Glucose Toxicity in β-Cells: Type 2 Diabetes, Good Radicals Gone Bad, and the Glutathione Connection

R. Paul Robertson, Jamie Harmon, Phuong Oanh Tran, Yoshito Tanaka, and Hiroki Takahashi

Chronic exposure to hyperglycemia can lead to cellular dysfunction that may become irreversible over time, a process that is termed glucose toxicity. Our perspective about glucose toxicity as it pertains to the pancreatic β-cell is that the characteristic decreases in insulin synthesis and secretion are caused by decreased insulin gene expression. The responsible metabolic lesion appears to involve a posttranscriptional defect in pancreas duodenum homeobox-1 (PDX-1) mRNA maturation. PDX-1 is a critically important transcription factor for the insulin promoter, is absent in glucotoxic islets, and, when transfected into glucotoxic β-cells, improves insulin promoter activity. Because reactive oxygen species are produced via oxidative phosphorylation during anaerobic glycolysis, via the Schiff reaction during glycation, via glucose autoxidation, and via hexosamine metabolism under supraphysiological glucose concentrations, we hypothesize that chronic oxidative stress is an important mechanism for glucose toxicity. Support for this hypothesis is found in the observations that high glucose concentrations increase intraislet peroxide levels, that islets contain very low levels of antioxidant enzyme activities, and that adenoviral overexpression of antioxidant enzymes in vitro in islets, as well as exogenous treatment with antioxidants in vivo in animals, protect the islet from the toxic effects of excessive glucose levels. Clinically, consideration of antioxidants as adjunct therapy in type 2 diabetes is warranted because of the many reports of elevated markers of oxidative stress in patients with this disease, which is characterized by imperfect management of glycemia, consequent chronic hyperglycemia, and relentless deterioration of β-cell function. *Diabetes* 52: 581–587, 2003

From the Pacific Northwest Research Institute, Seattle, Washington.

Address correspondence and reprint requests to R. Paul Robertson, Pacific Northwest Research Institute, 720 Broadway, Seattle, WA 98122.

Received for publication 5 September 2002 and accepted in revised form 11 November 2002.

DN, dominant-negative; GCL, γ-glutamylcysteine ligase; GSH, glutathione; GSSG, oxidized GSH; GPx, GSH peroxidase; JNK; c-Jun NH_2-terminal kinase; PDX-1, pancreas duodenum homeobox-1; ROS, reactive oxygen species; SOD, superoxide dismutase.

After type 2 diabetes patients initially respond to diet and oral hypoglycemic agents, why do they usually relapse into hyperglycemia, despite dietary compliance and maximal drug dosages? Is this a case of β-cell desensitization to the drugs, or is it something more sinister happening to the β-cell over time? Is there ongoing apoptosis of β-cells, and, if so, is this caused by aberrant genetic programming or something bad that invades the β-cell's microenvironment? Type 2 diabetes is characterized as a polygenic disorder and generally thought of as a syndrome, rather than a single specific entity. This suggests that a common adverse force is exerted on β-cells in all patients, regardless of the initial specific pathogenesis. One such force all patients experience in common is the chronic tendency toward developing abnormally high glucose levels on a daily basis. Even though fasting glucose and HbA_{1c} levels might be within the normal range, postprandial levels of glucose are often abnormal. The glucose toxicity theory proposes that continual exposure to modest increases in blood glucose over a long period of time could have adverse effects on β-cells. In essence, the consequence of type 2 diabetes, hyperglycemia, is proposed as a secondary cause of continued β-cell deterioration.

Definition of glucose toxicity. For the purposes of this article, glucose toxicity of the islet is defined as nonphysiological and potentially irreversible β-cell damage caused by chronic exposure to supraphysiological glucose concentrations. In its initial stages, this damage is characterized by defective insulin gene expression (1,2). Desensitization refers to a temporary physiological state of cellular refractoriness to glucose stimulation induced by repeated or prolonged exposure to high glucose concentrations. Desensitization is reversed in a time-dependent manner, usually minutes after restoration of normal glucose concentrations, and implies involvement of an intrinsic and reversible alteration in stimulus-secretion coupling. β-Cell exhaustion refers to a physical depletion of β-cell insulin stores secondary to prolonged, chronic stimulation with glucose or nonglucose secretatgogues, so that insulin secretion is not possible, even if the β-cell were to become resensitized to glucose. An important distinction between β-cell exhaustion and glucose toxicity is that the exhausted islet has no defects in insulin synthesis, and therefore cell function fully recovers as it rests. Glucose toxicity, on the other hand, implies the gradual, time-

dependent establishment of irreversible damage to cellular components of insulin production and, therefore, to insulin content and secretion. Clearly, glucose toxicity might also affect other important steps on the way from insulin gene expression to insulin release into the blood. Such candidates include decreased translational rates in insulin synthesis, suppression of glucokinase gene expression, decreased mitochondrial function, compromised exocytotic mechanisms, and accelerated apoptosis (3–5). In varying degrees, data exist that support each of these alternatives, but the preponderance of the reported experiments performed have not emphasized the critical dimension of time of exposure to supraphysiological glucose concentrations. Hence, arguably, some of the phenomena reported might be more accurately described as observations from experiments involving glucose desensitization or β-cell exhaustion rather than glucose toxicity.

