β-Cell Glucose Toxicity, Lipotoxicity, and Chronic Oxidative Stress in Type 2 Diabetes

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The relentless decline in β-cell function frequently observed in type 2 diabetic patients, despite optimal drug management, has variously been attributed to glucose toxicity and lipotoxicity. The former theory posits hyperglycemia, an outcome of the disease, as a secondary force that further damages β-cells. The latter theory suggests that the often-associated defect of hyperlipidemia is a primary cause of β-cell dysfunction. We review evidence that patients with type 2 diabetes continually undergo oxidative stress, that elevated glucose concentrations increase levels of reactive oxygen species in β-cells, that islets have intrinsically low antioxidant enzyme defenses, that antioxidant drugs and overexpression of antioxidant enzymes protect β-cells from glucose toxicity, and that lipotoxicity, to the extent it can be attributable to hyperlipidemia, occurs only in the context of preexisting hyperglycemia, whereas glucose toxicity can occur in the absence of hyperlipidemia. *Diabetes* 53 (Suppl. 1):S119–S124, 2004

Type 2 diabetes is generally viewed as a clinical syndrome with variable phenotypic expression rather than a single disease with a specific etiology. Phenotypic elements of the syndrome include β-cell insufficiency and insulin resistance. However, in most instances, the exact cause of type 2 diabetes seems to be polygenic in nature and is as yet unknown. Regardless of the primary causes of type 2 diabetes, a common clinical course is for patients to respond to therapy initially by normalizing their fasting glucose levels, but then to undergo gradual deterioration in glycemic control despite optimal medical management using a variety of drugs. Even the initial response in fasting glucose may be misleading because many patients still manifest abnormal glucose levels postprandially. This scenario gives rise to the concept of glucose toxicity, which envisions chronic exposure to abnormally high levels of glucose over many years as a pathogenic force that leads to toxic effects on the β-cell. Hence, chronic hyperglycemia, the adverse outcome of the initial polygenic disorder, in time becomes a secondary adverse force on the β-cell, leading to a vicious cycle of continuous deterioration of β-cell function. Similar logic has been advanced to posit abnormal lipid levels as a causative force in the relentless deterioration of the β-cell. Proponents of this theory point out that most individuals with type 2 diabetes are obese and tend to have elevated lipid levels in their blood that become higher as glycemia worsens and that intra-islet lipid accumulation can decrease β-cell function.

The mechanisms whereby chronic elevations of glucose and/or lipids might damage β-cells are subjects of intense clinical and laboratory investigation. Whether one or the other of these forces is more important or whether they may be interrelated is another important consideration. In this article, we will explore the views that chronic oxidative stress is a mechanism whereby glucose excess can damage β-cells and that lipotoxicity requires hyperglycemia to exert harmful effects, whereas glucose toxicity does not require concomitant hyperlipidemia to do so.

EVIDENCE FOR INCREASED OXIDATIVE STRESS IN TYPE 2 DIABETES

Many clinical studies suggest that patients with type 2 diabetes are subjected to chronic oxidative stress (1–6). Sophisticated laboratory techniques, including high-performance liquid chromatography, gas chromatography/mass spectrometry, and immunostaining of pancreas biopsies, have been used. Pro-oxidants and markers for oxidative tissue damage, such as 8-hydroxy-deoxyguanine, 4-hydroxy-2-nonenal (HNE) proteins, 8-epi-prostaglandin $F_{2\alpha}$, hydroperoxides, and oxidation of DNA bases, have been reported to be elevated in serum, plasma, white blood cells, and pancreas biopsies of patients with type 2 diabetes (Table 1). Compared with nondiabetic control subjects, these markers showed changes that have ranged to fivefold above normal. Glutathione (GSH), the primary intracellular antioxidant, has been studied extensively. Murakami et al. (7) reported that erythrocytes from diabetic patients contain low levels of the reduced form of GSH, high levels of the oxidized form (GSSG), and a 51% reduction in the GSH/GSSG ratio. Sharma et al. (8) also reported that type 2 diabetic patients in poor glycemic control had depressed erythrocyte GSH levels. After treatment with sulfonylureas decreased HbA_{1c} levels, the erythrocyte GSH concentration increased twofold, almost reaching the values found in a nondiabetic control group. Similar data were reported by Yoshida et al. (9), who in addition reported that the activity of the rate-limiting enzyme of GSH synthesis, γ-glutamylcysteine synthase, increased with improved glycemic control. This group also reported that erythrocyte thiol transport levels were sig-

