# Are Oxidative Stress–Activated Signaling Pathways Mediators of Insulin Resistance and β-Cell Dysfunction?

**Joseph L. Evans,[1] Ira D. Goldfine,[2] Betty A. Maddux,[2] and Gerold M. Grodsky[2]**

**In both type 1 and type 2 diabetes, diabetic complications in target organs arise from chronic elevations of glucose. The pathogenic effect of high glucose, possibly in concert with fatty acids, is mediated to a significant extent via increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and subsequent oxidative stress. ROS and RNS directly oxidize and damage DNA, proteins, and lipids. In addition to their ability to directly inflict damage on macromolecules, ROS and RNS indirectly induce damage to tissues by activating a number of cellular stress-sensitive pathways. These pathways include nuclear factor-κB, p38 mitogen-activated protein kinase, $NH_2$-terminal Jun kinases/stress-activated protein kinases, hexosamines, and others. In addition, there is evidence that in type 2 diabetes, the activation of these same pathways by elevations in glucose and free fatty acid (FFA) levels leads to both insulin resistance and impaired insulin secretion. Therefore, we propose here that the hyperglycemia-induced, and possibly FFA-induced, activation of stress pathways plays a key role in the development of not only the late complications in type 1 and type 2 diabetes, but also the insulin resistance and impaired insulin secretion seen in type 2 diabetes.** ***Diabetes*** **52:1–8, 2003**

Both type 1 and type 2 diabetes possess a significant genetic component (1–3). In the case of type 2 diabetes, additional environmental factors, including hormones, increased caloric intake, decreased physical inactivity, and adiposity (1,2), have a marked influence on the disease. Now there is evidence that elevated levels of metabolic substrates contribute to the diabetic phenotype.

There are considerable amounts of data indicating that the chronic elevation of plasma glucose causes many of the major complications of diabetes, including nephropathy, retinopathy, neuropathy, and macro- and microvascular damage (1,4). A causative role for elevated free fatty acid (FFA) levels in the development of microvascular complications remains to be established, however. Increased levels of FFAs are positively correlated with both insulin resistance (5,6) and the deterioration of β-cell function in the context of concomitant hyperglycemia (7,8). These latter effects may result from oxidative stress.

There is evidence that oxidative stress, defined as a persistent imbalance between the production of highly reactive molecular species (chiefly oxygen and nitrogen) and antioxidant defenses, leads to tissue damage (9). Oxidative stress results from increased content of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS). Examples of ROS include charged species such as superoxide and the hydroxyl radical, and uncharged species such as hydrogen peroxide (9). There are data indicating that ROS formation is a direct consequence of hyperglycemia (10); more recent studies have suggested that increased FFA levels may also result in ROS formation (see below).

Because of their ability to directly oxidize and damage DNA, protein, and lipid, ROS are believed to play a key direct role in the pathogenesis of late diabetic complications (9,11). In addition to their ability to directly inflict macromolecular damage, ROS can function as signaling molecules to activate a number of cellular stress-sensitive pathways that cause cellular damage, and are ultimately responsible for the late complications of diabetes. Furthermore, these same pathways are linked to insulin resistance and decreased insulin secretion. In this review, we propose that ROS and oxidative stress induced by elevations in glucose and possibly FFA levels play a key role in causing insulin resistance and β-cell dysfunction by their ability to activate stress-sensitive signaling pathways (Fig. 1).

## HYPERGLYCEMIA AND STRESS-ACTIVATED PATHWAYS

In vivo studies have revealed that oxidative stress caused by hyperglycemia (and perhaps FFAs) occurs before the complications of diabetes become clinically evident

From [1]Medical Research Institute, San Francisco, California; and [2]the University of California at San Francisco, San Francisco, California.

Address correspondence and reprint requests to Dr. Joseph L. Evans, Medical Research Institute, 444 De Haro St., Suite 209, San Francisco, CA 94107-2347. E-mail: jevansphd@earthlink.net.

Received for publication 8 January 2002 and accepted in revised form 15 April 2002.

J.L.E. has received consulting fees from Medical Research Institute.

AGE, advanced glycation end product; ERK, extracellular signal–related kinases; FFA, free fatty acid; IκB, inhibitory protein κB; IKK-β, IκB kinase-β; IR, insulin receptor; IRS, IR substrate; JNK, $NH_2$-terminal Jun kinase; LA, α-lipoic acid; NF-κB, nuclear factor-κB; MAP, mitogen-activated protein; MAPK, MAP kinase; NAC, *N*-acetyl-L-cysteine; PKC, protein kinase C; RAGE, receptor for AGEs; RNS, reactive nitrogen species; ROS, reactive oxygen species; SAPK, stress-activated protein kinases; VEGF, vascular endothelial growth factor.

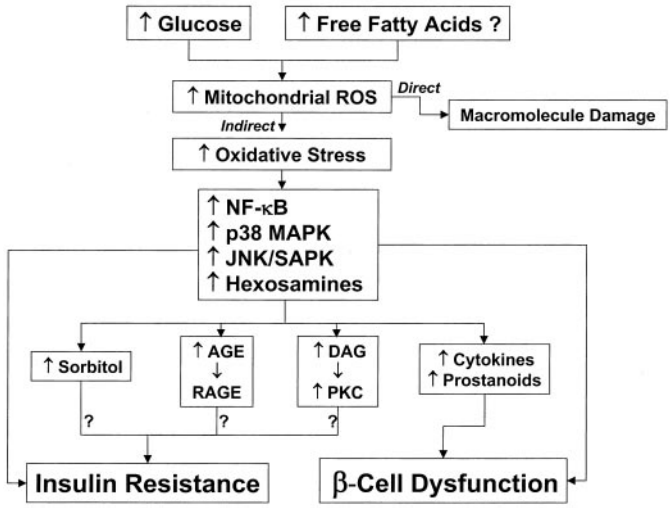


**FIG. 1. Proposed general theory of how elevated glucose and possibly FFA levels contribute to the pathophysiology of diabetes via the generation of ROS and consequent activation of numerous stress-sensitive pathways. The causative link among hyperglycemia, mitochondrial ROS generation, oxidative stress, and the development of diabetic complications has been previously suggested (10,11,22). ROS (and RNS), by inflicting macromolecular damage, may play a key direct role in the pathogenesis of diabetes. ROS also function as signaling molecules (analogous to second messengers) to activate several stress-sensitive pathways (indirect role). In addition, in type 2 diabetes, there is growing evidence that activation of stress-sensitive pathways, such as NF-kB, p38 MAPK, JNK/SAPK, and hexosamine, by elevations in glucose and possibly FFA levels leads to both insulin resistance and impaired insulin secretion. Thus ROS and oxidative stress, induced by elevations in glucose and possibly FFA levels, may play a key role in causing insulin resistance and β-cell dysfunction by their ability to activate stress-sensitive signaling pathways. The proposed sequence of events may also include other stress pathways, such as the increased production of AGE, sorbitol, cytokines, and prostanoids along with PKC activation. DAG, diacylglycerol.**

(9,12,13). Wolff and Dean (15) suggested that nonenzymatic protein glycation, a mechanism proposed early on to account for glucose cytotoxicity (14), was dependent on ROS (superoxide and hydroxyl) formation through transition metal-catalyzed glucose autoxidation. Research in numerous laboratories has indicated that hyperglycemia activates several major, well-characterized biochemical pathways that play a significant role in the etiology of diabetic complications. These pathways include advanced glycation end products (AGEs) and receptors for AGE (RAGE) (12), protein kinase C (PKC) (13), and the polyol pathway (16).