Mechanism of β-cell glucose toxicity involves pancreas duodenum homeobox-1 and insulin gene expression. In 1992, we observed that chronic exposure of HIT-T15 cells to supraphysiological concentrations of glucose over several months caused gradual loss of insulin gene expression (1). We then learned that the mechanism of this adverse event involves the loss of mRNA and protein levels of pancreas duodenum homeobox-1 (PDX-1), a critical regulator of insulin promoter activity (Fig. 1). In a series of studies, we observed that the loss of PDX-1 mRNA was associated with a posttranscriptional arrest in PDX-1 mRNA maturation, and that the consequent abnormality in insulin promoter activity in HIT-T15 cells could be partially corrected by transient transfection of PDX-1 cDNA (6–9). Decreased DNA binding of another important transcription factor, RIPE-3b1 activator, also appeared to be involved (8,10), which may explain why transfection of PDX-1 cDNA alone was not sufficient for complete restoration of insulin promoter activity (9). We extended this work to an in vivo model, the ZDF rat, and observed that these animals lost islet DNA binding activity of PDX-1 as well as insulin gene expression as they progressively developed more severe hyperglycemia (11). However, DNA binding activity and gene expression of PDX-1 and insulin were preserved and glycemic control was improved when we treated the animals with troglitazone (11), aminoguanidine (12), phlorizin (13), or *N*-acetylcysteine (Fig. 2) (12). In contrast, no beneficial effects were found when we treated the animals with a nitric oxide synthesis inhibitor (12) or bezafibrate (13), a drug that lowered circulating triglyceride but not glucose. Because troglitazone and phlorizin lower blood glucose levels, and because troglitazone, aminoguanidine, and *N*-acetylcysteine have antioxidant activity, we concluded that loss of insulin gene expression caused by chronic hyperglycemia and resultant glucose toxicity in the ZDF animal was at least in part explained by chronic oxidative stress.

Although the mechanism of decreased insulin gene expression can be largely attributed to decreased PDX-1 gene expression, what might account for the latter? Recently, Kaneto et al. (14) observed that adenoviral overexpression of dominant-negative c-Jun NH_2-terminal kinase (DN-JNK) protected both PDX-1 binding to DNA and insulin gene mRNA from hydrogen peroxide-induced oxidative stress as well as from the adverse effects of

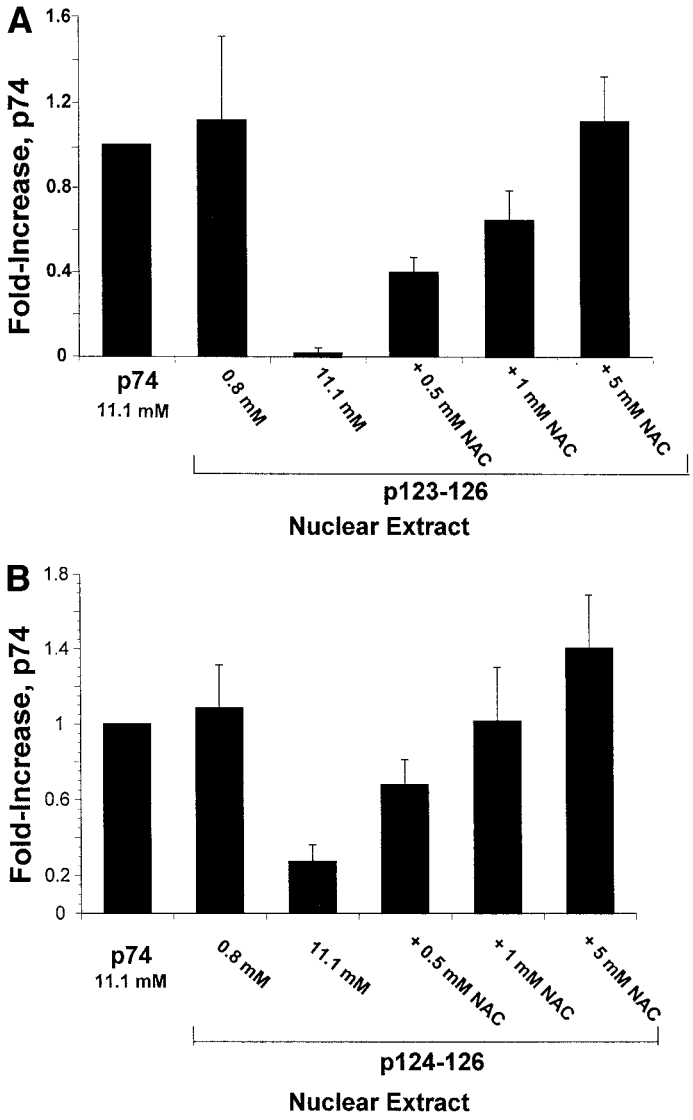

FIG. 1. *A*: Effect of increasing concentrations of *N*-acetylcysteine (NAC) on the disappearance of PDX-1 measured by Western blot analysis using nuclear extracts of HIT-T15 cells cultured in RPMI media containing either 0.8 or 11.1 mmol/l glucose for passage 74 (p74; control) and passages 123–126 (p123-126). *N*-acetylcysteine prevented the disappearance of PDX-1 protein that was associated with prolonged culturing with the higher glucose concentration. *B*: Effect of increasing concentrations of *N*-acetylcysteine on DNA binding of PDX-1 measured by electromobility shift assay of nuclear extracts in the same passages of HIT-T15 cells as described in *A*. *N*-acetylcysteine prevented the disappearance of PDX-1 binding that was associated with prolonged culturing with the higher glucose concentration. The methods are the same as in Tanaka et al. (12).

hyperglycemia in a transplantation model. However, the degree to which the hydrogen peroxide and DN-JNK effects on insulin mRNA and PDX-1 binding activity were clearly separated from apoptosis is not clear. Fluorescein diacetate fluorescence was used to demonstrate β-cell viability, but no quantitative analysis of cell death or β-cell mass was provided to document that these variables were quantitatively comparable under control and experimental conditions. Nonetheless, this work provides the potentially important clue that JNK may be involved in the adverse effects of glucose toxicity. Definitive demonstration of the actual mechanism by which JNK might interfere with PDX-1 gene expression and insulin mRNA level awaits further experimentation.