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GSH, glutathione; NAC, *n*-acetylcysteine; ROS, reactive oxygen species.

TABLE 1
Examples of studies documenting elevated markers of oxidative stress in blood elements and pancreas tissue in human type 2 diabetes

Substance	Method	Result	Reference
8-OH-guanine	Serum, HPLC	5× > Nl	1
ASA hydroxylation by ȮH	Plasma, HPLC	23% > Nl	2
8-epi-$PGF_{2\alpha}$	Plasma, GC/MS	3.5× > Nl	3
Hydroperoxides	Plasma, HPLC	2.4× > Nl	4
DNA base oxidation	White blood cells, GC/MS	2× > Nl	5
8-OH-deoxyguanine and 4-OH-2-nonenal proteins	Pancreas, β-cell immunochemistry	2× > Nl	6

GC/MS, gas chromatography/mass spectroscopy; HPLC, high-performance liquid chromatography; Nl, normal.

nificantly and inversely correlated with HbA_{1c}. Paolisso et al. (10) reported that intravenous infusion of GSH in type 2 diabetic patients improved insulin secretion and glucose tolerance during oral glucose tolerance tests.

EVIDENCE THAT CHRONIC HYPERGLYCEMIA CAN INCREASE β-CELL REACTIVE OXYGEN SPECIES LEVELS

Many investigators have used animal models in their research to assess the proposition that deteriorating glycemic control in type 2 diabetes causes oxidative stress on β-cells. For example, Ihara et al. (11) measured levels of 8-hydroxy-deoxyguanosine and 4-hydroxy-2-nonenal–modified proteins by immunohistochemical methods in β-cells of Goto-Kakizaki (GK) rats. They reported higher levels of these oxidative stress markers in GK rats compared with Wistar rats. Moreover, a diet rich in sucrose increased these two markers in GK rats, whereas inclusion of the hypoglycemic agent voglibose in the diet decreased marker levels. Tanaka et al. (12) reported elevated plasma concentrations of these two markers in Zucker diabetic fatty (ZDF) rats and that treatment prevented the increase. However, demonstration that glucose itself is capable of generating reactive oxygen species (ROS) in β-cells is essential to the hypothesis that glucose-induced oxidative stress is a mechanism for glucose toxicity. Potential glucose-related pathways through which ROS can be formed include autoxidation, oxidative phosphorylation, glycosylation, and the glucosamine pathways (13–17). Wolff and colleagues (13,14) were among the first to suggest that autoxidation of glucose could yield ROS. They showed that glyceraldehyde in the presence of heavy metals could form a radical anion via enolization. In the presence of oxygen, superoxide was generated, which in turn formed hydrogen peroxide via superoxide dismutase. In the presence of heavy metals, hydrogen peroxide generates highly toxic hydroxyl radicals. Evidence that this sequence of events might occur specifically in β-cells was provided by Ihara et al. (11), as mentioned above. Studies by Tanaka et al. used the peroxide-sensitive probe carboxyl dichlorodihydrofluorescein diacetate and flow cytometry to examine the consequences of exposing human isolated islets to 30 mmol/l glucose for 72 h (18). Compared with 5.6 mmol/l glucose, 30 mmol/l glucose caused an increase in intracellular peroxides (Fig. 1). The same

