

NOX2-Mediated Oxidative Stress Drives β -Cell Apoptosis and Insulin Secretory Dysfunction in an Experimental Streptozotocin-Induced Diabetic Rat Model

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Abstract

Background: Type 2 diabetes (T2D) is characterized by the combination of insulin resistance and progressive β -cell dysfunction, largely driven by oxidative stress. The present study addresses the β -cell oxidative-injury component of this process using a streptozotocin (STZ)-induced diabetic rat model, which reproduces glucotoxic β -cell damage but, in the absence of demonstrated peripheral insulin resistance, does not capture the insulin-resistance component of full T2D. NADPH oxidase 2 (NOX2) has recently emerged as a critical source of reactive oxygen species (ROS) in pancreatic β -cells, but its quantitative contribution to apoptosis and insulin secretory failure remains incompletely defined. **Objective:** To determine the contribution of NOX2 to β -cell apoptosis under glucotoxic stress and to evaluate the protective efficacy of broad-spectrum pharmacological NOX2-targeting agents. **Methods:** Isolated pancreatic islets from streptozotocin-induced diabetic rats ($n = 24$) were exposed to graded glucose concentrations (5.5 - 25 mM) with or without apocynin or diphenyleneiodonium (DPI). NOX2 and caspase-3 levels were quantified by ELISA in islet lysates, normalized to total protein content, and



insulin secretion was measured in culture supernatants. **Results:** Hyperglycemia induced a dose-dependent increase in NOX2 and caspase-3 expression, with a strong positive correlation between NOX2 concentration and caspase-3 concentration ($r = 0.93$; $p < 0.001$) and an inverse correlation between caspase-3 and insulin secretion ($r = -0.95$; $p < 0.001$). When apocynin and DPI were compared against untreated, glucose-matched controls at 15 μM , NOX2 and caspase-3 were consistently lower, and insulin secretion higher, in treated islets ($p < 0.05$ for 26 of 27 pairwise comparisons). **Conclusion:** NOX2 is a central determinant of β -cell apoptosis and insulin dysfunction in this experimental STZ-diabetic rat model. Broad-spectrum pharmacological perturbation of NOX2 activity attenuates oxidative stress-induced damage and represents a promising avenue for preserving β -cell mass, warranting further validation in models that also capture the insulin-resistance component of type 2 diabetes.

Keywords

NOX2, Oxidative Stress, β -Cell Apoptosis, Insulin Secretion, Streptozotocin-Induced Diabetes, Pharmacological Inhibition

1. Introduction

Type 2 diabetes (T2D) remains one of the most pressing global health challenges, currently affecting more than 463 million people worldwide, with projections indicating a continued rise over the coming decades [1] [2]. The disease results from the interplay of two mechanisms: insulin resistance in peripheral tissues and a progressive decline in pancreatic β -cell function, which together sustain persistent hyperglycemia and drive the onset of chronic metabolic complications [3]-[5].

Pancreatic β -cells, located within the islets of Langerhans, are central to glyce-mic regulation through the glucose-responsive production and release of insulin. Their intrinsically limited antioxidant capacity, however, leaves them particularly exposed to oxidative stress once hyperglycemia becomes chronic [6]-[8]. This redox imbalance accelerates β -cell apoptosis, which in turn worsens insulin deficiency and reinforces a vicious cycle that propels T2D forward [9]-[11].

Among the several sources of reactive oxygen species (ROS) implicated in this process, NADPH oxidase 2 (NOX2) has emerged as a particularly important contributor. First characterized in phagocytic immune cells, NOX2 is now known to be expressed in pancreatic β -cells as well, where glucotoxic conditions trigger its upregulation and amplify both ROS output and apoptotic signaling [12]-[14]. Several NOX isoforms, including NOX1, NOX2 and NOX4, have been detected in native pancreatic islets, and functional studies using isoform-specific knockout models have identified NOX2 as a predominant contributor to glucose-induced ROS production in β -cells [15]. Downstream of this cascade, caspase-3 operates as a key executioner of programmed cell death, and its expression correlates

closely with the severity of β -cell secretory failure [16]–[18].

Pharmacological agents such as apocynin and diphenyleneiodonium (DPI) have been widely used to probe NOX2 activity in experimental settings [19], although neither compound is NOX2-selective: DPI is a broad flavoprotein inhibitor that also affects other flavin-dependent enzymes, and apocynin has documented off-target antioxidant and enzyme-inhibitory effects at the concentrations commonly used experimentally [20] [21]. These agents are therefore best regarded as broad pharmacological perturbants of the NOX2 pathway rather than as NOX2-specific inhibitors, yet the quantitative relationship linking NOX2 upregulation, caspase-3-mediated apoptosis, and β -cell dysfunction has not been fully established. Clarifying this relationship is a necessary step toward validating NOX2 as a therapeutic target and toward assessing whether modulating its activity could realistically help preserve β -cell mass.

