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Modulation of Adipoinsular Axis in Prediabetic Zucker Diabetic Fatty Rats by Diazoxide

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Dysregulation of the adipoinsular axis in male obese Zucker diabetic fatty (ZDF; *fa/fa*) rats, a model of type 2 diabetes, results in chronic hyperinsulinemia and increased *de novo* lipogenesis in islets, leading to β-cell failure and diabetes. Diazoxide (DZ; 150 mg/kg·d), an inhibitor of insulin secretion, was administered to prediabetic ZDF animals for 8 wk as a strategy for prevention of diabetes. DZ reduced food intake ($P < 0.02$) and rate of weight gain only in ZDF rats ($P < 0.01$). Plasma insulin response to glucose load was attenuated in DZ-Zucker lean rats (ZL; $P < 0.01$), whereas DZ-ZDF had higher insulin response to glucose than controls ($P < 0.001$). DZ improved hemoglobin A_{1c} ($P < 0.001$) and glucose tolerance in ZDF ($P < 0.001$), but deteriorated hemoglobin A_{1c} in ZL rats ($P < 0.02$) despite normal tolerance in the fasted state. DZ lowered plasma leptin ($P < 0.001$), free fatty acid, and triglyceride ($P < 0.001$) levels, but increased adiponectin levels ($P < 0.02$) only in ZDF rats. DZ enhanced β_3-adrenoreceptor mRNA ($P < 0.005$) and adenylate cyclase activity ($P < 0.01$) in adipose tissue from ZDF rats only, whereas it enhanced islet β_3-adrenergic receptor mRNA ($P < 0.005$) but paradoxically decreased islet adenylate cyclase activity ($P < 0.005$) in these animals. Islet fatty acid synthase mRNA ($P < 0.03$), acyl coenzyme A carboxylase mRNA ($P < 0.01$), uncoupling protein-2 mRNA ($P < 0.01$), and triglyceride content ($P < 0.005$) were only decreased in DZ-ZDF rats, whereas islet insulin mRNA and insulin content were increased in DZ-ZDF ($P < 0.01$) and DZ-ZL rats ($P < 0.03$). DZ-induced β-cell rest improved the lipid profile, enhanced the metabolic efficiency of insulin, and prevented β-cell dysfunction and diabetes in diabetes-prone animals. This therapeutic strategy may be beneficial in preventing β-cell failure and progression to diabetes in humans. (*Endocrinology* 145: 5476–5484, 2004)

TYPE 2 DIABETES mellitus (DM) has become the most common complication of obesity worldwide (1). Obese humans and experimental animals characteristically manifest hyperinsulinemia, insulin resistance, and hyperlipidemia, which predispose to glucose intolerance and diabetes (2). Dysregulation of the adipoinsular axis in obese individuals due to defective leptin, an *Ob* gene product, reception by β-cells, is believed to result in chronic hyperinsulinemia and may contribute to the pathogenesis of adipogenic diabetes (2). Similarly, the inability of leptin to suppress insulin secretion in leptin-insensitive pancreatic islets of male obese Zucker diabetic fatty (ZDF *fa/fa*) male rats, a model of type 2 DM, results in chronic hyperinsulinemia and increased *de novo* lipogenesis in pancreatic islets (3, 4). Enhanced activity of fatty acid synthase (FAS), a key insulin-sensitive enzyme regulating lipogenesis, and overaccumulation of fat in pancreatic islets of obese ZDF rats cause β-cell failure with a predictable progression from the prediabetic to the diabetic state (5).

There is abundant evidence linking high plasma free fatty acid (FFA) levels of obesity to the increased risk of type 2 DM in humans and rodents (6, 7). Male ZDF rats develop progressive insulin resistance and glucose intolerance between 3 and 8 wk of age and become overtly diabetic usually between 8 and 10 wk (8). In obese prediabetic ZDF *fa/fa* rats, a rapid rise in plasma FFA levels 2 wk before the appearance of hyperglycemia (4) is associated with a 10-fold increase in islet triglyceride (TG) content, which coincides with the onset of islet dysfunction and overt diabetes (7). High tissue levels of fatty acyl coenzyme A interfere with glucose metabolism, not only in skeletal muscle (9), where they play an important role in insulin resistance (10), but also in β-cells, where they may cause the secretory dysfunction that characterizes type 2 DM (11). Over time, hyperglycemia worsens, a phenomenon that has been attributed to the deleterious effect of chronic hyperglycemia (glucotoxicity) (12, 13) or chronic hyperlipidemia (lipotoxicity) (14, 15) on β-cell function, and is often accompanied by increased TG content and decreased insulin gene expression (16, 17). A leptin receptor mutation in ZDF rats is believed to interfere with leptin's modulation of islet K^+_{ATP} channel and its antisteatotic effect with resultant chronic hyperinsulinemia and islet cell lipotoxicity, which lead to β-cell failure and diabetes (4, 18). Attenuation of insulin release by K^+_{ATP} channel openers like diazoxide (DZ) (19) can improve the insulin release pattern in both human and animal models of type 2 DM. This effect is likely mediated via hyperpolarization of β-cells, thereby providing β-cell rest by reducing insulin release (20, 21). Chronic suppression of hyperinsulinemia in mildly hyperglycemic obese Zucker (*fa/fa*) rats by DZ also reduces plasma FFA, TG, and leptin levels and down-regulates adipose tissue, FAS activity, and lipoprotein lipase mRNA (22). To assess the meta-

Abbreviations: AC, Adenylate cyclase; ACC, acyl coenzyme A carboxylase; ACRP30, adipocyte complement-related protein of 30 kDa; AUC, area under the curve; β_3AR, β_3-adrenergic receptor; BW, body weight; C-ZDF, control ZDF; C-ZL, control ZL; DM, diabetes mellitus; DZ, diazoxide; FAS, fatty acid synthase; FFA, free fatty acid; HbA_{1c}, hemoglobin A_{1c}; IPGTT, ip glucose tolerance test; PF, pair fed; TG, triglyceride; UCP-2, uncoupling protein-2; ZDF, Zucker diabetic fatty; ZL, Zucker lean.