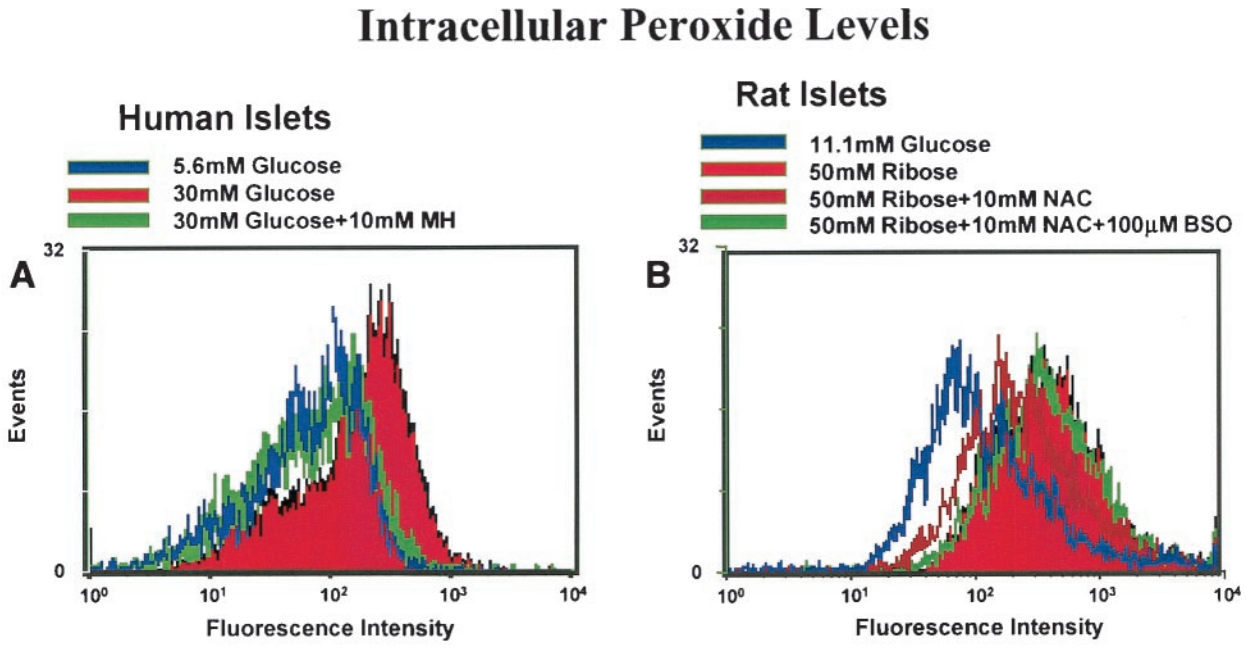

FIG. 1. Intracellular peroxide levels determined by a method involving dichlorodihydrofluorescein diacetate and flow cytometer. *A*: Isolated human islets were incubated in 5.6 or 30 mmol/l glucose with or without 10 mmol/l mannuloheptulose (MH) for 72 h to block glucose entry in the islets. Glucose (30 mmol/l) increased intra-islet peroxides, and MH blocked this increase. *B*: Isolated rat islets incubated with glucose, ribose, ribose + NAC, or ribose +NAC + buthionine sulfoxamine (BSO) for 72 h. Ribose generated increased levels of intracellular peroxides compared with glucose. The increase caused by ribose was greatly diminished by NAC, but the protective effect of NAC was completely abrogated by BSO. See the study by Tanaka et al. (18).

TABLE 2.
Antioxidant enzyme activities in islets and other tissues from rats

	Cu/Zn SOD	Mn SOD	Catalase	GPx
Liver	100 ± 7	100 ± 12	100 ± 9	100 ± 8
Kidney	104 ± 12	105 ± 12	64 ± 9	118 ± 7
Skeletal muscle	42 ± 5	50 ± 6	4 ± 1	31 ± 7
Fat	49 ± 5	84 ± 14	32 ± 5	67 ± 7
Islet	31 ± 3	25 ± 2	1 ± 0	21 ± 1

Data are means ± SD. Adapted from Tiedge et al. (20). SOD, superoxide dismutase.

phenomenon was observed in isolated rat islets, but only after pretreatment with buthionine sulfoximine, an agent that inhibits intracellular synthesis of GSH.