The present study addresses this gap by examining the role of NOX2 in glucotoxicity-induced β -cell apoptosis and by testing the protective potential of apocynin and DPI. Using isolated pancreatic islets from streptozotocin-induced diabetic rats, we assessed NOX2 concentration, caspase-3 concentration, and insulin secretion together, offering an integrated view of the molecular cascade connecting oxidative stress to β -cell failure. It should be noted that this study uses a single-dose STZ-induced diabetic rat model, which reproduces glucotoxic β -cell injury but, without demonstrated peripheral insulin resistance, does not fully recapitulate the T2D phenotype; findings are therefore most directly generalizable to the oxidative β -cell injury component shared by STZ-diabetes and T2D, rather than to T2D as a whole.

2. Materials and Methods

2.1. Experimental Model and Animals

Twenty-four male Wistar rats (8 - 10 weeks old, 250 - 300 g) were obtained from the breeding facility of the Faculty of Science and Technology, Marien Ngouabi University (Congo). Animals were housed under controlled conditions ($22^{\circ}\text{C} \pm 2^{\circ}\text{C}$; $55\% \pm 10\%$ humidity; 12 h light/dark cycle) with free access to standard chow and water.

Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 50 mg/kg body weight), a protocol that predominantly causes direct β -cell destruction and insulin-deficient hyperglycemia rather than the peripheral insulin-resistant phenotype that defines type 2 diabetes; no insulin-resistance testing (e.g., insulin tolerance test, HOMA-IR) was performed. Animals are therefore referred to throughout this manuscript as STZ-diabetic rats, and not as a type 2 diabetes model. Persistent fasting hyperglycemia > 250 mg/dL was used as the diagnostic criterion for successful induction.

2.2. Isolation and Culture of Pancreatic Islets

After anesthesia with isoflurane (5% induction, 2% maintenance), animals were

sacrificed by decapitation. Pancreata were rapidly removed and digested with collagenase P (0.5 mg/mL, 15 - 20 min, 37°C). Islets were purified by differential centrifugation on a Ficoll gradient, washed in HBSS, and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 µg/mL).

For each rat, approximately 10 islets were isolated. Twenty-four rats were allocated in three equal cohorts of eight to the three sub-experiments described in Section 2.3 (glucose dose-response, Sections 3.1 - 3.3; inhibitor dose-ranging, Section 3.4; and matched-control comparison, Section 3.5). Within each sub-experiment, islets from a given rat were distributed across the treatment conditions of that sub-experiment, rather than assigned in full to a single condition; the rat, and not the individual islet, was therefore treated as the independent biological unit for statistical analysis (Section 2.6), with islet-level measurements averaged within each rat/condition prior to group-level comparison. Islets were incubated for 24 h at 37°C (5% CO₂, saturated humidity) to allow recovery before experimental stimulation.

2.3. Experimental Design

Islets were exposed to different glucose concentrations representing normoglycemia (5.5 mM), moderate hyperglycemia (15 mM), and severe hyperglycemia (25 mM). To assess the effect of pharmacological NOX2 perturbation, islets were pretreated for 30 min with apocynin or diphenyleneiodonium (DPI) at 5, 15, or 25 µM (final DMSO ≤ 0.1% v/v). A vehicle-only control (DMSO ≤ 0.1% v/v, no apocynin/DPI) was included at each glucose concentration. Eight rats were allocated to this sub-experiment; islets pooled from these animals were processed into three independent biological islet preparations per condition, consistent with the biological triplicate design described in Section 2.6.

A second, independent experiment was carried out to test protection against glucotoxicity directly, rather than dose-response alone. Islets were exposed to normoglycemic (5.5 mM), moderately hyperglycemic (15 mM), or severely hyperglycemic (25 mM) medium and randomly assigned to one of four arms: an untreated, glucose-matched, vehicle-only (DMSO ≤ 0.1% v/v) control, apocynin (15 µM in vehicle), DPI (15 µM in vehicle), or apocynin combined with DPI (15 µM each in vehicle), with a 30 min inhibitor pretreatment as in the paragraph above. Islets were then cultured under these conditions for 48 h, and NOX2 and caspase-3 were measured in islet lysates (protein-normalized), and insulin in the culture supernatant, all collected at that endpoint (48 h); reported insulin values represent accumulated secretion over the 48 h culture period rather than an acute glucose-stimulated insulin secretion (GSIS) challenge. Including an inhibitor-free control at every glucose concentration made it possible to evaluate pharmacological NOX2 perturbation against untreated glucotoxicity, rather than only across increasing inhibitor doses. Eight rats were allocated to this sub-experiment, with islets pooled from all eight animals and processed into three independent biolog-

ical islet preparations per condition (Section 2.6).

