Involvement of SIRT1 in Zn\(^{2+}\), Streptozotocin, Non-Obese Diabetic, and Cytokine-Mediated Toxicities of \(\beta\)-cells

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Abstract

Zn\(^{2+}\) toxicity is implicated in pancreatic \(\beta\)-cell death that occurs secondarily to: streptozotocin exposure in vitro, and both autoimmune attack or streptozotocin in vivo models of T1DM. This is demonstrated by reduced \(\beta\)-cell death or diabetic incidence in vitro or in NOD mice after treatment with Zn\(^{2+}\) preferring chelators, pyruvate, nicotinamide, a reduced zinc diet, sirtuin inhibitors, or zinc transporter knockout. These therapeutics are also demonstrated to be efficacious against Zn\(^{2+}\) neurotoxicity.

Aims: To determine if the sirtuin pathway is involved in Zn\(^{2+}\), streptozotocin-, or cytokine-mediated \(\beta\)-cell death in vitro, and streptozotocin-, or NOD induced T1DM in vivo.

Methods: Sensitivity of MIN6 cells expressing empty vector, sirtuin protein-1 (SIRT1) or its siRNA, to Zn\(^{2+}\), streptozotocin, or cytokines, and effects on NAD\(^+\) levels were determined. Covariance of manipulating SIRT1 levels with diabetic incidence was tested in vivo.

Results: 1) sirtuin pathway inhibition or SIRT1 knockdown attenuated Zn\(^{2+}\)-, STZ-, and cytokine-mediated toxicity and NAD\(^+\) loss in \(\beta\)-cells, 2) SIRT1 overexpression potentiated these toxicities, 3) young SIRT1 \(\beta\)-cell transgenic mice have improved glucose tolerance under basal conditions, but upon aging showed increased sensitivity to streptozotocin compared to SIRT1 +/- mice, and 4) SIRT1 +/- mice in an NOD background or exposed to streptozotocin trended toward reduced diabetic incidence and mortality compared to wildtype.

Conclusions: These results have implicated SIRT1-mediated NAD\(^+\) loss in Zn\(^{2+}\), STZ, or cytokine toxicities of MIN6, and in NOD or streptozotocin T1DM animal models. Modulation of \(\beta\)-cell Zn\(^{2+}\) and NAD\(^+\) levels, and the sirtuin pathway could be novel therapeutic targets for T1DM.

Keywords: Siruins; NAD\(^{+}\); MIN6; SIRT1 knockout

Abbreviations: BESTO: \(\beta\)-cell SIRT1 Overexpressing mice; CaEDTA: Ethylene Diamine Tetra-Acetic acid-Calcium salt; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase; HD: High-Density cultures; IFN-\(\gamma\): Interferon Gamma; IL-1\(\beta\): Interleukin-1 Beta; MEM: Minimal Essential Medium; MLDS: Multiple Low-Dose Streptozotocin; mM: Millimol/L; MTT: 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium Bromide; Nampt: Nicotinamide Phosphoribosyl Transferase; Naph: 2-hydroxynaphthaldehyde; N: Nicotinamide; NAD\(^{+}\): Nicotinamide Adenine Dinucleotide; NOD: Non-Obese Diabetic; P: Pyruvate; PARP: Poly-ADP Ribose Polymerase; PDH: Pyruvate Dehydrogenase; ROS: Reactive Oxygen Species; S: Sirtinol; siRNA: Small inhibitory RNA; SIRT1: Sirtuin protein 1; STZ: Streptozotocin; TNF-\(\alpha\): Tumor Necrosis Factor-alpha; T1DM: Type-1 Diabetes; T2DM: Type-2 Diabetes; uM: Micromol/L; Zn\(^{2+}\): Zinc; [Zn\(^{2+}\)]: Intracellular Zinc concentration; ZnT5: Zinc Transporter 5

Introduction

Type-1 diabetes (T1DM) is an autoimmune disease resulting from specific T-lymphocyte-, ROS-, and cytokine-mediated destruction of the insulin-producing \(\beta\)-cells of the islets of Langerhans resulting in dysregulation of blood glucose [1]. The development of T1DM is reduced by treatment with T-cell and cytokine inhibitors, and ROS scavengers [2]. These oxidative processes are suggested to alter the NAD+/NADH ratio and inhibit proteins involved in energy metabolism and glycolysis causing the accumulation of triosephosphates [3,4]. Inhibitors of NAD+ catabolism have been demonstrated to attenuate diabetic incidence in models of T1DM [5,6]. NAD+ loss is linked to diabetes. Heterozygous knockout of the rate limiting enzyme in NAD+ synthesis (Nampt), causes reduced insulin secretion [7]. Also, Nampt and NAD+ levels are reduced in T2DM and the aging or high-fat diet models thereof. Restoration of NAD+, by nicotinamide mononucleotide precursor supplementation, attenuates diabetes in aging and high-fat diet mouse models of T2DM [8].