EVIDENCE FOR LOW ANTIOXIDANT CAPACITY OF ISLETS

Oxidative stress has been considered by many as an explanation for the tissue damage that accompanies chronic hyperglycemia. However, most of this research has been conducted in non-islet tissue. This is ironic because the islet has the lowest intrinsic antioxidant capacity compared with other metabolic tissues such as liver, kidney, skeletal muscle, and fat. Grankvist et al. (19) reported that islets contain relatively small activities for the major antioxidant enzymes Cu/Zn superoxide dismutase, Mn superoxide dismutase, catalase, and glutathione peroxidase (GPx). This observation was more recently reinforced by Tiedge et al. (20) (Table 2), who made the interesting observation that high glucose concentrations caused cellular stress in islets (as indicated by a rise in the heme oxygenase-1 protein), but did not induce higher activities of the antioxidant enzymes. This suggests that the islet is among the most vulnerable of tissues during times of oxidative stress.

EVIDENCE THAT ANTIOXIDANT DRUGS AND OVEREXPRESSION OF ANTIOXIDANT ENZYMES IN β-CELLS PROTECT AGAINST GLUCOSE TOXICITY

Many investigators have recognized the therapeutic implications of the adverse consequences on the β-cell from chronic oxidative stress in diabetes. This has led to many reports of experiments designed to assess whether antioxidant drugs can be used to protect against oxidative stress in models of type 1 and type 2 diabetes. For example, Mendola et al. (21) reported that nicotinamide and desferrioxamine partially protected islets from immune destruction during low-dose streptozotocin-induced insulitis—a process in which hydroxyl radicals play an important role. Nomikos et al. (22) demonstrated that in vivo treatment with superoxide dismutase and catalase protected islet tissue from oxygen metabolites induced by transplantation of islet allografts. Metformin or troglitazone, both of which have antioxidant properties, prevent hyperglycemia in the ZDF rat (23,24). Fukui et al. (25) examined lipid hydroperoxide concentrations and superoxide dismutase activity and determined that troglitazone prevented increased levels of lipid hydroperoxide and superoxide dismutase activity in the OLETF rat, an animal model of type 2 diabetes. Sano et al. (26) used electron spin resonance to determine levels of ROS in vivo in Wistar rats that received streptozotocin. They found that the spin clearance rate significantly correlated with malondialdehyde levels, a marker for lipid peroxidation, and that treatment with insulin decreased the spin clearance rate in diabetic rats. They concluded that the diabetic state enhances the generation of ROS in vivo and that glycemic control can reduce oxidative stress. Ho et al. (27) used electron spin resonance to document that alloxan generates ROS in the pancreas and that the antioxidant drug *n*-acetylcysteine (NAC) inhibits NF-κB activation and reduces hyperglycemia. Kaneto et al. (28) also demonstrated protective effects of NAC in C57BL/KsJ-*db*/*db* mice. As these mice developed hyperglycemia, their insulin content and insulin gene expression decreased. NAC treatment was associated with preserved insulin content and insulin mRNA as well as increased amounts of PDX-1, an important insulin gene transcription factor. The use of a weaker antioxidant, vitamin E, led to controversial results. Tajiri and Grill (29) found that vitamin E failed to restore glucose-induced insulin secretion in rat pancreatic islets transplanted under the kidney capsule in streptozotocin-induced diabetic rats. On the other hand, Ihara et al. (11), who had previously noted increased immunostaining for 8-OH-deoxyglucose and HNE-modified proteins in GK islets, found that vitamin E had beneficial effects on glycemic control in GK rats, which was accompanied by significant improvement in insulin secretion and lower levels of HbA_{1c} (30). In our studies with ZDF rats, animals were treated with the antioxidant NAC, the combined antioxidant and nitric oxide synthase inhibitor aminoguanidine, the selective nitric oxide synthase inhibitor *s*-methylisothiourea, or placebo (12). Treatment was begun at 6 weeks of age and carried through 12 weeks of age. Progressive worsening of hyperglycemia, glucose tolerance, insulin secretion, PDX-1 binding to the insulin promoter, and insulin gene expression were associated with the increase of oxidative stress markers. In contrast, the ZDF animals treated with NAC or aminoguanidine, but not *s*-methylisothiourea or placebo, developed much less hyperglycemia and had preserved PDX-1 binding, insulin gene expression, and nearly normal insulin secretion and glucose tolerance. These findings support chronic oxidative stress as a potential mechanism of action for glucose toxicity and point away from nitric oxide species as a candidate ROS in this animal model. In this context, it is interesting to recall that troglitazone, a drug that prevents glucotoxic decreases in insulin RNA levels in the ZDF animal (23,24), is also an antioxidant.

