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ISSN 2377-8369 Open Journal http://dx.doi.org/10.17140/GOJ-1-114

Review

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Volume 1 : Issue 3
Article Ref. #: 1000GOJ1114

Article History
Received: April 24th, 2015
Accepted: July 8th, 2015
Published: July 8th, 2015

Citation
Parmar MS, Meer AA, Syed I. Biphasic roles of a small G-protein, Rac1 in pancreatic β-cell. *Gastro Open J.* 2015; 1(3): 79-88. doi: 10.17140/GOJ-1-114

Biphasic Roles of a Small G-Protein, RAC1 in Pancreatic B-Cell

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ABSTRACT

Glucose-stimulated insulin secretion (GSIS) involves cross talk between small G-proteins and their regulating factors. These interactions results in translocation of insulin-laden granules to the plasma membrane for fusion and insulin release. Vesicular transport and fusion events are tightly regulated by signals which coordinate between vesicle- and membrane-associated docking proteins. It is now being accepted that small G-protein, Rac1-mediated Reactive Oxygen Species (ROS) functions as a second messenger in islet β-cell function. Further, evidence from multiple laboratories suggests a tonic increase in ROS generation is necessary for GSIS and fatty acid-induced insulin secretion. On the other hand, Rac1-mediated NADPH oxidase-activation and subsequent generation of excessive ROS under glucolipotoxic conditions and cytokines exposure has proven to be detrimental for islet β-cell function. In this review we overview the normal physiological effects (positive role) and adverse effects (negative role) of activated small G-protein, Rac1 in pancreatic β-cells.

KEYWORDS: Small G-protein; Rac; Insulin secretion; NADPH oxidase; Oxidative stress; Islets.

ABBREVIATIONS: GSIS: Glucose-stimulated insulin secretion; ROS: Reactive Oxygen Species; GEFs: Guanine exchange nucleotide factors; FPR: N-formyl peptide receptor; GDIs: GDP-dissociation inhibitors; GAPs: GTPase-activating proteins; DPI: Diphenyleneiodonium; ZDF: Zucker Diabetic Fatty.

INTRODUCTION

Diabetes is a metabolic disorder with multiple etiologies characterized by chronic hyperglycemia. This results from dysregulated insulin secretion and/or from the resistance to insulin action in peripheral tissues. In the settings of the metabolic disorder, disturbances in carbohydrate, fat and protein metabolism results in a diverse set of complications associated with pancreas, liver, kidney, heart and other vital organs. As per the National Diabetes Statistics Report, 2014, in 2012, 29.1 million Americans (2.9% of the population) have diabetes, which include 1.25 million type 1 diabetic children and adults. Further, 86 million people of age 20 and above are pre-diabetic, and are at increased risk for developing type 2 diabetes. Over decades of research, a greater understanding of the pancreatic β-cells in physiological insulin release has been made to therapeutically target and treat the metabolic disorder. Insulin secretion from islet β-cells is majorly regulated by glucose and other insulin secretagogues. This is mediated through fluctuations in the intracellular calcium, and interplay of soluble secondary messengers like reactive oxygen species (ROS), cyclic nucleotides and hydrolytic products generated from the phospholipases A2, C and D.[1-13] In addition, adenine nucleotides [e.g., ATP]

ISSN 2377-8369 Open Journal http://dx.doi.org/10.17140/GOJ-1-114

and guanine nucleotides [e.g., GTP;[14-17] regulate physiological insulin secretion. Even though many studies have shown the underlying mechanism[s] involved in stimulus-secretion coupling of glucose stimulated insulin secretion (GSIS), the precise molecular and cellular mechanism still remains unknown. However, role of guanine nucleotide-binding protein (G-protein) has been highly researched for their role in insulin release. The signal-transduction system (Adenylylcyclases, ion channels, and phospholipases) involved in insulin release are linked to the receptors for hormones or stimulatory agents *via* G-proteins.

CLASSIFICATION OF G-PROTEINS IN β-CELL

Till date three major classes of G-proteins have been identified in pancreatic β-cells.[18-22] The first class of G-proteins, heterotrimeric G-proteins assists in coupling membrane-associated receptors to their intracellular effectors adenylyl cyclases, ion channels, and phosphodiesterases.[23-25] The second class of G-proteins, small monomeric G-proteins [17-30 kDa] play a vital role in protein organization and trafficking of secretory vesicle.[26] These small G-proteins undergo posttranslational modifications [isoprenylation and methylation] at their C-terminal residues (CAAX motif)[26-30] for their active confirmation. The third class of G-proteins, the elongation factors and Tau proteins are implicated in protein synthesis.

