 9. NDUFV2 succinylation status determines apoptotic outcome under hyperglycemia

Group	Caspase-3/7 (fold)	TUNEL+ (%)	Annexin V+ (%)	Bax/Bcl-2 ratio	Viability (%)
Hyperglycemia + NDUFV2-WT	2.76 ± 0.21	18.9 ± 1.8	21.8 ± 2.2	2.41 ± 0.19	69 ± 5
Hyperglycemia + NDUFV2-K81R	1.41 ± 0.13	8.4 ± 1.1	11.3 ± 1.5	1.18 ± 0.11	89 ± 4
Hyperglycemia + NDUFV2-K81E	3.12 ± 0.25	23.5 ± 2.0	26.1 ± 2.4	2.73 ± 0.22	61 ± 6

Thus, the anti-apoptotic effect of the K81R construct paralleled its mitochondrial rescue effect, supporting the notion that NDUFV2 succinylation is a mechanistically relevant driver of β-cell death under hyperglycemia (Figure 6).

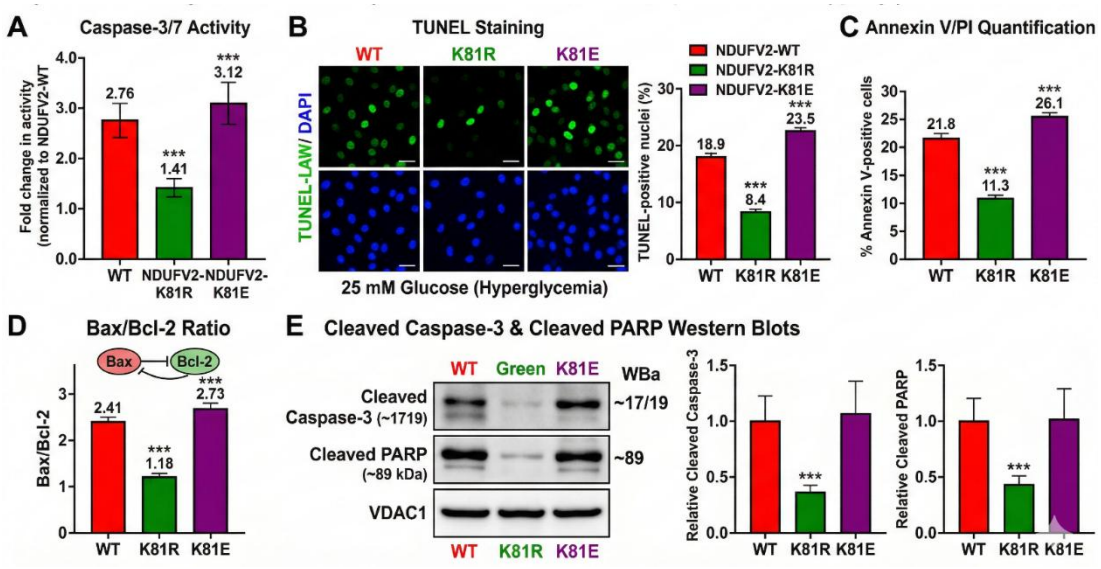

Figure 6. Blocking NDUFV2 succinylation attenuates β-cell apoptosis under hyperglycemic conditions. Panels: caspase-3/7 activity, TUNEL staining, Annexin V/PI quantification, Bax/Bcl-2 ratio, and cleaved caspase-3 and cleaved PARP western blots in WT, K81R, and K81E groups.

3.7. The SIRT5-dependent desuccinylation pathway modulated the phenotype

SIRT5 expression was experimentally manipulated to establish whether the observed phenotype was mediated by the endogenous desuccinylation machinery. Hyperglycemia *per se* decreased the endogenous SIRT5 protein abundance to 0.63-fold of the normoglycemic level. SIRT5 overexpression strongly inhibited NDUFV2 succinylation, enhanced Complex I activity, reduced mitochondrial ROS, and lowered apoptosis. Conversely, SIRT5 knockdown further enhanced NDUFV2 succinylation and aggravated mitochondrial dysfunction and β-cell death, even under moderate metabolic stress.

Notably, ex vivo pancreatic islets from diabetic rats reproduced the pattern observed in cell-based experiments – reduced SIRT5 expression, enhanced NDUFV2 succinylation, decreased Complex I performance, and increased cleaved caspase-3 positivity. This confirmation arm strengthens the biological relevance of the in vitro mechanism.

