

Antecedent Hyperglycemia, Not Hyperlipidemia, Is Associated With Increased Islet Triacylglycerol Content and Decreased Insulin Gene mRNA Level in Zucker Diabetic Fatty Rats

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Type 2 diabetes is caused by a combination of β -cell dysfunction and insulin resistance. Over time, hyperglycemia worsens, a phenomenon that has been attributed to deleterious effects of chronic hyperglycemia (glucotoxicity) or chronic hyperlipidemia (lipotoxicity) on β -cell function and is often accompanied by increased islet triacylglycerol (TAG) content and decreased insulin gene expression. To examine these two potentially pathogenic forces, we studied Zucker rats (leptin receptor wild type, +/+; heterozygous, +/-; and mutant, -/-). First, +/+ and +/- Zucker rats were compared metabolically. At 6 weeks of age, the +/- rats had a lower level of islet insulin mRNA compared with +/+. At 12 weeks of age, differences were found in body weight and islet TAG content; however, levels of insulin mRNA were equivalent. Second, we examined whether worsening of the diabetic state in the homozygous mutant (-/-) Zucker diabetic fatty (ZDF) rat is related more to chronic hyperglycemia or to hyperlipidemia. The ZDF rats were treated for 6 weeks with either bezafibrate, a lipid-lowering drug that does not affect plasma glucose levels, or phlorizin, a drug that reduces plasma glucose without lowering lipid levels. Bezafibrate treatment lessened the rise in plasma TAG observed in nontreated rats (239 ± 16 vs. 388 ± 36 mg/dl, treated versus nontreated; $P < 0.0001$) but did not prevent the rise in fasting plasma glucose. Despite lowering plasma TAG, bezafibrate was not effective in preventing an increased islet TAG content and did not prevent the associated decrease in insulin mRNA levels. Phlorizin treatment prevented hyperglycemia (61 ± 2 vs. 145 ± 7 mg/dl, treated versus nontreated; $P < 0.0001$) and lowered islet TAG content (32.7 ± 0.7 vs. 47.8 ± 2.7 ng/islet, treated versus nontreated; $P < 0.0001$) and preserved insulin mRNA levels without preventing hypertriglyceridemia. Plasma free fatty acid level did not correlate with changes in islet TAG or insulin mRNA levels. We conclude that antecedent elevated plasma glucose levels, not plasma lipid levels, are associated with elevated islet TAG content and decreased insulin mRNA levels in ZDF animals. *Diabetes* 50:2481–2486, 2001

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FFA, free fatty acid; LPL, lipoprotein lipase; PCR, polymerase chain reaction; PDX-1, pancreatic/duodenal homeobox-1; TAG, triacylglycerol.

After the onset of type 2 diabetes in experimental animals, chronic hyperglycemia is associated with defects in insulin gene expression (1–5). The mechanism of the decrease in insulin gene expression in Zucker diabetic fatty (ZDF) (2,4) and 90% pancreatectomized (4) rats, two animal models of type 2 diabetes, has been shown to be due in part to diminished DNA binding of the transcription factor pancreatic/duodenal homeobox-1 (PDX-1). We have reported that prevention of hyperglycemia and hyperlipidemia by troglitazone treatment in the ZDF rat preserves insulin and PDX-1 mRNAs and PDX-1 binding to DNA (2). Because increased triacylglycerol (TAG) content has been observed in the islets of ZDF rats (6,7), it has been posited to play a role in diabetes in this model (8,9). In support of this notion, islets cultured in high free fatty acid (FFA) media have increased TAG content (7,10,11), decreased glucose-stimulated insulin secretion and biosynthesis (12), and decreased insulin gene expression (13). Moreover, in addition to preventing hyperglycemia and hyperlipidemia, troglitazone treatment prevented increased islet TAG content in ZDF rats (14) and in islets cultured in high FFA and high glucose concentrations (15). Lee et al. (6) reported that plasma FFA levels precede the rise in plasma glucose levels in ZDF animals by 2 weeks and that correlations exist among plasma FFA, plasma glucose, and islet TAG. However, neither this nor other studies attempted to differentiate prospectively hyperglycemia versus hyperlipidemia in vivo as secondary metabolic forces that lead to further islet TAG accumulation and decreased insulin gene expression. To address this issue, we first genotyped and metabolically compared heterozygote (+/-) animals with the wild-type (+/+) animals. We next treated ZDF (-/-) rats with a drug that selectively decreases plasma lipids without affecting blood glucose levels (bezafibrate) or one that decreases plasma glucose without affecting plasma lipids (phlorizin). The mechanism of action of bezafibrate involves activation of peroxisome proliferator-activated receptor- α ; induction of lipoprotein lipase (LPL) activity, which increases hydrolysis of plasma TAG; stimulation of cellular fatty acid uptake; increased peroxisomal and mitochondrial β -oxidation; and decreased synthesis of fatty acids, TAG, and VLDL (reviewed in Schoonjans et al. [16]). Phlorizin inhibits glucose reabsorption by the renal tubular