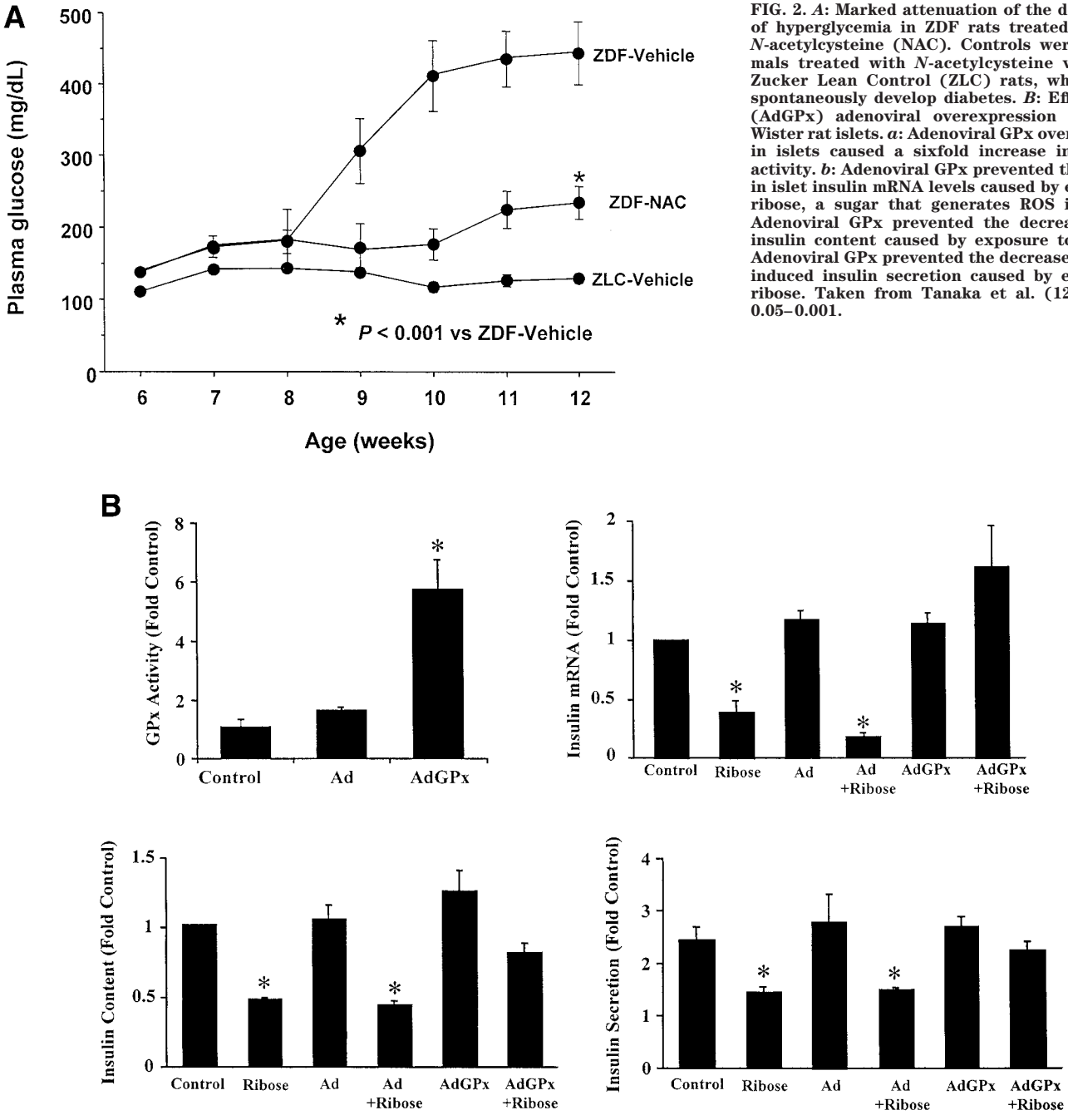

FIG. 2. ***A*****: Marked attenuation of the development of hyperglycemia in ZDF rats treated daily with *N*-acetylcysteine (NAC). Controls were ZDF animals treated with *N*-acetylcysteine vehicle and Zucker Lean Control (ZLC) rats, which do not spontaneously develop diabetes.** ***B*****: Effect of GPx (AdGPx) adenoviral overexpression in isolated Wister rat islets.** ***a*****: Adenoviral GPx overexpression in islets caused a sixfold increase in islet GPx activity.** ***b*****: Adenoviral GPx prevented the decrease in islet insulin mRNA levels caused by exposure to ribose, a sugar that generates ROS in islets.** ***c*****: Adenoviral GPx prevented the decrease in islet insulin content caused by exposure to ribose.** ***d*****: Adenoviral GPx prevented the decrease in glucose-induced insulin secretion caused by exposure to ribose. Taken from Tanaka et al. (12,23): *P < 0.05–0.001.**