SMALL G-PROTEINS

Based on the substantial evidences on the regulation of pancreatic islet β-cell function, small G-proteins are categorized into three major groups. Rho, Rac1, Cdc42 and ADP-ribosylation factor-6 [Arf6] fall under the first category of small G-proteins and these play an important role in cytoskeletal remodeling and vesicular fusion.[31-48] The second category of small G-proteins comprises of Rap1 and RabGTPases (Rab3A and Rab27).[49] These Rab GTPases assists in priming and docking of insulin-laden secretory granules on the plasma membrane. Unlike first category of small G-proteins, requisite for posttranslational modifications and mechanism[s] involved in the activation of Rab GTPases under the physiological insulin secretagogues remains elusive. However, Rap1 is activated transiently by glucose[50] and undergoes carboxymethylation.[18,51] The third group of small G-proteins consists of Rab2, Rhes and Rem2 which are under-studied,[52-55] whereas, RalA appears to draw direct regulatory effects in exocytosis.[56] Do you have data about small G-proteins expression in pancreas?

ACTIVATION AND DEACTIVATION CYCLE OF SMALL G-PROTEINS

Like heterotrimeric G-proteins, small G-proteins also shuttle between their inactive (GDP-bound) and active (GTP-bound) conformations, and are tightly regulated by various G-protein regulatory factors/proteins. Till date, three regulatory factors have been identified for small G-proteins, viz., Guanine exchange nucleotide factors [GEFs], GDP-dissociation inhibitors [GDIs] and GTPase-activating proteins [GAPs]. GEFs facilitate the translation of the inactive GDP-bound to their active GTP-bound forms, while, the GDIs avert the dissociation of GDP from the G-proteins, thereby keeping them in the inactive conformation (Figure 1). However, GAPs, convert the active GTP-bound to their inactive GDP-bound form by inactivating the intrinsic GTPase activity of the candidate G-proteins. The efficiency of the G-protein activation cascade depends on the relative amounts of active to inactive GTPase. The activity of GTPase can be altered either by accelerating GDP dissociation by GEFs or by inhibiting GDP dissociation by GDIs, or by accelerating GTP hydrolysis by GAPs. Any imbalance in either of the regulatory factors alters the hydrolytic cycle and physiological functions in pancreatic β-cells.[49,57,58]

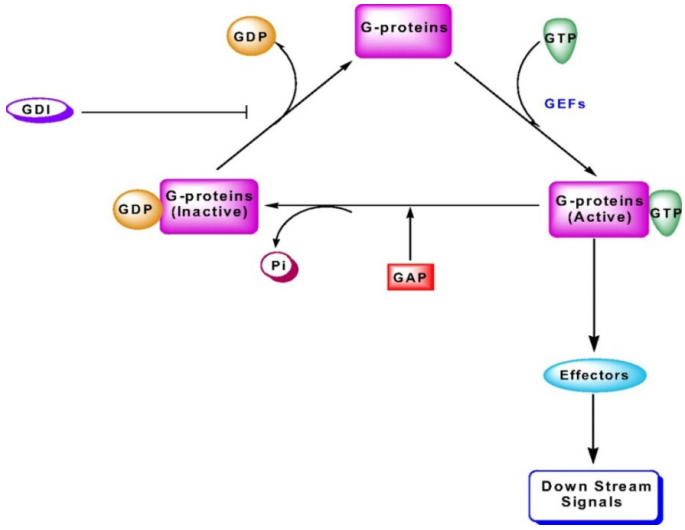

Figure 1: Activation and deactivation cascade of small G-proteins.[49]