More recently, hyperglycemia has been implicated in the activation of additional biochemical pathways, including the stress-activated signaling pathways of nuclear factor-κB (NF-κB), $NH_2$-terminal Jun kinases/stress activated protein kinases (JNK/SAPK), p38 mitogen-activated protein (MAP) kinase, and hexosamine (17–19). Data now indicate that activation of these pathways is linked not only to the development of the late complications of diabetes, but also to insulin resistance and β-cell dysfunction.

**NF-κB pathway.** The most extensively studied intracellular pathway that is a target of hyperglycemia, ROS, and oxidative stress is the transcription factor NF-κB (17,20,21). NF-κB plays a critical role in mediating immune and inflammatory responses and apoptosis. NF-κB regulates the expression of a large number of genes, including several of those linked to the complications of diabetes (e.g., vascular endothelial growth factor [VEGF] and RAGE) (20). Many of the gene products regulated by NF-κB in turn activate NF-κB (e.g., VEGF, RAGE), leading to a vicious circle. The aberrant regulation of NF-κB is associated with a number of chronic diseases, including diabetes and atherosclerosis. Activation of NF-κB involves the phosphorylation-induced, proteasome-mediated degradation of the inhibitory subunit, inhibitory protein κB (IκB). IκB is phosphorylated by an upstream serine kinase, IκB kinase β (IKK-β), which is phosphorylated and activated by additional upstream serine kinases.

A recent study in bovine endothelial cells found that exposure to hyperglycemia initially increased the production of intracellular ROS, followed by activation of NF-κB (22). Subsequently, PKC activity and AGE and sorbitol levels increased. Disruption of mitochondrial ROS production by several distinct approaches blocked the hyperglycemia-induced increase in ROS production. As a consequence, hyperglycemia-induced effects on NF-κB, PKC, and AGE and sorbitol levels were also suppressed. The effects of hyperglycemia on ROS formation and NF-κB activation preceded the stimulation of the other systems. Therefore, these data implicated NF-κB activation as the initial signaling event. If extended to other cell types and tissues, these findings would support the idea that ROS formation is a primary event followed by activation of the other systems.

**JNK/SAPK pathway.** The JNKs/SAPKs are members of the complex superfamily of MAP serine/threonine protein kinases. This superfamily also includes the p38 MAP kinases (p38 MAPKs) and the extracellular signal−related kinases (ERKs) (18). In contrast to ERKs (also referred to as MAPKs), which are typically activated by mitogens, JNK/SAPK and p38 MAPK are known as stress-activated kinases, and are responsive to a variety of exogenous and endogenous stress-inducing stimuli, including hyperglycemia, ROS, oxidative stress, osmotic stress, proinflammatory cytokines, heat shock, and ultraviolet irradiation. JNK/SAPK are activated by hyperglycemia-induced oxidative stress and are likely involved in apoptosis mediated by hyperglycemia in human endothelial cell (23,24). $H_2O_2$ generation, JNK/SAPK activity, and subsequent apoptosis induced by hyperglycemia could be suppressed by vitamin C (23).

**p38 MAPK pathway.** Activation of the p38 MAPK pathway occurs in response to hyperglycemia and in diabetes. In vascular smooth muscle cells, treatment with insulin and hyperglycemia induces the activation of p38 MAPK (25). In rat aortic smooth muscle cells, high glucose causes a fourfold increase in p38 MAPK (26). In a study of glomeruli of rats rendered diabetic by streptozotocin, p38 MAPK activity was increased compared with controls, followed by increased phosphorylation of heat shock protein 25, a downstream substrate of p38 MAPK (27). These effects were mediated by increased ROS production. Increases in total levels of JNK/SAPK and p38 MAPK have been reported in nerve tissue of patients with type 1 and type 2 diabetes (28), although a causative role in the pathophysiology has not been established.

**Hexosamine pathway.** The excessive flux of glucose or FFAs into a variety of cell types results in the activation of the hexosamine biosynthetic pathway (19,29), which in

turn leads to insulin resistance and the development of late complications of diabetes (19,29,30). Recent data have implicated a hyperglycemia-induced increase in ROS formation in the activation of the hexosamine pathway. In bovine endothelial cells, hyperglycemia induced a significant increase in the hexosamine pathway, an effect that was blocked by an inhibitor of electron transport, a mitochondrial uncoupling agent (CCCP), and the expression of either uncoupling protein 1 or MnSOD (31).

Taken together, there is strong evidence to indicate that the NF-κB, JNK/SAPK, p38 MAPK, and hexosamine pathways are stress-sensitive signaling systems that can be activated by hyperglycemia and ROS in vitro and in vivo. Chronic activation of these pathways is associated with the late complications of diabetes. This in an area worthy of continued research activity, and one that could yield new insights into the molecular pathogenesis of hyperglycemia as well as identify pharmacological targets for the treatment and/or prevention of the late complications of diabetes. What has become equally intriguing is the growing number of reports linking the activation of these same pathways to insulin resistance and β-cell dysfunction.

## OXIDATIVE STRESS AND INSULIN RESISTANCE

Both insulin resistance and decreased insulin secretion are major features of the pathophysiology of type 2 diabetes (1,32–34). Insulin resistance most often precedes the onset of type 2 diabetes by many years, is present in a large segment of the general population, and is multifactorial (1,32). It is clear that insulin resistance has a genetic component (1–3): insulin resistance is a feature of the offspring of parents with type 2 diabetes, aggregates in families, and, in longitudinal studies of families, has been implicated as a major risk factor for developing type 2 diabetes.

Insulin resistance is also caused by acquired factors, such as obesity, sedentary lifestyle, pregnancy, and the presence of excess hormones (1,33). Initially, insulin resistance is compensated for by hyperinsulinemia, through which normal glucose tolerance is preserved. Reaven (32) and others have reported that at least 25% of nondiabetic individuals exhibit insulin resistance that is in the range of that seen in patients with type 2 diabetes. Deterioration into impaired glucose tolerance occurs when either the insulin resistance increases or the compensatory insulin secretory responses decrease, or when both occur. An increase in insulin, FFA, and/or glucose levels can increase ROS production and oxidative stress as well as activate stress-sensitive pathways. This, in turn, can worsen both insulin action and secretion, thereby accelerating the progression to overt type 2 diabetes.

