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**G6PD Up-Regulation Promotes Pancreatic β-Cell Dysfunction**

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Increased reactive oxygen species (ROS) induce pancreatic β-cell dysfunction during progressive type 2 diabetes. Glucose-6-phosphate dehydrogenase (G6PD) is a reduced nicotinamide adenine dinucleotide phosphate-producing enzyme that plays a key role in cellular reduction/oxidation regulation. We have investigated whether variations in G6PD contribute to β-cell dysfunction through regulation of ROS accumulation and β-cell gene expression. When the level of G6PD expression in pancreatic islets was examined in several diabetic animal models, such as db/db mice and OLEFT rats, G6PD expression was evidently up-regulated in pancreatic islets in diabetic animals. To investigate the effect of G6PD on β-cell dysfunction, we assessed the levels of cellular ROS, glucose-stimulated insulin secretion and β-cell apoptosis in G6PD-overexpressing pancreatic β-cells. In INS-1 cells, G6PD overexpression augmented ROS accumulation associated with increased expression of prooxidative enzymes, such as inducible nitric oxide synthase and reduced nicotinamide adenine dinucleotide phosphate oxidase. G6PD up-regulation also caused decrease in glucose-stimulated insulin secretion in INS-1 cells and primary pancreatic islets. Moreover, elevated G6PD expression led to β-cell apoptosis, concomitant with the increase in proapoptotic gene expression. On the contrary, suppression of G6PD with small interference RNA attenuated palmitate-induced β-cell apoptosis. Together, these data suggest that up-regulation of G6PD in pancreatic β-cells would induce β-cell dysregulation through ROS accumulation in the development of type 2 diabetes. (Endocrinology 152: 793–803, 2011)

Type 2 diabetes is characterized by peripheral insulin resistance and progressive deterioration of pancreatic β-cell function (1). Accumulating evidence suggests that β-cell dysfunction in type 2 diabetes is closely associated with high levels of plasma glucose and nonesterified fatty acids (NEFAs) and consequent increases in proinflammatory cytokines, endoplasmic reticulum stress, and mitochondrial dysfunction (1, 2). In particular, several studies have suggested that elevated oxidative stress in obese and diabetic subjects would play a key role for metabolic abnormalities, including β-cell defects (3, 4).

A persistent imbalance between reactive oxygen species (ROS) production and antioxidative defense induces chronic oxidative stress (3, 4). Particularly, increased ROS production is critical for development of β-cell dysfunction, because pancreatic β-cells possess very low level of intrinsic antioxidative activity (5, 6). In β-cells, elevated levels of glucose (7, 8), NEFAs, and proinflammatory cytokines, such as IL-1β (9), potentiate cellular ROS pro-

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**Abbreviations:** Ad-G6PD, G6PD adenovirus; Ad-Mock, control mock adenovirus; Ad-SREBP, sterol regulatory element binding protein-1c adenovirus; DCF-DA, 2',7'-dichlorodihydrofluorescein diacetate; FBS, fetal bovine serum; GFP, green fluorescent protein; G6PD, glucose-6-phosphate dehydrogenase; GPx, glutathione peroxidase; GSIS, glucose-stimulated insulin secretion; HEK, human embryonic kidney; INOS, inducible nitric oxide synthase; KRB, Krebs-Ringer bicarbonate; Me, malic enzyme; NAC, N-acetyl cysteine; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NEFA, nonesterified fatty acid; NF-κB, nuclear factor κB; ROS, reactive oxygen species; RT-qPCR, quantitative real-time RT-PCR; siRNA, small interference RNA; SOD, superoxide dismutase; SREBP, sterol regulatory element binding protein; TUNEL, deoxyuridine triphosphate-biotin nick-end labeling.
duction and decrease β-cell function. β-Cell dysfunction is associated with exhaustion of insulin content, decreased insulin gene expression (10, 11), and β-cell apoptosis (12, 13). Interestingly, treatment with the antioxidant N-acetyl cysteine (NAC) rescues β-cell failure, indicating that homeostasis of reduction/oxidation regulation is critical for β-cell physiology (13, 14). Several studies have reported that induction of stress-sensitive signaling cascades, including nuclear factor κB (NF-κB), c-Jun N-terminal kinase, and p38 MAPK pathways are involved in the ROS-induced adverse cellular events, such as apoptosis in various cell types (4). Accordingly, activation of the NF-κB pathway stimulates proapoptotic events in β-cells (13). Also, c-Jun N-terminal kinase activation induced by hyperglycemia and oxidative stress inhibits binding of PDX-1 (pancreatic and duodenal homeobox-1) to the insulin promoter and reduces insulin gene expression, leading to β-cell defect (15). Nevertheless, although an elevated ROS level has been implicated in β-cell dysfunction, its underlying mechanism in the pathogenesis of type 2 diabetes has not been thoroughly studied.

Glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the pentose phosphate pathway, produces cellular reduced nicotinamide adenine dinucleotide phosphate (NADPH) (16, 17). NADPH is a cofactor for both prooxidative [i.e. NADPH oxidase and inducible nitric oxide synthase (iNOS)] and antioxidative [i.e. glutathione peroxidase (GPx) and superoxide dismutase (SOD)] enzymes (18). Thus, G6PD appears to have dual functions in the regulation of cellular oxidative changes, depending on cell and/or tissue types. For example, elevated G6PD contributes to the generation of superoxide via the activation of NADPH oxidase in failing hearts (19). Similarly, G6PD up-regulation in adipocytes stimulates ROS accumulation and proinflammatory responses (20). On the other hand, G6PD has been shown to reduce oxidative stress via activation of antioxidant enzymes in cardiomyocytes, kidney cortices, and ovary cells (21–23). Furthermore, a very recent study demonstrates that high glucose down-regulates G6PD expression in β-cells, which would lead to oxidative stress and β-cell abnormality (24). However, it is yet unanswered whether the expression and function of G6PD are indeed altered in pancreatic β-cells of diabetic animals and whether G6PD alteration mediate elevated oxidative stress as well as β-cell dysfunction in the progress of type 2 diabetes.

In this study, we demonstrate that G6PD is up-regulated in the pancreatic islets of diabetic animals. In INS-1 cells, G6PD overexpression increases the level of cellular ROS accumulation, concomitantly with NF-κB activation. Moreover, elevated G6PD expression impairs glucose-stimulated insulin secretion (GSIS) and induces β-cell apoptosis, whereas knockdown of G6PD prevents palmitate-induced β-cell apoptosis. These data suggest that up-regulation of G6PD in β-cells would promote oxidative stress and apoptosis, leading to β-cell dysfunction.

Materials and Methods

Reagents

NAC was purchased from Calbiochem (Darmstadt, Germany). Human TNFα was purchased from R&D Systems (Minneapolis, MN). A small interference RNA (siRNA) duplex [green fluorescent protein (GFP) siRNA and G6PD siRNA] was obtained from Bioneer (Daejeon, Korea).

Cell culture and siRNA transfection

Rat insulinoma INS-1 cells were grown in RPMI 1640 (HyClone, Logan, UT) containing 11.1 mM glucose, 10% fetal bovine serum (FBS)
(HyClone), 100 U/ml penicillin, and 100 mg/ml streptomycin. Human embryonic kidney (HEK)293 cells were grown in DMEM (HyClone) supplemented with 10% FBS and penicillin/streptomycin. The cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂.

To suppress G6PD expression, INS-1 cells were grown to 70% confluence and then transiently transfected with G6PD siRNA using Lipofectamine 2000 reagent according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA). After transfection, INS-1 cells were cultured in RPMI 1640 for 24 h followed by another 16 h of incubation under serum starvation. The INS-1 cells were then incubated with various conditions and analyzed for gene expression or apoptosis by using quantitative real-time RT-PCR (RT-qPCR) or fluorescence activated cell sorting, respectively. The nucleotide sequences of G6PD siRNA were 5' -CUUGAAGAAGUCGUGCUGCTT-3' (antisense) and 3' -HG11032-GCAGCACGACUUCUUCAAGTT-3' (sense) in addition, GFP siRNA was used for control experiments, with nucleotide sequences of G6PD-antisense (5' -CUGGAAACUUACACCAUCU-3') and G6PD-sense (5' -CUUGAAGAAGUCGUGCUGCTT-3') (antisense).