SMALL G-PROTEIN-RAC

Rac was first identified and implicated in cellular function with two cDNAs encoding proteins, Rac1 and Rac2.[59] So far three isoforms of Rac proteins, Rac1, Rac2, and Rac3 have been identified in mammals. Both Rac1 and Rac2 share over 90% homology. Rac1 and Rac3 are extensively expressed in diverse tissues, whereas as, Rac2 is restricted to hematopoietic cells. Rac1 and other small G-proteins, Cdc42 and Arf6 have been recognized as key regulatory molecules in vesicle trafficking and organelle dynamics coupled with proliferation and survival of a cell.[19,49] In addition, Rac1 has also been shown to play a vital role in various diseased states including cancer and neurological disorders,[60-62] liver fibrosis[63] and diabetes.[57,58] Furthermore, Rac1 protein has shown to be associated with GLUT4 translocation in the muscle of diabetic patients.[64,65] Furthermore, Rac1 has shown to play an important role in wound healing, bacterial clearance and cell adhesion/migration by regulating actin dynamics in the gut.[66] Rac1 together with cdc42 induces intestinal wound closure, mediated by N-formyl peptide receptor (FPR) stimulation leading to enhanced intestinal epithelial cell restitution.[67] In addition, many pathogenic bacteria secrete factors that

trigger the posttranslational modification and activation of Rho proteins like Rac1 and cdc42 leading to gut epithelial cell death *via* apoptosis.[68] Citalán-Madrid and group have clearly depicted the roles of small G proteins as important signaling molecules in the regulation of epithelial junctions.[69] Herein this review, we describe both the positive and negative roles for Rac1 small G-protein in islet β-cell pathophysiology.

A. Positive Role of Rac1 in Insulin Secretion: Like other small G-proteins, Rac also shuttles between inactive GDP and active GTP conformations to facilitate cellular function. These proteins undergo ADP ribosylation by C3 component of botulinum toxin prior to their association with membrane. However, potential roles for Rac1 in glucose stimulated insulin secretion (GSIS) was first demonstrated by using Clostridium difficile toxins A and B, which irreversibly monoglucosylate and inactivate specific G-proteins (Cdc42 and Rac1).[32] Like Cdc42, Rac1 also undergoes posttranslational carboxymethylation and membrane translocation in the presence of stimulatory glucose concentrations.[32] Expression of an inactive mutant of Rac1 (N17Rac1) in INS-1 cells resulted in significant morphological changes leading to inhibition of GSIS. These findings also confirmed the involvement of small G-protein Rac1 in cytoskeletal remodeling and reorganization.[41] As stated above, Rac1 also requires prenylation for its function. Experiments involving pharmacological and molecular biological inhibition of Rac1 prenylation indicated marked reduction in GSIS in a variety of insulin-secreting β-cells. For an instance, GGTI-2147, a specific inhibitor for geranylgeranylation, one of the post translational modifications, significantly augmented accumulation of Rac1 in cytosol and inhibited GSIS in insulin-producing β-Cell line INS 832/13. Over expression of the regulatory α-subunit of protein prenyltransferase also attenuated glucose-induced insulin secretion in clonal pancreatic β-cells.[31] In addition, a recent study has shown that siRNA-mediated knock down of small G-protein Rac1 attenuated GSIS significantly having no effect on the basal insulin secretion, suggesting a positive modulatory roles for Rac1 in insulin secretion.[70] The importance of these small G-proteins in insulin secretion has been extensively studied *in vitro*; however, studies concentrating on *in vivo* Rac1 knock out models are limited. As Rac1 small G-protein is involved in many physiological processes, knocking out Rac1 might have deleterious effects. In this context, epithelial-specific Rac1-Knockout mice showed epithelial hyperplasia and a reduced basal cell layer.[71] Recent study has shown that, Rac1 specific knockout in pancreatic β-cells has no difference in either β-cell mass or pancreatic islet density explaining the possible compensatory mechanisms by other Rho-GTPases.[72] However, glucose stimulated insulin secretion was attenuated in these mice lacking Rac1 in β-cells both *in vivo* and in isolated islets. Furthermore, Rac1-null mice [βRac1−/−] exhibited impaired glucose tolerance and hypoinsulinemia, suggesting key regulatory roles for Rac1 in normal insulin function.[43] Taken together, these evidences suggest a positive role for Rac1 protein in islet function.