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bolic effects of DZ-induced β-cell rest in prediabetic ZDF, as a strategy for prevention of diabetes in prediabetic ZDF rats, we measured insulin sensitivity, glucose tolerance, lipid profile, β_3-adrenoreceptor (β_3-AR) gene expression, and adenylate cyclase (AC) activity in pancreatic islets and adipose tissue and expression of liporegulatory genes and insulin gene expression in islets.

Materials and Methods

Animals

Six-week-old male prediabetic ZDF and Zucker lean (ZL) rats (Genetic Models, Inc., Indianapolis, IN) were provided rat chow, PMI LabDiet 5001 (Purina Mills Inc., St. Louis, MO), and water *ad libitum.* The obese and lean rats were divided into three subgroups (n = 6 per subgroup): DZ (DZ-ZDF), pair-fed (PF-ZDF), and control (C-ZDF). DZ [150 mg/kg body weight·d; Proglycem suspension 50 mg/ml (Baker-Norton Pharmaceuticals, Miami, FL)] or an equivalent volume of vehicle (to PF-ZDF and C-ZDF) was administered by gavage needle twice daily for a period of 8 wk.

Metabolic and biochemical studies

Rats were weighed twice weekly, and food consumption was measured in separate metabolic cages during the 2nd and 6th wk of the study. Blood samples from tail vein were obtained for analysis of glucose after an 18-hour fast once weekly. When animals were fasted, doses of DZ or vehicle were withheld for at least 18 h. At the end of the 8-wk period, ip glucose tolerance tests (IPGTTs; 1.0 g glucose/kg body weight) were performed after an overnight fast (18 h). Blood for glucose and insulin was drawn into heparinized tubes from the supraorbital sinus 0, 15, 30, and 60 min after glucose administration under anesthesia by im ketamine (65–100 mg/kg body weight). On a separate day, fasting blood samples were obtained for glucose, insulin, leptin, adiponectin/adipocyte complement-related protein of 30 kDa (ACRP30), FFAs, TGs, and hemoglobin A_{1c} (HbA_{1c}).

Pancreatic islet studies

After an overnight fast (18 h), a pancreatic islet isolation procedure was performed between 0900 and 1100 h. Rats were anesthetized by im injections of Nembutal (50 mg/kg) and ketamine (90 mg/kg). Pancreatic islets were isolated as previously described by Naber *et al.* (23). The pancreatic tissue was digested in collagenase buffer mix, and islets were separated from acinar tissue on a discontinuous Ficoll gradient. The RNA extraction was performed from freshly isolated islets in PBS (see below). Also, groups of 100–300 islets were immediately frozen in methanol-dry ice and stored at −80 C until used for TG, insulin, and AC assays. The animals were then euthanized by a terminal cardiac puncture and exsanguinations, omental adipose tissue was also harvested for RNA extraction, and the remainder was stored in −80 C. The Medical College of Wisconsin Animal Resource Center approved the animal procedures.

Assays

Plasma glucose, insulin, leptin, and adiponectin/ACRP30. Glucose level was measured by the glucose oxidase method (Sigma Chemical Co., St. Louis, MO). Plasma concentrations of insulin, leptin, and adiponectin were determined by RIA kits using a double-antibody method (LINCO Research, St. Louis, MO).

Plasma TGs and FFAs. Plasma TG level was measured by an enzymatic method (Sigma Diagnostics, St. Louis, MO). Plasma FFA was determined by an enzymatic colorimetric method (Wako Chemicals, Richmond, VA).

Hemoglobin A_{1c}. Glycosylated HbA_{1c} was measured using the DCA 2000 latex immunoagglutination method (Bayer, Tarrytown, CA).

Extraction of total RNA and multiple real-time RT-PCR quantitation. Total RNA was extracted by the TRIzol isolation method (Life Technologies, Gaithersburg, MD). The RNA yield from isolated pancreatic islets (~300–500) was 12–20 ìg per ZL and ZDF rat. RNA was treated with ribonuclease-free deoxyribonuclease (Promega, Madison, WI) and stored in Ambion RNA storage solution (Ambion, Austin, TX) at −80 C. First-strand cDNA was generated from 1 ìg RNA in a 10-μl solution containing 200 U Superscript II (Invitrogen Life Technologies, Carlsbad, CA), 1 mM deoxynucleotide triphosphate mix, 2 U Rnasin (Promega, Madison, WI), 2.5 μM random hexamers (Life Technologies, Inc.), 5× first-strand buffer (Invitrogen), and diethylpyrocarbonate water. After incubation for 1 h at 42 C, the reaction mixture was heated to 94 C for 2 min. The cDNA was then stored at −20 C.

PCR amplification and analysis was performed using a Smartcycler II instrument and software version 1.2 (Cepheid, Sunnyvale, CA) (24). The final reaction mix of 25 μl consisted of 2 μl cDNA, 0.5 μM each primer, 12.5 μl QuantiTect SYBR Green Master Mix (Qiagen, Valencia, CA), and 8.0 μl H_2O. For all primer pairs except 18S RNA (internal control), the cDNA was denatured by incubation at 95 C for 10 min. The template was amplified for 55 cycles of 30 sec at 95 C, 40 sec at 60 C, and 30 sec at 72 C. The 18S RNA primer pair was incubated at 95 C for 10 min; the template was then amplified for 65 cycles of 30 sec at 95 C, 40 sec at 55 C, and 30 sec at 72 C. Fluorescent data were acquired during each extension phase. A standard curve was created for each primer set using a serial dilution series of 1:5, 1:25, 1:125, and 1:625 using control sample cDNA. All samples and standard curve dilutions were run in duplicate. After all cycles were complete, a melting curve was generated by cooling samples to 60 C and slowly heating the samples at 0.2 C/sec to 95 C. Cycle threshold is defined as the first cycle in which there is significant increase in fluorescence above the background, which correlates to the log-linear phase of PCR amplification. Cycle threshold values were used to calculate relative amount values in samples.

Table 1 summarizes the sequences of the primers, β_3-adrenergic receptor (β_3AR), FAS, acyl coenzyme A carboxylase (ACC), uncoupling protein-2 (UCP-2), and insulin, which were used to amplify first-strand cDNA for gene products. The products were run on 1% agarose gel for confirmation. 18S rRNA (Quantum-RNA kit; Ambion) was used as an internal control, and levels of FAS, ACC, UCP-2, β_3AR, and insulin mRNA were expressed as the ratio for gene mRNA relative to 18S mRNA. All primers were obtained from Integrated Technologies (Coralville, IA).