We recently showed that just prior to becoming diabetic, NOD mice demonstrate increased punctate Zn\(^{2+}\) staining in islets which is attenuated by a reduced Zn\(^{2+}\) diet, or zinc transporter 5 (ZnT5) knockout. Triweekly pyruvate or nicotinamide injections, chronic treatment with a reduced zinc diet, or knockout of the zinc transporter 5 (ZnT5) gene delay onset of diabetic incidence and animal mortality by reducing pancreatic zinc and/or maintaining \(\beta\)-cell NAD+ levels and mass [9]. This complements the beneficial effects demonstrated for Zn2+ chelation against acute or multiple low dose streptozotocin exposures [10,11]. Zinc neurotoxicity in vitro or in vivo induces NAD+ loss and glycolytic inhibition resulting in increased triosephosphates and death in a manner exactly equivalent to that seen in \(\beta\)-cells. These results are...
Zn\textsuperscript{2+} and \(\beta\)-cell death

Zn\textsuperscript{2+} is present in the pancreas at the highest concentration anywhere in the body, and within the pancreas is concentrated in the secretory granules of \(\beta\)-cells [10]. In the \(\beta\)-cell, Zn\textsuperscript{2+} allows insulin processing and crystallization in the secretory granules [15,16]. Significant amounts of free Zn\textsuperscript{2+} are also released from \(\beta\)-cell secretory granules [17,18]. As \(\beta\)-cells contain most of the pancreatic Zn\textsuperscript{2+}, its toxic release, by the immune response or exposure to ROS/streptozotocin, would help explain the specificity of its toxic effects on NAD\textsuperscript{+} levels through activation of the sirtuin pathway. The NAD\textsuperscript{+} loss results in glycolytic inhibition which is prevented by restoration of NAD\textsuperscript{+} levels using nicotinamide, siRNA induction, and pyruvate, but not lactate (Figure 1). In these studies, we examined the deleterious effects that Zn\textsuperscript{2+}-mediated NAD\textsuperscript{+} loss, had in facilitating \(\beta\)-cell death. We propose that immune- and ROS-mediated dysfunction of energy metabolic pathways could be potentiated by Zn\textsuperscript{2+} release, and sirtuin-mediated loss of NAD\textsuperscript{+}. We studied the effects of SIRT1 overexpression or knockdown in MIN6 cultures on NAD\textsuperscript{+} loss and Zn\textsuperscript{2+}, STZ, or cytokine toxicities, and the effect of SIRT1 expression on the streptozotocin or NOD in vivo models of T1DM.