**Antioxidants and type 2 diabetes.** As discussed above, oxidative stress has long been associated with the late complications of diabetes, and has been implicated in their etiology (9,11,35). More recently, studies have linked ROS production and oxidative stress to insulin resistance (36–40). Through in vitro studies and in animal models of diabetes, it has been found that antioxidants, especially α-lipoic acid (LA), improve insulin sensitivity (40–42). Several clinical trials, albeit small and of short duration, have also demonstrated that treatment with vitamin E, vitamin C, or glutathione improves insulin sensitivity in insulin-resistant individuals and/or patients with type 2 diabetes (43,44). The effect of LA has been quantitated by the euglycemic-hyperinsulinemic clamp (Fig. 2). For LA, the magnitude of this increased insulin sensitivity compares favorably with the currently available medications metformin and rosiglitazone. These insulin sensitizers produced an ~25 and ~20% improvement in insulin-stimulated glucose metabolism, respectively (45,46). Recently it has been shown that oral administration of a controlled release formulation of LA for 6 weeks lowered plasma fructosamine levels in patients with type 2 diabetes (47). Also, noncontrolled-release LA recently has been reported to increase insulin-mediated glucose disposal in patients with type 2 diabetes (48).

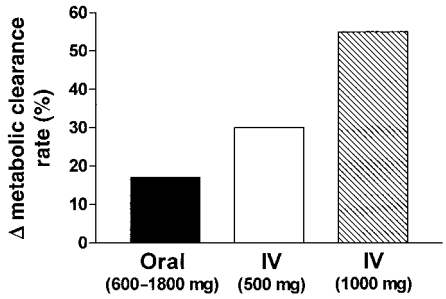


**FIG. 2. LA increases insulin-stimulated glucose metabolism in patients with type 2 diabetes. Intravenous (IV) administration of LA is able to significantly increase insulin sensitivity (as judged by the percent change [Δ] in the metabolic clearance rate [MCR]) in patients with type 2 diabetes, whereas oral administration exerts a lesser effect. ■, 17% increase in MCR ($P < 0.05$) (106); □, 30% increase in MCR ($P < 0.05$) (107); ▧, 55% increase in MCR ($P < 0.05$) (108). Figure reprinted with permission from Evans and Goldfine (44).**

LA's site of action has not yet been defined. Several laboratories have reported that use of LA in vitro at high concentrations (2.5 mmol/l) has a direct stimulatory effect on GLUT4 translocation or activation (49–51). However, these data should be interpreted with caution, as this concentration of LA is ~10- to ~100-fold greater than the level sufficient to protect against oxidative stress–induced insulin resistance in cells (40,41) and to increase insulin sensitivity in patients with type 2 diabetes (44,47).

**Activation of stress-sensitive signaling systems, insulin receptor substrate serine phosphorylation, and insulin resistance.** In vitro, ROS and oxidative stress lead to the activation of multiple serine kinase cascades (18). The insulin signaling pathway offers a number of potential targets (substrates) of these activated kinases, including the insulin receptor (IR) and the family of IR substrate (IRS) proteins. For IRS-1 and -2, an increase in serine phosphorylation decreases the extent of tyrosine phosphorylation and is consistent with the attenuation of insulin action (52,53) (Fig. 3).

In Chinese hamster ovary cells, stress activation of JNK/SAPK increased serine phosphorylation (at Ser307) and inhibited insulin-stimulated tyrosine phosphorylation of IRS-1 (54). In L6 muscle cells, $H_2O_2$-mediated inhibition of insulin-stimulated glucose transport was accompanied by activation of p38 MAPK by $H_2O_2$ (40,55). Insulin-stimulated glucose transport could be restored by LA and a specific inhibitor of p38 MAPK (40,55). To determine whether the protective effects of LA could also be observed under more physiological conditions, we have used hyperglycemia to induce oxidative stress and blunt the

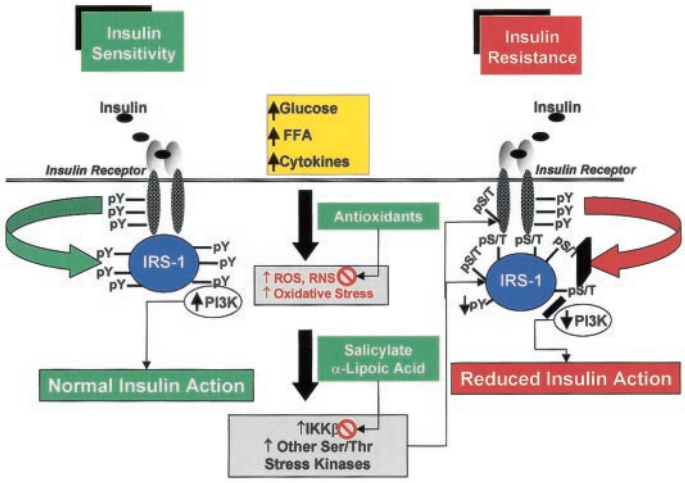


**FIG. 3. The role of serine kinase activation in oxidative stress–induced insulin resistance. A variety of stimuli, including hyperglycemia, elevated FFA levels, cytokines, and others, increase ROS (and RNS) production and oxidative stress. This results in the activation of multiple stress-sensitive serine/threonine (Ser/Thr) kinase signaling cascades such as IKK-β and others (see text for details). Once activated, these kinases are able to phosphorylate multiple targets, such as the IR and IRS proteins (including IRS-1 and IRS-2). Increased phosphorylation of IR or IRS proteins on discrete serine or threonine sites (pS/T) decreases the extent of insulin-stimulated tyrosine phosphorylation (pY) (52,53). Consequently, the association and/or activities of downstream signaling molecules (e.g., phosphatidylinositol 3-kinase [PI3K]) are decreased, resulting in reduced insulin action (insulin resistance). The protective effects of antioxidants (e.g., LA) on oxidative stress–induced insulin resistance could relate to their ability to preserve the intracellular redox balance (neutralizing ROS) or, analogous to pharmacological agents (e.g., salicylates, p38 MAPK inhibitors), to block the activation of stress-sensitive kinases (40,55,56).**

effects of insulin. In one study, chronic incubation of L6-GLUT4-IR cells (L6 cells that express both human GLUT4 and IR) with 20 mmol/l glucose caused a marked decrease in insulin-stimulated glucose transport (B.A.M. and I.D.G., unpublished observations). Coincubation with LA (100 μmol/l) completely protected the cells against hyperglycemia-induced insulin resistance.

