In Vivo Prevention of Hyperglycemia Also Prevents Glucotoxic Effects on PDX-1 and Insulin Gene Expression

Jamie S. Harmon, Catherine E. Gleason, Yoshito Tanaka, Elizabeth A. Oseid, Kimberly K. Hunter-Berger, and R. Paul Robertson

Chronic exposure of pancreatic islet β-cell lines to supraphysiologic glucose concentrations causes defects in insulin gene expression and insulin secretion. To determine whether these in vitro phenomena have pathophysiologic relevance in vivo, we studied the Zucker diabetic fatty (ZDF) rat, an animal model of type 2 diabetes. The ZDF animals had relatively higher levels of glycemia and islet insulin mRNA at 6 weeks of age than age-matched Zucker lean control (ZLC) rats. As glycemia increased in 12- and 16-week-old ZDF rats, we observed decrements in glucose-induced insulin secretion during static incubations of pancreatic islets and in insulin mRNA levels, PDX-1 mRNA levels, and PDX-1 protein binding to the insulin promoter compared with age-matched ZLC rats and 6-week-old ZDF rats. To determine whether normalization of blood glucose levels would prevent these defects, ZDF rats were treated with troglitazone beginning at 6 weeks of age. Troglitazone prevented ZDF rats from becoming hyperglycemic and preserved glucose-induced insulin responses. Furthermore, troglitazone-treated ZDF animals had greater levels of insulin and PDX-1 mRNAs compared with untreated ZDF animals of the same ages at 12 and 16 weeks. Our results demonstrate that chronic and progressive hyperglycemia resulting from type 2 diabetes in ZDF rats is associated with loss of insulin and PDX-1 mRNAs and loss of glucose-stimulated insulin secretion. Prevention of hyperglycemia prevented the associated defects in insulin and PDX-1 gene expression and improved insulin secretion. These findings provide the first in vivo evidence that prevention of progressive hyperglycemia in a model of type 2 diabetes preserves insulin and PDX-1 gene expression. *Diabetes* 48:1995–2000, 1999

From Pacific Northwest Research Institute and the Division of Metabolism, Endocrinology, and Nutrition, Departments of Medicine and Pharmacology, University of Washington, Seattle, Washington.

Address correspondence and reprint requests to R. Paul Robertson, MD, Pacific Northwest Research Institute, 720 Broadway, Seattle, WA 98122. E-mail: rpr@u.washington.edu.

Received for publication 26 January 1999 and accepted in revised form 16 June 1999.

C/EBP-β, CCAAT/enhancer-binding protein β; EMSA, electrophoretic mobility shift assay; FBS, fetal bovine serum; FFA, free fatty acid; HBSS, Hanks' balanced salt solution; PDX-1, pancreatic/duodenal homeobox-1; RPA, ribonuclease protection assay; TG, triglyceride.

Type 2 diabetes is characterized by insulin resistance and β-cell dysfunction. It has been suggested that progressive hyperglycemia exacerbates β-cell abnormalities through a process known as glucose toxicity (1,2). Our laboratory has reported that chronic exposure of HIT-T15 (3) and βTC-6 (4) cells, two transformed β-cell lines, to supraphysiologic glucose concentrations decreases insulin gene promoter activity. This decrease is accompanied by decreases in the binding of pancreatic/duodenal homeobox-1 (PDX-1) (also known as somatostatin transactivating factor-1 [STF-1], islet duodenum HOX-1 [IDX-1], and insulin promoter factor-1 [IPF-1]) (3) and rat insulin promoter element-3b1 (RIPE-3b1) activator (4,5), two important stimulators of promoter activity, to the insulin gene promoter. Insulin secretion and mRNA levels are preserved when HIT-T15 cells are exposed to physiologic concentrations of glucose (6) and are reversed in INS-1 cells (7) or partially reversed in HIT-T15 cells (8) when switched from a supraphysiologic glucose concentration back to a physiologic concentration. Recently, we reported that reconstitution of PDX-1 gene expression in glucotoxic HIT-T15 cells partially restores insulin promoter activity (9). Exposure of human islets to high glucose was found by Eizirik et al. (10) to decrease insulin content and impair insulin responsiveness to glucose. In partially (90%) pancreatectomized rats, a model of hyperglycemia, Zangen et al. (11) found a reduction in insulin mRNA and PDX-1 protein.

A shortcoming of most of these previous studies is that they have been conducted using β-cell lines and islets in vitro. To ascertain the relevance of these findings to the pancreatic islet in vivo, we conducted studies using an animal model of type 2 diabetes. The Zucker diabetic fatty (ZDF) rat has many features in common with human type 2 diabetes, such as obesity, insulin resistance, and hyperglycemia (12). The ZDF rat typically develops glucose intolerance by 8 weeks and overt diabetes by 10–12 weeks of age. In the prediabetic stage, the animals are obese and insulin resistant but maintain normal blood glucose levels (12,13). Their lean (+/*fa* and +/+) littermates do not develop obesity or insulin resistance, thereby providing appropriate controls.