Experimental Procedures

Cell culture and toxicity studies

The \(\beta\)-cell line, MIN6, was used to generate stably transformed cell lines overexpressing SIRT1 (760% of control, MIN6-Sir2OE1), an siRNA to SIRT1 (resulting in 28% of control expression, MIN6-Sir2KD1), and their empty vector control lines [21]. These lines were maintained in modified Dulbecco’s medium (DMEM) + 15% supplemented bovine serum, 1% L-glutamine, 0.1% penicillin/streptomycin, 200 micromolar/L of G418, and 29 micromolar/L \(\beta\)-mercaptoethanol. Waxes were passaged at 50-70% confluent using 0.05% trypsin/0.02% EDTA, and Zn\textsuperscript{2+} loading was achieved by supplementation with 10 micromolar/L Zn\textsuperscript{2+} in the growth medium. High-density cultures (HD) were used for streptozotocin and cytokine toxicities. MIN6 cells were collected, counted, resuspended in DMEM + 15% FBS and plated at HD/low extracellular volume (7.5 x 10\textsuperscript{6} cells/L in 0.04 milliliter) in 24-well plates [10]. These HD cultures were plated in 7.5 millimolar/L streptozotocin (STZ) or a mixture of cytokines (250 microgram/L IL-1\(\beta\), 8 microgram/L TNF-\(\alpha\), and 200 microgram/L IFN-\(\gamma\)) with coexposure to the compounds tested. High cytokine levels were required due to the HD cultures and short exposure (6 h). Exposure to 15-40 micromolar/L Zn\textsuperscript{2+} in serum-free MEM, or to 200-300 micromolar/L Zn\textsuperscript{2+} in DMEM + 15% FBS under low density conditions were also utilized. Optimized concentrations of compounds were included during the toxicity exposure as indicated. 2-hydroxy naphthaldheyde (30-60 micromolar/L Naph), or sirtinol is sirtuin inhibitors; and pyruvate, nicotinamide (10-20 millimolar/L), and NAD\textsuperscript{+} (6 millimolar/L), restore NAD\textsuperscript{+} levels [13]. Cell viability was assayed at varying times later by 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium Bromide (MTT) staining (0.1% final) of individual wells of a tissue culture plate, and the absorbance at 595 nm was then measured (n = 8-20 wells of cells from at least three independent experiments). Staining with propidium iodide (2.5 milligram/L) for normal density Zn\textsuperscript{2+} toxicity studies followed by measurement of fluorescence was also used. HD cultures were only assayed by MTT because a monolayer culture is required for PI staining [22,23].

Determination of levels of NAD\textsuperscript{+}, and NADH

Measurements of NAD\textsuperscript{+} and NADH were made on cell lysates prepared immediately after 4 hr Zn\textsuperscript{2+}, STZ or cytokine exposures. Cells were washed three times to remove compounds followed by lysis in NaOH/EDTA. This lysis was split and part of it directly hydrolyzed at 80°C for 20 min, and the other part acidified followed by hydrolysis at 80°C for 20 min. Alkaline hydrolysis destroys NADH, whereas acid hydrolysis destroys NAD\textsuperscript{+} allowing for linked enzymatic cycling reactions. 2 \(\mu\)l of acid extract (~5000 cells) were added directly to 100 \(\mu\)l of NAD\textsuperscript{+} cycling reagent (100 millimolar/L Tris-HCl, pH 8.1, 2 millimolar/L \(\beta\)-mercaptoethanol, 2 millimolar/L oxaloacetate, 0.3 millimolar/L ethanol, 0.02% BSA, and yeast alcohol dehydrogenase and 0.5 \(\mu\)g/mL malic dehydrogenase) and incubated at 25°C to obtain 500 cycles of NAD\textsuperscript{+} amplification. Termination by heating at 100°C for 5 min was followed by addition of 1 \(\mu\)l of malate indicator reagent (50 millimolar/L amino-methylpropanol (pH 9.9), 5 millimolar/L L-glutamate, 0.2 millimolar/L NAD\textsuperscript{+}, 5 \(\mu\)g/mL malic dehydrogenase, and 2 \(\mu\)L glutamate oxaloacetate transaminase). This reaction was incubated for 10 min at 25°C. The NADH generated from malate was measured fluorimetrically (excitation at 365 nm, emission monitored at 460 nm) [13,24]. For NAD\textsuperscript{+} additions, a correction was made based on NAD\textsuperscript{+} addition and washout from control cultures.

SIRT1 gene expression

Total RNA from harvested MIN6 cells (10\textsuperscript{5}) exposed to 0 or 40 micromolar/L Zn\textsuperscript{2+} in serum-free MEM for 3 h was extracted as detailed [25]. The RNA concentration and integrity were verified, and reverse transcription was performed using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA) on 1 \(\mu\)g of total cellular RNA. Real-time RT-PCR specific primers for SIRT1 and \(\beta\)-actin were designed (26).

The reactions were run in duplicate with iQSYBR green Supermix (Bio-Rad) as per manufacturer’s instructions. A melt-curve analysis was

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Injured \(\beta\)-cell

Immune-Cell Response

[\(\text{Zn}\textsuperscript{2+}\)]

