A Genetic Defect in β-Cell Gene Expression Segregates Independently From the *fa* Locus in the ZDF Rat

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Type 2 diabetes is a strongly genetic disorder resulting from inadequate compensatory insulin secretion in the face of insulin resistance. The Zucker diabetic fatty (ZDF) rat is a model of type 2 diabetes and, like the human disease, has both insulin resistance (from a mutant leptin receptor causing obesity) and inadequate β-cell compensation. To test for an independently inherited β-cell defect, we examined β-cell function in fetuses of ZDF-lean rats, which have wild-type leptin receptors. β-Cell number and insulin content do not differ among wild-type, heterozygous, and homozygous ZDF-lean fetuses. However, insulin promoter activity is reduced 30–50% in homozygous ZDF-lean fetal islets, and insulin mRNA levels are similarly reduced by 45%. This is not a generalized defect in gene expression nor an altered transfection efficiency, because the islet amyloid polypeptide promoter and viral promoters are unaffected. Insulin promoter mapping studies suggest that the defect involves the critical A2-C1-E1 region. This study demonstrates that the ZDF rat carries a genetic defect in β-cell transcription that is inherited independently from the leptin receptor mutation and insulin resistance. The genetic reduction in β-cell gene transcription in homozygous animals likely contributes to the development of diabetes in the setting of insulin resistance. *Diabetes* 50:63–68, 2001

The most common form of diabetes in adults, type 2 diabetes, is a genetic disease in which there is relative insulin insufficiency. It is currently believed that the disease is polygenic, requiring a combination of genetic defects in conjunction with environmental influences to result in the diabetic phenotype (1–3). Most individuals with the disease are resistant to the actions of insulin and cannot overcome this resistance by producing more insulin. This mismatch of insulin requirement and insulin supply probably results from two defects: insulin resistance and inadequate compensatory insulin production. However, it remains unresolved whether insulin resistance and insulin deficiency are independent defects or whether one defect is secondary to the other.

The Zucker diabetic fatty (ZDF) rat provides a model for common human type 2 diabetes (4,5). The ZDF rat was originally derived from the Zucker fatty rat, which carries a spontaneous mutation in the leptin receptor (*fa* gene) (6) that causes hyperphagia and obesity. Although the Zucker fatty rats show evidence of insulin resistance, they compensate for the resistance by increasing insulin production and secretion and therefore do not become hyperglycemic. In this regard, these animals are similar to most obese humans. The ZDF subline was developed from the Zucker fatty line by selectively inbreeding those animals with the highest blood sugars (4). Animals of the ZDF line that are heterozygous for the *fa* gene mutation (ZDF-lean *fa*/+) or wild-type (ZDF-lean +/+) at this gene locus do not become obese and hence do not develop diabetes. In contrast, obese rats in the ZDF subline (ZDF *fa*/*fa*) are as insulin resistant as the nondiabetic Zucker fatty rats, but develop frank diabetes because they cannot adequately compensate by increasing insulin production and secretion by the β-cell. This suggests that the β-cell defect that results in diabetes in the obese ZDF rat does so only in the setting of obesity and insulin resistance.

We hypothesized that the ZDF rats might have a defect in β-cell gene transcription that limits their ability to compensate for insulin resistance. To test for such a defect, and to determine whether it is present in the absence of obesity, we used the insulin promoter to probe the transcriptional machinery of β-cells from fetal rats of the ZDF line that do not carry the *fa* mutation (ZDF-lean +/+ rats). We found that even at this early stage of development—and in the absence of insulin resistance or hyperglycemia—the ZDF rat β-cells have an autosomal recessive defect in β-cell gene transcription.

RESEARCH DESIGN AND METHODS

Animals. Twenty-one-day pregnant ZDF-lean rats (ZDF-lean/Gmi +/+) were obtained from Genetic Models (GMI, Indianapolis, IN). L/AN rats were obtained from Dr. C. Hansen (National Institutes of Health, Bethesda, MD). LA/N rats were chosen as wild-type controls because they do not develop overt diabetes when a leptin receptor mutation is introduced, despite developing obesity and insulin resistance (7). Sprague-Dawley rats (Simonsen, Gilroy, CA) were also used as a second wild-type control. Three different matings were performed as shown in Fig. 1. Wild-type fetuses were obtained from L/AN females bred with L/AN males. Homozygous ZDF-lean fetuses were obtained from ZDF-lean females bred with ZDF-lean males. Heterozygous ZDF-lean fetuses were obtained from ZDF-lean females bred with L/AN males, thus keeping the maternal in utero environment constant for both the homozygous and heterozygous ZDF-lean fetuses. Of note, all animals used for these experiments have two wild-type leptin receptor alleles.