2.4. Biochemical Analyses

2.4.1. NOX2 Quantification

NOX2 levels were measured using a specific sandwich ELISA kit (MyBiosource, San Diego, CA, USA), according to the manufacturer's protocol. Results were expressed as ng/mg total protein.

2.4.2. Caspase-3 Quantification

Caspase-3 levels were quantified with a commercial ELISA kit (SunLong Biotech, Hangzhou, China; ref. SL0152Ra), in the same islet lysates as NOX2. Concentrations were calculated from a standard curve and corrected for protein content (Bradford assay), and expressed as ng/mg protein.

2.4.3. Insulin Secretion

Culture supernatants were collected at 1 h, 24 h, and 48 h post-stimulation. Insulin concentrations were determined by rat-specific ELISA (Mercodia, Uppsala, Sweden) and expressed as ng/mg protein. Unless otherwise stated, values reported in Section 3 correspond to the 48 h collection point and represent insulin accumulated in the medium over the culture period under sustained glucose exposure, rather than an acute glucose-stimulated insulin secretion (GSIS) response to a step change in glucose concentration.

2.5. Ethical Considerations

All experimental procedures involving animals were conducted in accordance with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines and institutional regulations on animal care and use, and were approved by the Health Ethics Committee under approval number 053-25/MESRSIT/DGRST/CERSSA/-25.

2.6. Statistical Analysis

All experiments were performed in biological triplicates with technical duplicates and analyzed using GraphPad Prism 5 software (GraphPad Software, Inc., CA, USA). Data are expressed as mean $\pm$ standard deviation (SD). Group comparisons were performed using Student's t-test for pairwise comparisons, or one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Non-normally distributed data were analyzed with the Mann-Whitney or Kruskal-Wallis test, as appropriate. Correlations were assessed using Spearman's coefficient. Differences were considered statistically significant at $p < 0.05$.

Sample size was determined from preliminary data and a power analysis ($\alpha = 0.05$, power $(1 - \beta) = 0.80$, Cohen's $d \approx 0.6$), which justified the inclusion of 8 animals per group.

Because islets from a given rat were distributed across treatment conditions within a sub-experiment, islet-level measurements are not statistically independ-

ent within a rat. The rat was therefore treated as the biological experimental unit for all comparisons: islet-level values were averaged within each rat and condition prior to group-level analysis. The 24 rats were allocated in equal cohorts of eight to each of the three sub-experiments (Sections 3.1 - 3.3, 3.4, and 3.5). For the glucose dose-response experiment (Sections 3.1 - 3.3), the *n* reported in each figure legend refers directly to these 8 rats. For the two inhibitor sub-experiments (Sections 3.4 and 3.5), islets pooled from the corresponding 8-rat cohort were processed into three independent biological islet preparations per condition, consistent with the biological triplicate design noted above; this is why the figure legends for those sections report *n* = 3 biological islet preparations per condition, each preparation drawing on the shared 8-rat cohort.

3. Results

3.1. Caspase-3 Levels in Response to Glucose

ELISA analysis revealed a significant increase in caspase-3 concentration proportional to glucose concentration. Levels remained low under normoglycemic conditions (5.5 mM), whereas severe hyperglycemia (25 mM) induced a marked increase in protein expression, confirming a direct glucotoxic impact on pancreatic β -cells (**Figure 1**).

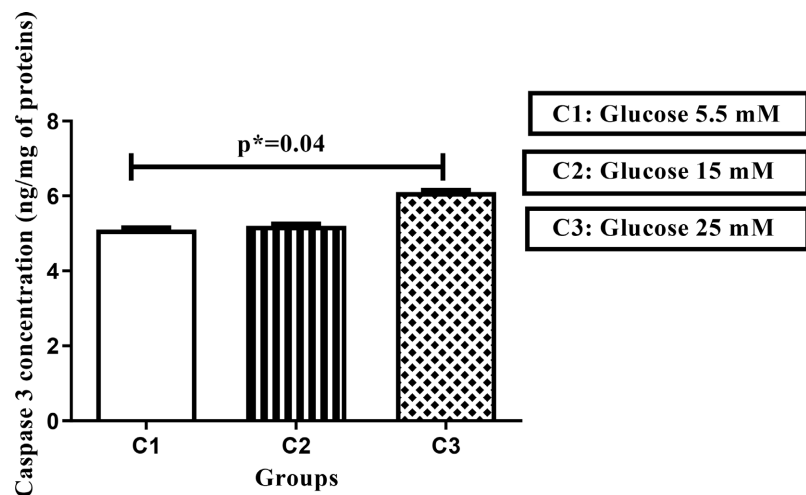


Figure 1. Caspase-3 concentration in pancreatic islets (*n* = 8 rats, 80 islets per condition, with islets from each rat distributed across the three glucose conditions). Results are expressed as mean $\pm$ standard deviation.