Animals and pancreatic islet isolation

All animal experiments were approved by the Seoul National University Animal Experiment Ethics Committee. Male db/db and db/+ mice (13 wk old), OLETF/LETO rats (28 wk old), and Sprague Dawley rats (200–300 g) were used. Pancreatic islets were isolated from db/db and db/+ mice, or OLETF/LETO rats or Sprague Dawley rats (200–300 g) by collagenase (Sigma-Aldrich, St. Louis, MO) digestion and purified using different densities of Ficoll solution [29, 24, and 15% (wt/vol) mixed with Hanks’ balanced salt solution]. After 30 min of centrifugation, the layer between 24 and 15% density was collected and rinsed with cold Hanks’ balanced salt solution twice. Islets were then handpicked. Isolated primary islets from Sprague Dawley rats were cultured in RPMI 1640 containing 10% FBS, antibiotics, and 11.1 mm glucose before analysis of glucose GSIS. Primary islets were collected to isolate total RNA (db/db and db/+ mice or OLETF/LETO rats) and total cell lysates (db/db and db/+ mice) for RT-qPCR and Western blot analysis, respectively.

Quantitative real-time RT-PCR

Total RNA was isolated using TRizol (Invitrogen) according to the manufacturer’s protocol. For RT-qPCR analysis, cDNAs were synthesized with RevertAid M-MuLV reverse transcriptase (Fermentas, Glen Burnie, MD) using oligo-dT and subjected to PCR amplification with gene-specific primers using the Cycler real-time PCR detection system (Bio-Rad, Hercules, CA) and SYBR Green (BioWhittaker Molecular Applications, Rockland, ME). The relative abundance of all reaction products was normalized to the level of cyclophilin. The primer sequences used for real-time PCR analysis are described in Supplemental Table 1.

Western blotting

Equal amounts of total cell lysates were separated on SDS-PAGE gels and then transferred to nitrocellulose membrane. The membrane blots were blocked with Tris-buffered saline with 0.1% Tween 20 containing 5% nonfat milk and sequentially hybridized with primary antibodies and horseradish peroxidase-conjugated secondary antibodies (Sigma-Aldrich). After washing, the membrane was visualized by chemiluminescence (Fuji, Tokyo, Japan).

Adenovirus infection

Recombinant G6PD adenovirus (Ad-G6PD), sterol regulatory element binding protein (SREBP)-1c adenovirus (Ad-SREBP-1c), and control mock adenovirus (Ad-Mock) were obtained from Boram Pharmaceutical Co. (Seoul, Korea). INS-1 cells were infected with 10 multiplicity of infection Ad-Mock, Ad-G6PD, or Ad-SREBP-1c and cultured in serum-free RPMI 1640 for 3 h. Cells were then incubated with fresh RPMI 1640 supplemented with 10% FBS for another 72 h before performing experiments. Isolated primary islets were infected with Ad-Mock.
or Ad-G6PD (10^7 plaque-forming units per islet) in serum-free RPMI 1640 for 24 h. After infection, islets were transferred into fresh RPMI 1640 containing 10% FBS. Each experiment was performed at 3 d after viral infection. Adenoviral infection was assessed by determining the abundance of G6PD mRNA using RT-qPCR. For high glucose experiments, adenovirus-infected cells and primary islets were incubated with fresh RPMI 1640 supplemented with 10% FBS and different concentrations of glucose.

**ROS measurement**

Cellular ROS level was measured using 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) (Invitrogen-Molecular Probes, Eugene, OR). INS-1 cells were washed with PBS and incubated with DCF-DA (2.5 µM) in the dark for 10 min. DCF-DA green fluorescence was viewed under a fluorescence microscope (Olympus, Center Valley, PA) or quantitated by Envision 2102 multilabel reader at an excitation wavelength of 492 nm and an emission wavelength of 530 nm.