While the ZDF rat has been studied previously, it is not known whether loss of insulin mRNA and deterioration of glucose-stimulated insulin secretion in these diabetic animals is associated with the loss of PDX-1 or whether these losses can be avoided by preventing hyperglycemia in the ZDF animal.

The present study examined insulin secretion and insulin and PDX-1 mRNA levels in the ZDF rat in its prediabetic and diabetic phases to ascertain whether prevention of progressive hyperglycemia with troglitazone (14–16) would prevent these abnormalities in insulin gene expression.

RESEARCH DESIGN AND METHODS

Animals. Male Zucker lean control (ZLC) (ZLC/Gmi +/*fa* or +/+) and ZDF (ZDF/Gmi *fa*/*fa*) rats were purchased from Genetic Models (Indianapolis, IN) at 5 weeks of age. Animals were maintained on an ad libitum diet consisting of commercial powdered chow (Purina 5008). Starting at 6 weeks of age, the ZLC and ZDF rats were *1*) not treated, *2*) treated with an admixture of 0.2% troglitazone (a gift of Sankyo, Tokyo) for 6 weeks, or *3*) treated with troglitazone for 10 weeks. Animals were studied at 6, 12, and 16 weeks of age. After an overnight fast, animals were anesthetized with intraperitoneal injections of 0.9 ml/kg of a ketamine-HCl (500 mg)/xylazine (32 mg) solution. Blood samples were collected from the tail vein. Plasma glucose concentrations were determined using a Glucose Analyzer 2 (Beckman Instruments, Fullerton, CA). Plasma triglyceride (TG) levels were measured by the GPO-Trinder method (Sigma, St. Louis, MO). Plasma free fatty acid (FFA) levels were measured in an enzymatic colorimetric assay using the Free Fatty Acid, Half-micro Test (Boehringer Mannheim, Indianapolis, IN).

Islet isolation. The pancreas was distended by injection of 8–10 ml Hanks' balanced salt solution (HBSS) containing 0.09% collagenase type V (Sigma), 1% fetal bovine serum (FBS), and 2 U/ml RQ1 DNase (Promega, Madison, WI). The pancreas was removed, minced, and incubated with mild shaking at 37°C for 12–20 min. After 15 s of vigorous shaking, the tissue was washed twice with ice-cold HBSS followed by centrifugation at 250*g* for 4 min. The pellet was resuspended in 2 ml of 35% bovine serum albumin, incubated on ice for 5 min, and separated on a Dextran gradient as described by Field et al. (17). Isolated islets were hand-picked and cultured overnight at 37°C in RPMI 1640 containing 11.1 mmol/l glucose with 10% FBS. Islets were cultured with high-glucose medium overnight to maintain the high-glucose environment of the ZDF animal. To be consistent, and to provide equal stimulation, high glucose concentration was also used for the ZLC islets. Due to the small numbers of islets (100–200) that could be isolated from each rat, islets were pooled as indicated in Table 1 to obtain sufficient sample material to harvest nuclear fractions.

Glucose-stimulated insulin secretion. Insulin secretion was measured during static incubations of 8–10 islets/well in Krebs-Ringer buffer containing increasing glucose concentrations of 0.5–24 mmol/l for 60 min at 37°C in 5% CO_2, 95% O_2 as previously described (18). Medium samples were collected and assayed for insulin using the Sensitive Rat Insulin RIA Kit (Linco Research, St. Louis, MO).

Analysis of insulin and PDX-1 mRNAs. The abundance of PDX-1 and insulin mRNAs in pancreatic islets was determined by ribonuclease protection assay (RPA) using the Direct Protect RPA Kit (Ambion, Austin, TX). Riboprobes were transcribed from template DNA using the MAXIscript In Vitro Transcription Kit (Ambion) incorporating [^{32}P]CTP. The PDX-1 template DNA was generated by inserting the *Mlu*I to *Spe*I fragment of PDX-1/CMV (a gift from Dr. Roland Stein, Vanderbilt University) into the pSPORT 1 vector (Life Technologies, Grand Island, NY). The insulin DNA template was generated by inserting the 307-bp *Pst*I fragment of the human preproinsulin genomic DNA (phins 214; ATCC, Rockville, MD) into the pSPORT 1 vector. The PDX-1 template was linearized with *Fsp*I and transcribed with SP6 RNA polymerase to generate a 371-bp RNA fragment (331-bp protected fragment). The insulin template was linearized with *Kpn*I and transcribed with T7 RNA polymerase to generate a 330-bp RNA fragment (186-bp protected fragment). β-Actin was used for the control, since Tokuyama et al. (13) observed no significant differences in the levels of β-actin mRNA between ZDF and age-matched ZLC rats. The β-actin DNA was transcribed with T7 RNA polymerase from a linearized pTRIPLEscript plasmid containing a 250-bp mouse β-actin gene fragment to generate a 304-bp RNA fragment (245-bp protected fragment). The probes were gel-purified on a 5% polyacrylamide/8 mol/l urea gel and eluted overnight at 37°C in elution buffer (0.5 mol/l NH_4OAc, 1 mmol/l EDTA, 0.1% SDS). Probes were precipitated from the elution buffer with 10 µg yeast tRNA and 1 ml of 100% ethanol on ice for 15 min and resuspended in an appropriate volume of lysis/denaturation solution to generate a specific activity of 10^4–10^5 cpm/µl.