3.2. Modulation of NOX2 Concentration

ELISA measurements showed that NOX2 concentration increased in parallel with rising glucose concentrations, peaking at 25 mM and reflecting the heightened oxidative stress experienced by islets under hyperglycemia (**Figure 2**).

3.3. Insulin Secretion under Glucotoxic Stress

Exposure of islets to hyperglycemia resulted in a marked decrease in insulin se-

cretion. Secretion remained effective under physiological glucose conditions but progressively collapsed under severe glucotoxic stress, illustrating the functional decline of β -cells (**Figure 3**).

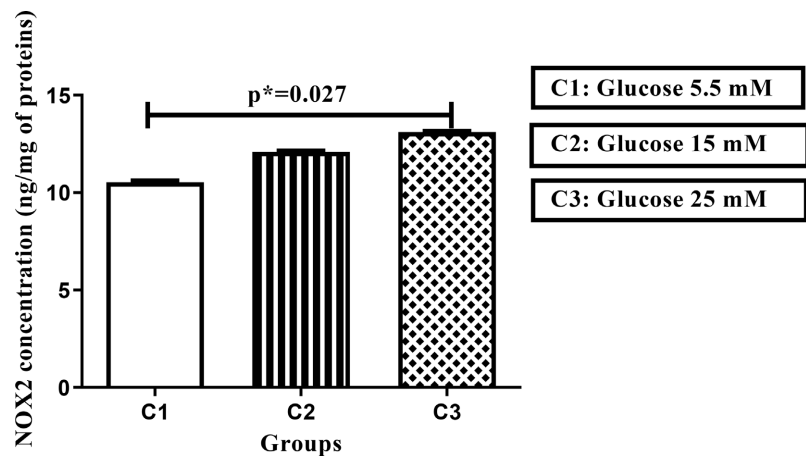


Figure 2. NOX2 concentration (ng/mg total protein) measured by ELISA in islet lysates. Means $\pm$ SD, $n = 8$ rats (80 islets per condition).

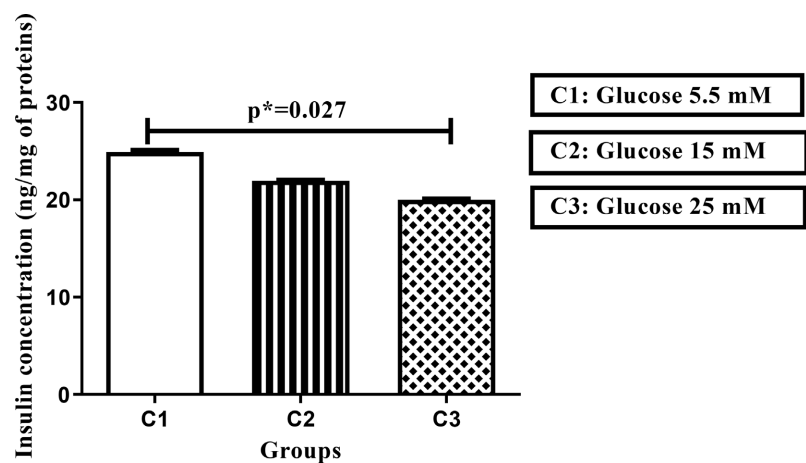


Figure 3. Effect of glucose variation on accumulated insulin secretion at 48 h. Means $\pm$ SD, $n = 8$ rats/condition (80 islets per condition).

3.4. Dose-Range Effects of Pharmacological Inhibitors on Apoptosis and Insulin Secretion

To characterize the dose-response relationship of pharmacological NOX2 perturbation, islets under severe hyperglycemia (25 mM) were pretreated with apocynin or DPI across a 5 - 25 μ M range. Both agents lowered NOX2 concentration in a clearly dose-dependent manner (**Figure 4(c)**, **Figure 4(f)**). This reduction did not, however, translate into a parallel benefit downstream. Insulin secretion declined progressively as the concentration of either agent increased (**Figure 4(b)**, **Figure 4(e)**), and caspase-3 concentration actually rose across the DPI series (**Figure 4(a)**), with only a modest decline for apocynin (**Figure 4(d)**). NOX2 was being suppressed, yet islet function was not improving, and for DPI the apoptotic