Activation of IKK-β, a serine kinase that regulates the NF-κB pathway, inhibits insulin action (56). Salicylates lower blood glucose (rev. in 56), augment glucose-induced insulin secretion in normal subjects, and restore insulin secretion in patients with type 2 diabetes (57,58). In addition, salicylates inhibit IKK-β activity and restore insulin sensitivity, both in vitro and in vivo (56,59). Treatment with aspirin or salicylates alters the phosphorylation patterns of IRS proteins, resulting in decreased serine phosphorylation, increased tyrosine phosphorylation, and improved insulin action (56,59). Further support for the importance of IKK-β in insulin resistance is provided by results of recent gene knockout experiments in mice. IKK-β (+/−) heterozygotes were more insulin sensitive compared with their normal (+/+) littermates (56). Treatment of nine type 2 diabetic patients for 2 weeks with high dosages of aspirin (7 g/day) resulted in reduced hepatic glucose production and fasting hyperglycemia and increased insulin sensitivity (60). Although these latter data are preliminary and require confirmation in an expanded study, they are consistent with a role for activation of IKK-β in the pathogenesis of insulin resistance. Furthermore, they suggest that inhibition of IKK-β might be an attractive pharmacological approach to increasing insulin sensitivity.

**Obesity, fatty acids, and insulin resistance.** Because insulin resistance is evident before the development of chronic (fasting) hyperglycemia (1,32), it is unlikely that insulin resistance at the prediabetic stage results from oxidative stress triggered by hyperglycemia per se. However, the strong association of obesity and insulin resistance suggests that a mediator of oxidative stress–induced insulin resistance at the prediabetic stage might be an adipocyte-derived factor.

In this regard, several possible candidate molecules have been suggested including tumor necrosis factor-α (61), leptin (62), FFAs (5,6,63), and, most recently, resistin (64). However, the evidence is strongest that FFAs are the most likely link between obesity and insulin resistance (5,6,65). Several mechanisms of how elevated FFA levels decrease insulin sensitivity have been proposed, including the Randle hypothesis (63) along with a more recent alternative concerning inhibition of insulin-stimulated glucose transport (65). It also should be noted that FFAs and many of their metabolites interact directly with transcription factors to regulate gene expression, especially those involved in lipid and carbohydrate metabolism (66).

**Fatty acids, redox balance, and activation of NF-κB.** In patients with type 2 diabetes, there is a significant inverse correlation between the fasting plasma FFA concentration and ratio of reduced/oxidized glutathione (the major endogenous antioxidant) (36). In healthy subjects, infusion of FFAs (as intralipid) causes increased oxidative stress, as judged by increased malondialdehyde levels and a decline in the plasma reduced/oxidized glutathione ratio (36). Malondialdehyde, a highly toxic by-product generated in part by lipid oxidation and ROS, is increased in diabetes (67). In both normal individuals and in type 2 diabetic patients, restoration of redox balance by infusion of glutathione improves insulin sensitivity along with β-cell function (68).

Evidence in vitro indicates that elevated FFA levels have numerous adverse effects on mitochondrial function, including the uncoupling of oxidative phosphorylation (69) and the generation of ROS, including superoxide (70). This latter situation is exacerbated because FFAs are not only capable of inducing oxidative stress, but also impair endogenous antioxidant defenses by reducing intracellular glutathione (36,71,72). Numerous in vitro studies have reported FFA-mediated activation of NF-κB, a likely consequence of the ability of FFAs to increase ROS formation and reduce glutathione (72–75). This effect might be also linked to FFA-mediated activation of PKC-θ (76), which has the unique ability among PKC isoforms to activate NF-κB (77). FFA-induced activation of NF-κB can be prevented by vitamin E (72), suggesting that the alteration in cellular redox status is a contributory component of the proinflammatory effects of FFAs. The association of obesity, fatty acids, and oxidative stress with insulin action clearly merits further attention, with a particular focus on identifying the molecular mechanisms.

## OXIDATIVE STRESS AND β-CELL DYSFUNCTION

An additional target of oxidative stress is the β-cell. β-Cells are responsible for sensing and secreting the

appropriate amount of insulin in response to a glucose stimulus (78). Although this process is complex and dependent on many factors (rev. in 34), the critical importance of mitochondrial glucose metabolism in linking stimulus to secretion is well established (78–80). Therefore, the ability of oxidative stress ($H_2O_2$) to damage mitochondria and markedly blunt insulin secretion is not surprising (80).

Many studies have suggested that β-cell dysfunction is the result of prolonged exposure to high glucose, elevated FFA levels, or a combination of the two. There is considerable evidence that chronic hyperglycemia in patients with type 2 diabetes contributes to impaired β-cell function (34,81). However, in vitro evidence for a direct toxic effect of glucose has been conflicted because, in large part, of variations in the definition of toxicity along with subtle differences in experimental design (34). For example, evidence of impaired secretion may simply reflect a normal decrease in β-cell insulin content caused by prior exposure to elevated glucose levels (34,82). Moreover, recent data have suggested that the combined effects of elevations in glucose and FFA levels, acting by the generation of ROS, may be particularly toxic. As discussed above, chronic exposure to these molecules can result in increased production of ROS and RNS, and activation of stress-sensitive pathways.

β-Cells are sensitive to ROS and RNS because they are low in free-radical quenching (antioxidant) enzymes such as catalase, glutathione peroxidase, and superoxide dismutase (83). Overexpression of the antioxidant enzymes in islets or transgenic mice prevents many of the deleterious effects noted above (84,85). Oxygen stress generated by short exposure of β-cell preparations to $H_2O_2$ increases production of p21 (an inhibitor of cyclin-dependent kinase), decreases insulin mRNA, cytosolic ATP, and calcium flux in cytosol and mitochondria, and causes apoptosis (rev. in 80). Insulin secretion stimulated by glucose or methyl succinate can be inhibited within 30 min, whereas the response to $K^+$ remains normal (80). These results indicate that the mitochondrial processes involved in glucose-mediated insulin secretion are particularly affected by oxidative stress.

Inhibition of insulin secretion and glucose oxidation also occurs when islets are exposed to lipid peroxidation products (86). Conversely, antioxidants such as *N*-acetyl-L-cysteine (NAC), aminoguanidine, zinc, and the spin-trapping agent α-phenyl-tert-butylnitrone, can protect against β-cell toxicity and the generation of glycation end products and inhibit the activation of NF-κB (87–91). Recently, β-cell function was evaluated in islets after overexpression of glutamine:fructose-6-phosphate amidotransferase, the rate-limiting enzyme of hexosamine biosynthesis (92). Activation of the hexosamine pathway resulted in significant deterioration of glucose-stimulated insulin secretion along with other indexes of β-cell function, coincident with an increase in $H_2O_2$ (92). These effects were counteracted by treatment with the antioxidant NAC.