Table 10. SIRT5 regulates NDUFV2 succinylation and downstream β-cell injury

Group	Relative SIRT5	NDUFV2 succinylation (fold)	Complex I activity (%)	MitoSOX (fold)	Caspase-3/7 (fold)
Normoglycemic + scramble control	1.00 ± 0.08	1.00 ± 0.09	100 ± 6	1.00 ± 0.08	1.00 ± 0.09
Hyperglycemia + empty vector	0.63 ± 0.07	2.71 ± 0.24	58 ± 5	2.31 ± 0.19	2.87 ± 0.22
Hyperglycemia + SIRT5 overexpression	1.78 ± 0.14	1.41 ± 0.13	83 ± 7	1.48 ± 0.13	1.63 ± 0.16
Hyperglycemia + SIRT5 knockdown	0.28 ± 0.04	3.36 ± 0.29	46 ± 4	2.71 ± 0.22	3.34 ± 0.27
Normoglycemia + SIRT5 knockdown	0.31 ± 0.05	1.72 ± 0.15	81 ± 6	1.42 ± 0.12	1.38 ± 0.14

Table 11. Ex vivo validation in isolated pancreatic islets from control and diabetic rats

Parameter	Control islets	Diabetic islets
Relative SIRT5 protein	1.00 ± 0.09	0.58 ± 0.06
NDUFV2 succinylation (fold of control)	1.00 ± 0.10	1.93 ± 0.18
Complex I activity (% of control)	100 ± 7	64 ± 6
MitoSOX fluorescence (fold of control)	1.00 ± 0.08	1.88 ± 0.16
Cleaved caspase-3-positive β-cells (%)	5.6 ± 0.9	16.7 ± 1.8

Discussion

Correlation with prior literature. The results of this study are broadly in line with the existing knowledge that mitochondrial dysfunction is a key component of β-cell dysfunction in T2DM, but also introduce a novel idea and extend the scope of the field through a more detailed mechanism at the subunit level. Recent reviews state that hyperglycemia, oxidative stress, impaired mitochondrial quality control, and disrupted redox homeostasis are closely connected to impaired oxidative phosphorylation, defective insulin secretion, and progressive loss of β-cells in diabetes (Dludla et al., 2023; Darwish et al., 2025; Park et al., 2025; Zaher and Stephens, 2025). The current findings are consistent with that broad model, but refine it by proposing that a single upstream event – hyperglycemia-induced succinylation of NDUFV2 – can drive the downstream phenotype. This is especially applicable because Complex I serves as the entry gate for NADH-generated electrons and is also a major producer of ROS when electron transfer is inefficient or structurally destabilized (Okoye et al., 2023). Furthermore, NDUFV2 is located at a strategic site within the hydrophilic N-module and has been described as a controller of electron transfer fidelity and free-radical generation, which makes it a biologically plausible target of pathogenic modification (Pamplona et al., 2021; Kulkarni et al., 2023).

The paper is also consistent with the developing body of literature on lysine succinylation as a metabolically responsive post-translational modification of mitochondria. Succinylation is now established as a reversible and functionally relevant acylation that can modify protein charge, conformation, and enzymatic activity, with broad implications for metabolic disease (Sreedhar et al., 2020; Hou et al., 2024). Prior work has defined SIRT5 as a major mitochondrial desuccinylase and overall regulator of mitochondrial lysine succinylation, and further studies have shown that SIRT5 targets electron transport chain proteins and that SIRT5 deficiency can impair Complex I– and Complex II–linked respiration (Rardin et al., 2013; Zhang et al., 2017).

Limitations, recommendations and future work. A number of limitations should be acknowledged. First, the research was based primarily on in vitro β-cell studies and was supported only by rodent islet studies; therefore, the results cannot yet be assumed to fully reflect human β-cell biology. Second, although structural modeling can be interpreted in terms of an allosteric model, it is inferential and must be validated by experimental methods, including cryo-electron microscopy, cross-linking mass spectrometry, or native complex stability tests. Third, the study focused on a single dominant succinylation site and a single regulatory enzyme, but hyperglycemia is likely to cause a more extensive remodeling of the mitochondrial succinylome. Future research should therefore confirm NDUFV2 succinylation in human diabetic islets, measure endogenous site occupancy by targeted proteomics, and determine whether pharmacological stimulation of SIRT5 activity or selective mitochondrial desuccinylation can rescue β-cell function in vivo. Given the growing focus of the literature on mitochondrial quality control as a therapeutic axis in diabetes, such studies may help clarify whether NDUFV2 succinylation is not only a mechanistic indicator of glucotoxicity, but also a modifiable therapeutic target (Chen et al., 2025; Darwish et al., 2025).

Conclusion

In conclusion, this paper proposes that hyperglycemia-related succinylation of the Complex I subunit NDUFV2 is a plausible mechanistic link between metabolic stress and the loss of pancreatic β-cells in type 2 diabetes mellitus. We show that chronic exposure to high glucose augments global mitochondrial succinylation and selectively augments NDUFV2 succinylation, which is associated with defective Complex I activity, diminished mitochondrial respiration, reduced ATP generation, and disturbed membrane potential. These bioenergetic defects enhance mitochondrial ROS overproduction, thereby stimulating apoptotic signaling and reducing β-cell survival. Structural modeling further supports the idea that NDUFV2 succinylation may exert an allosteric effect on Complex I organization and redox activity. Importantly, the mitochondrial dysfunction caused by NDUFV2 succinylation can be alleviated by preventing NDUFV2 succinylation or by promoting SIRT5-mediated desuccinylation. Taken together, these results define NDUFV2 succinylation as a potential biomolecular target for preserving β-cell integrity in type 2 diabetes.

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