Islet TG content. Fifty to 100 islet homogenates were prepared as previously described by Danno *et al.* (25). The homogenates underwent chloroform/methanol extraction before TG measurement. Measurements were made in duplicate using the Sigma (GPO-Trinder) kit as previously described (7).

Islet insulin content. For measurement of islet insulin content, 10 islets in 0.5 ml PBS were sonicated three times for 15 sec, and then the homogenate underwent acid extraction before determination of insulin content,

TABLE 1. Sequences of PCR primers

Gene	Sense (5′–3′)	Antisense (3′–5′)	Size (bp)	GenBank accession no.
β_3AR	ACCTTGGCGCTGACTGG	ATGGGCGCAAACGACAC	233	S56152
ACC	ATATGTTCGAAGAGCTTATATCGCCTAT	TGGGCAGCATGAACTGAAATT	105	J03808
FAS	GGAACAACTCATCCGTTCTCTGT	GGACCGAGTAATGCCGTTCA	105	M76767
UCP-2	TTGCCCGAATGCCATTG	GCAAGGGAGGTCGTCTGTCA	92	AF039033
Insulin	GCCCAGGCTTTTGTCAAACA	CTCCCCACACACCAGGTAGAG	90	J00747

as previously described (11). Insulin content was determined by using a double-antibody RIA with rat standards (LINCO Research, St. Louis, MO).

Islet AC activity in islets and adipose tissue. Before the assay, the 10–50 islets were homogenized in a medium consisting of 0.25 M sucrose and 5 mM Tris-HCl at pH 8.0. Adipose tissues were minced and plasma membranes were prepared as previously described (26). The enzyme activity was assayed using a bioluminescent assay for AC activity, which was expressed as cAMP (picomoles per minute per milligram of protein) (27). Protein content of islet and adipose tissue fractions was estimated using the Lowry method (28).

Statistical analysis

The reported values represent the mean ± SE. Statistical comparisons between subgroups were assessed by one-way ANOVA and Dunnett's and Tukey's tests. $P < 0.05$ was considered statistically significant.

Results

Effect of DZ on food intake and body weight

Table 2 shows weight and food intake in ZDF and ZL rats. C-ZDF rats exhibited higher initial weight and greater weight gain over the 8-wk period than ZL animals ($P < 0.001$). The final body weight and average weight gain in DZ-ZDF animals were reduced compared with PF-ZDF ($P < 0.02$) and C-ZDF rats ($P < 0.001$), respectively. Also, PF-ZDF rats had lower final weight ($P < 0.02$) and rate of weight gain ($P < 0.02$) than C-ZDF animals. Body weight differences between DZ-ZDF and C-ZDF ($P < 0.02$) and PF-ZDF and C-ZDF ($P < 0.02$) first emerged at ages 8 and 9 wk, respectively (data not shown). Body weight differences between DZ-ZDF and PF-ZDF ($P < 0.02$) were first observed at 13 wk of age. DZ treatment did not affect body weight in ZL rats. When food intake was measured during the treatment period (2nd and 6th wk), C-ZDF rats consumed larger amounts of food than C-ZL animals ($P < 0.001$). DZ treatment reduced food intake in ZDF rats compared with PF-ZDF ($P < 0.02$) and C-ZDF rats ($P < 0.001$) without a significant effect in ZL animals.

Effect of DZ on fasting plasma glucose, insulin, leptin, adiponectin, lipid concentrations, and HbA_{1c}

Figure 1 illustrates weekly fasting plasma glucose in obese ZDF and ZL animals. DZ-treated ZDF rats had lower fasting plasma glucose concentrations than C-ZDF and PF-ZDF rats ($P < 0.0001$) at the completion of the study. Fasting plasma glucose differences between DZ-ZDF and C-ZDF ($P < 0.005$) initially appeared at age 8 wk. Also, PF-ZDF had lower fasting plasma glucose levels than C-ZDF ($P < 0.02$) during the last 2 wk of the study.

Table 3 shows fasting plasma levels of glucose, insulin, leptin, adiponectin, FFAs, and TG, after 8 wk of DZ or vehicle treatment. Fasting plasma glucose and insulin concentrations were higher among ZDF animals compared with ZL rats ($P < 0.001$). Fasting plasma glucose was lower in DZ-ZDF compared with PF-ZDF ($P < 0.002$) and C-ZDF ($P < 0.001$). However, PF-ZDF had lower plasma glucose than C-ZDF rats ($P < 0.02$). Fasting plasma insulin levels were higher in DZ-ZDF ($P < 0.001$) compared with PF-ZDF and

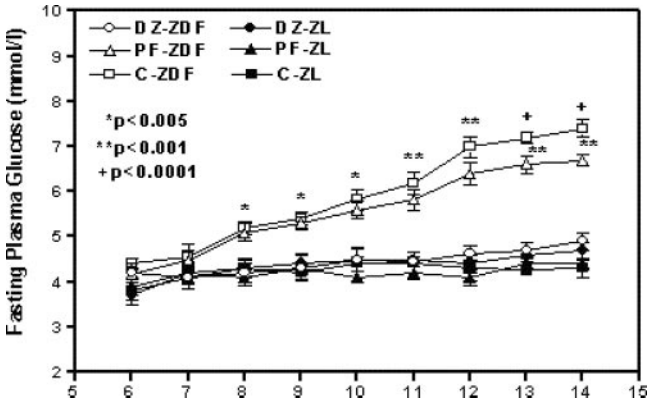

FIG. 1. Weekly fasting blood glucose levels (mean ± SE) in obese ZDF (*open symbols*) and ZL (*closed symbols*) rats. Blood glucose data from PF-ZDF and C-ZDF are compared with those of DZ-ZDF rats.