Evidence that the islet is uniquely at risk for oxidative damage. The concept that the islet is unusually at risk for damage by pro-oxidant forces is not a new one (Fig. 3). Oxidative phosphorylation during anaerobic glycolysis generates reactive oxygen species (ROS), a process that might become excessive in hyperglycemic states (15). The following are metabolic pathways that excess glucose might be shunted into when it accumulates beyond the levels that glycolytic enzymes can handle and that can form ROS: glycosylation (Schiff reaction) (16), glucose autoxidation (17,18), and the glucosamine pathway (19). ROS that might be formed include superoxide, hydrogen peroxide, nitric oxide, and hydroxyl radicals. Among these, the hydroxyl radical is the most toxic because it easily passes through membrane barriers to the cell's nucleus and strongly reacts mutagenically with DNA.

The concept of glucose autoxidation and the consequent excess generation of ROS in relation to diabetes were proposed as early as 1987 by Wolff and Dean (17), although its relevance to defective β-cell function has not received a great deal of investigation. In 1988, Hunt et al. (18) demonstrated that α-hydroxyaldehyde products of glucose (such as glyceraldehyde) can undergo enolization to produce an enediol radical anion in the presence of heavy metals. In the presence of oxygen, these anions generate superoxide anions that combine to form hydrogen peroxide in a reaction catalyzed by superoxide dismutase (SOD). In the presence of heavy metals, hydrogen peroxide forms the highly toxic hydroxyl radical. Hunt et al. also demonstrated that hydroxyl radical scavengers protect against glucose-induced fragmentation of protein.

Earlier work by Grankvist et al. (20) demonstrated that

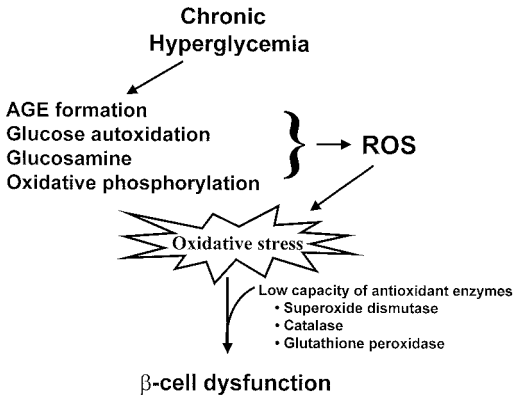

FIG. 3. Four biochemical pathways that are sensitive to glucose and produce ROS (see text). The islet is particularly at risk for chronic oxidative stress when exposed to long-term hyperglycemia because it expresses very low levels of antioxidant mRNA, protein, and activity (see text and Table 1.).

pancreatic islets contain relatively small amounts of the antioxidant enzymes CuZn-SOD, Mn-SOD, catalase, and glutathione peroxidase (GPx). In 1982, Malaisse et al. (21) demonstrated that β-cells in albino rats were sensitive to peroxide and that the activity of GPx was low. At about the same time, Grankvist et al. (22) demonstrated that treatment with SOD prevented alloxan-induced diabetes in mice. These and many other observations have reinforced the notion that the intrinsically low levels of antioxidant activity of islets render them particularly at risk for ROS-induced damage (Table 1).

The pathophysiological implication of the islet's intrinsically low level of antioxidant enzyme expression and activity is that the β-cell is at greater risk of oxidative damage than other tissues with higher levels of antioxidant protection. To explore this concept in greater depth, we recently examined whether glucose could increase oxidative stress in human islets. Compared with a low concentration of glucose, a higher concentration was shown to increase the level of intracellular peroxide levels within the islet (23). This was prevented by coincubation with mannoheptulose, indicating that glucose metabolism was essential for the event. These observations agree with those of Ihara et al. (24), who reported elevated levels of oxidative stress markers (8-hydroxy-2′-deoxyguanosine and 4-hydroxy-2-nonenal-modified proteins) in β-cells of Goto Kakazaki rats. Because GPx levels are known to be diminished in islets, we also examined whether interference with the existing level of GPx activity would facilitate the adverse effects of oxidative stress on the islet. Incubation of islets with buthionine sulfoximine, an inhibitor of γ-glutamylcysteine ligase (GCL) and thereby glutathione (GSH) synthesis, negated the ameliorative effect of *N*-acetylcysteine on ribose-induced decreases in insulin gene expression, emphasizing the importance of endogenous GSH and GPx activity in protecting the islet (23). In additional experiments, we observed that infection of isolated islets with adenovirus encoding GPx increased enzymatic activity sixfold. This protected the islet against the effects of ribose to increase intraislet peroxide levels and to decrease insulin gene expression, insulin content, and glucose-induced insulin secretion. These results led us to suggest that therapeutic steps designed to increase intrinsic antioxidant activity within the islet may protect it against the oxidative stress associated with glucose toxicity (23).

The islet, antioxidant enzymes, and GSH metabolism. The generation of ROS per se seems likely to be a physiological event that contributes to the regulation of general metabolic health, such as mediating phagocyte killing of bacteria. Another physiological function is to modulate transcription of genes, such as nuclear factor-κB (NF-κB), a well-known redox-sensitive transcription factor. However, the elegantly designed host-defense mechanisms against ROS provided by antioxidant enzymes and GSH synthesis (Fig. 4) (rev. in 54,55) imply that ROS levels need to be finely regulated to keep good radicals from going bad and thereby avoid oxidative damage to cellular processes (56).