Islets (100–150) were hand-picked after isolation and incubated overnight in RPMI 1640 containing 11.1 mmol/l glucose at 37°C. Islets were washed with phosphate-buffered saline, resuspended in 1 µl lysis/denaturation solution per islet, and sonicated for 10 s. The appropriate probe (5 µl) was hybridized to 50 µl lysate for PDX-1 and β-actin mRNA determination and 5 µl lysate for insulin mRNA determination overnight at 45°C. The unprotected mRNA fragments were then degraded with 10 µl RNase cocktail and 200 U RNase T1 in the PDX-1/β-actin hybridization and insulin hybridization reactions, respectively, for 30 min at 37°C. The protected mRNA fragments were precipitated with 0.5 ml isopropanol and resuspended in 10 µl gel loading buffer. The fragments were separated on a 5% polyacrylamide/8 mol/l urea gel that was then fixed in 5% glacial acetic acid/5% methanol for 15 min and dried for 2 h. Gels were exposed to X-ray film and quantitated by densitometry. Density of the band of interest was divided by the density of the β-actin band within the same lane to correct for loading.

Analysis of PDX-1 binding to the insulin promoter. The binding of PDX-1 protein to the insulin promoter was analyzed by electrophoretic mobility shift assay (EMSA). An average of 500 pooled islets were used to obtain nuclear extracts (in some cases, islets from up to 10 animals were required; see Table 1). Nuclear extracts of the islets were prepared essentially as described by Vaisse et al. (19) using a Microcon-30 ultrafiltration column (Amicon, Beverly, MA). The oligodeoxynucleotide probe consisted of the human insulin CT2 (–230 to –210) (3) that was annealed and end-labeled with [^{32}P]dCTP using the Klenow fragment of *Escherichia coli* DNA polymerase-I. The binding reaction of the EMSA contained 30,000 cpm of the CT2 probe and 40–45 µg protein in a total volume of 30 µl containing 15 mmol/l HEPES, pH 7.5, 60 mmol/l KCl, 5 mmol/l $MgCl_2$, 2 mmol/l EDTA, 12% glycerol, 3.3 mmol/l dithiothreitol, and 100 ng poly(dI-dC). The reaction mixture was incubated for 30 min at room temperature, loaded onto a 4% acrylamide gel, and run in 0.5× TBE (44.5 mmol/l Tris, 44.5 mmol/l boric acid, 1 mmol/l EDTA) for 2 h at 4°C. To identify the band specific for PDX-1 binding,

TABLE 1
Experimental design

Measurement and age	Animal	Pools	No. of animals/pool
mRNA			
6 weeks	ZLC	2	5, 5
	ZDF	2	5, 5
12 weeks	ZLC	3	5, 5, 5
	ZLC-T	3	5, 10, 10
	ZDF	2	10, 10
	ZDF-T	3	5, 5, 10
16 weeks	ZLC	2	5, 10
	ZLC-T	3	5, 5, 10
	ZDF	2	10, 10
	ZDF-T	3	10, 10, 10
EMSA			
6 weeks	ZLC	2	5, 5
	ZDF	2	5, 5
12 weeks	ZLC	5	3, 3, 3, 5, 5
	ZLC-T	4	5, 5, 5, 5
	ZDF	5	4, 4, 5, 5, 10
	ZDF-T	2	5, 5
16 weeks	ZLC	2	5, 5
	ZLC-T	1	10
	ZDF	2	1, 1
	ZDF-T	3	1, 1, 5

ZLC-T, treated ZLC rat; ZDF-T, treated ZDF rat.