RAC1-NOX SIGNALING IN INSULIN SECRETION

Recent evidence suggests that NADPH oxidase derived tonic increase in reactive oxygen species (ROS) is required for glucose stimulated insulin secretion.[50,73-76] NADPH oxidase (Nox) represent a group of superoxide-generating enzymes which transport electrons through membranes and catalyze the cytosolic NADPH-dependent reduction of molecular oxygen to O_2 •-.[77] Till date, seven Nox family members have been identified i.e., Nox1, Nox2, Nox3, Nox4, Nox5, DUOX1 and DUOX2.[78] The Phagocytic Nox is a multicomponent enzyme complex, composed of membrane components [catalytic glycosylated $gp91^{phox}$ and the regulatory non-glycosylated $p22^{phox}$], cytosolic proteins [$p47^{phox}$, $p67^{phox}$, $p40^{phox}$] and a small GTPase, Rac ½.[78] Activation of Nox requires translocation of cytosolic components to the membrane and association with $gp91^{phox}$/$p22^{phox}$ complex (Figure 2).[79] Furthermore, Nox1 is the first homologue of $gp91^{phox}$ to be described and requires small GTPase Rac for activation.[80-83] In contrast to Nox1, 2 and 3, Nox4 is a constitutively active enzyme and is activated without the necessity for GTPase Rac or the cytosolic components.[84]

In this setting, the functional activation of Rac1 has shown to be critical in holoenzyme assembly and activation of Nox.[78,85-88] In support of this, Gorzalczany and associates have

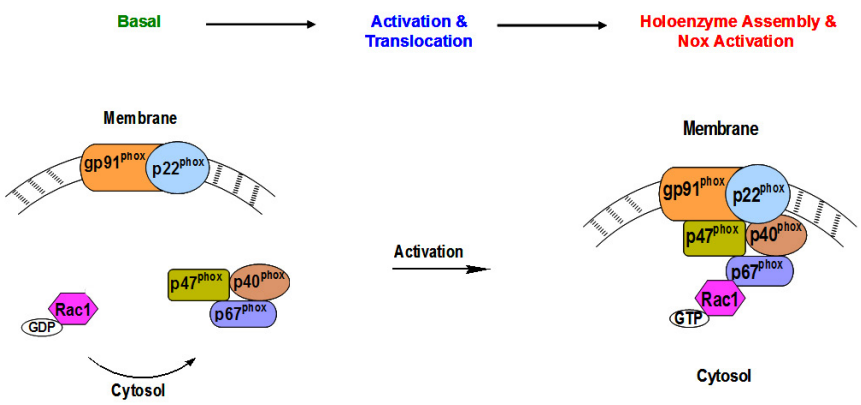

Figure 2: Activation of NADPH oxidase holoenzyme.

shown the activation of Nox and subsequent generation of ROS by targeting Rac1 to the membrane fraction.[89] They also demonstrated that prenylated Rac1 but not the unprenylated form binds to the phagocyte membrane more efficiently to facilitate the superoxide generation. Along these lines, Pi and Collins have overviewed the existing evidence in supporting "secondary messenger" roles of ROS in physiological insulin secretion.[50] In addition, studies have also emphasized roles for Nox in physiological insulin secretion. For example, Diphenyleneiodonium [DPI], a selective inhibitor of Nox, inhibited glucose-induced Nox activity and GSIS.[75] These observations were further confirmed by Morgan and associates suggesting that DPI or $p47^{phox}$ antisense-induced inhibition of Nox attenuated GSIS under static or perifusion conditions.[90] Graciano and co-workers demonstrated regulatory roles for Nox in palmitate-induced superoxide generation and insulin secretion in rat islets.[50] Furthermore, recent findings suggests that prenylation and activation of Rac1 are critical for glucose- and mitochondrial fuel-induced Nox-dependent ROS generation in clonal pancreatic β-cells and rodent islets.[91] In summary, a tonic increase in intracellular ROS is necessary for normal physiological insulin secretion and Rac1 initiates subsequent signaling steps including Nox activation and insulin release.[92]

B. RAC1-Nox Signaling and Metabolic Dysfunction: In addition to the above described beneficial roles for Rac1 in Nox-mediated ROS signaling in islet function, recent evidence also confirmed negative roles for ROS in islet β-cell dysfunction.[91] Excessive ROS generation is considered central to the development of diabetes and its associated complications. Under normal physiological conditions, generation of free radicals is relatively low; however increased levels of circulating glucose promote intracellular accumulation of superoxides leading to metabolic dysfunction. Although, mitochondria remain the primary source for free radicals, emerging evidence implicates Nox as one of the major sources of extra-mitochondrial ROS. Immunological localization and functional regulation of Nox have been described in clonal β-cells, rat and human islets.[50,75,90,92] Studies by Shen and associates in cardiac myocytes have also suggested regulatory roles for Rac1 in the activation of Nox and associated generation of ROS in animal models of diabetes.[93] In addition, significant increase in Nox-mediated oxidative stress and subsequent metabolic dysfunction has been clearly reviewed in a recent article by Kowluru.[70] However, very little is known with regard to regulatory roles of Rac1 in the holoenzyme assembly and activation of Nox in islet β-cells following chronic exposure to glucose, saturated fatty acids or cytokines.