**β-Cell glucose-induced toxicity.** In patients with type 2 diabetes, reducing hyperglycemia with diet, insulin, or sulfonylureas results in improved insulin release (rev. in 34; 93). Conversely, in healthy individuals, high glucose infused as a clamp reduces insulin release (93). In vitro, long-term culture of either HIT-T15 or βTC-6 cells with elevated glucose decreases insulin release, insulin mRNA, and binding of insulin mRNA transcription factors (94,95). The antioxidants NAC and aminoguanidine markedly prevent glucotoxic effects on insulin gene activity (87). These antioxidants have been shown to partially prevent glucose-induced decreases in insulin mRNA, DNA-binding of pancreatic duodenal homeobox-1, insulin content, and glucose-stimulated insulin secretion (87).

**β-Cell lipid-induced toxicity.** Increased sensitivity to low glucose after prolonged high FFA levels (96–98) and coculture of normal islets with high FFA levels and moderate glucose causes increased secretory response during a test stimulus (96–99). In contrast, prolonged culture of β-cell preparations with FFAs causes decreased mitochondrial membrane potential and increased uncoupling proteins, leading to the opening of $K^+$-sensitive ATP channels and selective impairment of glucose-stimulated, but not $K^+$-stimulated, insulin secretion (100,101). Impaired insulin secretion has been associated with an FFA-induced increase in ROS (96).

Prolonged culture of β-cell preparations from animals with a predilection for type 2 diabetes, particularly those with impaired leptin production or leptin receptors, results in consistently demonstrable impaired secretion as well as other deleterious effects on β-cell function (rev. in 99). Therefore, genetic defects may amplify the toxic effects of FFAs that are not evident with normal insulin secreting cells.

**β-Cell combined glucose/lipid toxicity.** Because both glucose and FFA levels are elevated in type 2 diabetes, it is possible that their combination is required to maximize β-cell toxicity. This hypothesis is supported by recent studies showing that when either isolated islets or HIT cells were exposed to chronic elevated glucose and FFA levels, there was a clear decrease in both insulin mRNA and the activation of an insulin-gene reporter construct (102). In other studies, coculture of islets with high levels of glucose and palmitate resulted in almost complete impairment of glucose-stimulated insulin secretion, despite partially sustained stored insulin (96). Recent studies have suggested that β-cell lipotoxicity is an amplifying effect only if mediated by concurrent hyperglycemia (7,8).

## CONCLUSIONS AND IMPLICATIONS FOR FUTURE RESEARCH

As discussed above, there is considerable evidence from in vitro and in vivo studies that in a variety of tissues, hyperglycemia and possibly elevated FFA levels (both alone and in combination) result in the generation of ROS and RNS and consequently increased oxidative stress. In the absence of an appropriate compensatory response from the cell's endogenous antioxidant network, the system becomes overwhelmed, resulting in redox imbalance, thereby further exacerbating the situation. The reactive species not only directly damage cells by oxidizing DNA, protein, and lipids, but indirectly damage cells by activating a variety of stress-sensitive intracellular signaling pathways such as NF-κB, p38 MAPK, JNK/SAPK, hexosamine, PKC, AGE/RAGE, sorbitol, and others. Activation of these pathways results in the increased expression

of numerous gene products that also cause cellular damage and play a major role in the etiology of the late complications of diabetes.

In addition, recent data in vitro and in vivo suggest that activation of the same or similar stress pathways results in insulin resistance and impaired insulin secretion. Accordingly, we propose the existence of a link among the hyperglycemia- and FFA-induced increases in ROS and oxidative stress, activation of stress-sensitive pathways, and the eventual development of not only the late complications of diabetes, but also insulin resistance and β-cell dysfunction

Although our understanding of how hyperglycemia-induced oxidative stress ultimately leads to tissue damage has advanced considerably in recent years (7,10,13,103), effective therapeutic strategies to prevent or delay the development of this damage remain limited. We believe that research needs to be carried out on several fronts. First, antioxidant therapy needs to be improved. Either older antioxidants such as vitamin E, LA, and NAC need to be reformulated, or newer antioxidants need to be identified. At this juncture, the general use of antioxidant mixtures may not prove useful and could even interfere with other therapies and, therefore, is not advised (104). Moreover, screening tests to monitor oxidative stress need to be standardized and used in patients with diabetes.

Second, strategies to interrupt the stress pathways need to be studied more thoroughly. There has been some progress in this area. The specific inhibitor of PKC-β, LY333531, is active in cell and animal models, and is now being used in clinical trials in humans. In addition, the recent finding that salicylates, which inhibit IKK, improve insulin action in both cells and animal models is a major advance. A major challenge, however, is to obtain a more detailed understanding of the nature of the stress pathways, and to develop effective modulators that can be used clinically.

Third, additional research is clearly needed to firmly establish whether either the reduction of ROS formation activated by hyperglycemia and elevated FFA levels and/or the blockade of the ROS-induced stress pathways will result in improved insulin action and/or secretion. Analysis of the effects of hyperglycemia and hypertriglyceridemia in muscle, fat, and pancreatic islets on the development of oxidative stress and activation of stress pathways is urgently needed. Although small clinical studies with antioxidants such as vitamin E, LA, and NAC provide support for a role for oxidative stress in these conditions, several prospective clinical studies evaluating the effectiveness of vitamin E on cardiovascular outcomes have yielded disappointing results (rev. in 105). Nonetheless, the totality of available data provide support for conducting more extensive clinical studies evaluating the effectiveness of antioxidant treatment.

## ACKNOWLEDGMENTS

This work was supported in part by the American Diabetes Association, the Diabetes Action Research and Education Foundation, and the following Mt. Zion funds: Jay Gershow, M.H. Fishbon, and Lee K. Schwartz.

The authors thank Dr. Jack Youngren for his comments and suggestions regarding this manuscript. This review is dedicated to the memory of J. Denis McGarry.