TABLE 2. Body weight and food consumption data in male ZDF and ZL rats

Subgroup	n	Initial wt (g)	Final wt (g)	wt gain (per 100 g BW)	Cal/d	
					2nd wk	6th wk
DZ-ZDF	6	168 ± 2	356 ± 8[a,b,c]	53 ± 1[a,b,c]	92 ± 5[b,c]	104 ± 5[b,c]
PF-ZDF	6	169 ± 2	381 ± 8[c,d]	56 ± 1[c,d]	92 ± 5[c,d]	104 ± 6[c,d]
C-ZDF	6	168 ± 2	408 ± 9[c]	59 ± 1[c]	104 ± 2[c]	120 ± 3[c]
DZ-ZL	6	118 ± 3	219 ± 6	40 ± 2	65 ± 2	60 ± 3
PF-ZL	6	117 ± 3	206 ± 4	43 ± 2	65 ± 2	60 ± 3
C-ZL	6	119 ± 2	218 ± 2	42 ± 1	71 ± 2	66 ± 1
P						
ZDF						
DZ *vs.* PF		NS	<0.02	<0.01	NS	NS
DZ *vs.* C		NS	<0.001	<0.001	<0.02	<0.02
PF *vs.* C		NS	<0.02	<0.01	<0.02	<0.02
ZL						
DZ *vs.* PF		NS	NS	NS	NS	NS
DZ *vs.* C	NS	NS	NS	NS	NS	
PF *vs.* C	NS	NS	NS	NS	NS	
ZDF *vs.* ZL[c]		<0.001	<0.001	<0.001	<0.001	<0.001

Data are mean ± SE and were analyzed by one-way ANOVA. wt, weight; BW, body weight; Cal, calories; NS, not significant.
[a] Only DZ *vs.* PF.
[b] Only DZ *vs.* C.
[c] Male ZDF (DZ, PF, and C) *vs.* ZL (DZ, PF, and C).
[d] Only PF *vs.* C.

TABLE 3. Biochemical changes in male ZDF and ZL rats

Subgroup	n	Plasma glucose (mmol/liter)	Plasma IRI (pmol/ml)	Plasma leptin (ng/ml)	Plasma adiponectin (μg/ml)	Plasma FFA (mEq/l)	Plasma TG (mmol/liter)	HbA_{1c} (%)
DZ-ZDF	6	$4.9 \pm 0.2^{a,b}$	$1.7 \pm 0.10^{a,b,c}$	$30.8 \pm 0.8^{a,b,c}$	$5.1 \pm 0.3^{a,b}$	$1.0 \pm 0.1^{a,b,c}$	$2.1 \pm 0.05^{a,b,c}$	$3.4 \pm 0.2^{a,b}$
PF-ZDF	6	$6.6 \pm 0.2^{c,d}$	$1.3 \pm 0.05^{c,d}$	38.7 ± 1.6	3.9 ± 0.2	1.3 ± 0.1^{c}	5.2 ± 0.7^{c}	5.9 ± 0.3
C-ZDF	6	7.4 ± 0.3^{c}	1.0 ± 0.10^{c}	41.0 ± 1.5^{c}	3.7 ± 0.5	1.4 ± 0.1^{c}	6.9 ± 0.7^{c}	6.5 ± 0.3
DZ-ZL	6	$4.7 \pm 0.3^{a,b}$	$0.25 \pm 0.02^{a,b}$	$2.0 \pm 0.5^{a,b}$	7.6 ± 1.0	0.7 ± 0.05	1.5 ± 0.05	3.1 ± 0.1
PF-ZL	6	4.4 ± 0.2	0.38 ± 0.02	4.0 ± 0.3	6.3 ± 0.6	0.8 ± 0.10	1.6 ± 0.1	2.5 ± 0.2
C-ZL	6	4.3 ± 0.3	0.42 ± 0.03	4.6 ± 0.2	6.5 ± 0.3	0.9 ± 0.05	1.6 ± 0.05	2.6 ± 0.1
P								
ZDF								
DZ *vs.* PF		<0.001	<0.001	<0.001	<0.03	<0.02	<0.0001	<0.001
DZ *vs.* C		<0.0001	<0.001	<0.001	<0.02	<0.005	<0.0001	<0.001
PF *vs.* C		<0.02	<0.01	NS	NS	NS	NS	NS
ZL								
DZ *vs.* PF		NS	<0.01	<0.005	NS	NS	NS	<0.02
DZ *vs.* C		NS	<0.01	<0.001	NS	NS	NS	<0.02
PF *vs.* C		NS	NS	NS	NS	NS	NS	NS
ZDF *vs.* ZL[c]		<0.0001	<0.0001	<0.0001	<0.001	<0.02	<0.0001	<0.0001

Data are mean ± SE and were analyzed by one-way ANOVA.
IRI, Immunoreactive insulin; NS, not significant.
[a] Only DZ *vs.* PF.
[b] Only DZ *vs.* C.
[c] ZDF (DZ, PF, and C) *vs.* ZL (DZ, PF, and C).
[d] Only PF *vs.* C.

C-ZDF animals ($P < 0.001$). PF-ZDF also had higher fasting plasma insulin levels than C-ZDF rats ($P < 0.01$).

Fasting plasma leptin levels were higher in ZDF than in ZL animals ($P < 0.001$). Although DZ treatment reduced plasma leptin in ZDF animals ($P < 0.001$), it did not affect leptin level in ZL rats. Furthermore, ZL animals showed higher fasting plasma adiponectin levels than ZDF rats ($P < 0.005$). DZ resulted in higher plasma adiponectin levels in ZDF rats compared with controls ($P < 0.02$), without any effect in ZL rats.

Fasting plasma levels of FFA and TG were higher in ZDF than in ZL animals ($P < 0.001$). The plasma FFA levels were decreased in DZ-ZDF compared with PF-ZDF ($P < 0.02$) and C-ZDF ($P < 0.001$) rats. DZ-ZDF animals also had lower plasma TGs than PF-ZDF and C-ZDF ($P < 0.0001$) rats. Fasting plasma FFAs and TGs were not altered in DZ-ZL rats compared with their controls.

C-ZDF animals had higher HbA_{1c} levels compared with C-ZL ($P < 0.0001$). Whereas DZ lowered HbA_{1c} in ZDF rats compared with controls ($P < 0.001$), it increased HbA_{1c} level in ZL rats ($P < 0.02$).