TABLE 1
Characteristics of ZLC rats

| | 6-week-old | | | 12-week-old | | |
|--------------------------------|-------------|-------------|----------|-------------|-------------|----------|
| | ZLC (+/+) | ZLC (+/-) | <i>P</i> | ZLC (+/+) | ZLC (+/-) | <i>P</i> |
| Body weight (g) | 158.8 ± 3.1 | 145.2 ± 2.0 | NS | 321.1 ± 5.8 | 294.8 ± 5.4 | <0.003 |
| Plasma glucose (mg/dl) | 78.9 ± 3.2 | 84.7 ± 1.7 | NS | 92.4 ± 1.6 | 87.6 ± 1.2 | NS |
| Plasma FFA (mmol/l) | 0.53 ± 0.06 | 0.61 ± 0.06 | NS | 0.32 ± 0.02 | 0.46 ± 0.04 | NS |
| Plasma TAG (mg/dl) | 38.6 ± 5.2 | 32.9 ± 2.9 | NS | 29.2 ± 2.0 | 40.3 ± 1.9 | NS |
| TAG content (ng/islet) | 32.0 ± 0.8 | 35.3 ± 2.2 | NS | 12.0 ± 1.3 | 34.3 ± 0.8 | <0.0001 |
| Plasma insulin (μU/ml) | 2.99 ± 0.47 | 3.61 ± 0.28 | NS | 5.53 ± 0.37 | 5.79 ± 0.57 | NS |
| Insulin content (μU/ml/islet) | 919 ± 81 | 734 ± 31 | NS | 1903 ± 118 | 1786 ± 89 | NS |
| Insulin mRNA (arbitrary units) | 1.5 ± 0.7 | 0.5 ± 0.1 | <0.02 | 0.8 ± 0.1 | 1.0 ± 0.4 | NS |

Data are means ± SE. *n* = 4–10. NS, not significant; data obtained from fasted rats.

cells. Our results indicate that wild-type and heterozygous Zucker rats are metabolically similar and that defective insulin mRNA level, which occurs only in the homozygous mutant ZDF animal, is associated with antecedent chronic hyperglycemia but not chronic hyperlipidemia.

RESEARCH DESIGN AND METHODS

Animals. Male Zucker lean control (ZLC/Gmi +/- and +/+) rats and Zucker diabetic fatty (ZDF/Gmi -/-) rats were purchased from Genetic Models (Indianapolis, IN) at 5 weeks of age. Animals were maintained on an ad libitum diet of Purina 5008 chow (7.5% fat) as recommended by Genetic Models.