## REFERENCES

1. DeFronzo RA: Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. *Diabetes Review* 5:177–269, 1997
2. Kahn CR, Vicent D, Doria A: Genetics of non-insulin-dependent (type-II) diabetes mellitus. *Annu Rev Med* 47:509–531, 1996
3. Unger RH, Foster DW: Diabetes mellitus. In *Williams Textbook of Endocrinology*. Wilson JD, Foster DW, Kronenberg HM, Larsen PR, Eds. Philadelphia, Saunders, 1998, p. 973–1059
4. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
5. McGarry JD: Banting Lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes* 51:7–18, 2002
6. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* 46:3–10, 1997
7. Poitout V, Robertson RP: Minireview: secondary beta-cell failure in type 2 diabetes—a convergence of glucotoxicity and lipotoxicity. *Endocrinology* 143:339–342, 2002
8. 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
9. Rosen P, Nawroth PP, King G, Moller W, Tritschler HJ, Packer L: The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by UNESCO-MCBN, the American Diabetes Association, and the German Diabetes Society. *Diabetes Metab Res Rev* 17:189–212, 2001
10. Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 414:813–820, 2001
11. Nishikawa T, Edelstein D, Brownlee M: The missing link: a single unifying mechanism for diabetic complications. *Kidney Int* 58:26–30, 2000
12. Brownlee M: Advanced protein glycosylation in diabetes and aging. *Annu Rev Med* 46:223–234, 1995
13. Koya D, King GL: Protein kinase C activation and the development of diabetic complications. *Diabetes* 47:859–866, 1998
14. Brownlee M, Cerami A: The biochemistry of the complications of diabetes mellitus. *Annu Rev Biochem* 50:385–432, 1981
15. Wolff SP, Dean RT: Glucose autoxidation and protein modification: the potential role of 'autoxidative glycosylation' in diabetes. *Biochem J* 245:243–250, 1987
16. Stevens MJ, Obrosova I, Feldman EL, Greene DA: The sorbitol-osmotic and sorbitol-redox hypothesis. In *Diabetes Mellitus: A Fundamental and Clinical Text*. LeRoith D, Taylor SI, Olefsky JM, Eds. Philadelphia, Lippincott Williams & Wilkins, 2000, p. 972–983
17. Barnes PJ, Karin M: Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 336:1066–1071, 1997
18. Kyriakis JM, Avruch J: Sounding the alarm: protein kinase cascades activated by stress and inflammation. *J Biol Chem* 271:24313–24316, 1996
19. Marshall S, Garvey WT, Traxinger RR: New insights into the metabolic regulation of insulin action and insulin resistance: role of glucose and amino acids. *FASEB J* 5:3031–3036, 1991
20. Mohamed AK, Bierhaus A, Schiekofer S, Tritschler H, Ziegler R, Nawroth PP: The role of oxidative stress and NF-κB activation in late diabetic complications. *Biofactors* 10:157–167, 1999
21. Bierhaus A, Schiekofer S, Schwaninger M, Andrassy M, Humpert PM, Chen J, Hong M, Luther T, Henle T, Kloting I, Morcos M, Hofmann M, Tritschler H, Weigle B, Kasper M, Smith M, Perry G, Schmidt AM, Stern DM, Haring HU, Schleicher E, Nawroth PP: Diabetes-associated sustained activation of the transcription factor nuclear factor-κB. *Diabetes* 50:2792–2808, 2001
22. 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, 2000
23. Ho FM, Liu SH, Liau CS, Huang PJ, Lin-Shiau SY: High glucose-induced apoptosis in human endothelial cells is mediated by sequential activations of c-Jun NH(2)-terminal kinase and caspase-3. *Circulation* 101:2618–2624, 2000
24. Natarajan R, Scott S, Bai W, Yerneni KKV, Nadler J: Angiotensin II