Gene overexpression experiments have been designed to test whether increased levels of antioxidant enzymes in pancreatic islets might protect against oxidative stress. Kubisch et al. (31) reported that a transgenic model with increased levels of Cu/Zn superoxide dismutase had enhanced tolerance of β-cells to oxidative stress induced by alloxan. In a later study, enhanced superoxide dismutase expression in transgenic mice was targeted specifically to pancreatic β-cells. In other experiments, the expression of the transgene significantly enhanced resistance to alloxan-induced diabetogenesis, which the authors concluded provided direct in vivo evidence that genetic variation in ROS metabolism can affect susceptibility to oxidative stress–mediated diabetogenesis (32). Benhamou et al. (33) dem-

onstrated that overexpression of catalase in human islets protected against oxidative stress induced by xanthine oxidase-hypoxanthine. Similarly, Xu et al. (34) overexpressed catalase in mouse islets and found that it protected islet insulin secretion against hydrogen peroxide and significantly reduced the diabetogenic effect of streptozotocin in vivo.

The beneficial effects of antioxidant gene overexpression in protecting β-cell function in a ribose-related model of type 2 diabetes was recently reported by Tanaka et al. (18). These studies focused primarily on GSH as an intracellular antioxidant. Evidence to support the importance of GSH was found in experiments wherein buthionine sulfoximine was used to inhibit GSH synthesis. This drug potentiated the adverse consequences of ribose-induced ROS generation on rat islet β-cell insulin mRNA and glucose-induced insulin secretion. Adenoviral overexpression of GSH peroxidase protected the islets from ribose-induced formation of intracellular ROS and decreases in insulin gene expression and glucose-induced insulin secretion (Fig. 2). Therefore, there is ample evidence in animal models that type 2 diabetes is associated with increased markers of oxidative stress, that glucose can increase β-cell levels of ROS, that antioxidant treatment can ameliorate hyperglycemia, and that overexpression of antioxidant enzymes in the β-cell protects against increases in oxidants in its microenvironment.

EVIDENCE THAT GLUCOSE TOXICITY AND LIPOTOXICITY ARE INTERRELATED RATHER THAN SEPARATE ADVERSE FORCES ON THE β-CELL

Because type 2 diabetes in humans is frequently associated with obesity and hyperlipidemia as well as hyperglycemia, many investigators have examined whether high levels of free fatty acids or other lipids might be harmful to islet function. Indeed, there is ample evidence that fatty acids, which under normal circumstances are physiological fuels for the β-cell, become toxic when present at elevated concentrations for prolonged periods of time (35). Adverse effects of chronic exposure of the β-cell to elevated fatty acid concentrations include decreased glucose-induced insulin secretion (36–41), impaired insulin gene expression (42–45), and increased cell death (46–52).