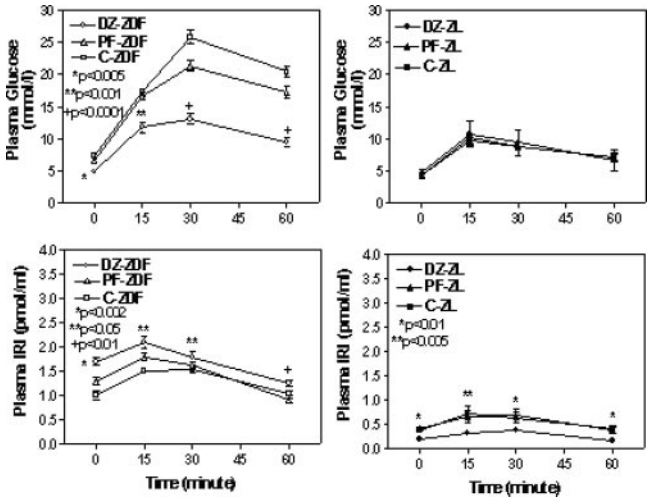

FIG. 2. Plasma glucose (*top*) and immunoreactive insulin (IRI) (*bottom*) responses (mean ± SE) to IPGTT (1 g/kg) in ZDF (*open symbols*) and ZL (*closed symbols*) rats after an 18-hour fast. Blood glucose data from DZ-ZDF and DZ-ZL rats are compared with their controls (PF and C) at each time point.

Effect of DZ on glucose tolerance

Figure 2 illustrates the glucose and insulin responses to ip glucose load (1 g/kg) after an 18-hour fast in ZDF and ZL animals at the termination of the 8-wk study. The area under the curve (AUC) for glucose (mM*min) and insulin (pmol*min) data were analyzed for each subgroup of ZDF and ZL rats. DZ treatment prevented the development of diabetes in ZDF rats (AUC glucose: 928 ± 55 *vs.* 1504 ± 53 and 1748 ± 64 mM*min; $P < 0.0005$) and preserved insulin response to IPGTT (AUC insulin: 149 ± 7 *vs.* 116 ± 6 and 112 ± 7 pmol*min; $P < 0.01$) compared with PF and C rats. The PF-ZDF rats also showed improved glucose response ($P < 0.02$) because of a decreased rate of weight gain but only a modest improvement (not significant) in insulin response compared with controls. DZ-ZL rats showed suppression of insulin response (AUC insulin: 24.0 ± 2 *vs.* 49 ± 7 and 47 ± 8 pmol*min; $P < 0.01$) compared with PF and C rats, without any effect on glucose response during IPGTT (AUC glucose: 719 ± 54 *vs.* 691 ± 45 and 694 ± 30 mM*min).

Effect of DZ treatment on islet β_3AR mRNA content and AC activity

Figure 3 illustrates the islet β_3AR mRNA levels and basal AC activity. C-ZDF rats showed lower β_3AR expression than C-ZL animals ($P < 0.0001$) (Fig. 3A). Whereas β_3AR mRNA was higher in DZ-ZDF than PF and C-ZDF rats ($P < 0.005$),

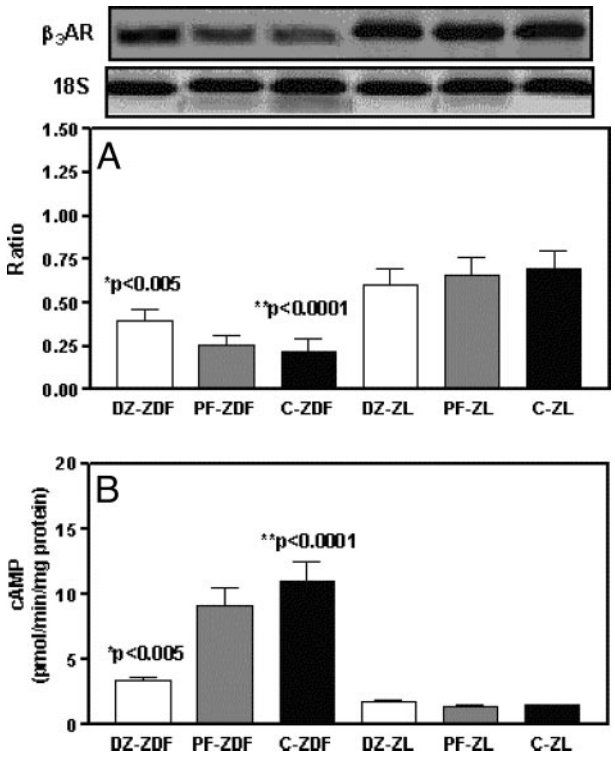

FIG. 3. β_3AR mRNA levels (A) and AC activity (B) (mean ± SE) of pancreatic islets in ZDF and ZL rats. Islet β_3AR mRNA contents and AC activity from DZ-ZDF and DZ-ZL rats are compared with their controls (PF and C), and C-ZDF data are compared with C-ZL.

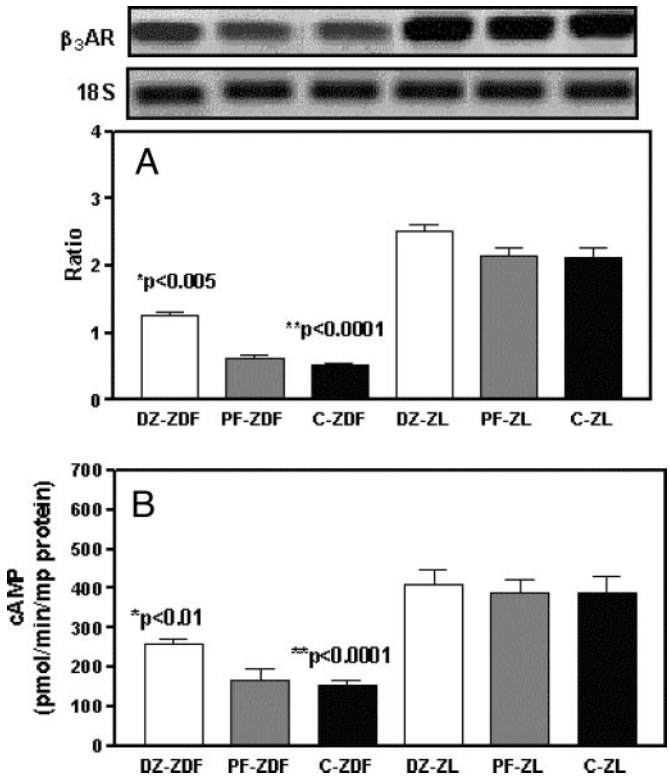

FIG. 4. β_3AR mRNA levels (A) and AC activity (B) (mean ± SE) in adipose tissues from ZDF and ZL rats. Adipose tissue β_3AR mRNA contents and AC activity from DZ-ZDF and DZ-ZL rats are compared with their controls (PF and C), and C-ZDF data are compared with C-ZL.

it did not increase in DZ-ZL rats. However, C-ZDF rats had higher basal AC activity than ZL animals ($P < 0.0001$). DZ treatment decreased AC activity only in ZDF rats ($P < 0.005$) but not in ZL rats (Fig. 3B).