The major antioxidant enzymes are catalase, GPx, and the two SODs (Fig. 4). Mn-SOD is active in the mitochondria, and Cu/Zn-SOD is active in the cytosol. SOD catalyzes the reaction of superoxides with hydrogen to form hydrogen peroxide. Catalase catalyzes dismutation of hydrogen peroxide to water and molecular oxygen. In mammals, at least four species of GPx exist that catalyze the reduction of hydrogen peroxide by GSH. GPx can also reduce lipid peroxides to alcohols, which catalase cannot do. GCL, formerly termed glutamylcysteine synthetase, is a holoenzyme made up of a catalytic (heavy) and catalytic (light) subunit. GCL catalyzes the cysteine substrate–limiting reaction that forms γ-glutamyl cysteine, the substrate for the second enzyme regulating GSH synthesis, GSH synthetase. GSH is regenerated for future use from its oxidized form, GSSG, through a reaction involving GSH reductase and NADPH. To protect itself from the highly toxic hydroxyl radical, the β-cell must metabolize hydrogen peroxide via catalase and GPx. However, a potentially major problem for the β-cell is its unusually low complement of SOD, catalase, and GPx. This unusual situation sets up the β-cell as an easy target for ROS, whether generated by interactions with cytokines or too much glucose.

Therapeutic implications. The major thesis of this article is that the unrelenting deterioration of β-cell function over time in type 2 diabetes may be explained by incompletely treated hyperglycemia, which in turns forms exces-

TABLE 1
Literature supporting chronic oxidative stress as a mechanism for glucose toxicity of the β-cell

	References
ROS can be formed from glucose	17, 18, 23, 24
Islets contain unusually low concentrations of antioxidant enzymes and activity	20, 23, 25–29
Antioxidant drugs protect the β-cell from the effects of chronic exposure to supraphysiological glucose levels	12, 22, 30–37
Antioxidant gene transfer techniques enhance protection of β-cells from oxidative stress	23, 38–45
Type 2 diabetes in humans is associated with increased markers of oxidative stress	46–53

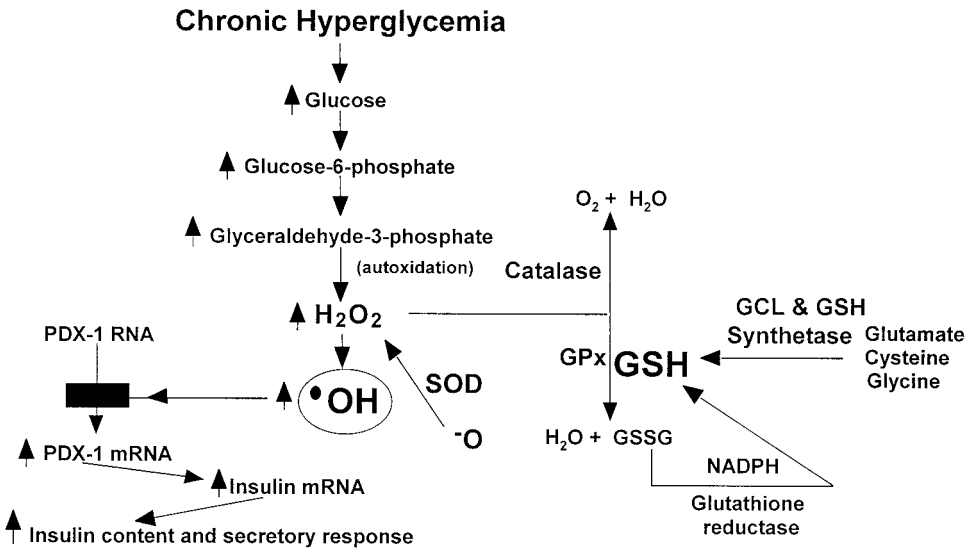

FIG. 4. Mechanism of action of β-cell glucose toxicity. In our hypothesis, hyperglycemia presents such excessively high concentrations of glucose to islet glycolytic enzymes that glucose is shunted to the alternative pathway of enolization, with subsequent formation of superoxide, H_2O_2, and eventually hydroxyl radicals (see text). Hydroxyl radicals interfere with normal processing of PDX-1 mRNA, a necessary transcription factor for insulin gene expression and glucose-induced insulin secretion. The two major defense mechanisms against H_2O_2 formation are catalase, which metabolizes H_2O_2 into water and oxygen, and GPx, which depends on islet GSH to convert H_2O_2 into water and GSSG. GSH is regenerated by islet NADPH and GSH reductase. The enzymes responsible for de novo GSH synthesis are GCL and GSH synthetase (see text).

sive levels of ROS that continually bombard and damage the β-cell. Clinical evidence supporting this line of thinking includes the many reports of elevated markers of oxidative stress in the plasma and urine of type 2 diabetic patients, as well as decreased levels of GSH in their blood cells (Table 1). The glucose levels and duration of exposure that might be required for this sequence of events have not been defined at a clinical level. However, under in vitro conditions using a cell line, we have established that glucose toxicity is a continuous rather than a threshold function of glucose concentration, and that the shorter the period of antecedent glucose toxicity, the more likely that full recovery of β-cell function will occur (57). Although difficult to design, clinical studies are clearly needed to determine whether these conclusions hold true for diabetic patients, or whether there is a threshold of glucose concentration (or time of exposure to hyperglycemia) above which glucose toxicity begins to occur in vivo.