**Electrophoretic mobility shift assay**

Nuclear extracts were isolated from INS-1 cells for EMSA, as described in a previous study (25), with minor modifications. The following target DNA sequence of NF-κB was used as a probe (the sequence of only one strand is shown): 5'-AGTTGAGGGGACTTTCCCAGGC-3'. Double-stranded oligonucleotides were end-labeled with [γ-32P]ATP by T4 polynucleotide kinase. Nuclear extracts were mixed with radiolabeled probes (1 pmol/30,000 cpm) in the reaction buffer [4 mM Tris (pH 7.9), 23 mM HEPES, 66 mM NaCl, 5 mM MgCl2, 0.7 mM EDTA, 1 mM dithiothreitol, 14% (vol/vol) glycerol, and 4 µg of poly(deoxyinosinic-deoxyxycytidyllic)]. After incubation at room temperature for 25 min, the samples were resolved on a native polyacrylamide gel (4%).

**Insulin secretion**

After adenoviral infection, INS-1 cells or primary islets from Sprague Dawley rats (similar-sized 10 islets per dish) were statically incubated in Krebs-Ringer bicarbonate (KRB) buffer supplemented with 0.1% BSA and containing 2.8 mM glucose at 37 C for 1 h. After that, the cells or primary islets were incubated in KRB buffer with 25 mM glucose at 37 C for 1 h. KRB samples were harvested at the end of each static incubation, and secreted insulin in KRB samples was measured by using a RIA kit (Linco, St. Charles, MO).

**NEFA solution preparation**

Each NEFA stock solution (lauric, palmitic, and stearic acids dissolved in ethanol) was complexed with fatty acid-free 10% BSA solution by stirring at 37 C for 1 h and then diluted in culture...
medium to a final concentration as indicated in the legend of Fig. 6. The final molar ratio of fatty acid to BSA was 5:1. A vehicle solution (ethanol mixed with BSA solution having no free fatty acid content) was used in all control conditions.

Luciferase report assay and measurement of apoptotic cells

HEK293 cells were transiently cotransfected with a luciferase reporter containing G6PD promoter (−1.1 to +1.1 kb) and SREBP-1c-expressing vector according to the calcium phosphate protocol. After transfection, cells were incubated with fresh culture medium for 24 h. Cells were then lysed and subjected to luciferase assays (26).

Apoptotic cells were detected by flow cytometric analysis (FACS Aria) after staining with Annexin V (BD Biosciences, San Jose, CA) or by using a deoxyuridine triphosphate-biotin nick-end labeling (TUNEL) (Roche, Indianapolis, IN) assay. Annexin V staining and TUNEL assay were performed according to the manufacturers’ instructions (27). Fluorescein-labeled images were viewed under a fluorescence microscope (Olympus) using an excitation wavelength in the range of 450–500 nm and a detection wavelength in the range of 515–565 nm.

Statistical analysis

All data represent multiple experiments independently performed at least three times. Data are presented as means ± se. Data were analyzed using Student’s t test. *P < 0.05 was considered statistically significant.

Results

Up-regulation of G6PD expression in pancreatic islets of diabetic animals

Because G6PD dysregulation has been implicated in metabolic disorders, such as obesity, diabetes, and cardiovascular diseases (20, 21, 28, 29), we investigated whether G6PD expression is altered in pancreatic islets of diabetic animals. In pancreatic islets of diabetic animal model, such as db/db mice and OLETF rats, which exhibit clear insulin resistant and hyperglycemic (Supplemental Fig. 1 and data not shown), the level of G6PD mRNA was
higher (2.1- and 6.3-fold, respectively) than that of their nondiabetic counterparts (Fig. 1, A and B). Furthermore, G6PD protein was markedly increased in pancreatic islets of db/db mice compared with lean counterparts (Fig. 1C). Consistent with a previous report (30), an increase in iNOS expression, which affects NO production known to be deleterious to β-cell function, was also detected in both diabetic mice and rats (Fig. 1, A–C). On the other hand, expression levels of other NADPH-producing genes, including 6-phosphogluconate dehydrogenase (6pgd), isocitrate dehydrogenase (Idh), and malic enzyme (Me), were not significantly changed in diabetic animals (Fig. 1, A and B). These results suggest that increased G6PD expression might be associated with the defects in pancreatic β-cells of type 2 diabetic animals.