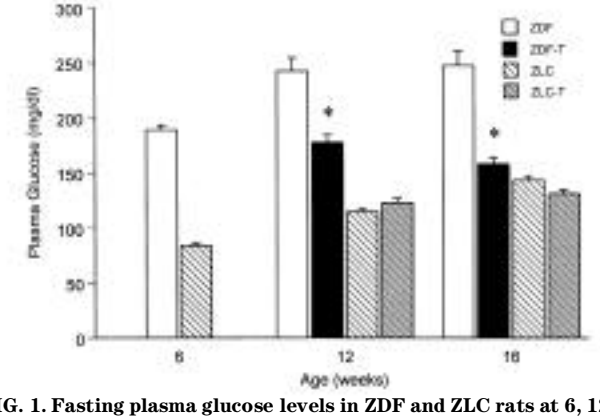

FIG. 1. Fasting plasma glucose levels in ZDF and ZLC rats at 6, 12, and 16 weeks of age. Animals were either untreated or treated (T) with troglitazone for 6 or 10 weeks beginning at 6 weeks of age. *P < 0.0001 vs. untreated.

TABLE 2
Characterization of body weight and fasting plasma levels of TG and FFA

Age and type of animal	Weight (g)	TG (mg/dl)	FFA (mmol/l)
6 weeks			
ZLC	149.9 ± 2.7	58.4 ± 6.4	1.66 ± 0.09
ZDF	185.3 ± 3.7*	211.6 ± 10.5*	3.25 ± 0.09*
12 weeks			
ZLC	314.1 ± 2.3†	51.2 ± 3.0	1.22 ± 0.08‡
ZLC-T	316.8 ± 2.7†	58.1 ± 4.2	1.23 ± 0.05‡
ZDF	370.8 ± 4.1†§	439.5 ± 17.1*†	2.60 ± 0.16*†
ZDF-T	527 ± 4.1†§‖	95.2 ± 11.3†§‖	2.00 ± 0.11†§‖
16 weeks			
ZLC	368.5 ± 4.5†	71.9 ± 5.6	1.12 ± 0.07¶
ZLC-T	367.1 ± 4.1†	51.6 ± 1.9	1.05 ± 0.08¶
ZDF	387.3 ± 7.2†#	400.7 ± 14.4*†	1.60 ± 0.07†#
ZDF-T	684.3 ± 6.5†§‖	275.6 ± 13.4§‖¶	1.44 ± 0.07†**

Data are means ± SE. ZLC-T, treated ZLC rats; ZDF-T, treated ZDF rats. *ZDF different from age-matched ZLC, $P < 0.0001$; †different from 6-week-old counterpart, $P < 0.0001$; ‡different from 6-week-old counterpart, $P < 0.008$; §treated different from age- and group-matched untreated, $P < 0.0001$; ‖ZDF-T different from age-matched ZLC, $P < 0.0001$; ¶different from 6-week-old counterpart, $P < 0.002$; #ZDF different from age-matched ZLC, $P < 0.006$; **ZDF-T different from age-matched ZLC, $P < 0.05$.

supershift assay was performed with the antibody to PDX-1 (a gift from Dr. Roland Stein). Autoradiograms were analyzed and quantitated by densitometry.
Statistics. Data are presented as means ± SE and analyzed by one-way analysis of variance.

RESULTS

Glucose, TG, and FFA levels. Fasting plasma glucose levels of the untreated rats were relatively elevated in 6-week-old ZDF rats (189.3 ± 4.4; $n = 26$) compared with ZLC rats (83.5 ± 3.1; $n = 25$). Although both continued to rise through 16 weeks of age (Fig. 1), the plasma glucose levels remained significantly higher in the ZDF animals. Troglitazone treatment significantly reduced the glucose level by 12 weeks of age and brought it to within normal levels by 16 weeks of age in ZDF rats. Other biochemical characteristics of these rats are compared with untreated and 6-week-old rats and summarized in Table 2. At 6 weeks of age, the ZDF rats had significantly higher weights and elevated levels of plasma TG and FFAs compared with their lean littermates, suggesting that the defects known to be present in ZDF rats can already be detected in the prediabetic stage of life. Twelve- and 16-week-old ZDF rats were also observed to have significantly higher TG and FFA levels compared with the age-matched ZLC rats. Plasma FFA levels declined dramatically with age, however, whereas the TG levels rose at 12 weeks then appeared to decline by 16 weeks of age. The plasma levels of TG and FFAs were significantly reduced with troglitazone treatment in the ZDF rats but did not reach those seen in the age-matched ZLC rats. The treatment had little effect in the ZLC rats.

Insulin secretion. Insulin secretion in response to increasing concentrations of glucose in a 60-min static incubation was measured from islets isolated from 6- and 12-week-old ZDF (Fig. 2*A*) and ZLC (Fig. 2*B*) rats. Islets isolated from 6-week-old ZDF rats had higher insulin responses compared with islets from ZLC animals. In contrast, insulin secretion from islets isolated from 12-week-old ZDF animals failed to respond to glucose, whereas insulin secretion from ZLC islets continued to secrete appropriately over the range of glucose concentrations. Treatment of ZDF rats with troglitazone for 6 weeks partially restored glucose-stimulated insulin secretion; no obvious effects were observed in ZLC rats treated with the drug (Fig. 2*B*).