Genotyping. A method by Phillips et al. (17), modified by Dr. Elizabeth Rutledge at the Molecular Genetics Core of the University of Washington Diabetes Center, was used to genotype the Zucker rats. DNA was isolated from 3-mm tail snips. The tissue was placed in 0.5 ml of extraction buffer (100 mmol/l Tris-HCl [pH 8.5], 5 mmol/l EDTA, 0.2% SDS, 200 mmol/l NaCl, and 200 μg/ml proteinase K) and incubated overnight at 55°C in a shaking water bath. After a 15-min centrifugation at 15,500g, the supernatant was added to 0.5 ml of ice-cold isopropanol and the tubes were shaken to form a precipitant. The tubes were centrifuged at 15,500g for 3 min, and the supernatant was discarded. The pellets were washed two times with cold 70% ethanol and air-dried. The DNA was dissolved in 500 μl of H₂O at 55°C for 1 h, and the concentration was determined spectrophotometrically. Polymerase chain reactions (PCRs) were performed using the Promega PCR Core System I, 100 ng of DNA, 14 pmol of each primer, and 30 units of *Taq* enzyme with the following cycling conditions: 92°C for 2 min, 49 cycles of 92°C for 30 s, 65°C for 30 s, 68°C for 3 min, then 5 min at 68°C. The amplified product of 1.8 kb was digested with *MspI* (New England Biolabs, Beverly, MA), generating a 1.1-kb band for the wild type (+/+), a 1.0-kb band for the mutant (-/-), and 1.1- and 1.0-kb bands for the heterozygote (+/-).

Treatments. Starting at 6 weeks of age, the rats were nontreated, were treated orally with an admixture of 0.3% bezafibrate (Sigma) for 6 weeks, or received an injection of 0.4 g/kg phlorizin (Sigma) (in 40% propylene glycol) every 12 h for 6 weeks. Starting at 7 weeks of age, another subset of rats received Linplant, a subcutaneous insulin implant that releases 2 units/24 h of insulin (Linshin Canada, Ontario, Canada); additional insulin pellets were implanted as needed over the course of the study in an attempt to obtain glucose levels similar to those seen in lean animals. Control ZDF rats received the same number of mock implants. Each animal received between three and six implants total over the course of 5 weeks. Animals were anesthetized, and blood samples were collected from the tail vein after an overnight fast (~16 h), except for those that were treated with insulin, which were not fasted to avoid severe hypoglycemia. Plasma glucose concentration was determined using a Glucose Analyzer 2 (Beckman Instruments, Fullerton, CA). Blood was also collected via cardiac puncture at the time that the rats were killed for plasma insulin, TAG, and FFA level determinations. The Sensitive Rat Insulin RIA KIT (Linco Research, St. Louis, MO) was used to measure plasma insulin levels. Plasma TAG levels were measured by the GPO-Trinder (#339) method (Sigma Diagnostics, St. Louis, MO). Plasma FFA levels were measured immediately using the Half-micro test (Boehringer-Mannheim).

Islet isolation. Islets were isolated essentially as described previously (2). After removal of the pancreas, collagenase digestion occurred at 37°C for 17 min. After 15 s of vigorous shaking, the undigested tissue was removed by a 500-μm screen. Isolated islets were handpicked twice. Because of the small numbers of islets that could be isolated from each ZDF rat, islets (~100–200)

were pooled from two to three animals to obtain sufficient sample material for the various assays.

TAG content. Islet TAG content was measured after chloroform:methanol extraction as described by Briaud et al. (11).

Insulin content. Islet insulin content was measured in 10 islets after acid extraction as described by Zhou and Grill (12).

Analysis of insulin mRNA. Isolated islets (100–200) were cultured overnight at 37°C in RPMI 1640 containing 11.1 mmol/l glucose with 10% fetal bovine serum. Islets were cultured with high-glucose media overnight to mimic the high-glucose environment of the ZDF animal. To be consistent and to provide equal stimulation of insulin gene expression, we also used high-glucose concentration for the ZLC islets. Islets were washed with phosphate-buffered saline, and total RNA was extracted according to the method of Chomczynski and Sacchi (18). One-step reverse transcriptase-PCR was carried out using an ABI Prism 7700 sequence detector equipped with a thermocycler (Taqman Technology) as described by Tanaka et al. (19). Insulin mRNA levels are expressed relative to β-actin mRNA levels. Primer and probe sequences are as follows: probe, rat insulin 6FAM-AGCTTCCACCAAGTGAGAACCACAAAGGT-TAMRA; forward primer, rat insulin GCCCAGGCTTTGTCAAACA; reverse primer, rat insulin CTCCCCACACACCAGGTAGAG; probe, rat β-actin 6FAM-AGGCATCCTGACCCTGAAGTACCCCA-TAMRA; forward primer, rat β-actin ACAGAGGCCAGAGCAAGA; reverse primer, rat β-actin TTGGTTACAATGC CGTGTTC.