As developed above, both defective insulin gene expression and excessive apoptosis of β-cells in type 2 diabetes are considered by many to be major mechanisms of continuing deterioration of insulin secretion. If true, it seems possible that chronic oxidative damage is a major contributor to both processes. One means of testing this hypothesis would be to determine whether better maintenance of glucose levels in hyperglycemic patients is accompanied by lower levels of ROS and improved insulin secretion. Another would be to add antioxidants to conventional therapy to determine whether this maneuver would prevent continued deterioration in β-cell function, despite continued hyperglycemia. Arguments against this hypothesis can be mounted using the largely negative data from studies with antioxidant vitamins, which do not reliably improve glycemic control. However, a more intensive test of this hypothesis would involve much stronger antioxidants, such as *N*-acetylcysteine, along with indexes of β-cell function that are more sensitive than fasting glucose or HbA_{1c} levels. Should it be the case that the steady decline in β-cell function in type 2 diabetes is attributable to any significant extent to ongoing apoptosis via chronic oxidative stress with no deterioration in β-cell replication, interference with apoptosis by antioxidants might provide a much needed new ancillary approach to conventional treatment that could stabilize β-cell mass. The same reasoning can be applied to islet transplantation. The process of islet procurement from a cadaveric donor and subsequent intrahepatic transplantation is a long and challenging one for β-cells that involves stress from hypothermia, exposure to collagenase, physical separation from exocrine tissue, and deprivation of adequate oxygenation in the early days posttransplantation. These stresses seem likely to generate unhealthy levels of ROS. If so, introduction of antioxidants into the various stages of islet isolation might prove valuable.

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health Grant NIDDK RO1 38325 (to R.P.R.) and an American Diabetes Association mentor-based grant (to R.P.R.).

REFERENCES

1. Robertson RP, Zhang HJ, Pyzdrowski KL, Walseth TF: Preservation of insulin mRNA levels and insulin secretion in HIT cells by avoidance of chronic exposure to high glucose concentrations. *J Clin Invest* 90:320–325, 1992
2. Robertson RP, HJ, Tanaka Y, Sacchi G, Tran POT, Gleason C, Poitout V: Glucose toxicity of the β-cell: cellular and molecular mechanisms. In *Diabetes Mellitus*. LeRoith D, Olefsky JM, Taylor S, Eds. New York, Lippincott Williams & Wilkins, 2000, p. 125–132
3. Kajimoto Y, Matsuoka T, Kaneto H, Fujitani Y, Kishimoto M, Sakamoto K, Matsuhisa M, Kawamori R, Yamasaki Y, Hori M: Induction of glycation suppresses glucokinase gene expression in HIT-T15 cells. *Diabetologia* 42:1417–1424, 1999
4. Maechler P, Jornot L, Wollheim CB: Hydrogen peroxide alters mitochondrial activation and insulin secretion in pancreatic beta cells. *J Biol Chem* 274:27905–27913, 1999

5. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC: β-Cell deficit and increased β-cell apoptosis in humans with type-2 diabetes mellitus. *Diabetes* 52:102–110, 2003
6. Olson LK, Redmon JB, Towle HC, Robertson RP: Chronic exposure of HIT cells to high glucose concentrations paradoxically decreases insulin gene transcription and alters binding of insulin gene regulatory protein [see comments]. *J Clin Invest* 92:514–519, 1993
7. Olson LK, Sharma A, Peshavaria M, Wright CV, Towle HC, Rodertson RP, Stein R: Reduction of insulin gene transcription in HIT-T15 beta cells chronically exposed to a supraphysiologic glucose concentration is associated with loss of STF-1 transcription factor expression [published erratum in *Proc Natl Acad Sci U S A* 92:11322, 1995]. *Proc Natl Acad Sci U S A* 92:9127–9131, 1995
8. Sharma A, Olson LK, Robertson RP, Stein R: The reduction of insulin gene transcription in HIT-T15 beta cells chronically exposed to high glucose concentration is associated with the loss of RIPE3b1 and STF-1 transcription factor expression. *Mol Endocrinol* 9:1127–1134, 1995
9. Harmon JS, Tanaka Y, Olson LK, Robertson RP: Reconstitution of glucotoxic HIT-T15 cells with somatostatin transcription factor-1 partially restores insulin promoter activity. *Diabetes* 47:900–904, 1998
10. Poitout V, Olson LK, Robertson RP: Chronic exposure of betaTC-6 cells to supraphysiologic concentrations of glucose decreases binding of the RIPE3b1 insulin gene transcription activator. *J Clin Invest* 97:1041–1046, 1996
11. Harmon JS, Gleason CE, Tanaka Y, Oseid EA, Hunter-Berger KK, Robertson RP: In vivo prevention of hyperglycemia also prevents glucotoxic effects on PDX-1 and insulin gene expression. *Diabetes* 48:1995–2000, 1999
12. Tanaka Y, Gleason CE, Tran PO, Harmon JS, Robertson RP: Prevention of glucose toxicity in HIT-T15 cells and Zucker diabetic fatty rats by antioxidants. *Proc Natl Acad Sci U S A* 96:10857–10862, 1999
13. Harmon JS, Gleason CE, Tanaka Y, Poitout V, Robertson RP: Antecedent hyperglycemia, not hyperlipidemia, is associated with increased islet triacylglycerol content and decreased insulin gene mRNA level in Zucker diabetic fatty rats. *Diabetes* 50:2481–2486, 2001
14. Kaneto H, Xu G, Fujii N, Kim S, Bonner-Weir S, Weir GC: Involvement of c-Jun N-terminal kinase in oxidative stress-mediated suppression of insulin gene expression. *J Biol Chem* 277:30010–30018, 2002
15. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M: Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 404:787–790, 1999
16. Baynes JW: Role of oxidative stress in development of complications in diabetes (Review). *Diabetes* 40:405–412, 1991
17. Wolff SP, Dean RT: Glucose autoxidation and protein modification: the potential role of "autoxidative glycosylation" in diabetes. *Biochem J* 245: 243–250, 1987
18. Hunt JV, Dean RT, Wolff SP: Hydroxyl radical production and autoxidative glycosylation: glucose autoxidation as the cause of protein damage in the experimental glycation model of diabetes mellitus and ageing. *Biochem J* 256:205–212, 1988
19. Kaneto H, Xu G, Song KH, Suzuma K, Bonner-Weir S, Sharma A, Weir GC: Activation of the hexosamine pathway leads to deterioration of pancreatic beta-cell function through the induction of oxidative stress. *J Biol Chem* 276:31099–31104, 2001
20. Grankvist K, Marklund SL, Taljedal IB: CuZn-superoxide dismutase, Mn-superoxide dismutase, catalase and glutathione peroxidase in pancreatic islets and other tissues in the mouse. *Biochem J* 199:393–398, 1981
21. Malaisse WJ, Malaisse-Lagae F, Sener A, Pipeleers DG: Determinants of the selective toxicity of alloxan to the pancreatic B cell. *Proc Natl Acad Sci U S A* 79:927–930, 1982
22. Grankvist K, Marklund S, Taljedal IB: Superoxide dismutase is a prophylactic against alloxan diabetes. *Nature* 294:158–160, 1981
23. Tanaka Y, Tran PO, Harmon J, Robertson RP: A role for glutathione peroxidase in protecting pancreatic beta cells against oxidative stress in a model of glucose toxicity. *Proc Natl Acad Sci U S A* 99:12363–12368, 2002
24. Ihara Y, Toyokuni S, Uchida K, Odaka H, Tanaka T, Ikeda H, Hiai H, Seino Y, Yamada Y: Hyperglycemia causes oxidative stress in pancreatic β-cells of GK rats, a model of type 2 diabetes. *Diabetes* 48:927–932, 1999
25. Welsh N, Margulis B, Borg LA, Wiklund HJ, Saldeen J, Flodstrom M, Mello MA, Andersson A, Pipeleers DG, Hellerstrom C, et al.: Differences in the expression of heat-shock proteins and antioxidant enzymes between human and rodent pancreatic islets: implications for the pathogenesis of insulin-dependent diabetes mellitus. *Mol Med* 1:806–820, 1995
26. Wacker T, Jahr H, Weinand S, Brandhorst H, Brandhorst D, Lau D, Hering BJ, Federlin K, Bretzel RG: Different toxic effects of hydrogen peroxide, nitric oxide, and superoxide on human, pig, and rat islets of Langerhans. *Exp Clin Endocrinol Diabetes* 103 (Suppl. 2):133–135, 1995
27. Lenzen S, Drinkgern J, Tiedge M: Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. *Free Radic Biol Med* 20:463–466, 1996
28. Tiedge M, Lortz S, Munday R, Lenzen S: Complementary action of antioxidant enzymes in the protection of bioengineered insulin-producing RINm5F cells against the toxicity of reactive oxygen species. *Diabetes* 47:1578–1585, 1998
29. Kralik PM, Xu B, Epstein PN: Catalase transfection decreases hydrogen peroxide toxicity in a pancreatic beta cell line. *Endocr Res* 24:79–87, 1998
30. Mendola J, Wright JR Jr, Lacy PE: Oxygen free-radical scavengers and immune destruction of murine islets in allograft rejection and multiple low-dose streptozocin-induced insulitis. *Diabetes* 38:379–385, 1989
31. Nomikos IN, Wang Y, Lafferty KJ: Involvement of O2 radicals in "autoimmune" diabetes. *Immunol Cell Biol* 67:85–87, 1989
32. Sreenan S, Sturis J, Pugh W, Burant CF, Polonsky KS: Prevention of hyperglycemia in the Zucker diabetic fatty rat by treatment with metformin or troglitazone. *Am J Physiol* 271:E742–E747, 1996
33. Fukui T, Noma T, Mizushige K, Aki Y, Kimura S, Abe Y: Dietary troglitazone decreases oxidative stress in early stage type II diabetic rats. *Life Sci* 66:2043–2049, 2000
34. Ho E, Chen G, Bray TM: Supplementation of N-acetylcysteine inhibits NFkappaB: activation and protects against alloxan-induced diabetes in CD-1 mice. *FASEB J* 13:1845–1854, 1999
35. Kaneto H, Kajimoto Y, Miyagawa J, Matsuoka T, Fujitani Y, Umayahara Y, Hanafusa T, Matsuzawa Y, Yamasaki Y, Hori M: Beneficial effects of antioxidants in diabetes: possible protection of pancreatic β-cells against glucose toxicity. *Diabetes* 48:2398–2406, 1999
36. Tajiri Y, Grill VE: Interactions between vitamin E and glucose on B-cell functions in the rat: an in vivo and in vitro study. *Pancreas* 18:274–281, 1999
37. Ihara Y, Yamada Y, Toyokuni S, Miyawaki K, Ban N, Adachi T, Kuroe A, Iwakura T, Kubota A, Hiai H, Seino Y: Antioxidant alpha-tocopherol ameliorates glycemic control of GK rats, a model of type 2 diabetes. *FEBS Lett* 473:24–26, 2000
38. Tiedge M, Lortz S, Munday R, Lenzen S: Protection against the co-operative toxicity of nitric oxide and oxygen free radicals by overexpression of antioxidant enzymes in bioengineered insulin-producing RINm5F cells. *Diabetologia* 42:849–855, 1999
39. Hohmeier HE, Thigpen A, Tran VV, Davis R, Newgard CB: Stable expression of manganese superoxide dismutase (MnSOD) in insulinoma cells prevents IL-1beta-induced cytotoxicity and reduces nitric oxide production. *J Clin Invest* 101:1811–1820, 1998
40. Lortz S, Tiedge M, Nachtwey T, Karlsen AE, Nerup J, Lenzen S: Protection of insulin-producing RINm5F cells against cytokine-mediated toxicity through overexpression of antioxidant enzymes. *Diabetes* 49:1123–1130, 2000
41. Moriscot C, Pattou F, Kerr-Conte J, Richard MJ, Lemarchand P, Benhamou PY: Contribution of adenoviral-mediated superoxide dismutase gene transfer to the reduction in nitric oxide-induced cytotoxicity on human islets and INS-1 insulin-secreting cells. *Diabetologia* 43:625–631, 2000
42. Kubisch HM, Wang J, Luche R, Carlson E, Bray TM, Epstein CJ, Phillips JP: Transgenic copper/zinc superoxide dismutase modulates susceptibility to type I diabetes. *Proc Natl Acad Sci U S A* 91:9956–9959, 1994
43. Kubisch HM, Wang J, Bray TM, Phillips JP: Targeted overexpression of Cu/Zn superoxide dismutase protects pancreatic beta-cells against oxidative stress. *Diabetes* 46:1563–1566, 1997
44. Benhamou PY, Moriscot C, Richard MJ, Beatrix O, Badet L, Pattou F, Kerr-Conte J, Chroboczek J, Lemarchand P, Halimi S: Adenovirus-mediated catalase gene transfer reduces oxidant stress in human, porcine and rat pancreatic islets. *Diabetologia* 41:1093–1100, 1998
45. Xu B, Moritz JT, Epstein PN: Overexpression of catalase provides partial protection to transgenic mouse beta cells. *Free Radic Biol Med* 27:830–837, 1999
46. Nourooz-Zadeh J, Tajaddini-Sarmadi J, McCarthy S, Betteridge DJ, Wolff SP: Elevated levels of authentic plasma hydroperoxides in NIDDM. *Diabetes* 44:1054–1058, 1995
47. Yoshida K, Hirokawa J, Tagami S, Kawakami Y, Urata Y, Kondo T: Weakened cellular scavenging activity against oxidative stress in diabetes mellitus: regulation of glutathione synthesis and efflux. *Diabetologia* 38: 201–210, 1995
48. Dandona P, Thusu K, Cook S, Snyder B, Makowski J, Armstrong D, Nicotera T: Oxidative damage to DNA in diabetes mellitus. *Lancet* 347: 444–445, 1996
49. Ceriello A, Falleti E, Bortolotti N, Motz E, Cavarape A, Russo A, Gonano F,