Effect of DZ treatment on adipose β_3AR mRNA content and AC activity

Figure 4 shows the adipose tissue β_3AR mRNA levels in ZDF and ZL rats. C-ZDF rats showed lower β_3AR expression than C-ZL animals ($P < 0.0001$) (Fig. 4A). Whereas β_3AR mRNA was higher in DZ-ZDF than PF and C-ZDF rats ($P < 0.005$), it did not increase in DZ-ZL rats. C-ZDF rats had lower basal AC activity than ZL animals ($P < 0.0001$). DZ treatment increased basal AC activity in adipose tissue only in ZDF rats ($P < 0.01$) but not in ZL rats (Fig. 4B).

Effect of DZ treatment on pancreatic islet FAS and ACC mRNA contents

Figure 5 illustrates the pancreatic islet FAS and ACC mRNA levels in ZDF and ZL rats. C-ZDF rats showed higher islet FAS mRNA than ZL rats ($P < 0.0001$) (Fig. 5A). DZ caused lower islet FAS mRNA content compared with PF-ZDF ($P < 0.03$) and C-ZDF ($P < 0.01$) rats (Fig. 4A) without any effect in ZL rats. Similarly, ZDF rats had higher islet ACC mRNA than ZL rats ($P < 0.0001$) (Fig. 5B). Whereas DZ-ZDF animals showed lower ACC mRNA levels than PF-ZDF ($P < 0.01$) and C-ZDF animals ($P < 0.005$), islet ACC mRNA was not altered in DZ-ZL rats.

Effect of DZ treatment on pancreatic islet UCP-2 and insulin mRNA contents

Figure 6 shows the pancreatic islet UCP-2 and insulin mRNA levels in ZDF and ZL rats. C-ZDF rats showed higher islet UCP-2 mRNA content than ZL rats (Fig. 6A). DZ treatment caused lower islet UCP-2 mRNA levels in ZDF (DZ *vs.* PF, $P < 01$; DZ *vs.* C, $P < 0.007$) animals, without any effect in ZL islets. C-ZDF had higher islet insulin mRNA than ZL rats ($P < 0.0001$) (Fig. 6B). DZ treatment increased islet insulin mRNA content in ZDF (DZ *vs.* PF and C, $P < 0.01$) and ZL (DZ *vs.* PF and C, $P < 0.02$).

Effect of DZ treatment on pancreatic islet TG and insulin contents

Figure 7 illustrates TG and insulin contents in freshly isolated pancreatic islets of ZDF and ZL rats. Control ZDF rats had higher islet TG than ZL rats ($P < 0.0001$) (Fig. 7A). DZ treatment reduced islet TG content in ZDF animals ($P < 0.005$), without any effect in their PF-ZDF and ZL rats. Control ZDF rats demonstrated higher islet insulin content than ZL rats ($P < 0.01$) (Fig. 7B). DZ treatment increased islet insulin content in ZDF ($P < 0.001$) and ZL ($P < 0.03$) rats compared with their controls.

Discussion

In our study, DZ treatment of prediabetic male ZDF rats decreased food consumption and the rate of weight gain. In

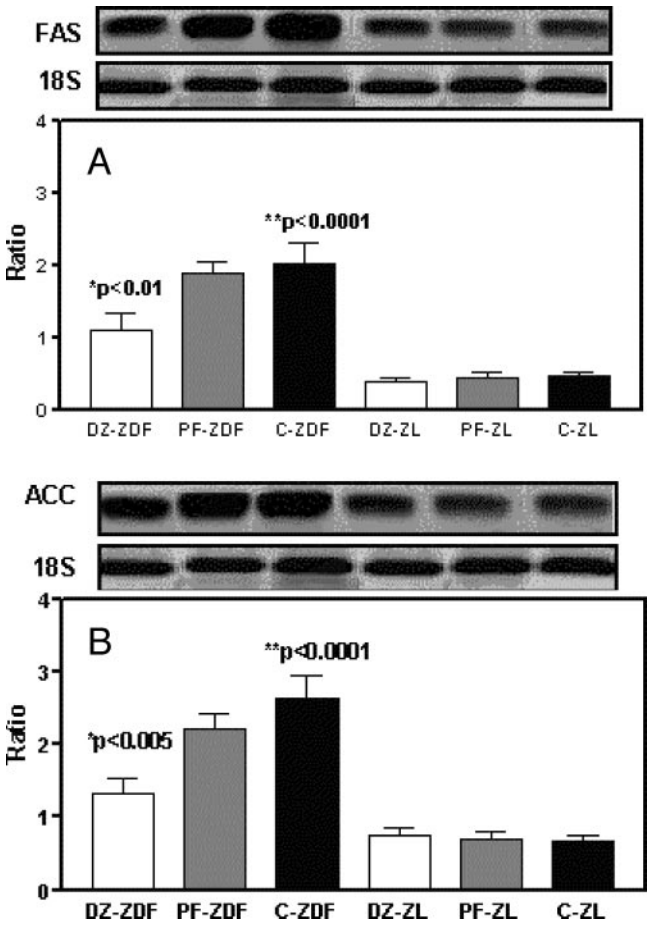

FIG. 5. FAS (A) and ACC (B) mRNA contents (mean ± SE) of pancreatic islets in ZDF and ZL animals. Islet FAS and ACC mRNA contents from DZ-ZDF and DZ-ZL rats are compared with their controls (PF and C), and C-ZDF data are compared with C-ZL.