Statistics. Data are presented as means ± SE and analyzed by one-way analysis of variance.

RESULTS

ZLC rats. To ascertain whether rats heterozygous for the mutation of one allele for the leptin receptor (ZLC, +/-) have a moderate or intermediate phenotype of type 2 diabetes, we compared their characteristics with the wild-type rats (ZLC, +/+; Table 1). At 6 weeks of age, the only observed difference between the two groups was a lower level of insulin mRNA in the ZLC (+/-) compared with ZLC (+/+) rats (*P* < 0.02). However, at 12 weeks of age, the level of insulin message was similar in the two groups. In the older animals, a significant increase in islet TAG content was observed between ZLC (+/+) and (+/-) (*P* < 0.0001), which was accompanied by a decreased body weight.

ZDF rats. The body weight of ZDF rats at 12 weeks of age remained unchanged with bezafibrate and phlorizin treatments and increased with insulin treatment (Table 2). The fasting plasma glucose level of ZDF rats was significantly elevated at 12 weeks of age compared with the level observed at 6 weeks of age (Fig. 1). The glucose level continued to rise in the ZDF animals that were treated with bezafibrate for 6 weeks, whereas phlorizin treatment was effective not only in preventing an increased glucose level but also in lowering it below that observed in control animals. Insulin treatment significantly reduced the fed

TABLE 2
Characteristics of ZDF rats

| Age (weeks) | Treatment | Body weight (g) | Plasma insulin ($\mu\text{U/ml}$) | Insulin content ($\mu\text{U/ml/islet}$) |
|-------------|-------------|-------------------|-------------------------------------|--|
| 6 | Non | 181.4 \pm 4.9 | 19.79 \pm 1.99 | 1,885 \pm 215 |
| 12 | Non | 348.5 \pm 5.7* | 37.32 \pm 3.55* | 717 \pm 155* |
| 12 | Bezafibrate | 345.7 \pm 5.7* | 25.23 \pm 3.26§ | 1,787 \pm 250§ |
| 12 | Phlorizin | 344.4 \pm 6.5* | 12.00 \pm 2.43†‡ | 2,408 \pm 233†‡ |
| 12 | Insulin | 482.7 \pm 4.5*‡ | NA | 1,157 \pm 201† |

Data are means \pm SE. Different from 6-week-old counterpart, * $P < 0.0004$, † $P < 0.05$; treated different from age-matched untreated, ‡ $P < 0.0001$, § $P < 0.005$. Measurements were taken from fasted animals, except for insulin-treated animals.

glucose level compared with nontreatment (227 \pm 22 vs. 559 \pm 12 mg/dl, respectively; $P < 0.0001$). However, this level was not reduced to that observed in fed lean animals (143 \pm 6 mg/dl, $n = 13$) and thus was not as effective as phlorizin in preventing hyperglycemia. As expected, the plasma TAG level of the 12-week-old nontreated ZDF rats was higher than at 6 weeks of age (388 \pm 36 vs. 180 \pm 9 mg/dl, respectively; $P < 0.0001$; Fig. 2). Treatment with bezafibrate was effective in preventing the rise in plasma TAG (240 \pm 16 mg/dl; $P < 0.0001$ vs. nontreatment), whereas phlorizin treatment was not (559 \pm 51 mg/dl). Insulin treatment had no significant effect on the fed level of plasma TAG. In the nontreated ZDF group, the FFA level declined at 12 weeks of age compared with 6 weeks of age (0.51 \pm 0.05 vs. 0.96 \pm 0.05 mmol/l, respectively; $P < 0.0001$; Fig. 3). This is consistent with recently reported data (2) but in contrast to the levels reported by Lee et al. (6), which likely is due to a difference in the source of rat colony. In ZDF rats, treatment with bezafibrate and phlorizin were associated with a significant elevation of the FFA level (0.73 \pm 0.72 mmol/l [$P < 0.005$] and 0.96 \pm 0.05 mmol/l [$P < 0.0001$], respectively) compared with 12-week-old nontreated rats, whereas insulin