signaling in vascular smooth muscle cells under high glucose conditions. *Hypertension* 33:378–384, 1999
25. Begum N, Ragolia L: High glucose and insulin inhibit VSMC MKP-1 expression by blocking iNOS via p38 MAPK activation. *Am J Physiol* 278:C81–C91, 2000
26. Igarashi M, Wakasaki H, Takahara N, Ishii H, Jiang ZY, Yamauchi T, Kuboki K, Meier M, Rhodes CJ, King GL: Glucose or diabetes activates p38 mitogen-activated protein kinase via different pathways. *J Clin Invest* 103:185–195, 1999
27. Dunlop ME, Muggli EE: Small heat shock protein alteration provides a mechanism to reduce mesangial cell contractility in diabetes and oxidative stress. *Kidney Int* 57:464–475, 2000
28. Purves T, Middlemas A, Agthong S, Jude EB, Boulton AJ, Fernyhough P, Tomlinson DR: A role for mitogen-activated protein kinases in the etiology of diabetic neuropathy. *FASEB J* 15:2508–2514, 2001
29. Boden G, Chen X, Ruiz J, White JV, Rossetti L: Mechanisms of fatty acid-induced inhibition of glucose uptake. *J Clin Invest* 93:2438–2446, 1994
30. Schleicher ED, Weigert C: Role of the hexosamine biosynthetic pathway in diabetic nephropathy. *Kidney Int* 58 (Suppl. 77):S13–S18, 2000
31. Du XL, Edelstein D, Rossetti L, Fantus IG, Goldberg H, Ziyadeh F, Wu J, Brownlee M: Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc Natl Acad Sci U S A* 97:12222–12226, 2000
32. Reaven GM: Insulin resistance and its consequences: type 2 diabetes mellitus and coronary heart disease. In *Diabetes Mellitus: A Fundamental and Clinical Text*. LeRoith D, Taylor SI, Olefsky JM, Eds. Philadelphia, Lippincott Williams & Wilkins, 2000, p. 604–615
33. Kahn CR: Insulin action, diabetogenes, and the cause of type 2 diabetes. *Diabetes* 43:1066–1084, 1994
34. Grodsky GM: Kinetics of insulin secretion: underlying metabolic events in diabetes mellitus. In *Diabetes Mellitus: A Fundamental and Clinical Text*. Le Roith D, Taylor SI, Olefsky JM, Eds. Philadelphia, Lippicott Williams & Wilkins, 2000, p. 2–11
35. West IC: Radicals and oxidative stress in diabetes. *Diabet Med* 17:171–180, 2000
36. Paolisso G, Giugliano D: Oxidative stress and insulin action. Is there a relationship? *Diabetologia* 39:357–363, 1996
37. Rudich A, Kozlovsky N, Potashnik R, Bashan N: Oxidant stress reduces insulin responsiveness in 3T3–L1 adipocytes. *Am J Physiol* 35:E935–E940, 1997
38. Ceriello A: Oxidative stress and glycemic regulation. *Metabolism* 49:27–29, 2000
39. Yaworsky K, Somwar R, Klip A: Interrelationship between oxidative stress and insulin resistance. In *Antioxidants in Diabetes Management*. Packer L, Rösen P, Tritschler HJ, King GL, Eds. New York, Marcel Dekker, 2000, p. 275–302
40. Maddux BA, See W, Lawrence JC Jr, Goldfine AL, Goldfine ID, Evans JL: Protection against oxidative stress-induced insulin resistance in rat L6 muscle cells by micromolar concentrations of α-lipoic acid. *Diabetes* 50:404–410, 2001
41. Rudich A, Tirosh A, Potashnik R, Khamaisi M, Bashan N: Lipoic acid protects against oxidative stress induced impairment in insulin stimulation of protein kinase B and glucose transport in 3T3–L1 adipocytes. *Diabetologia* 42:949–957, 1999
42. Packer L, Rosen P, Tritschler H, King GL, Azzi A (Eds): *Antioxidants and Diabetes Management*. New York, Marcel Dekker, 2000
43. Jacob S, Lehmann R, Rett K, Häring H-U: Oxidative stress and insulin action: a role for antioxidants. In *Antioxidants in Diabetes Management*. Packer L, Rösen P, Tritschler HJ, King GL, Eds. New York, Marcel Dekker, 2000, p. 319–338
44. Evans JL, Goldfine ID: α-Lipoic acid: a multi-functional antioxidant that improves insulin sensitivity in patients with type 2 diabetes. *Diabetes Technol Ther* 2:401–413, 2000
45. Inzucchi SE, Maggs DG, Spollett GR, Page SL, Rife FS, Walton V, Shulman GI: Efficacy and metabolic effects of metformin and troglitazone in type II diabetes mellitus. *N Engl J Med* 338:867–872, 1998
46. Mayerson AB, Hundal RS, Dufour S, Lebon V, Befroy D, Cline GW, Enocksson S, Inzucchi SE, Shulman GI, Petersen KF: The effects of rosiglitazone on insulin sensitivity, lipolysis, and hepatic and skeletal muscle triglyceride content in patients with type 2 diabetes. *Diabetes* 51:797–802, 2002
47. Evans JL, Heymann CJ, Goldfine ID, Gavin LA: Pharmacokinetics, tolerability, and fructosamine-lowering effect of a novel, controlled release formulation of α-lipoic acid. *Endocr Pract* 8:29–35, 2002
48. Pjodkowaki RA, Grotz VL, Mudalar S, Henry RR: The effects of alpha-lipoic acid on glucose homeostasis in type 2 diabetes. *J Invest Med* 50:71A, 2002
49. Estrada DE, Ewart HS, Tsakiridis T, Volchuk A, Ramlal T, Tritschler HJ, Klip A: Stimulation of glucose uptake by the natural coenzyme α-lipoic acid/thioctic acid: participation of elements of the insulin signaling pathway. *Diabetes* 45:1798–1804, 1996
50. Konrad D, Somwar R, Sweeney G, Yaworsky K, Hayashi M, Ramlal T, Klip A: The antihyperglycemic drug alpha-lipoic acid stimulates glucose uptake via both GLUT4 translocation and GLUT4 activation: potential role of p38 mitogen-activated protein kinase in GLUT4 activation. *Diabetes* 50:1464–1471, 2001
51. Ramrath S, Tritschler HJ, Eckel J: Stimulation of cardiac glucose transport by thioctic acid and insulin. *Horm Metab Res* 31:632–635, 1999
52. Paz K, Hemi R, LeRoith D, Karasik A, Elhanany E, Kanety H, Zick Y: A molecular basis for insulin resistance: elevated serine/threonine phosphorylation of IRS-1 and IRS-2 inhibits their binding to the juxtamembrane region of the insulin receptor and impairs their ability to undergo insulin-induced tyrosine phosphorylation. *J Biol Chem* 272:29911–29918, 1997
53. Birnbaum MJ: Turning down insulin signaling. *J Clin Invest* 108:655–659, 2001
54. Aguirre V, Uchida T, Yenush L, Davis R, White MF: The c-jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). *J Biol Chem* 275:9047–9054, 2000
55. Blair AS, Hajduch E, Litherland GJ, Hundal HS: Regulation of glucose transport and glycogen synthesis in L6 muscle cells during oxidative stress: evidence for cross-talk between the insulin and SAPK2/p38 mitogen-activated protein kinase signaling pathways. *J Biol Chem* 274: 36293–36299, 1999
56. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, Shoelson SE: Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of IKKβ. *Science* 293:1673–1677, 2001
57. Robertson RP, Chen M: A role for prostaglandin E in defective insulin secretion and carbohydrate intolerance in diabetes mellitus. *J Clin Invest* 60:747–753, 1977
58. Chen M, Robertson RP: Restoration of the acute insulin response by sodium salicylate: a glucose dose-related phenomenon. *Diabetes* 27:750–756, 1978
59. Kim JK, Kim YJ, Fillmore JJ, Chen Y, Moore I, Lee J, Yuan M, Li ZW, Karin M, Perret P, Shoelson SE, Shulman GI: Prevention of fat-induced insulin resistance by salicylate. *J Clin Invest* 108:437–446, 2001
60. Hundal RS, Mayerson AB, Petersen KF, Rife FS, Randhawa PS, Inzucchi SE, Shoelson SE, Shulman GI: Potential for a novel class of insulin sensitizing agents by inhibition of IKKβ activity (Abstract). *Diabetes* 50 (Suppl. 2):A117, 2001
61. Hotamisligil GS, Spiegelman BM: Tumor necrosis factor α: a key component of the obesity-diabetes link. *Diabetes* 43:1271–1278, 1994
62. Cohen B, Novick D, Rubinstein M: Modulation of insulin activities by leptin. *Science* 274:1185–1188, 1996
63. Randle PJ, Kerbey AL, Espinal J: Mechanisms decreasing glucose oxidation in diabetes and starvation: role of lipid fuels and hormones. *Diabetes Metab Rev* 623:638, 1988
64. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA: The hormone resistin links obesity to diabetes. *Nature* 409:307–312, 2001
65. Shulman GI: Cellular mechanisms of insulin resistance. *J Clin Invest* 106:171–176, 2000
66. Duplus E, Glorian M, Forest C: Fatty acid regulation of gene transcription. *J Biol Chem* 275:30749–30752, 2000
67. Slatter DA, Bolton CH, Bailey AJ: The importance of lipid-derived malondialdehyde in diabetes mellitus. *Diabetologia* 43:550–557, 2000
68. Paolisso G, Di Maro G, Pizza G, D'Amore A, Sgambato S, Tesauro P, Varricchio M, D'Onofrio F: Plasma GSH/GSSG affects glucose homeostasis in healthy subjects and non-insulin-dependent diabetics. *Am J Physiol* 263:E435–E440, 1992
69. Wojtczak L, Schonfeld P: Effect of fatty acids on energy coupling processes in mitochondria. *Biochim Biophys Acta* 1183:41–57, 1993
70. Bakker SJ, IJzerman RG, Teerlink T, Westerhoff HV, Gans RO, Heine RJ: Cytosolic triglycerides and oxidative stress in central obesity: the missing link between excessive atherosclerosis, endothelial dysfunction, and beta-cell failure? *Atherosclerosis* 148:17–21, 2000
71. Toborek M, Hennig B: Fatty acid-mediated effects on the glutathione redox cycle in cultured endothelial cells. *Am J Clin Nutr* 59:60–65, 1994
72. Hennig B, Meerarani P, Ramadass P, Watkins BA, Toborek M: Fatty