Zn\textsuperscript{2+}

Secretery Granules

Ca\textsuperscript{2+}

Channel

CZn\textsuperscript{2+}

Chelators

Zn\textsuperscript{2+}

NAD\textsuperscript{+}

PDH

SIRT1 siRNA

Nicotinamide

GAPDH

Pyruvate

Lactate

Figure 1: Model of zinc toxicity and prevention in Type-1 Diabetes

We hypothesize that Zn15 and Zn18 mediate Zn\textsuperscript{2+} accumulation in secretory granules. The immune cell response injures \(\beta\)-cells causing Zn\textsuperscript{2+} release from granules, and re-uptake, through Ca\textsuperscript{2+} channels, in neighboring \(\beta\)-cells. Additionally, the ROS may oxidize metallothionein causing Zn\textsuperscript{2+} release. The resulting increased [Zn\textsuperscript{2+}] may cause direct inhibition of mitochondria, and GAPDH, or their indirect inhibition by a reduction in NAD\textsuperscript{+} levels induced by the NAD\textsuperscript{+} catabolizing enzyme SIRT1. Pyruvate, nicotinamide, and sirtuin inhibition prevent NAD\textsuperscript{+} loss and glycolytic inhibition. Black = Toxic, Gray = Therapeutic.
performed at the end of each experiment to verify that a single product per primer pair was amplified, and the sizes of the amplified DNA fragments were verified. Samples were compared using the relative CT (the cycle number at the threshold level of log-based fluorescence) method. The percent increase was determined relative to an untreated control culture after normalizing to β-actin expression using 2-ΔΔCT (Livak) method.

Colony maintenance and trials

The NOD inbred mouse strain (Taconic), the β-cell SIRT1 overexpressing mice (BESTO), and the SIRT1 +/- mice were maintained at LSUHSC’s transgenic animal facility. The SIRT1 +/- and BESTO mice were backcrossed onto a C57Bl6/J background for maintenance. The BESTO mice, line 431-2, showed a 12-fold overexpression of SIRT1 predominantly in β-cells which resulted in transcriptional regulation of target genes [21]. Housing and anesthesia concurred with the institutional Animal Studies Committee guidelines, the PHS Guide for the Care and Use of Laboratory Animals, USDA Regulations, and the AVMA Panel on Euthanasia. SIRT1 heterozygous knockout animals [27] were backcrossed to NOD mice for 10 generations to syngeneity; SIRT1 +/-/NOD animals develop diabetes and mortality at equivalent ages to the parental NOD animals. Upon interbreeding heterozygous animals, SIRT1 +/- animals die peri-natally whether on an SV129 or an NOD background, but survive on a CD1 background [27,28]. This was a double blind trial (both handler and histologist were blind to genotype) of age-matched female NOD mice with either a SIRT1 +/- or a SIRT1 +/- genotype. Water and food ingestion, and body weight were monitored weekly and did not vary between groups. Fed blood glucose was monitored every Monday afternoon, and fasted blood glucose was also determined periodically (glucose oxidase). Fed and fasted blood glucose gave qualitatively similar results. Animals demonstrating continued akinesia with prodding or inability to eat and drink were euthanized and mortality recorded. MLDs was performed at 4-7 months of age (0.055 g/kg i.p. injection each day for 5 days), and blood glucose was measured on days 0, 1, 4, 7, 14, and 21 (n = 20).

Reagents

Unless otherwise stated, all reagents were from Sigma Chemical Co (St. Louis, MO).

Results

In MIN6 cultures, knockdown of SIRT1 reduced Zn2+ toxicities, and overexpression of SIRT1 potentiated Zn2+ toxicities

As shown in Figure 2, MIN6 cultures over expressing SIRT1 or an overexpression empty vector control were exposed to A) Zn2+ in the absence of serum or B) Zn2+ in the presence of serum. SIRT1 overexpression (760% of control) significantly potentiated Zn2+ toxicity at all levels of exposure in the presence or absence of serum in β-cells, and sirtuin inhibitors (sirtinol or Naph) attenuated this death. As shown in Figure 3, MIN6 cultures over expressing an siRNA to SIRT1 or an siRNA empty vector control were exposed to A) Zn2+ in the absence of serum or B) Zn2+ in the presence of serum. SIRT1 knockdown (28% of control) significantly attenuated Zn2+ toxicity at all levels of exposure in the presence or absence of serum in β-cells and sirtuin inhibitors did not further reduce this toxicity. Real-time PCR for β-cell SIRT1 expression was performed on control MIN6 cultures 3 h after exposure to 40 micromol/L Zn2+, resulting in a 39 ± 4.5% significant increase in expression relative to untreated control at P < 0.05 by student t-test (n = 6).
NAD+ was isolated and measured. Zn2+, streptozotocin, and cytokines toxicities, with nicotinamide and NAD+ having the best efficacy. In shown). These compounds each attenuated STZ and mixed cytokine inducement. Pyruvate, nicotinamide, NAD+, sirtinol, and Naph were applied at optimized concentrations as determined by dose responses (data not shown). These compounds each attenuated STZ and mixed cytokine toxicities, with nicotinamide and NAD+ having the best efficacy. In SIRT1 overexpressing MIN6 cells, 2x naphthaldehyde (60 micromol/L) was required for efficacy.