Immunohistochemistry. Three or four 21-day fetal pancreata from wild-type (L/AN × L/AN), heterozygous, and homozygous ZDF-lean rats were harvested, fixed in 4% paraformaldehyde, and imbedded in paraffin. Sections

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CAT, chloramphenicol acetyltransferase; CMV, cytomegalovirus; IAPP, islet amyloid polypeptide; MODY, maturity-onset diabetes of the young; PDX-1, pancreatic duodenal homeobox transcription factor 1; RIP, rat insulin promoter; RSV, Rous Sarcoma virus.

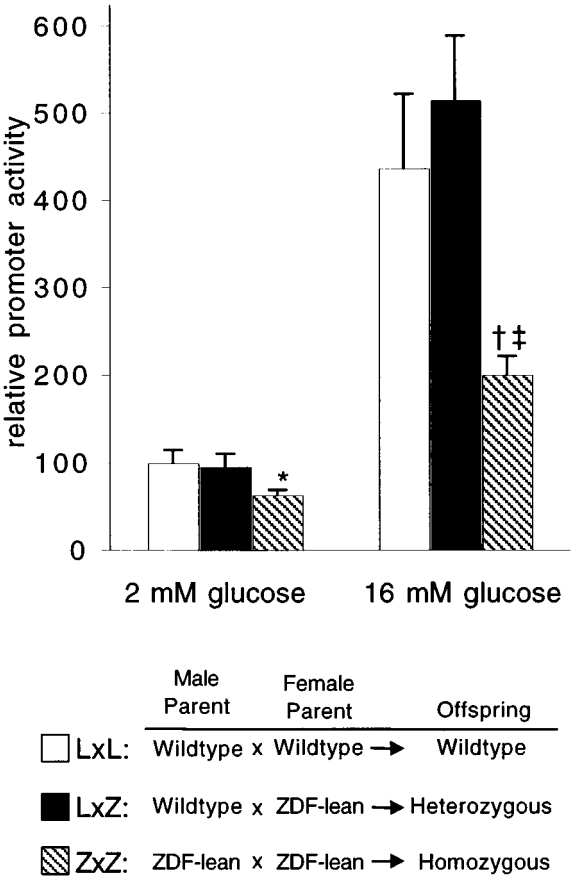

FIG. 1. Activity of the insulin promoter is reduced in ZDF-lean islets. ZDF-lean female rats were bred with either wild-type or ZDF-lean male rats to generate heterozygous or homozygous ZDF-lean offspring, respectively. The maternal environment was the same for both of these crosses. In addition, wild-type males and females were bred to generate wild-type offspring as a control group. Insulin promoter activity was compared at low and high glucose among the three crosses by using the proximal 410 bases of the rat insulin I promoter driving the expression of the reporter gene CAT and incubating in either 2 or 16 mmol/l glucose. □, wild-type (L/AN × L/AN); ■, ZDF-lean heterozygous (L/AN × ZDF); ⊠, ZDF-lean homozygous. CAT activity in the wild-type islets grown at 2 mmol/l glucose was set at 1.0. Each data point represents the mean of at least six independent transfections ± SE. *P < 0.05 vs. wild type; †P < 0.02 vs. wild type; ‡P < 0.01 vs. ZDF-lean heterozygous group.

were stained for insulin (rabbit anti-pig insulin primary antibody, Cappel, Durham, NC; biotin conjugated goat anti-rabbit secondary and streptavidin aminohexanol-biotin HRP complex, Vector, Burlingame, CA; 3,3-diaminobenzidine tetrahydochloride, Sigma, St. Louis, MO). The numbers of insulin-positive β-cells were counted in every fifth section on a total of five slides (total of 25 sections) for each pancreas (8).