acid-mediated activation of vascular endothelial cells. *Metabolism* 49: 1006–1013, 2000
73. Dichtl W, Nilsson L, Goncalves I, Ares MP, Banfi C, Calara F, Hamsten A, Eriksson P, Nilsson J: Very low-density lipoprotein activates nuclear factor-kappaB in endothelial cells. *Circ Res* 84:1085–1094, 1999
74. Hennig B, Meerarani P, Toborek M, McClain CJ: Antioxidant-like properties of zinc in activated endothelial cells. *J Am Coll Nutr* 18:152–158, 1999
75. Lee JY, Sohn KH, Rhee SH, Hwang D: Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through toll-like receptor 4. *J Biol Chem* 276:16683–16689, 2001
76. Griffin ME, Marcucci MJ, Cline GW, Bell K, Barucci N, Lee D, Goodyear LJ, Kraegen EW, White MF, Shulman GI: Free fatty acid-induced insulin resistance is associated with activation of protein kinase C θ and alterations in the insulin signaling cascade. *Diabetes* 48:1270–1274, 1999
77. Coudronniere N, Villalba M, Englund N, Altman A: NF-kappa B activation induced by T cell receptor/CD28 co-stimulation is mediated by protein kinase C-theta. *Proc Natl Acad Sci U S A* 97:3394–3399, 2000
78. Meglasson MD, Matschinsky FM: Pancreatic islet glucose metabolism and regulation of insulin secretion. *Diabetes Metab Rev* 2:163–214, 1986
79. Malaisse WJ: Physiology, pathology and pharmacology of insulin secretion: recent acquisitions. *Diabetes Metab* 23:6–15, 1997
80. 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
81. Robertson RP, Harmon JS, Tanaka Y, Sacchi G, Tran PO, Gleason CE, Poitout V: Glucose toxicity of the β-cell: cellular and molecular mechanisms. In *Diabetes Mellitus*: *A Fundamental and Clinical Text*. Le Roith D, Taylor SI, Olefsky JM, Eds. Philadelphia, Lippincott Williams & Wilkins, 2000, p. 125–132
82. Bolaffi JL, Bruno L, Heldt A, Grodsky GM: Characteristics of desensitization of insulin secretion in fully in vitro systems. *Endocrinology* 122: 1801–1809, 1988
83. Tiedge M, Lortz S, Drinkgern J, Lenzen S: Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes* 46:1733–1742, 1997
84. 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
85. 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
86. Miwa I, Ichimura N, Sugiura M, Hamada Y, Taniguchi S: Inhibition of glucose-induced insulin secretion by 4-hydroxy-2-nonenal and other lipid peroxidation products. *Endocrinology* 141:2767–2772, 2000
87. 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
88. Ho E, Bray TM: Antioxidants, NF-kappaB activation, and diabetogenesis. *Proc Soc Exp Biol Med* 222:205–213, 1999
89. Tajiri Y, Moller C, Grill V: Long-term effects of aminoguanidine on insulin release and biosynthesis: evidence that the formation of advanced glycosylation end products inhibits β-cell function. *Endocrinology* 138: 273–280, 1997
90. Ho E, Chen G, Bray TM: Supplementation of N-acetylcysteine inhibits NF-kappaB activation and protects against alloxan-induced diabetes in CD-1 mice. *FASEB J* 13:1845–1854, 1999
91. Ho E, Chen G, Bray TM: Alpha-phenyl-tert-butylnitrone (PBN) inhibits NFkappaB activation offering protection against chemically induced diabetes. *Free Radic Biol Med* 28:604–614, 2000
92. 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
93. Boden G, Ruiz J, Kim CJ, Chen X: Effects of prolonged glucose infusion on insulin secretion, clearance, and action in normal subjects. *Am J Physiol* 270:E251–E258, 1996
94. 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
95. Poitout V, Olson LK, Robertson RP: Chronic exposure of beta TC-6 cells to supraphysiologic concentrations of glucose decreases binding of the RIPE3b1 insulin gene transcription activator. *J Clin Invest* 97:1041–1046, 1996
96. Carlsson C, Borg LA, Welsh N: Sodium palmitate induces partial mitochondrial uncoupling and reactive oxygen species in rat pancreatic islets in vitro. *Endocrinology* 140:3422–3428, 1999
97. Zhou YP, Grill VE: Long-term exposure of rat pancreatic islets to fatty acids inhibits glucose-induced insulin secretion and biosynthesis through a glucose fatty acid cycle. *J Clin Invest* 93:870–876, 1994
98. Bollheimer LC, Skelly RH, Chester MW, McGarry JD, Rhodes CJ: Chronic exposure to free fatty acid reduces pancreatic beta cell insulin content by increasing basal insulin secretion that is not compensated for by a corresponding increase in proinsulin biosynthesis translation. *J Clin Invest* 101:1094–1101, 1998
99. Unger RH, Zhou YT: Lipotoxicity of β-cells in obesity and in other causes of fatty acid spillover. *Diabetes* 50 (Suppl. 1):S118–S121, 2001
100. Lameloise N, Muzzin P, Prentki M, Assimacopoulos-Jeannet F: Uncoupling protein 2: a possible link between fatty acid excess and impaired glucose-induced insulin secretion? *Diabetes* 50:803–809, 2001
101. Segall L, Lameloise N, Assimacopoulos-Jeannet F, Roche E, Corkey P, Thumelin S, Corkey BE, Prentki M: Lipid rather than glucose metabolism is implicated in altered insulin secretion caused by oleate in INS-1 cells. *Am J Physiol* 277:E521–E528, 1999
102. Jacqueminet S, Briaud I, Rouault C, Reach G, Poitout V: Inhibition of insulin gene expression by long-term exposure of pancreatic beta cells to palmitate is dependent on the presence of a stimulatory glucose concentration. *Metabolism* 49:532–536, 2000
103. Nadler JL, Natarajan R: Oxidative stress, inflammation, and diabetic complications. In *Diabetes Mellitus: A Fundamental and Clinical Text*. LeRoith D, Taylor SI, Olefsky JM, Eds. Philadelphia, Lippincott Williams & Wilkins, 2000, p. 1008–1016
104. Brown BG, Zhao XQ, Chait A, Fisher LD, Cheung MC, Morse JS, Dowdy AA, Marino EK, Bolson EL, Alaupovic P, Frohlich J, Albers JJ: Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N Engl J Med* 345:1583–1592, 2001
105. Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P: Vitamin E supplementation and cardiovascular events in high-risk patients: the Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med* 342:154–160, 2000
106. Jacob S, Ruus P, Hermann R, Tritschler HJ, Maerker E, Renn W, Augustin HJ, Dietze GJ, Rett K: Oral administration of RAC-alpha-lipoic acid modulates insulin sensitivity in patients with type 2 diabetes mellitus: a placebo-controlled pilot trial. *Free Radic Biol Med* 27:309–314, 1999
107. Jacob S, Henriksen EJ, Tritschler HJ, Augustin HJ, Dietze GJ: Improvement of insulin-stimulated glucose-disposal in type 2 diabetes after repeated parenteral administration of thioctic acid. *Exp Clin Endocrinol Diabetes* 104:284–288, 1996
108. Jacob S, Henriksen EJ, Schiemann AL, Simon I, Clancy DE, Tritschler HJ, Jung WI, Augustin HJ, Dietze GJ: Enhancement of glucose disposal in patients with type 2 diabetes by alpha-lipoic acid. *Arzneimittelforschung* 45:872–874, 1995