Insulin and PDX-1 gene expression. Insulin mRNA was significantly higher in ZDF animals at 6 weeks compared

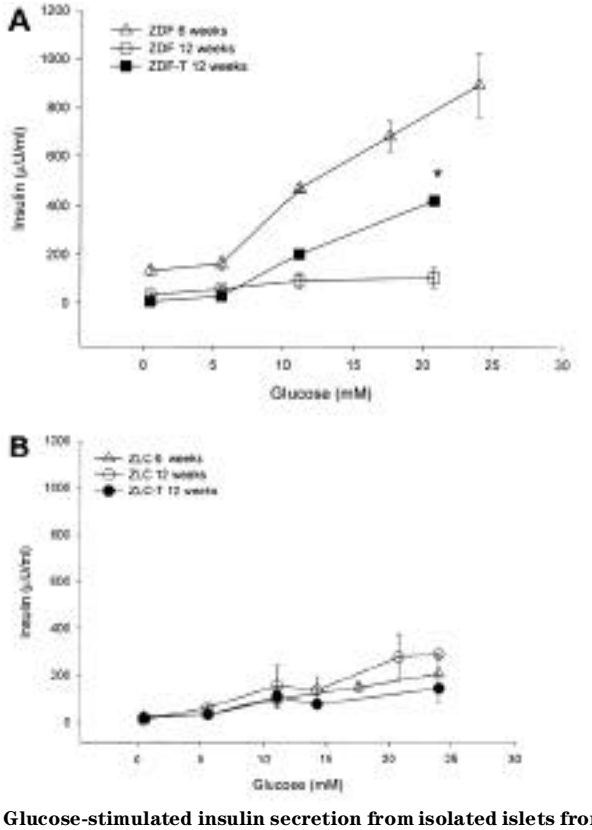

FIG. 2. Glucose-stimulated insulin secretion from isolated islets from Zucker rats exposed to glucose for 1 h at concentrations ranging from 0.5 to 24 mmol/l. *A*: Glucose-stimulated insulin secretion from ZDF rats at 6 (△) and 12 (□) weeks of age and after troglitazone treatment for 6 weeks (■). *B*: Glucose-stimulated insulin secretion from ZLC rats at 6 (△) and 12 (○) weeks of age and after troglitazone treatment for 6 weeks (●). Each value represents the mean ± SE of triplicate measurements, each consisting of 8–10 islets/well. Some error bars lie within the boundaries of the symbol. *$P < 0.003$ vs. untreated.

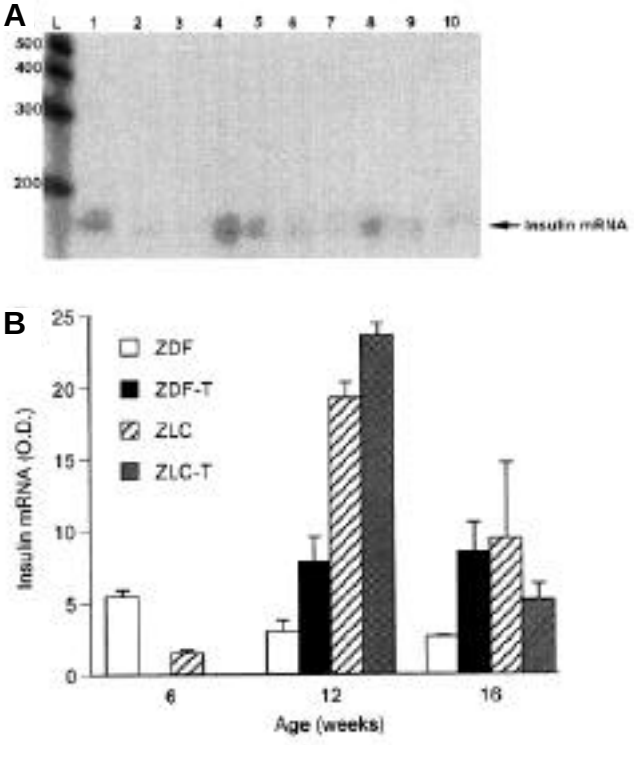

FIG. 3. Measurement of insulin mRNA in islets isolated from Zucker rats. *A*: Representative autoradiograms of insulin mRNA as measured by RPA at 6, 12, and 16 weeks of age. *Lane L*, ladder; *lane 1*, 6-week-old ZDF; *lane 2*, 6-week-old ZLC; *lane 3*, 12-week-old ZDF; *lane 4*, 12-week-old ZDF-T; *lane 5*, 12-week-old ZLC; *lane 6*, 12-week-old ZLC-T; *lane 7*, 16-week-old ZDF; *lane 8*, 16-week-old ZDF-T; *lane 9*, 16-week-old ZLC; *lane 10*, 16-week-old ZLC-T. *B*: Average optical densities of insulin mRNA measured in treated and untreated ZDF and ZLC rats. Data from 12- and 16-week-old animals were pooled, and the ZDF-T mRNA level (n = 7) was found to be significantly greater than the ZDF mRNA level (n = 5), P < 0.006. ZDF-T, treated ZDF rat; ZLC-T, treated ZLC rat.