Bartoli E: Increased circulating intercellular adhesion molecule-1 levels in type II diabetic patients: the possible role of metabolic control and oxidative stress. *Metabolism* 45:498–501, 1996
50. Thornalley PJ, McLellan AC, Lo TW, Benn J, Sonksen PH: Negative association between erythrocyte reduced glutathione concentration and diabetic complications. *Clin Sci (Colch)* 91:575–582, 1996
51. Santini SA, Marra G, Giardina B, Cotroneo P, Mordente A, Martorana GE, Manto A, Ghirlanda G: Defective plasma antioxidant defenses and enhanced susceptibility to lipid peroxidation in uncomplicated IDDM. *Diabetes* 46:1853–1858, 1997
52. Leinonen J, Lehtimaki T, Toyokuni S, Okada K, Tanaka T, Hiai H, Ochi H, Laippala P, Rantalaiho V, Wirta O, Pasternack A, Alho H: New biomarker evidence of oxidative DNA damage in patients with non-insulin-dependent diabetes mellitus. *FEBS Lett* 417:150–152, 1997
53. Rehman A, Nourooz-Zadeh J, Moller W, Tritschler H, Pereira P, Halliwell B: Increased oxidative damage to all DNA bases in patients with type II diabetes mellitus. *FEBS Lett* 448:120–122, 1999
54. Nordberg J, Arner ES: Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic Biol Med* 31:1287–1312, 2001
55. Will Y: Overview of glutathione function and metabolism. In *Current Protocols in Toxicology*. New York, John Wiley & Sons, 1999, p. 6.1.1–6.1.18
56. Evans JL, Goldfine ID, Maddux BA, Grodsky GM: Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev* 23:599–622, 2002
57. Gleason CE, Gonzalez M, Harmon JS, Robertson RP: Determinants of glucose toxicity and its reversibility in the pancreatic islet beta-cell line, HIT-T15. *Am J Physiol Endocrinol Metab* 279:E997–E1002, 2000