Pancreatic insulin content. Twenty-one-day fetal pancreata from wild-type (L/AN × L/AN), heterozygous, and homozygous ZDF-lean rats were harvested and placed in acid alcohol for 24 h at 4°C to extract insulin. Insulin content was measured by radioimmunoassay (Linco, St. Charles, MO) and corrected for protein content as determined by the Bradford method (BioRad, Hercules, CA) (8).

Fetal islet transfection. Twenty-one-day fetal pancreata from wild-type, heterozygous, and homozygous ZDF-lean rats were harvested; minced; digested with collagenase; plated in RPMI (Gibco BRL, Gaithersburg, MD) with 11 mmol/l glucose, 10% fetal bovine serum, and antibiotics; and incubated at 37°C with 5% CO_2 for 4 h. The digested pancreatic tissue was rinsed off the plate, washed with phosphate-buffered saline, digested with trypsin, and plated under the same conditions for an additional 3 h. Subsequently, dispersed islet cells were washed with phosphate-buffered saline and transfected with 25 μg of plasmid DNA by electroporation (9). Transfected islets were incubated for an additional 36 h in RPMI with 10% fetal bovine serum, antibiotics, and either 2 or 16 mmol/l glucose, after which 10–25 μg of extracted protein, depending on the reporter construct used (determined by the Bradford method), was assayed for chloramphenicol acetyltransferase (CAT) activity (10).

Plasmids. All plasmids except cytomegalovirus (CMV)-CAT were constructed in the pFOXCAT2 vector backbone, allowing the cloning of a specific promoter or promoter element upstream of the bacterial reporter gene CAT (11). The pFOXCAT2 includes two polyadenylation signals upstream of the promoter and the human β-globin intron between the promoter construct and the reporter gene, factors that are not included in the CMV-CAT plasmid.

Promoter constructs were as follows:

- CMV: cytomegalovirus immediate early gene promoter.
- RSV: Rous Sarcoma virus long terminal repeat promoter.
- IAPP: 3.6 kb islet amyloid polypeptide promoter.
- –410 RIP1: proximal 410 bases of rat insulin 1 promoter.
- –249 RIP1: proximal 249 bases of rat insulin 1 promoter.
- –249 RIP1 ΔS20: proximal 249 bases of rat insulin 1 promoter with mutant A3/4 element (12).
- –249 RIP1 ΔS22ΔS10: proximal 249 bases of rat insulin 1 promoter with mutant E2 and E1 elements (12).
- 5× E2-A3/4: 5 copies of the E2-A3/4 insulin promoter mini-enhancer linked to the –85bases of the rat insulin 1 promoter (13).
- 5× E1-A1: 5 copies of the E1-A1 insulin promoter elements linked to the –85bases of the rat insulin 1 promoter (13).
- 4× A2-C1-E1: 4 copies of the A2-C1-E1 insulin promoter elements linked to the –85bases of the rat insulin 1 promoter (13).

mRNA measurement. Twenty-one-day fetal pancreata from wild-type (L/AN × L/AN), heterozygous, and homozygous ZDF-lean rats were harvested, placed immediately in ice-cold RNAzol (Tel-Test "B", Friendswood, TX) and sonicated. Total RNA was extracted with phenol and chloroform. RNA was precipitated with isopropranol and sodium acetate. Insulin mRNA and β-actin mRNA content was measured in triplicate for each sample using the branched DNA assay (Bayer Diagnostics, Emeryville, CA) (14).

Statistical analysis. Data are presented as the average ± SE. Statistical significance was evaluated by unpaired Student's t test. Results were considered significant at $P < 0.05$.

RESULTS

β-cell mass and insulin content in fetal pancreas from ZDF-lean rats. Figure 1 outlines the design of the ZDF-lean crosses. In initial experiments, three crosses were performed, as diagrammed, to generate wild-type fetuses as a control group and heterozygous and homozygous ZDF-lean fetuses. To limit the numbers of animals used in these experiments, the ZDF-lean homozygous fetuses were compared with the ZDF-lean heterozygous fetuses alone in later studies. Importantly, only ZDF-lean rats—which are homozygous for the wild-type *fa* allele and thus do not have a defect in the leptin receptor—were used for this study. In addition, both the homozygous and heterozygous ZDF-lean fetuses were bred in ZDF-lean females, assuring identical intrauterine metabolic environments.