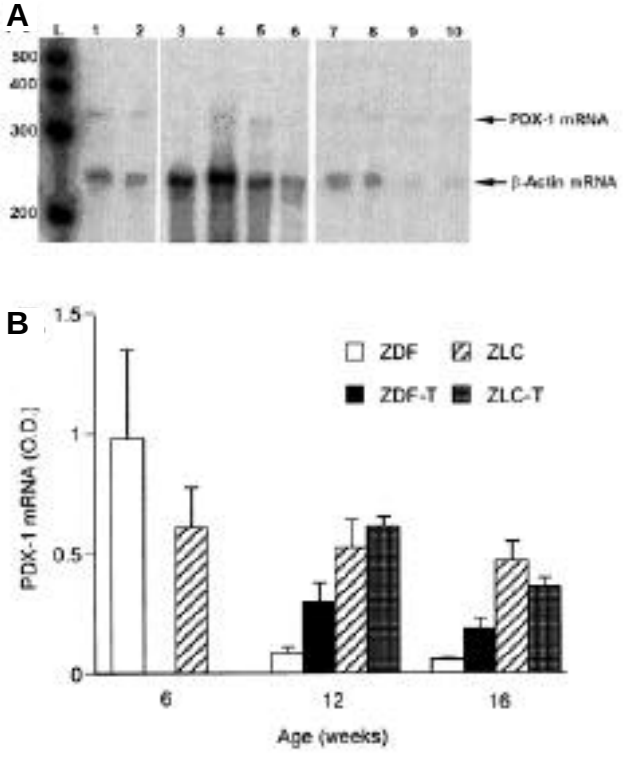

FIG. 4. Measurement of PDX-1 mRNA in islets isolated from Zucker rats. *A*: Representative autoradiograms of PDX-1 mRNA as measured by RPA at 6, 12, and 16 weeks of age. *Lane L*, ladder; *lane 1*, 6-week-old ZDF; *lane 2*, 6-week-old ZLC; *lane 3*, 12-week-old ZDF; *lane 4*, 12-week-old ZDF-T; *lane 5*, 12-week-old ZLC; *lane 6*, 12-week-old ZLC-T; *lane 7*, 16-week-old ZDF; *lane 8*, 16-week-old ZDF-T; *lane 9*, 16-week-old ZLC; *lane 10*, 16-week-old ZLC-T. *B*: Average optical densities of PDX-1 mRNA measured in treated and untreated ZDF and ZLC rats. Data from 12- and 16-week-old animals were pooled, and the ZDF-T mRNA level (n = 6) was found to be significantly greater than the ZDF mRNA level (n = 4), P < 0.03. ZDF-T, treated ZDF rat; ZLC-T, treated ZLC rat.

with ZLC animals. However, it was significantly lower in the 12- and 16-week-old ZDF animals compared with the age-matched control animals (Fig. 3). Compared with untreated ZDF animals, ZDF rats treated with troglitazone for 6 and 10 weeks had greater insulin mRNA levels. Treatment had no effect on the mRNA levels in the ZLC rat islets.

A similar pattern emerged for PDX-1 mRNA. ZDF and ZLC levels were not different at 6 weeks of age, but they were lower in ZDF rats at 12 and 16 weeks (Fig. 4). Troglitazone treatment in the 12- and 16-week-old ZDF rats significantly elevated the levels of PDX-1 mRNA compared with the untreated ZDF animals. No treatment effect was observed in the control animals.

PDX-1 binding to the insulin gene promoter. PDX-1 binding to the insulin promoter was examined in islets from the same pools used to assess insulin and PDX-1 mRNA levels. Troglitazone-treated ZDF animals had greater binding than untreated controls (optical density [OD] 54.17 ± 24.9 vs. 8.57 ± 4.3, mean ± SE; P < 0.06), although there was great variation in binding within the groups (n = 12, data not shown).

DISCUSSION

The glucotoxicity hypothesis, as it relates to regulatory processes in the pancreatic islet, maintains that progressive hyperglycemia caused by type 2 diabetes secondarily causes defects in insulin gene expression and decreased insulin content (1,2). The rationale for this hypothesis is based primarily on work carried out in β-cell lines demonstrating that chronic exposure to supraphysiologic glucose concentrations can decrease insulin gene expression, insulin content, insulin secretion, and gene expression of transcription factors that stimulate insulin gene expression (3–9,20,21). The experiments described herein were designed to assess the relevance of this evidence to type 2 diabetes as it occurs in vivo. Our studies are the first to examine whether treatment of diabetic animals with a drug that prevents hyperglycemia (troglitazone) can also prevent deterioration of insulin gene expression and insulin secretion. Our 6-week-old ZDF animals were relatively hyperglycemic but still had greater insulin mRNA levels and insulin secretion than 6-week-old ZLC rats. Troglitazone treatment arrested hyperglycemia and partially prevented losses in insulin and PDX-1 gene expression and glucose-induced insulin secretion. These findings are consistent with the hypothesis that chronic hyperglycemia in vivo secondarily leads to deterioration in insulin and PDX-1 gene expression and insulin secretion.