The mechanisms by which fatty acids impair β-cell function are largely unknown. Initially, the possibility was raised that elevated fatty acid oxidation might decrease glucose oxidation (36,38), similar to the "glucose-fatty acid cycle" described in cardiac muscles (51). However, several observations argue against such a phenomenon being operative in β-cells. Experimental evidence suggests that the deleterious effects of fatty acids are mediated via lipid-derived intracytosolic metabolites rather than by fatty acid oxidation (52). Our hypothesis, initially proposed by Prentki and Corkey (53), is that in the presence of physiological glucose concentrations, excessive fatty acids are readily disposed of through mitochondrial β-oxidation. In contrast, when both fatty acids and glucose are elevated, accumulation of metabolites derived from fatty acid esterification impairs β-cell function. This model is supported by several observations. In vitro, prolonged exposure of isolated rat islets to palmitate inhibits insulin gene expression only in the presence of high glucose (44).

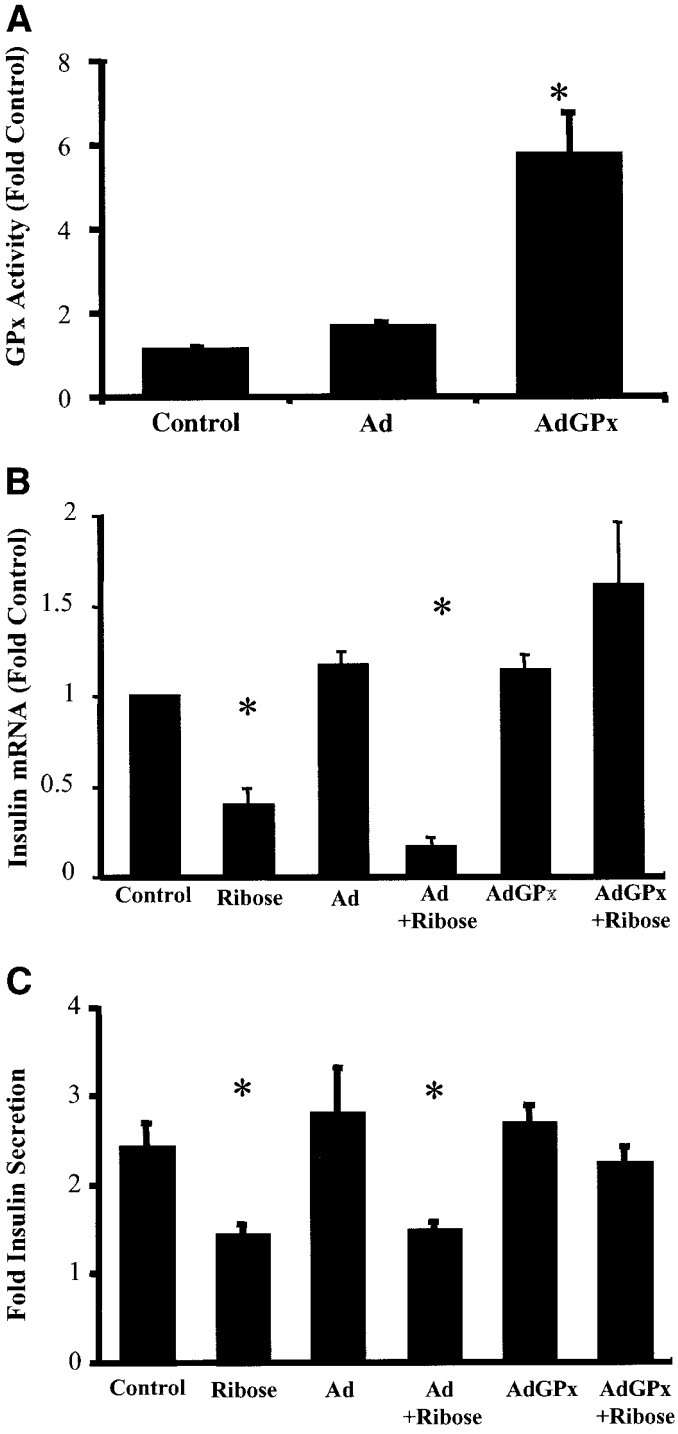

FIG. 2. Effect of overexpression of GPx by adenoviral infection for 72 h on adverse effects of oxidative stress caused by ribose on insulin gene expression and insulin secretion in isolated rat islets. *A*: Infection with adenovirus encoding GPx increased GPx activity approximately six-fold. *B*: Overexpression of GPx prevented the decrease in insulin gene expression caused by a 72-h exposure to ribose. *C*: Overexpression of GPx prevented the decrease in glucose-induced insulin secretion caused by a 72-h exposure to ribose. Ad, adenovirus control; AdGPx, adenovirus encoding GPx. *Statistically significant differences from respective controls. See the study by Tanaka et al. (18).