A. Fasted Levels

B. Fed Levels

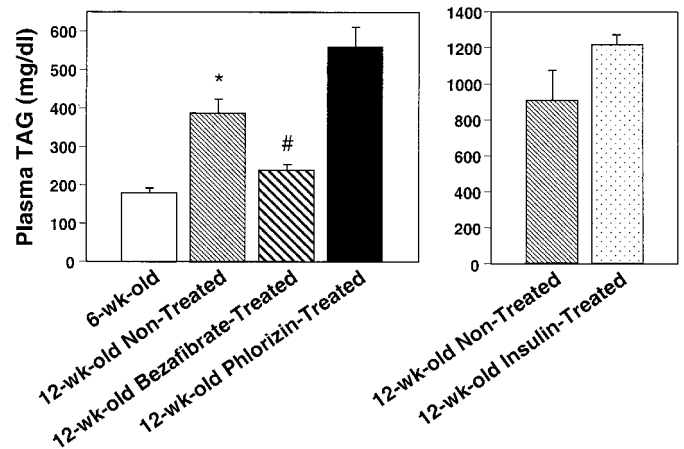


FIG. 2. Levels of plasma TAG at 6 and 12 weeks of age in fasted ZDF rats that were either nontreated or treated for 6 weeks with bezafibrate or phlorizin (A) and in fed ZDF rats that were treated for 5 weeks with mock (nontreated) or insulin implants (B; $n = 9-20$). * $P < 0.0001$ versus 6-week-old; # $P < 0.0001$ versus 12-week-old nontreated. Animals in the insulin treatment group were not fasted to avoid hypoglycemia; note difference in scale in A and B.

treatment was not. Plasma insulin levels were closely associated with plasma glucose; the highest insulin levels occurred in the animals with the highest glucose (Table 2). The insulin content of the islets was inversely proportional to the plasma insulin level. The TAG content (Fig. 4) of ZDF islets was similar at 6 and 12 weeks of age. Insulin treatment had no effect on islet TAG content (48.2 \pm 2.7 ng/islet; NS), whereas it was significantly reduced in the phlorizin-treated group (32.7 \pm 0.7 ng/islet; $P < 0.001$) compared with nontreated animals (47.8 \pm 2.7 ng/islet). There was no decrease in islet TAG content in the bezafibrate-treated group. Islet insulin mRNA was significantly reduced in ZDF rats at 12 weeks of age compared with 6 weeks of age (0.23 \pm 0.1 vs. 1.0 \pm 0.1, arbitrary units, respectively; $P < 0.05$; Fig. 5). Phlorizin treatment pre-