Accumulation of ROS in G6PD-overexpressing β-cells

To examine the effects of G6PD up-regulation in β-cells, INS-1 insulinoma cells were infected with Ad-Mock or Ad-G6PD. Elevated G6PD mRNA and protein, as well as its enzymatic activity, were confirmed (Supplemental Fig. 2). Given that oxidative stress is one of the major factors contributing to β-cell dysfunction in type 2 diabetes (1, 4, 31), it led us to investigate whether elevated G6PD in β-cells might affect pro- or antioxidative signals. Interestingly, G6PD-overexpressing β-cells promoted the expression of several prooxidative enzyme genes, such as iNOS and NADPH oxidase (gp91phox and p22phox) (Fig. 2A). In contrast, the levels of antioxidative enzymes were not changed (manganese SOD and GPx1) or decreased (catalase and catalytic subunit of glutamate cysteine ligase) by G6PD overexpression (Fig. 2A). To test the idea that G6PD up-regulation in β-cells would potentiate prooxidative signals, eventually leading to ROS generation, we examined cellular ROS accumulation in β-cells upon G6PD overexpression. Simultaneously, we treated INS-1 cells with H₂O₂ (10 mM) as a positive control. Compared with Ad-Mock-infected β-cells, the level of ROS in Ad-G6PD-infected β-cells was elevated, whereas such an effect was diminished by a ROS-scavenging agent, NAC (Fig. 2, B and C). In accordance with these results, G6PD up-regulation in primary pancreatic islets promoted NO release (Fig. 2D). Therefore, it is feasible that elevated G6PD in β-cells would stimulate cellular ROS accumulation, which may consequently lead to β-cell dysfunction.

Activation of NF-κB signals in G6PD-overexpressing β-cells

It is well established that elevated ROS in β-cells activates NF-κB signaling, which impairs insulin secretion and induces β-cell apoptosis (32). Because we observed that G6PD overexpression in β-cells augmented prooxidative genes and ROS accumulation, we investigated whether G6PD overexpression would trigger NF-κB activation. DNA-binding activity of NF-κB was promoted in G6PD-overexpressing β-cells (Fig. 3A), and the level of cytoplasmic p65 protein was slightly but substantially decreased in Ad-G6PD-infected β-cells, similar to TNFα-treated cells (Supplemental Fig. 3). Moreover, the expression of NF-κB target genes, such as TNFα, IL-1β, and IL-6, the key proinflammatory cytokines, was stimulated in G6PD-overexpressing β-cells (Fig. 3B). These data indicate that the increased G6PD in β-cells would activate NF-κB signaling to induce inflammatory signals, which might be associated with ROS accumulation and β-cell defects.

Impaired insulin secretory response in G6PD-overexpressing β-cells

To determine whether up-regulated G6PD in β-cells is responsible for β-cell dysfunction, we measured GSIS with or without G6PD overexpression. In both INS-1 cells and isolated primary islets, G6PD up-regulation caused a significant decrease in GSIS (Fig. 4, A and B). Additionally, the expression level of β-cell-specific genes, such as insulin and Pdx1, was decreased by G6PD overexpression (Fig. 4C). Further, the levels of cellular insulin contents were decreased by G6PD up-regulation in INS-1 cells and primary islets (Supplemental Fig. 4, A and B). Therefore, these data suggest that increased level of G6PD in β-cells could impair β-cell functions, including GSIS and β-cell-specific gene expression.