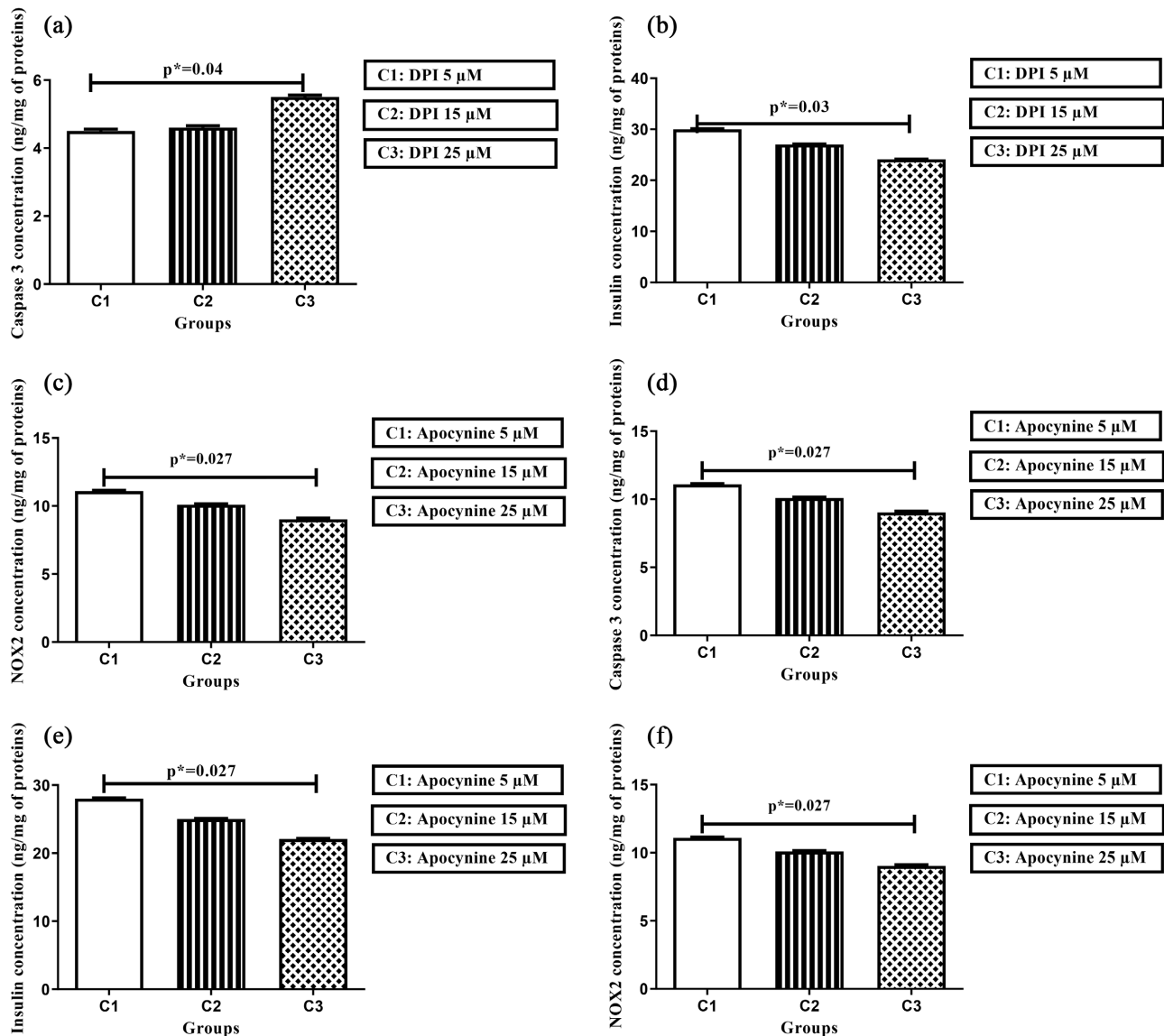


Figure 4. (a) Effect of DPI inhibitor on caspase-3 concentration. (b) Effect of DPI inhibitor on insulin secretion. (c) Effect of DPI inhibitor on NOX2 concentration. (d) Effect of apocynin inhibitor on caspase-3 concentration. (e) Effect of apocynin inhibitor on insulin secretion. (f) Effect of apocynin inhibitor on NOX2 concentration. Means $\pm$ SD, $n = 3$ biological islet preparations/condition, drawn from a cohort of 8 rats (Section 2.6). Note: this experiment compares inhibitor doses under fixed severe hyperglycemia (25 mM) and has no untreated, glucose-matched control; **Figure 5** provides that comparison.

signal appeared to worsen at the highest concentration tested. A plausible explanation is a biphasic, dose-dependent response, in which off-target or cytotoxic effects of these agents at higher concentrations offset or outweigh the benefit of NOX2 inhibition itself; this would be consistent with reports that DPI broadly inhibits other flavoproteins beyond NOX2, and that apocynin can act non-specifically above the low micromolar range [20] [21]. It should be noted that this dose-ranging experiment did not include an untreated, glucose-matched control, so on their own these data cannot establish whether any of the concentrations tested conferred net protection relative to unopposed glucotoxicity. They do, however,

define a dose range in which NOX2 suppression alone is a poor predictor of islet outcome, which is what motivated the controlled comparison described in Section 3.5.