In this context, recent findings demonstrated that prenylation of Rac1 is necessary for glucose-induced Nox activation and ROS generation in isolated β-cells.[91] In addition, studies have also implicated Nox in metabolic dysfunction of the islet β-cell under conditions of glucolipotoxicity and exposure to cytokines.[57,94] Generation of ROS under these conditions appears to be largely due to the activation of Nox, since inhibition of Nox [e.g., DPI, apocynin or siRNA-$p47^{phox}$] or Rac1 activation [e.g., GGTI-2147, NSC23766] markedly attenuated deleterious effects on pancreatic β-cells. In addition, the activation status of Rac1 was shown to be under precise control of Tiam1, a known guanine nucleotide exchange factor for Rac1, but not Cdc42 and Rho G-proteins in isolated β-cells.[95] In further support of this, a marked reduction in high glucose-, high palmitate- cytokine-induced Rac1 and Nox activation and ROS generation in isolated β-cells was observed following treatment with NSC23766, a selective inhibitor of Tiam1/Rac1 signaling axis.[57,94] Using selective inhibitors of protein prenylation, Subasinghe, et al. demonstrated a critical requirement of prenylation of Rac1 for Nox-mediated β-cell dysfunction.[94]

Taken together, these *in vitro* findings clearly implicate participatory roles of Nox in exerting effects at the mitochondrial level including loss in membrane potential, cytochrome C release and activation of caspase-3 culminating in islet β-cell dysfunction.[94,96] In addition, recent studies from Sidarala and colleagues present the evidence that the Rac1-Nox2 signaling is vital in high glucose induced activation of stress activated kinases and loss in GSIS causing islet β-cell dysfunction.[97] Despite these *in vitro* evidences, potential roles for Nox in islet dysfunction in animal models of type 2 diabetes are minimal. However, a recent study systematically examined the functional status of Nox in islets from Zucker Diabetic Fatty [ZDF] rat, which develops obesity, hyperinsulinemia, hyperglycemia and a decline in β-cell function.[58] These *in vitro* observations supported by findings in islets derived from the diabetic rodents [the ZDF rat] and diabetic human islets, form basis for the development of small molecule inhibitors for Rac1 and Nox activation in halting the metabolic defects, thereby retaining normal β-cell mass. In addition, a recent study from Zhou and colleagues also confirmed that the treatment with selective inhibitor NSC23766 attenuated Rac1 expression and oxidative stress in the pancreas in ob/ob mice.[98] These findings provide insights into potential therapeutic targets and interventional modalities to prevent the metabolic defects.

POTENTIAL THERAPEUTIC TARGETS AND INTERVENTIONAL MODALITIES

Based on the above discussion and published evidences, it is clear that Nox-derived reactive oxygen species have both positive and negative roles in the islet β-cell function. Targeting Nox holoenzyme complex could be beneficial in subsiding the excessive generation of ROS during oxidative stress milieu. In this context, a recent study proposed that $gp91^{phox}$, $p47^{phox}$ and $p67^{phox}$ might serve as potential drug targets due to their selective association in the Nox holoenzyme complex.[99] On the contrary, peptide inhibitors blocking Rac1/2 activation and $p47^{phox}$ translocation might not be a good approach, since they are integral members of other NADPH oxidasecomplexes too. However, Mizrahi, et al. developed $p47^{phox}$-$p67^{phox}$-Rac1 chimera as a quintessential single molecule activator of Nox[100] to study the