β-Cell apoptosis induced by G6PD

In β-cells, it is well known that increased ROS accumulation and NF-κB activation stimulates proapoptotic events (32). To determine whether G6PD elevation leads to β-cell apoptosis in the progress of β-cell dysfunction, we assessed the expression levels of apoptotic genes and the degree of apoptosis upon G6PD overexpression. In G6PD-overexpressing β-cells, the mRNA levels of proapoptotic genes, including Fas ligand, Fas, and Bax, were increased,
whereas that of Bcl-2, a key antiapoptotic molecule, was decreased (Fig. 5A). It is of interest to note that both Fas ligand and Fas are well-known target genes of NF-κB, and both the genes play important roles in apoptosis (33, 34).

To directly monitor whether G6PD overexpression would indeed increase β-cell apoptosis, Ad-Mock- or Ad-G6PD-infected INS-1 cells were stained with Annexin V. Consistent with gene expression profiles, the apoptosis rate in Ad-G6PD-overexpressing cells was promoted (Fig. 5B), and TUNEL assays revealed the similar results (Fig. 5C). These data imply that chronic G6PD up-regulation in β-cells would promote β-cell apoptosis, which may possibly result in a reduction in β-cell mass during the progression of type 2 diabetes.

**Suppression of G6PD prevents palmitate-induced β-cell apoptosis**

When β-cells are exposed to high levels of NEFAs, they undergo apoptosis via oxidative stress; this phenomenon is termed lipotoxicity. Such lipid-induced β-cell apoptosis is one of the key arms in β-cell dysfunction (13). Similar to adipocytes (20), G6PD expression in β-cells was increased by NEFAs such as lauric, palmitic, and stearic acids (Fig. 6A). The observations that elevated G6PD in β-cells promoted ROS accumulation (Fig. 2) and apoptosis (Fig. 5) prompted us to determine whether G6PD in β-cells is also involved in NEFA-induced ROS generation and apoptosis. To investigate the role of G6PD in β-cell lipotoxicity, G6PD expression was suppressed using siRNA, and the cells were challenged with palmitate, a well-known proapoptotic NEFA (35). Consistent with previous reports (13, 35), palmitate treatment in β-cells increased ROS levels (Fig. 6B). More importantly, we observed that G6PD knockdown in β-cells remarkably suppressed palmitate-induced β-cell apoptosis (Fig. 6, C and D), indicating that G6PD appears to be important in mediating lipotoxicity as a part of the β-cell dysfunction.

**Regulation of G6PD expression by SREBP-1c**

SREBP-1c, a key lipogenic transcription factor, is increased in pancreatic β-cells of obese and diabetic animals (27, 36, 37). In addition, the promoter region of G6PD has been reported to contain SRE and E-box motifs, which are potential binding sites of SREBP-1c (38). Thus, we speculated whether increased levels of SREBP-1c in β-cells might regulate G6PD expression. We observed that SREBP-1c effectively transactivated the G6PD promoter containing a luciferase reporter (Fig. 7A). Ectopic expression of SREBP-1c in β-cells consistently elevated G6PD expression levels (Fig. 7B). Thus, it is likely that the G6PD expression in pancreatic β-cells is possibly stimulated by elevated SREBP-1c in diabetic animals.

**Effect of high glucose challenge on G6PD regulation**

Very recently, Zhang et al. (24) reported that high glucose reduces G6PD expression, which would lead to β-cell defects. Because hyperglycemia is one of the crucial factors in β-cell dysfunction, we assessed the effect of high glucose on G6PD expression and its consequent changes in β-cell functions. In INS-1 cells, chronic incubation (72 h) with...
high glucose (25 mM) clearly stimulated G6PD mRNA and protein, compared with low glucose (5.6 mM) treatment (Fig. 8, A–E). Moreover, under high glucose (25 mM) challenging condition, G6PD-overexpression additively decreased the expression of β-cell-specific genes, such as insulin and Pdx1 (Fig. 8D), and further impaired GSIS (Fig. 8F). On the contrary, in INS-1 cells, chronic high glucose challenge (25 mM, 72 h) potently elevated ROS accumulation as well as other β-cell defects, such as increase of proinflammatory genes and apoptosis, regardless of G6PD ectopic expression (Supplemental Fig. 5, A–C). Additionally, in INS-1 cells, G6PD knockdown via siRNA did not restore high glucose-induced β-cell dysfunction, including expression of insulin and Pdx1 genes as well as apoptosis (Supplemental Fig. 5, D and E). Although these data did not completely provide mechanistic understandings between G6PD and glucotoxicity, it appears that both high glucose and G6PD up-regulation would contribute to pancreatic β-cell dysfunction. Nonetheless, it is possible that β-cell defects upon hyperglycemia are attributed to the increased oxidative stress, which might be resulted from, at least in part, elevated G6PD.