3.5. Protective Effect of NOX2 Perturbation against Glucotoxicity under Matched Control Conditions

To test directly whether NOX2 perturbation protects islets against glucotoxicity, we ran a second, independent experiment comparing apocynin (15 μ M) and DPI (15 μ M), alone or combined, with an untreated, glucose-matched, vehicle-only control at each glucose concentration (5.5, 15, and 25 mM; $n = 3$ biological islet preparations per condition, drawn from a cohort of 8 rats (Section 2.6)), analyzed by one-way ANOVA with Tukey's post-hoc test at each glucose level, as described in Section 2.6. All three treatment arms showed lower NOX2 concentration and lower caspase-3 concentration than the untreated control at every glucose level, together with higher insulin secretion. Of the 27 pairwise comparisons against the untreated control (3 treatment arms $\times$ 3 outcome measures $\times$ 3 glucose concentrations), 26 reached statistical significance (Tukey-adjusted $p < 0.05$, and $p < 0.001$ for most); the sole exception was apocynin versus control for caspase-3 concentration at 15 mM glucose ($p = 0.14$). Combining apocynin and DPI gave numerically the strongest protection at each glucose concentration, although no formal interaction analysis was performed; this combined effect should therefore be described as more pronounced rather than as a formally demonstrated additive effect (**Figures 5(a)-(c)**). At 25 mM glucose, for instance, insulin secretion rose from 20.0 ± 0.2 ng/mg protein in untreated islets to 26.0 ± 0.2 ng/mg protein with combined treatment (Tukey-adjusted $p < 0.001$), while caspase-3 concentration fell from 6.1 ± 0.1 to 5.1 ± 0.1 ng/mg protein over the same comparison ($p < 0.001$).

Because this experiment includes the glucose-matched control missing from the dose-ranging study in Section 3.4, it provides more direct support for a genuine protective effect of NOX2 perturbation, at least at a defined, moderate

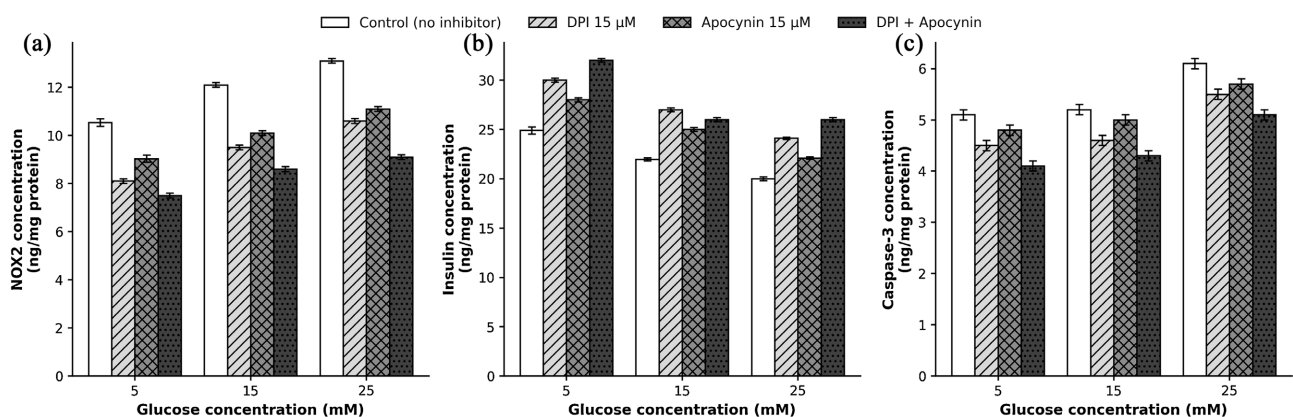


Figure 5. Effect of apocynin (15 μ M), DPI (15 μ M), and their combination on (a) NOX2 concentration, (b) insulin secretion, and (c) caspase-3 concentration, relative to untreated, glucose-matched, vehicle-only controls, at increasing glucose concentrations (5.5, 15, 25 mM). Means $\pm$ SD, $n = 3$ biological islet preparations/condition, drawn from a cohort of 8 rats (Section 2.6).

concentration (15 μ M) of either agent. It also suggests that this protection does not necessarily keep improving as the dose is increased further.

3.6. Correlations between Oxidative Stress, Apoptosis, and Secretory Function

Correlation analysis demonstrated a robust positive relationship between NOX2 concentration and caspase-3 concentration ($r = 0.93$; $p = 0.0004$), underscoring the pivotal role of oxidative stress in driving apoptotic signaling in β -cells (**Figure 6(a)**). This correlation was calculated using individual rat-level values (averaged per rat and condition, as described in Section 2.6) across the 8 rats and 3 glucose conditions of the dose-response experiment ($n = 24$ paired observations; Sections 3.1 - 3.3), rather than from only 3 condition-level means, which would be too few points to support the reported p -value. Even so, because all 8 rats contributed data at all 3 glucose levels, observations are not fully independent of one another, and the correlation should be interpreted as an association within this dose-response design rather than as a fully independent-sample effect-size estimate.