The inability of the ZDF rat β-cell to adequately compensate for insulin resistance by increasing insulin secretion could be due simply to a reduction in the number of β-cells. To exclude this possible explanation, we quantified insulin-expressing cells in the pancreata of fetuses from all three crosses. Consistent with previous observations in prediabetic adult ZDF rats (15), we found no significant differences in β-cell number (Table 1). In addition, we measured pancreatic insulin content by radioimmunoassay and again found no significant differences among the three sets of fetuses (Table 1). Because fetal islets are less well formed than adult islets, we were not able to accurately measure insulin content per islet in the fetuses. However, since both β-cell number and total pancreatic insulin content are similar for all three groups, the insulin content per β-cell is also the same.

Activity of the insulin gene promoter in ZDF-lean rat fetuses. To test the transcriptional machinery in the ZDF-lean β-cells, we transiently transfected dispersed fetal islets with

TABLE 1
β-cell mass and insulin content

	Number of β-cells per section	Insulin content (μg/mg total protein)
WT × WT	524.3 ± 97.1	152.3 ± 36.9
WT × ZDF lean*	605.5 ± 44.4	90.3 ± 4.3
ZDF lean × ZDF lean†	718.6 ± 115.6	129.1 ± 35.4

For number of β-cells per section, cells were stained for insulin by immunohistochemistry in 25 sections of at least three 21-day fetal rats from each cross. Pancreatic insulin content was determined by radioimmunoassay and corrected for total pancreatic protein determined by the Bradford method in four 21-day fetal rats from each group. *ZDF-lean heterozygous; †ZDF-lean homozygous.

the proximal 410 base pair of the rat insulin I promoter driving CAT expression. Insulin promoter activity is the same in islets from both wild-type and heterozygous fetuses. In contrast, promoter activity is significantly reduced both at 2 mmol/l glucose (~37% reduction relative to wild-type fetuses) and at 16 mmol/l glucose (~55% reduction relative to wild-type fetuses) in the ZDF-lean homozygous islets (Fig. 1). A similar reduction in insulin promoter activity was found when fetal islets from Sprague-Dawley rats were used as wild-type controls (data not shown).

This reduction could result from a general defect in gene expression or transfection efficiency in the ZDF-lean β-cells. To exclude these explanations, and to determine whether the defect was specific for the insulin promoter, we tested the activity of several other promoters in ZDF-lean homozygous and heterozygous islets. We tested two powerful viral promoters, the RSV and CMV promoters, as well as the glucose-responsive β-cell promoter from the human IAPP gene (13). None of these promoters differ in activity in the two crosses (Fig. 2). Indeed, the IAPP promoter appears to have slightly higher activity in the homozygous ZDF-lean islets, although this difference does not reach statistical significance ($P = 0.08$). These transfection studies demonstrate a defect in ZDF islet gene transcription that is not generalized, but rather selectively affects the insulin gene.

Insulin mRNA levels in ZDF-lean islets. To determine whether the decrease in insulin promoter activity translates into a decrease in steady-state mRNA levels, we compared endogenous insulin mRNA levels in pancreata from ZDF-lean homozygous and heterozygous fetuses. Similar to the results from the transfection experiments, there is a marked reduction in insulin mRNA levels in the homozygous ZDF-lean β-cells (Fig. 3). Despite this obvious reduction in mRNA levels, insulin content is the same in the two groups (Table 1), suggesting some degree of posttranscriptional compensation for the transcriptional defect in the ZDF-lean rat. This compensation may explain the ZDF-lean rat's ability to maintain normal blood sugar levels in the absence of insulin resistance.

Mapping the insulin promoter defect. The proximal insulin promoter has been studied in detail in both cell lines and cultured fetal islets. These studies have identified many of the key sequence elements and transcription factors that control the function of the promoter (8,13,16–19). If the transcription defect could be localized to a single element or type of element, it would suggest potential transcription factors or interacting proteins that might be involved. We therefore attempted to map the specific regions of the insulin promoter that are involved in the transcriptional defect in the ZDF-lean rat.