Discussion

Pancreatic β-cell dysregulation and loss have been implicated in overt type 2 diabetes. Although increased ROS generation and subsequent oxidative stress have been considered as key causative factors for β-cell impairment elicited by glucotoxicity in concert with lipotoxicity, the exact etiology of oxidative stress-induced β-cell dysfunction in diabetes has not been completely understood. In this study, we demonstrate that up-regulated G6PD in β-cells would contribute to β-cell dysfunction through increase in ROS accumulation.

In pancreatic β-cells, elevated glucose, NEFAs, and proinflammatory cytokines are known to stimulate NADPH oxidase and iNOS, leading to β-cell defects due to elevated cellular ROS (39, 40). Because the activities of NADPH oxidase and iNOS are modulated by cellular NADPH levels, altered NADPH production is closely associated with regulation of oxidative stress (40, 41). In this aspect, increased G6PD expression and activity have been shown to contribute to deleterious effects on several tissues under metabolically dysregulated conditions. For instance, in liver and heart of obese and diabetic Zucker fa/fa rats, G6PD expression and NADPH oxidase-derived superoxide production were increased (42, 43). Moreover, elevated G6PD promotes oxidative stress in the adipose tissue of obese animals, ultimately resulting in insulin resistance (20, 29). Here, we reveal that G6PD expression is increased in pancreatic islets of obese and diabetic animals without altered Idh and Me2, the other sources of cellular NADPH production. We have also demonstrated that G6PD overexpression in β-cells stimulated the expression of prooxidative enzymes as well as accumulation of intracellular ROS, implying that G6PD-induced prooxidative signals would be one of the key mechanisms inducing β-cell defect. In accordance with these findings, in RINm5F β-cells, IL-1B has been reported to increase the expression of G6PD without affecting Me and to induce iNOS-mediated NO production via G6PD-dependent NADPH production (40). Thus, these data suggest that G6PD in β-cells would play an important role in NADPH production, and altered G6PD expression would confer β-cell impairments.

It is well established that ROS-induced NF-κB activation is tightly linked with pancreatic β-cell defects (44, 45). β-Cells exposed to high levels of glucose or NEFAs activate NF-κB, accompanied by ROS production and apoptotic β-cell death (13, 46). Conversely, supplementation of NAC, a potent antioxidant chemical, inhibits NF-κB activation in pancreas and alleviates β-cell dysfunction (45). In this study, we demonstrated that G6PD overexpression in β-cells stimulates NF-κB activation, thereby inducing β-cell apoptosis, indicating that chronically elevated G6PD might cause β-cell dysregulation through oxidative stress and NF-κB activation.

It is well known that autoimmune-induced inflammation mediated by resident islet lymphoid cells and macrophages has been considered as the primary etiology of β-cell destruction in type 1 diabetes (47). Recent studies have reported that increased inflammatory response is also observed in pancreatic islets of type 2 diabetic subjects (48). Thus, it is likely that augmented proinflammatory response is involved in β-cell defects in both the types 1 and 2 diabetes. Here, we observed that up-regulated G6PD in β-cells potentiated the expression of proinflammatory cytokines, including IL-1β, IL-6, and TNFα, that would be associated with ROS production and β-cell impairments. Recently, it has been reported that increased ROS mediates NLR family, Pyrin domain-containing 3 inflammasome activation by releasing thioredoxin-interacting protein from thioredoxin. This allows cleavage of pro-IL-1β by caspase-1 activation, ultimately resulting in elevated IL-1β (49). Therefore, it is feasible that in G6PD-overexpressing β-cells increased IL-1β might contribute to G6PD-dependent ROS accumulation. Further, increased NF-κB signaling possibly through elevated ROS in G6PD-overexpressing β-cells would facilitate elevated inflammatory cytokines and β-cell inflammation, which would lead to β-cell dysfunction. Together, these data suggest that up-regulated G6PD in β-cells would affect pathogenesis of β-cell dysregulation through multiple pathways, such as...
prooxidative, inflammatory, and apoptotic cascades (Supplemental Fig. 6).