Conversely, caspase-3 concentration was strongly and inversely correlated with insulin secretion ($r = -0.95$; $p = 0.0002$) (calculated on the same $n = 24$ rat-level, per-condition observations described above), a pattern consistent with, but not proof of, a contribution of apoptosis to secretory loss. Because glucose exposure can independently drive both increased caspase-3 concentration and decreased insulin secretion, this association is best described as a treatment-associated relationship rather than as evidence that caspase-3-driven apoptosis is the principal cause of reduced insulin output (**Figure 6(b)**).

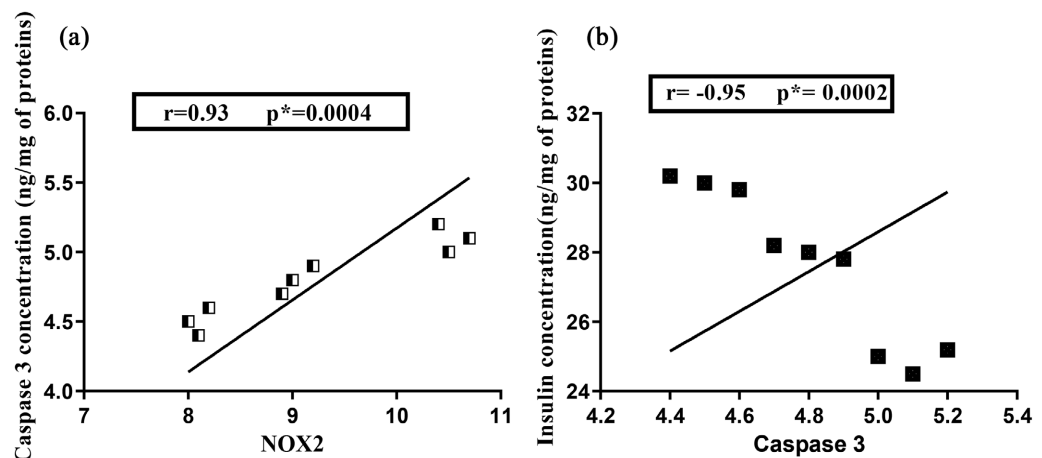


Figure 6. (a) Positive correlation between NOX2 concentration and caspase-3 concentration. (b) Inverse correlation between caspase-3 concentration and insulin secretion.

4. Discussion

This study provides quantitative evidence for the role of NADPH oxidase 2 (NOX2) in β -cell apoptosis under glucotoxic stress and demonstrates the protective effect of broad-spectrum pharmacological perturbation with apocynin and

diphenyleiiodonium (DPI). By combining measurements of NOX2 concentration, caspase-3 concentration, and insulin secretion within the same experimental system, our findings outline a pathogenic cascade linking hyperglycemia, oxidative stress, apoptosis, and β -cell dysfunction in this STZ-diabetic rat model.

A dose-dependent increase in NOX2 concentration was observed in response to increasing glucose concentrations, in line with earlier reports identifying this enzyme as a major source of ROS in β -cells [12]–[14]. The strong positive correlation between NOX2 concentration and caspase-3 concentration ($r = 0.93$) is compatible with NOX2 acting as a contributor to apoptotic intensity, extending earlier, largely qualitative, observations [22] [23], although, as noted in Section 3.6, this correlation was calculated on condition-level means and should be treated as hypothesis-generating.

Caspase-3 concentration was inversely correlated with insulin secretion ($r = -0.95$), consistent with the possibility that secretory failure in this model tracks the loss of viable β -cells, in addition to any reversible functional block, although the present correlational data cannot establish this directly (Section 3.6). This two-step pattern, an initial impairment of stimulus-secretion coupling followed by an apoptosis-driven decline in insulin output, has already been described in other experimental diabetes models [24] [25], and our data add a quantitative dimension to that picture.