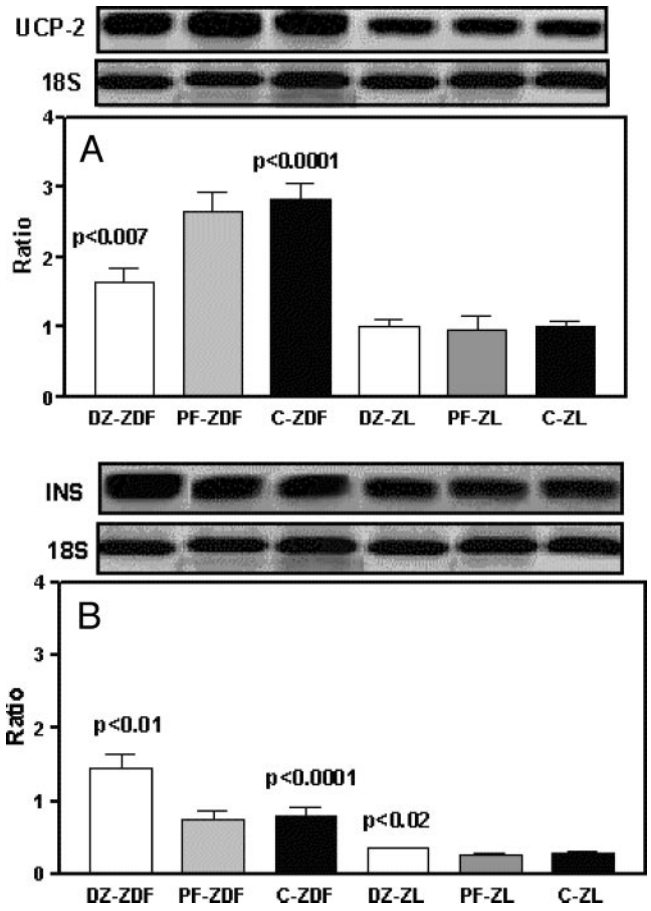

FIG. 6. UCP-2 mRNA (A) and insulin mRNA (B) levels (mean ± SE) of pancreatic islets in ZDF and ZL rats. Islet UCP-2 and insulin mRNA contents from DZ-ZDF and DZ-ZL rats are compared with their controls (PF and C), and C-ZDF data are compared with C-ZL.

addition, DZ improved lipid profile and insulin sensitivity, restored adipose tissue β_3AR function, preserved insulin response to glucose, and prevented the development of diabetes in these animals. These metabolic changes were accompanied by altered β_3AR function and decreased expression of the key genes regulating lipogenesis and islet TG content in pancreatic islets, but increased islet gene expression and insulin content.

Because insulin acts centrally to decrease body weight (29), a possible hypothesis is that the obesity state and hyperphagia observed in Zucker rats are in part due to insulin resistance in the brain, as manifested by reduced capillary insulin binding, which is thought to mediate the transport of insulin into the brain. DZ treatment has significant anorectic and antiobesity effects only in obese hyperphagic Zucker rats (30, 31), which are, in part, attributed to enhancement of central nervous system insulin sensitivity and uptake (32) and reduction of lipogenesis, leading to a decrease in food intake and rate of weight gain. In our study, DZ-treated ZDF rats had a lower rate of weight gain than PF-ZDF and C-ZDF animals, suggesting that the antiobesity effect of DZ is due not only to decreased food intake but also to down-regulation of key lipogenic enzymes (FAS and lipoprotein lipase) in adipose tissue (22).

In the obesity state, adipose cellular enlargement is associated with a decreasing maximum capacity for total glucose utilization (33), whereas glucose carbons are increasingly diverted to TG glycerol synthesis at the expense of fatty acid synthesis and glucose oxidation through the hexose-monophosphate shunt (34). These alterations in relative activities of the pathway for glucose metabolism are probably mediated by an observed increase in intracellular FFA with increasing adipocyte cell size (34, 35). DZ treatment of obese Zucker rats reduces plasma TG and enhances glucose transport into adipocytes (31), suggesting reversal of increased TG glycerol synthesis and enhanced glucose oxidation. Indeed, the lipid-lowering effect of DZ in ZDF rats is partially due to reduction in rate of weight gain and lower plasma glucose, given that PF-ZDF rats showed only a modest reduction in body weight and plasma glucose without significant reduction in plasma TG levels.

Consistent with our previous studies in obese Zucker rats, DZ-ZDF animals were normoglycemic and showed higher insulin sensitivity than controls (30, 31), whereas their higher fasting insulin and insulin response to glucose compared with controls appeared paradoxical. Induction of β-cell rest by short-term DZ dosing in insulin-treated patients with type 2 DM has been shown to improve β-cell function without a

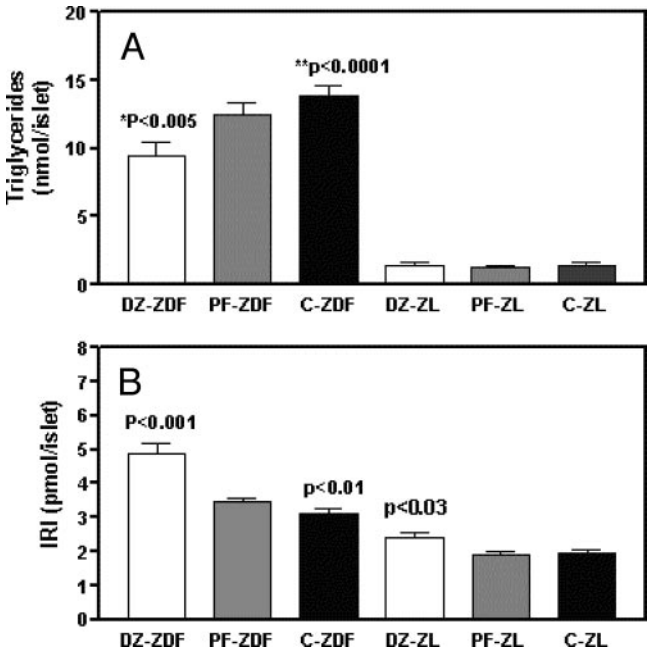

FIG. 7. TG (A) and insulin (B) contents (mean ± SE) of pancreatic islets in ZDF and ZL rats. Islet TG and insulin contents from ZDZ-DF and DZ-ZL rats are compared with their controls (PF and C); and C-ZDF data are compared with C-ZL.

significant change in mean plasma glucose levels and body weight (20). Song *et al.* demonstrated that *in vitro* DZ treatment of human islets resulted in attenuation of glucose-induced defects in first-phase insulin release and pulsatile insulin secretion (36). Also, Aizawa *et al.* (37) demonstrated that chronic suppression of hyperinsulinemia by DZ in Otsuka Long-Evans Tokushima Fatty rats resulted in marked improvement of glucose tolerance and disappearance of exaggerated β-cell insulin response *in vitro*. Therefore, reduction of β-cell workload by DZ in ZDF rats improved glucose tolerance and insulin secretory responsiveness similar to a recent study (21). However, DZ resulted in relative hypoinsulinemia and deterioration in ambient glucose in ZL rats during the fed state.