Support for our findings can be found in previous work by others who have investigated adverse effects of supraphysiologic glucose concentrations on insulin gene expression and β-cell function. Eizirik et al. (10) reported decreased

insulin secretion from human islets cultured in 28 mmol/l glucose for 7 days. Zangen et al. (11) noted reduction in insulin content, insulin mRNA, and PDX-1 protein in islets from rats rendered hyperglycemic for 28 days by 90% pancreatectomy. Lu et al. (20) examined HIT-T15 cells cultured for 16 weeks in 25 mmol/l glucose and observed increased protein levels of CCAAT/enhancer-binding protein β (C/EBP-β), a repressor of insulin gene transcription. Seufert et al. (21) observed hyperglycemia and diminished insulin and PDX-1 gene expression and increased C/EBP-β gene expression by semiquantitative reverse transcription–polymerase chain reaction in 9-week-old ZDF rats. In none of these studies, however, was a therapeutic maneuver used to stem the development of hyperglycemia to assess whether prevention of progressive hyperglycemia would preserve expression of insulin gene and insulin gene transcription factors. Such studies as we now report are essential to evaluate potential cause-and-effect relationships between elevated glycemia and dysfunctional islet gene expression.

Potentially confounding events that could complicate interpretation of our results would be a decrease in islet mass associated with the development of diabetes in ZDF animals and direct effects of troglitazone to increase islet cell mass or insulin gene expression. In this regard, Pick et al. (22) reported that β-cell mass in 12-week-old ZDF rats is double, not less than, that of 5- to 7-week-old animals. Ohtani et al. (23) observed inhibition, not stimulation, of β-cell proliferation rate and no increase in preproinsulin mRNA in HIT-T15 cells treated with troglitazone.

The ameliorative effects of troglitazone on β-cell function in ZDF rats have been ascribed by Shimabukuro et al. (24) to troglitazone's ability to decrease intra-islet triglyceride concentrations after in vitro incubation for 48 h. Gremlich et al. (25) reported that exposure of isolated islets to palmitic acid for 48 h decreased PDX-1 and insulin mRNA levels. It is not clear, however, whether such phenomena over these short experimental time intervals represent islet toxicity or short-term β-cell exhaustion or desensitization. In this regard, Bollheimer et al. (26) observed that the effect of high circulating FFA levels, which increased insulin secretion and decreased islet insulin content, could be blocked by somatostatin, a potent inhibitor of insulin secretion. This finding is more consistent with β-cell exhaustion than a toxic effect (that is, an irreversible effect secondary to chronic exposure to a toxin) caused by FFAs. In the study of chronic hyperglycemia reported herein, we observed that troglitazone was more efficacious in preventing increases in glucose and losses of insulin mRNA than it was in preventing increases in FFAs and TG in ZDF animals. Nonetheless, since long-term elevated glucose levels, as well as elevated FFA levels, are common in type 2 diabetes in humans, and since both can potentially lead to increased islet triglycerides, glucotoxicity and lipotoxicity may both contribute to the decrease in insulin and PDX-1 gene expression we observed.

In conclusion, we report data indicating that the loss of insulin mRNA in ZDF rats is at least partially attributable to a loss of PDX-1, that these losses contribute to the accompanying defects in glucose-stimulated insulin secretion, and that the losses can be at least partially stemmed by preventing progression of hyperglycemia in this animal. These findings provide novel evidence that in vivo prevention of progressive hyperglycemia in a model of type 2 diabetes partially preserves insulin and PDX-1 gene expression, a linkage that supports the glucotoxicity hypothesis.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grant RO1-DK-38325 (R.P.R.), the Juvenile Diabetes Foundation International (J.S.H.), and the American Diabetes Association Mentor Based Post Doctoral Fellowship Program (R.P.R., Y.T.).

We thank Dr. Roland Stein for the generous gifts of plasmid and antibody. We also thank Elizabeth Kilpatrick for aiding in the development of islet isolation.