Our two inhibitor experiments, considered together, help clarify the relationship between NOX2 perturbation and islet outcome. When apocynin and DPI were compared directly against untreated, glucose-matched controls at a fixed, moderate concentration (15 μ M), both agents reduced NOX2 concentration and caspase-3 concentration while increasing insulin secretion at nearly every glucose level tested (26 of 27 comparisons; Section 3.5), and their combination was numerically more protective than either agent alone, although no formal interaction analysis was performed. This is fairly direct evidence that broad-spectrum pharmacological NOX2 perturbation protects islets from glucotoxic injury, while underscoring that apocynin and DPI should be regarded as non-selective pharmacological tools rather than NOX2-specific inhibitors (Section 3.5). The picture looks different, however, when the same agents are tested across a wider dose range (5 - 25 μ M) under fixed severe hyperglycemia without a matched control: NOX2 concentration still falls progressively with dose, but insulin secretion declines in parallel, and for DPI, caspase-3 rises rather than falls at the highest concentration tested (Section 3.4). Read together, these results suggest that NOX2 perturbation is protective within a defined concentration range, but that the benefit does not simply increase with dose. At higher concentrations, off-target or cytotoxic effects of apocynin and DPI, both documented at supra-micromolar concentrations, may offset or even reverse the benefit of NOX2 suppression itself [20] [21]. This matters for how the findings should be interpreted: our data support NOX2 as a valid pharmacological target, but they also caution against assuming that more inhibition of NOX2 is automatically better for islet protection, and

they highlight why glucose-matched controls are needed when evaluating candidate NOX2 perturbants. With that caveat in mind, NOX2 perturbation at an appropriately defined dose still appears to be a credible therapeutic avenue for preserving β -cell mass, particularly earlier in the course of diabetic β -cell injury, when a meaningful reserve of functional β -cells remains [18], consistent with earlier work showing that modulating oxidative stress can slow disease progression [19].

Beyond the individual pathway components examined here, our data are compatible with a self-reinforcing cycle in which hyperglycemia sustains NOX2 concentration, which in turn perpetuates oxidative injury and apoptotic signaling, summarized schematically in the Graphical Abstract. Framing the results this way may be useful for identifying additional intervention points beyond NOX2 itself.

This study has several limitations worth acknowledging. First, isolated islets offer controlled experimental conditions but do not fully reproduce the *in vivo* pancreatic environment, including its vascular, neural, and immune components. Second, the 48 h observation window precludes any conclusion about the long-term consequences of NOX2 perturbation. Third, although apocynin and DPI remain valuable experimental tools, neither has a pharmacokinetic or safety profile currently suited to clinical use, and neither is NOX2-selective. Fourth, NOX2 and caspase-3 were quantified by concentration-based ELISA rather than by direct functional or apoptosis-specific assays such as TUNEL or Annexin V staining; future work using these complementary methods, together with a NOX2-selective genetic or pharmacological tool, would further strengthen the mechanistic specificity of these findings. Fifth, this study used a single-dose STZ-induced diabetic rat model without demonstrated insulin resistance; the findings should therefore be interpreted as applying to glucotoxic β -cell oxidative injury rather than to the full T2D phenotype, and extrapolation to human T2D, which combines insulin resistance with β -cell failure, should be made cautiously. Sixth, the correlations reported in Section 3.6 were derived from a limited number of condition-level observations and should be considered exploratory rather than confirmatory. Future work should also prioritize *in vivo* validation of these findings, longer-term assessment of NOX2 perturbation, and the development of next-generation inhibitors with improved selectivity and bioavailability. Circulating NOX2-related biomarkers could also be explored as early indicators of β -cell dysfunction, potentially opening new avenues for clinical monitoring. Finally, the dose-ranging experiment (Section 3.4) and the controlled comparison (Section 3.5) were performed on separate batches of islets, and absolute values differ somewhat between the two, which is not unexpected given normal biological variability between independent preparations. For this reason, values should be compared within each experiment rather than across them.

5. Conclusion

Taken together, our findings indicate that NOX2 is a central mediator of glucotoxic β -cell apoptosis and insulin dysfunction in this STZ-diabetic rat model.

The quantitative correlations observed between NOX2 concentration, caspase-3 concentration, and insulin secretion (interpreted with the caveats noted in Section 3.6) position this enzyme as both a candidate biomarker and a therapeutic target. At an appropriately defined and controlled dose, broad-spectrum pharmacological perturbation of NOX2 activity attenuates oxidative stress-induced damage and represents a promising avenue for preserving β -cell mass, warranting further validation in models that also capture the insulin-resistance component of type 2 diabetes, and delaying the progression of diabetic β -cell failure.

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Author Contributions

Conceptualization, F.K.H.M. and D.M.; methodology, F.K.H.M. and C.L.E.B.; software, F.K.H.M.; validation, F.K.H.M., C.L.E.B. and G.L.V.; formal analysis, F.K.H.M.; investigation, F.K.H.M., C.L.E.B., G.L.V., J.B.K.G., C.N.K., E.B., J.J.M.M., F.E.B.G., G.L.E.M. and V.A.J.A.B.; resources, D.M.; data curation, F.K.H.M.; writing original draft preparation, F.K.H.M.; writing review and editing, F.K.H.M. and D.M.; visualization, F.K.H.M.; supervision, D.M.; project administration, F.K.H.M.; funding acquisition, F.K.H.M. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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