A. Fasted Levels

B. Fed Levels

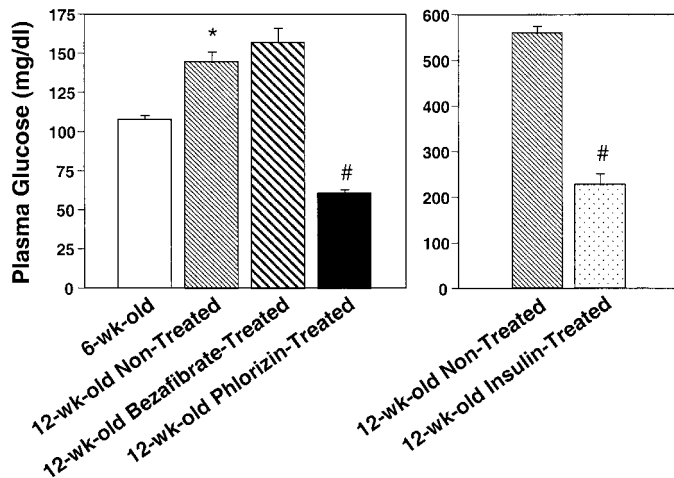


FIG. 1. Levels of plasma glucose at 6 and 12 weeks of age in fasted ZDF rats that were either nontreated or treated for 6 weeks with bezafibrate or phlorizin (A) and in fed ZDF rats that were treated for 5 weeks with mock (nontreated) or insulin implants (B; $n = 9-20$). * $P < 0.0001$ versus 6-week-old; # $P < 0.0001$ versus 12-week-old nontreated. Animals in the insulin treatment group were not fasted to avoid hypoglycemia; note difference in scale in A and B.

A. Fasted Levels

B. Fed Levels

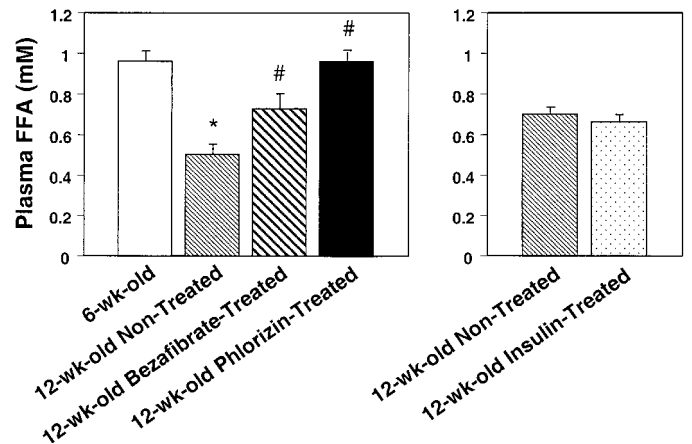


FIG. 3. Levels of plasma FFA at 6 and 12 weeks of age in fasted ZDF rats that were either nontreated or treated for 6 weeks with bezafibrate or phlorizin (A) and in fed ZDF rats that were treated for 5 weeks with mock (nontreated) or insulin implants (B; $n = 9-20$). * $P < 0.0001$ versus 6-week-old; # $P < 0.005$ for bezafibrate treated and $P < 0.0001$ for phlorizin-treated versus 12-week-old nontreated. Animals in the insulin treatment group were not fasted to avoid hypoglycemia.

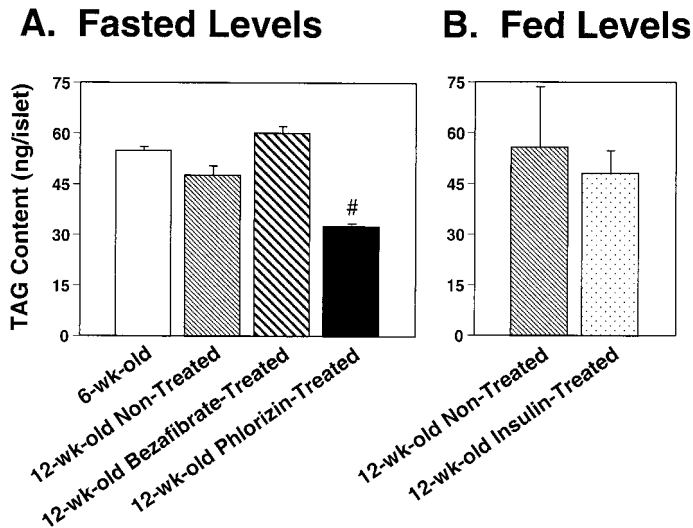


FIG. 4. Levels of islet TAG content at 6 and 12 weeks of age in fasted ZDF rats that were either nontreated or treated for 6 weeks with bezafibrate or phlorizin (A) and in fed ZDF rats that were treated for 5 weeks with mock (nontreated) or insulin implants (B; $n = 4-5$ [each n contains islets pooled from two to three rats]). $\#P < 0.001$ for phlorizin-treated versus 12-week-old nontreated.

vented this fall in insulin mRNA, whereas treatments with bezafibrate or insulin did not.