ISSN 2377-8369 Open Journal http://dx.doi.org/10.17140/GOJ-1-114

effects of Nox activation regulatory roles for Rac1. These observations are in agreement with the findings, where researchers have demonstrated a decrease in glucose-mediated Nox-induced ROS generation in the presence of prenylation inhibitors. Developing inhibitors for such quintessential single molecule activators might provide a novel therapeutics to minimize excessive generation of ROS Nox-mediated pancreatic β-cell dysfunction. Furthermore, an alternate approach to minimize the excessive generation of ROS is to enrich the antioxidant capacity of the islet β-cells. As reviewed by Acharya and Ghaskadbi,[101] pancreatic islet β-cells hold a poor antioxidant defense mechanism. And counterbalancing oxidative environment by antioxidant treatment or overexpressing antioxidant enzymes might prove to be successful in regulating islet β-cell function. Indeed, such modalities have been shown to work efficiently both *in vivo* and *in vitro*. Along these lines, treatment with antioxidant, α-lipoic acid has been demonstrated to improve insulin sensitivity in type 2 diabetic subjects.[102] Moreover, researchers have also shown that vitamin E treatment improves pancreatic physiology under diabetic state.[103] Asayama, et al. found that rats deficient in vitamin E, selenium, or both had decreased insulin secretory reserves, suggesting that vitamin E status can directly affect pancreatic islet function. In a mouse model of type 2 diabetes, treatment with vitamin E combined with vitamin C and n-acetyl cysteine resulted in large number of pancreatic islets than controls.[104] Furthermore, a recent study in humans has shown that, taurine affectively restored β-cell function and improved insulin sensitivity.[105] Together these studies further highlight antioxidant therapy as one of the feasible options in attenuating excessive generation of ROS and subsequent reduction in oxidative stress environment in the islet β-cells.

In addition to the above mentioned strategies for attenuating oxidative stress, inhibitors blocking Tiam1/Rac1/Nox signaling axis,[57,94,106] polyphenolic extracts supplementation,[107] stress activated kinase inhibitors,[108-111] and angiotensin receptor antagonists[112] have proven efficaciously to reduce oxidative stress and improve islet β-cell function.

CONCLUSION

Glucose stimulate insulin secretion (GSIS) involves a series of metabolic events involving interaction between a variety of signaling pathways to facilitate the transport of insulin-laden granules to the plasma membrane for fusion and subsequent insulin release. Compelling evidence supports involvement of small G-proteins like Rac1 and Cdc42 in the cytoskeletal reorganization, which is necessary for GSIS to occur. Recent findings further validate that Tiam1 represents one of the GEFs for Rac1 and that Tiam1/Rac1 signaling axis is requisite for GSIS. Nox appears to be an effector protein for Tiam1/Rac1 signaling and that its activation leads to a tonic increase in the generation of ROS under the stimulatory conditions of glucose and fatty acids leading to insulin release. In addition to this, Tiam1/Rac1 signaling axis appears to play a vital role in Nox-mediated ROS generation under the duress of excessive glucose, palmitate, ceramide and cytokines culminating in oxidative stress and metabolic dysfunction of islet β-cells. Together, these findings suggest positive and negative modulatory roles for Tiam1-Rac1-Nox signaling pathway in islet function. The Figure 3 depicted below is indicative of potential effects of ROS on islet β-cells at different stages. Low levels of generated ROS have a positive effect on glucose stimulated insulin secretion, and as the levels of the ROS increases it causes detrimental effects and β-cell dysfunction.[113] Therefore, it may be challenging to draw a line as to how much of ROS generation is beneficial for the normal function of islets as opposed to how much is bad to elicit damaging effects on the pancreatic β-cell. It is likely that there may be a "window of opportunity" or "point of return" for the islet β-cell to recover from the noxious effects of excessive ROS due to accelerated Tiam1-Rac1-Nox signaling pathway in the diabetic states.

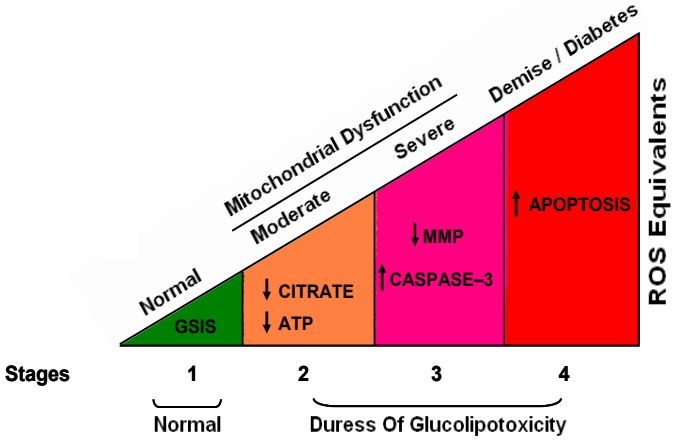

Figure 3: Hypothetical model for ROS generation in identifying the effects on pancreatic islet β-cells.

CONFLICTS OF INTEREST

The authors declare that they dont have any conflicts of interest or any acknowledgements for this submission.

FINANCIAL INTERESTS: None.

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