Zn2+, STZ, and cytokines reduced NAD+ levels which could be restored by SIRT1 knockdown, nicotinamide, and NAD+

MIN6 cultures were exposed to 10 millimol/L streptozotocin, 40 micromol/L Zn2+, or mixed cytokines for 4 hrs (prior to cell death); NAD+ was isolated and measured. Zn2+, streptozotocin, and cytokines induced a significant decrease in NAD+ levels and nicotinamide or NAD+ restored these levels. In addition, SIRT1 knockdown attenuated the loss of NAD+ for Zn2+ and streptozotocin (Figure 6). We previously showed that sirtuin inhibition attenuated the loss of NAD+ for Zn2+ in unmodified MIN6 cells [9].

Mice overexpressing SIRT1 in β-cells had increased susceptibility to STZ compared to SIRT1 +/-, and SIRT1 +/- bred onto an NOD background had reduced mortality

Two more BESTO mice were susceptible to multiple low dose streptozotocin exposure than their wildtype littermates (Table 1). SIRT1 knockout mice survive on a CD1 background, but are smaller and sick-
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**Discussion**

In these studies we demonstrated that: 1) sirtuin pathway inhibition attenuated Zn\(^{2+}\), STZ-, and cytokine-mediated toxicity and NAD\(^{+}\) loss in \(\beta\)-cells, 2) SIRT1 overexpression in MIN6 cells line potentiated NAD\(^{+}\) loss, and Zn\(^{2+}\), STZ, and cytokine toxicities, 3) SIRT1 knockdown using an siRNA expressing MIN6 cell line attenuated NAD\(^{+}\) loss and these toxicities, 4) diabetic incidence and mortality induced in vivo by streptozotocin or NOD, showed covariance with SIRT1 expression levels upon genetic manipulation.

Zn\(^{2+}\) is toxic to insulinoma cells and to isolated islets, and that zinc chelation and pyruvate can prevent toxicity. Zn\(^{2+}\) chelation and pyruvate attenuate hyperglycemia in the acute high dose streptozotocin model though the NAD\(^{+}\)-dependent mechanism of action was questioned [10,20]. We have utilized several in vitro and in vivo models of T1DM. These include in vitro exposure of \(\beta\)-cells to zinc, mixed cytokines, or STZ; and in vivo exposure to STZ, or the immune-mediated NOD mouse model. STZ and the NOD mouse cause selective death of insulin-secreting \(\beta\)-cells, inducing reductions in nicotinamide cofactor levels, glucose oxidation, and glucose-induced insulin secretion [29-32]. The NAD\(^{+}\) precursor, nicotinamide, reduces diabetic incidence in both the acute high dose, and the multiple low dose streptozotocin injection paradigms [33,34]. Zn\(^{2+}\) preferring chelators (CaEDTA and clioquinol) reduce diabetic incidence in the acute high dose and multiple low dose streptozotocin injection models as demonstrated by the reduction in Zn\(^{2+}\) staining, \(\beta\)-cell death and diabetic symptoms achieved [10,11,20]. We recently demonstrated that pyruvate attenuates multiple low-dose streptozotocin exposure (MLDS) induced-, and NOD-induced diabetes, and that a zinc reduced diet also attenuates NOD-induced diabetes [9].