DISCUSSION

The concept of glucose toxicity of the β -cell was considered as early as 15 years ago (20,21) and has received support from many investigators (22–28). Its potential mechanisms of action include chronic oxidative stress (19,29) and participation with fatty acids in the formation of islet lipids. The latter mechanism has led to the concept of lipotoxicity (30), which has received support from many investigators as well (6–10,13,31). The possibility that toxic effects of lipids may take place only in the setting of high-glucose concentrations has been advanced by in vitro studies (11,13,32). However, this possibility has not been examined previously in vivo. For example, two reports (2,14) describing the beneficial effects of troglitazone in ZDF rats established that treatment with this drug is associated with lower TAG content and prevention of islet apoptosis (14) as well as preservation of PDX-1 and insulin gene expression and glucose-stimulated insulin secretion (2), yet troglitazone treatment lowers plasma levels of both glucose and TAG in ZDF rats (2). This prompted us to conduct the studies described herein to examine the consequences of preventing either hyperglycemia or hyperlipidemia as independent variables. Although apoptosis of β -cells is clearly an important concern, it likely is not a variable in this study as Pick et al. (33) reported an increase in β -cell mass and Ohneda et al. (34) demonstrated an increase in β -cell volume, both in 12-week-old ZDF rats.

Phlorizin treatment for 6 weeks in ZDF animals prevented hyperglycemia but not hypertriglyceridemia and also prevented the increase in islet TAG content and the decrease in insulin mRNA observed in nontreated animals. Similar observations have been made in partially pancreatectomized animals (35) in which phlorizin treatment reversed hyperglycemia and normalized islet gene expression of several mRNAs without changes in plasma fatty

acids, whereas treatment with vehicle alone had no effect. Rossetti et al. (36) were able to normalize insulin sensitivity with phlorizin treatment in diabetic animals and, in a later study (21), found that the associated improvements in β -cell function could not be attributed to an increased β -cell mass or insulin content. However, TAG levels in the plasma and islets were not reported in these previous studies. In contrast to our results with phlorizin, which completely prevented hyperglycemia, incomplete prevention of hyperglycemia in the insulin-treated group had no beneficial effects on elevated islet TAG or decreased insulin mRNA. Bezafibrate successfully prevented hypertriglyceridemia but not hyperglycemia and failed to preserve insulin mRNA. Our observation that insulin mRNA levels are dramatically decreased in the bezafibrate group at 12 weeks is consistent with the onset of defective insulin gene expression. Despite these low levels of insulin mRNA, improvement in insulin content was observed. We have no explanation for this, but it seems possible that bezafibrate may have independent effects on translation or processing of preproinsulin. Regardless, the animals remained hyperglycemic, suggesting that their insulin secretory reserve was insufficient to match the insulin resistance associated with obesity. Plasma FFA levels did not correlate with changes in islet TAG content or insulin mRNA in any treatment group. This lack of correlation agrees with our previous report (2) but not with others (6). This difference could be due to differences in ZDF colonies and/or differences in sample handling. The latter is an important consideration because delay in assay can lead to artifactual increases in FFA concentration (37). Thus, our data indicate that progressive islet dysfunction in this type 2 diabetic animal is more related to chronic hyperglycemia than chronic elevations in plasma TAG or FFA. In contrast to our findings with bezafibrate treatment of ZDF animals, treatment with this drug in Otsuka Long Evans Tokushima fatty rats, another model of type 2 diabetes, preserved