REFERENCES

1. Robertson RP, Olson LK, Zhang H-J: Differentiating glucose toxicity from glucose desensitization: a new message from the insulin gene. *Diabetes* 43:1085–1089, 1994
2. Yki-Jarvinen H: Glucose toxicity. *Endocrine Rev* 13:413–431, 1992
3. 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. *J Clin Invest* 92:514–519, 1993
4. Poitout V, Olson LK, Robertson RP: Chronic exposure of βTC-6 cells to supraphysiologic concentrations of glucose decreases binding of the RIPE3b1 insulin gene transcription activator. *J Clin Invest* 97:1041–1046, 1996
5. Sharma A, Olson LK, Robertson RP, Stein R: The reduction of insulin gene transcription in HIT-T15 β 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
6. Robertson RP, Zhang H-J, 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
7. Olson LK, Qian J, Poitout V: Glucose rapidly and reversibly decreases INS-1 cell insulin gene transcription via decrements in STF-1 and C1 activator transcription factor activity. *Mol Endocrinol* 12:207–219, 1998
8. Moran A, Zhang H-J, Olson LK, Harmon JS, Poitout V, Robertson RP: Differentiation of glucose toxicity from beta cell exhaustion during the evolution of defective insulin gene expression in the pancreatic cell line, HIT-T15. *Diabetes* 99:534–539, 1997
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. Eizirik DL, Korbutt GS, Hellerstrom C: Prolonged exposure of human pancreatic islets to high glucose concentrations in vitro impairs the β-cell function. *J Clin Invest* 90:1263–1268, 1992
11. Zangen DH, Bonner-Weir S, Lee CH, Latimer JB, Miller CP, Habener JF, Weir GC: Reduced insulin, GLUT2, and IDX-1 in β-cells after partial pancreatectomy. *Diabetes* 46:258–264, 1997
12. Peterson RG, Shaw WN, Neel M-A, Little LA, Eichberg J: Zucker Diabetic Fatty rat as a model for non-insulin-dependent diabetes mellitus. *ILAR News* 32: 16–19, 1990
13. Tokuyama Y, Sturis J, DePaoli AM, Takeda J, Stoffel M, Tang J, Sun X, Polonsky KS, Bell GI: Evolution of β-cell dysfunction in the male Zucker Diabetic Fatty rat. *Diabetes* 44:1447–1457, 1995
14. Fujiwara T, Wada M, Fukuda K, Fukami M, Yoshioka S, Yoshioka T, Horikoshi H: Characterization of CS-045, a new oral antidiabetic agent. II. Effects on glycemic control and pancreatic islet structure at a late stage of the diabetic syndrome in C57BL/KsJ-db/db mice. *Metabolism* 40:1213–1218, 1991
15. 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
16. Prigeon RL, Kahn SE, Porte D Jr: Effect of troglitazone on B cell function, insulin sensitivity, and glycemic control in subjects with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 83:819–823, 1998
17. Field J, Farney A, Sutherland DER: Improved islet isolation from rat pancreas using 35% bovine serum albumin in combination with dextran gradient separation. *Transplantation* 61:1554–1556, 1996
18. Zhang H-J, Walseth TF, Robertson RP: Insulin secretion and cAMP metabolism in HIT cells. *Diabetes* 38:44–48, 1989
19. Vaisse C, Halaas JL, Horvath CM, Darnell JE Jr, Stoffel M, Friedman JM: Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. *Nature Genet* 14:95–97, 1996

20. Lu M, Seufert J, Habener JF: Pancreatic β-cell-specific repression of insulin gene transcription by CCAAT/enhancer-binding protein β. *J Biol Chem* 272: 28349–28359, 1997
21. Seufert J, Weir GC, Habener JF: Differential expression of the insulin gene transcriptional repressor CCAAT/enhancer-binding protein β and transactivator islet duodenum homeobox-1 in rat pancreatic β cells during the development of diabetes mellitus. *J Clin Invest* 101:2528–2539, 1998
22. Pick A, Clark J, Kubstrup C, Levisetti M, Pugh W, Bonner-Weir S, Polonsky KS: Role of apoptosis in failure of beta-cell mass compensation for insulin resistance and beta-cell defects in the male Zucker diabetic fatty rat. *Diabetes* 47:358–364, 1998
23. Ohtani K-I, Shimizu H, Sato N, Mori M: Troglitazone (CS-045) inhibits β-cell proliferation rate following stimulation of insulin secretion in HIT-T15 cells. *Endocrinology* 139:172–178, 1998
24. Shimabukuro M, Zhou Y-T, Lee Y, Unger RH: Troglitazone lowers islet fat and restores beta cell function of Zucker Diabetic Fatty rats. *J Biol Chem* 273: 3547–3550, 1998
25. Gremlich S, Bonny C, Waeber G, Thorens B: Fatty acids decrease IDX-1 expression in rat pancreatic islets and reduce GLUT2, glucokinase, insulin, and somatostatin levels. *J Biol Chem* 272:30261–30269, 1997
26. Bollheimer LC, Skelly RH, Chester MW, McGarry JD, Rhodes CJ: Chronic exposure to free fatty acid reduces pancreatic β 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