Our studies in neurons show that an increase in intracellular Zn\(^{2+}\) causes a loss of NAD\(^{+}\) levels that may be partially mediated by sirtuin or poly-ADP ribosyl polymerase (PARP) activation depending on cell type [13,35]. The resultant decrease in the NAD\(^{+}\)/NADH ratio inhibits the energy metabolic pathway at the susceptible enzymes GAPDH and PDH. Pyruvate, nicotinamide, or exogenous NAD\(^{+}\) restore NAD\(^{+}\) levels upon genetic manipulation.
levels, and glycolytic flux, and thereby attenuate death. Pyruvate is converted to lactate regenerating NAD+ at the expense of NADH [12]. Nicotinamide induces increased synthesis of NAD+, or decreases its degradation by NAD+-catabolizing enzymes [35]. Nicotinamide is effective in diabetic models [5,36], with therapeutic effects observed only if it is given to prediabetic patients [37–40]. Its mechanism of action is not well defined, with suggestions that it prevents PARP-activation and NAD+ depletion, thereby reducing apoptosis of β-cells induced by DNA damage [5]. PARP induced NAD+ depletion is also a mechanism for GAPDH inhibition, resulting in triosephosphate accumulation, and β-cell death in diabetes [41]. However, no reduction in diabetes occurs in the NOD/PARP-/- mouse arguing against a causative role for PARP in this animal model of diabetes [42]. The ability of exogenous NAD+ to attenuate Zn2+, STZ, and cytokine toxicities in insulinoma cultures argues that NAD+ levels are involved in the mechanism. The protective effects of sirtinol and SIRT1 knockdown on NAD+ levels and beta-cell death suggested that the sirtuin pathway may be involved.

The sirtuin family (SIRT) and the pancreas

Inhibition of the sirtuin pathway attenuates Zn2+ or STZ toxicities of β-cells in part by preventing NAD+ depletion [9]; as we have also shown for Zn2+ neurotoxicity: the sirtuin pathway is involved in Zn2+-induced neuronal, and β-cell NAD+ depletion and toxicities [9,13]. The sirtuin family of proteins are NAD+-dependent protein deacetylases resulting in NAD+-catabolism, transcriptional silencing, and transcriptional regulation [43]. SIRT1 is ubiquitously expressed; within the pancreas, SIRT1 is expressed strongly in the cytoplasm of β-cells, and weakly in both the nucleus and cytoplasm of β-cells [21]. Young β-cell SIRT1 transgenic (BESTO) mice have increased glucose tolerance under basal conditions [21], but lose this effect with age [44]. This suggests that under young physiologic conditions, SIRT1 overexpression may be beneficial, but under pathophysiologic aged T1DM diabetic conditions (MLDS), SIRT1 may be detrimental perhaps due to potentiation of zinc toxicity. Sirtuins appear to mediate part of the NAD+ loss after Zn2+ and STZ exposures of MIN6 cells, as evidenced by the partial restoration of NAD+ levels by sirtuin inhibition for zinc and STZ exposures, but not for cytokine exposures [9]. Sirtuins also act through transcriptional modulation, which may be the predominant mechanism in Zn2+-neurotoxicity, and cytokine toxicity in β-cells. SIRT1 overexpression was shown to attenuate IL-1β and IFN-γ induced toxicity in RIN β-cells [45]. However, this study was not done in the presence of TNF-α, or under high-density conditions where zinc release and toxicity could play a role. Recently, SIRT1 was shown to attenuate pancreatic β-cell expansion [46], and to decrease hepatic insulin responsiveness [47]. However, the SIRT1 and AMP kinase activator, resveratrol, suppresses T-cell immune responses, and attenuates diabetic incidence in NOD mice [48]. These effects of resveratrol may be SIRT1 independent, mediated instead by oxidant scavenger or AMP kinase activation mediated mechanisms [49,50]. In age or high-fat diet models of T2DM, SIRT1 activation partially mediates the beneficial effects of NAD+ restoration in the peripheral tissues affected by T2DM (liver, WAT, skeletal muscle), though effects on the pancreas or beta-cells were not presented [8]. Similar effects in models of T1DM were not presented.

In these studies, we have implicated the sirtuin pathway and SIRT1 in the pancreatic NAD+ loss and beta-cell death induced by the in vitro models of T1DM: Zn2+, STZ, or mixed cytokine exposures. We have also suggested that SIRT1 plays a role in the diabetic incidence and mortality induced by the STZ or NOD in vivo models of T1DM. These results differ from those reported for SIRT1 on T2DM induced affects in peripheral tissues, where SIRT1 activity appears to mediate the effects of reducing or increasing NAD+ levels [8]. The roles of SIRT1 in pancreatic versus peripheral tissue, and in T1DM versus T2DM appear to be varied and complex, and will require further studies to unravel.

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Author Contributions

C.T.S. is the guarantor of this manuscript, performed all aspects of this paper, and has no conflicts to report.

References