This is accompanied by a glucose-dependent increase in neutral lipid synthesis (45). Further, forcing triglyceride synthesis in islets by overexpression of diacylglycerol acyltransferase inhibits glucose-induced insulin secretion

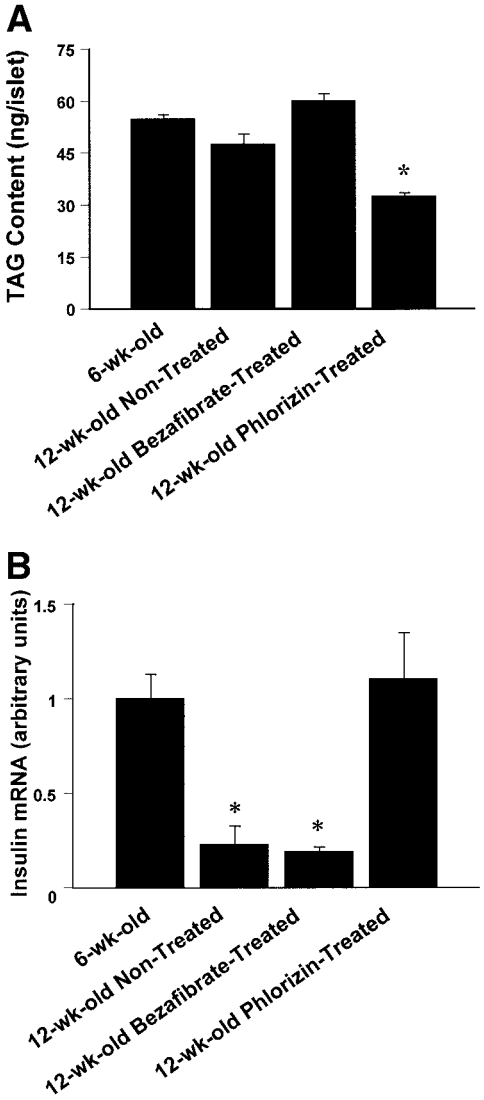

FIG. 3. Consequences of treating ZDF rats with the lipid-lowering drug bezafibrate or the glucose-lowering agent phorizin agent for 6 weeks beginning at 6 weeks of age. Bezafibrate treatment was associated with lower plasma triglyceride (TAG), but not glucose, levels compared with nontreated controls, and phlorizin treatment was associated with lower plasma glucose, but not triglyceride, levels compared with nontreated controls at 12 weeks of age. *A*: Islet TAG content. Islet TAG content was decreased by phlorizin, but not by bezafibrate. *B*: Islet insulin mRNA levels. The decrease in islet insulin mRNA levels normally found in 12-week-old hyperglycemic control rats was prevented by phlorizin but not by bezafibrate treatment. *Statistically significant differences from 6-week-old animals. See the study by Harmon et al. (55).

after culture in high glucose, but not in low glucose (54). In vivo, the glucose-lowering agent phlorizin, but not the lipid-lowering agent bezafibrate, lowers islet triglyceride content and prevents the decrease in insulin mRNA levels in ZDF rats (Fig. 3) (55). Finally, high-fat feeding impairs glucose-induced insulin secretion in hyperglycemic GK rats, but not in normoglycemic Wistar rats, an effect prevented by normalization of blood glucose in GK rats (56). Overall, these findings support the hypothesis that lipotoxicity only occurs in the context of chronic hyperglycemia, consistent with the observation that most individuals with increased circulating lipid levels have normal β-cell function.

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