Glucose is the major physiological activator of insulin secretion from pancreatic β-cells. Although the regulation of this activation is complex, elevated intracellular levels of cAMP potentiate insulin secretion in a glucose-dependent manner (38). The intracellular concentration of cAMP is established by the balance between its synthesis by AC and degradation by phosphodiesterases (39). Stimulation of AC through β_3ARs results in marked increase in the plasma insulin concentrations in rats (40). Increasing levels of intracellular calcium further augment β-cell AC activity (41). On the other hand, β_3AR agonists are believed to have an antidiabetic effect by modulating carbohydrate and lipid metabolism in rodents' adipose tissue (42). Surwit *et al.* showed that a diet-induced animal model of diabetes and hyperinsulinemic obesity is associated with blunting of β_3AR function in adipocytes, which is then reversed by DZ suppression of insulin (43). In our study, DZ treatment enhanced basal β_3AR expression in adipose tissue of ZDF rats with a parallel increase in AC activity. The absence of a DZ effect on basal β_3AR and AC in ZL rats may be due to unaltered ambient glucose during the fasting state compared with their controls. On the other hand, DZ treatment enhanced β_3AR expression but lowered AC activity in pancreatic islets of ZDF rats. It is possible that decreased islet AC activity in only DZ-treated ZDF rats was due to improved ambient glucose but not to an inhibitory effect of DZ on calcium channels (44) given that no changes in islet AC activity were observed in DZ-ZL rats. We speculate that paradoxical up-regulation of islet β_3AR expression in ZDF rats is a compensatory response to a marked increase in islet insulin content, whereas unaltered β_3AR expression in ZL islets is likely due to only a modest increase in islet insulin content. Our data suggest that chronic insulin suppression restored β_3-adrenergic function in both pancreatic islets and adipose tissue, leading to increased insulin response to glucose and enhanced metabolic efficiency of insulin.

Long-term exposure of pancreatic β-cells to FFA could alter the coupling of glucose metabolism to insulin secretion by increasing basal insulin secretion, through changes in the cytosolic ATP-to-ADP ratio leading to the closure of K^+_{ATP} channels (45), but markedly decreasing insulin secretion in response to glucose (44, 45). Chronic FFA exposure also alters the energy metabolism of the β-cell, elevates the redox state, and increases basal oxygen consumption; this suggests an effect at the level of ATP generation (46) or beyond. The UCP-2 is located in the inner mitochondrial membrane of the islets and acts as a proton channel or transporter (47) and uncouples the electrochemical gradient produced by the respiratory chain from ATP synthesis. Fatty acids or dietary fats increase UCP-2 mRNA and protein levels in several tissues (48, 49). Indeed, chronic exposure of the islets to FFAs increases the expression level of the UCP-2 gene and decreases the effects of glucose on the ATP-to-ADP ratio and on the plasma membrane and mitochondrial membrane potentials (50). However, changes in plasma FFA level did not correlate with changes in islet TG or insulin mRNA level by Harmon *et al.* (51), implying that antecedent hyperglycemia, not hyperlipidemia, increases islet lipogenesis, with a resultant decrease in islet insulin mRNA levels in ZDF rats. In our study, a decrease in islet UCP-2 mRNA of DZ-ZDF rats was accompanied by increased islet insulin content and insulin mRNA. These metabolic effects in islets are, in part, due to improved ambient glucose (as evidenced by lower HbA_{1c} in ZDF rats) and lipids. On the other hand, the increased islet insulin and insulin mRNA contents in DZ-ZL rats during the fasted (basal) state are likely due to induction of β-cell rest despite deterioration of glycemic control secondary to relative hypoinsulinemia during the fed state.

However, because DZ-ZL rats had higher ambient glucose during the fed state (elevated HbA_{1c}) than control lean rats, the novel adipokine, adiponectin/ACRP30, appears to play an important role in modulating glucose tolerance and insulin sensitivity (52), without a compensatory effect on insulin secretion. Adiponectin is the most abundant adipose-specific protein and is exclusively expressed and secreted from adipose tissue (53). Plasma adiponectin levels are decreased in obesity and type 2 DM (54, 55) and are inversely correlated with fasting insulin and plasma TG (56). In our study, plasma adiponectin levels were higher in DZ-ZDF rats

than in their controls, which corresponded to higher insulin sensitivity, normoglycemic state, and improved plasma lipids.

Insulin stimulates the synthesis of leptin in adipose tissue (57), whereas leptin inhibits the production of insulin in β-cells by modulation of K^+_{ATP} channel (19) and activation of cyclic nucleotide phosphodiesterase 3B and subsequent suppression of cAMP levels (58). Consistent with our previous studies, chronic suppression of insulin by DZ in prediabetic Zucker rats reduced plasma leptin levels (22, 31) without any alteration in DZ-treated ZL rats. The plasma leptin reduction in DZ-ZDF rats appears to be due to a significant role of body fat and adipose tissue leptin production on plasma leptin levels (59).

In conclusion, prophylactic DZ treatment in prediabetic ZDF rats decreased rate of weight gain, improved lipid profile, increased insulin sensitivity, restored β_3AR function in adipose tissue, enhanced islet insulin content, and preserved the pancreatic insulin response to glucose. This was accompanied by alteration of β_3AR function and decreased lipogenesis in pancreatic islets. These findings suggest that induction of β-cell by DZ in the prediabetic state may serve as a valuable therapeutic strategy in the prevention of β-cell failure and progression to diabetes in humans.

Acknowledgments

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