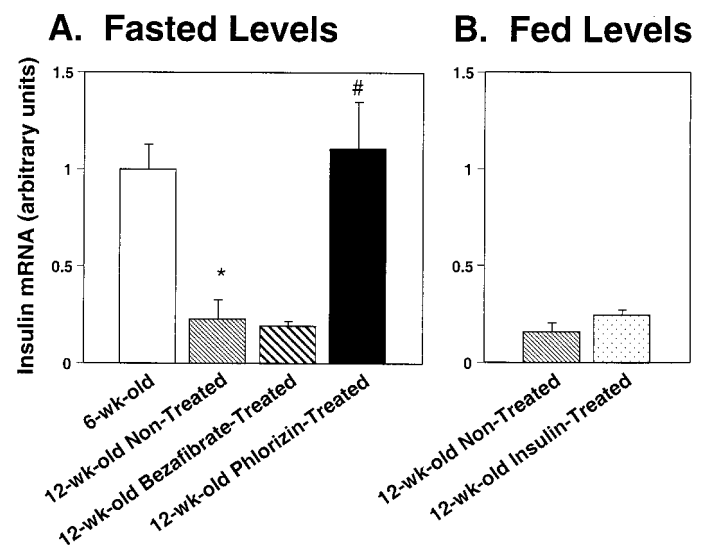


FIG. 5. Levels of islet insulin mRNA at 6 and 12 weeks of age in fasted ZDF rats that were either nontreated or treated for 6 weeks with bezafibrate or phlorizin (A) and in fed ZDF rats that were treated for 5 weeks with mock (nontreated) or insulin implants (B). Insulin mRNA levels are expressed relative to the level of β -actin ($n = 3-9$ [each n contains islets pooled from two to three rats]), $*P < 0.05$ versus 6-week-old; $\#P < 0.04$ treated versus 12-week-old nontreated.

glucose-induced insulin secretion compared with the non-treated control group (38). However, this treatment also significantly lowered the blood glucose level in this animal, again making it difficult to distinguish between the effects of lowering glucose versus lipids.

In the first study to document the capacity of β -cells to store and use TAG (39), increasing the glucose concentration from 3.5 to 17 mmol/l resulted in twofold and fivefold increases in the rate of glucose incorporation into TAG and phospholipids, respectively, in cultured mice islets. Similarly, Briaud et al. (11) demonstrated that islets exposed to palmitate have a glucose-dependent increase in esterification of fatty acids into neutral lipids. This was associated with inhibition of insulin gene expression. Inhibitory effects on insulin secretion from human islets cultured for 48 h in 250 μ mol/l palmitate were additive to the inhibitory effects of previous exposure to high glucose (40). Furthermore, in a study by Jacqueminet et al. (13) in which rat islets were cultured for up to 7 days with palmitate, insulin content and mRNA level were not affected by palmitate at basal glucose concentrations (2.8 mmol/l); however, both were significantly decreased in the presence of 16.7 mmol/l glucose. In agreement, Prentki and Corkey (32) proposed that only under conditions of both high glucose and high lipids will metabolic abnormalities become apparent. They suggested that the toxic effects of glucose and lipids are synergistic and the mechanism may be via elevated levels of cytosolic long-chain acyl CoA, resulting in altered insulin secretion.

Our study suggests that the increase in ZDF rat islet TAG content is related to antecedent increases in glucose but not lipid levels in plasma. In keeping with this concept, high-glucose concentrations have been shown to stimulate lipid esterification and TAG deposition in INS-1 cells by Roche et al. (41), who reported conversion of glucose into lipids along with an inhibition of fatty acid oxidation. However, it is not yet clear what adverse effects, if any, TAG itself has on β -cells. It may be possible that TAG acts as a storage pool for fatty acids that can be used in ceramide formation (30,42), thus leading to an increase in β -cell apoptosis (7,14). However, Listenberger et al. (29) observed recently that generation of oxygen species and palmitate-induced apoptosis occurred independent of ceramide synthesis. In any event, we conclude that increased ZDF rat islet TAG is a result of exposure to elevated glucose concentrations and that associated adverse consequences on insulin mRNA levels are likely to be consequences of chronic hyperglycemia rather than hyperlipidemia.

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