Lipotoxicity is one of the major arms of β-cell dysfunction, which is related to the enhanced oxidative stress in type 2 diabetes. In pancreatic β-cells, palmitate elevates ROS production (13, 39), along with the increase of NADPH oxidase, and causes β-cell apoptosis (13, 50). In the present study, we found that G6PD would mediate lipotoxicity by increasing ROS levels in β-cells. Suppression of G6PD via siRNA apparently protected palmitate-induced β-cell apoptosis, indicating the engagement of G6PD in β-cell dysregulation during lipotoxicity. In this regard, we also observed that palmitate promoted expression of SREBP1c in β-cells (Supplemental Fig. 7), and in turn, SREBP-1c stimulated G6PD expression. These data propose that up-regulation of G6PD by SREBP-1c in β-cells might play a role in hyperlipidemia-mediated lipotoxicity.

While this article was being prepared, Zhang et al. (24) very recently reported that G6PD expression is reduced in pancreatic islets cultured with long-term (72 h) treatment of high glucose (25 mM), which would lead to increased oxidative stress and apoptosis in β-cells. In addition, they have demonstrated that hemizygous G6PD-deficient animals are glucose intolerant (24). On the contrary, we observed that G6PD expression is elevated in pancreatic islets from several diabetic animals (Fig. 1), as reported in other metabolic tissues, such as liver, adipose tissue, and heart (29, 42, 43). Moreover, we clearly demonstrated that G6PD overexpression increased oxidative stress and decreased GSIS capacity in both pancreatic islets and INS-1 cells (Figs. 2 and 4). To elucidate the discrepancy between our work and the recent report (24), we have carefully investigated the effect of chronic high glucose challenge (25 mM, 72 h) on G6PD expression and many aspects of β-cell functions. However, unlike Zhang’s report (24), we observed that high glucose increased G6PD expression in INS-1 cells (Fig. 8). In addition, hyperglycemic condition additively suppressed expression of certain genes, such as insulin and Pdx-1, and impaired GSIS in the presence of G6PD overexpression. On the contrary, chronic high glucose challenge in β-cells dominantly promoted ROS production and apoptosis (Supplemental Fig. 5). However, certain β-cell dysfunctions induced by high glucose challenges were not reversed by G6PD knockdown via siRNA, suggesting a possibility that G6PD may not be sufficient for glucose-induced β-cell dysfunctions. Although we cannot exclude the possibility that the different outcomes between our report and the previous report (24) might result from distinct experimental conditions and approaches, it is very likely that G6PD expression and its function in β-cells would be regulated by complex mechanisms under hyperglycemic condition. To clarify this controversy, it is definitely required to explore the exact role of elevated G6PD on β-cell functions through analyzing β-cell-specific G6PD-overexpressing transgenic animals or β-cell-specific G6PD knockout animals in the future studies.

In summary, we show that up-regulated G6PD in β-cells would mediate β-cell dysfunction through oxidative stress and NF-κB activation. Role of G6PD in β-cell dysfunction is associated with the amplified inflammatory responses resulted from proinflammatory cytokine production, probably by the activation of NF-κB signaling pathway. Furthermore, it is plausible to propose that elevated G6PD would be a crucial factor, at least, in part, contributing to lipotoxicity and/or glucotoxicity-mediated pathogenesis of β-cell dysfunction in type 2 diabetes.

Acknowledgment

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