Expression of the 249 proximal bases of the rat insulin 1 promoter was reduced in the ZDF-lean homozygous cross to a similar degree as found with –410-bp RIP1 (Fig. 4*A* vs. *B*). This suggests that the defect in the ZDF-lean islets is related to alterations in the activity of this key region of the insulin promoter. Multiple elements within this region have been demonstrated to have important regulatory functions (20,21). Studies using mutant promoters as well as studies using isolated sequence elements (mini-enhancers) have demonstrated the critical importance of the E and A elements within the insulin promoter for both basal function and responsiveness to glucose (12,13). The E and A elements synergistically activate transcription, due to cooperative interactions between the basic helix-loop-helix proteins binding to the E elements and the homeodomain proteins binding to the A elements.

Surprisingly, the potent glucose-responsive E2-A3/4 mini-enhancer from the rat insulin I promoter (9) appears to function normally in both homozygous and heterozygous ZDF-lean islets (Fig. 4*C*). In addition, the more proximal E-A pair, the E1-A1 mini-enhancer, is similarly unaffected by the transcriptional defect. Together, these data suggest that the insulin gene transcription defect in the ZDF-lean rat is not solely dependent on the function of the E-A mini-enhancers.

The A2-C1 (RIPE3b) region has also been strongly implicated in the normal function and glucose responsiveness of the insulin promoter (11,22,23), although to date the factors binding the C1 region have not been identified. The activity of a multimeric construct of the A2-C1-E1 mini-enhancer

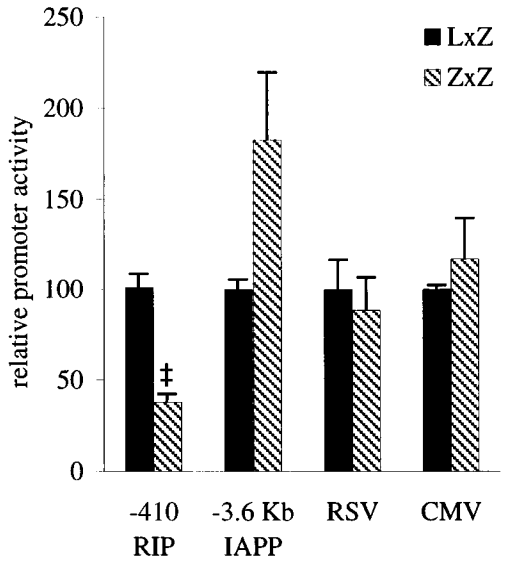

FIG. 2. The ZDF-lean transcriptional defect is relatively specific for the insulin promoter. To test the specificity of the transcriptional defect, islets from ZDF-lean homozygous and heterozygous fetuses were transfected with either the –410-bp rat insulin 1, the RSV, the CMV, or the IAPP promoter driving the expression of CAT and incubated in 16 mmol/l glucose. ■, ZDF-lean heterozygous group (L/AN × ZDF); ▨, ZDF-lean homozygous group. CAT activity in the ZDF-lean heterozygous islets grown at 16 mmol/l glucose was set at 100% for each promoter. Each data point represents the mean of at least three independent transfections ± SE. ‡$P < 0.01$ vs. ZDF-lean heterozygous group. Although the trend for the IAPP promoter is toward greater activity in the ZDF-lean homozygous islets (100.0 ± 5.31 vs. 182.8 ± 36.8, $P = 0.08$), it does not reach statistical significance.

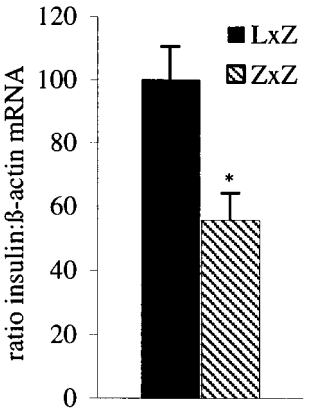

FIG. 3. Insulin mRNA levels are reduced in ZDF-lean islets. Insulin and β-actin mRNA (a housekeeping gene) levels were measured in triplicate from freshly isolated fetal pancreas and are presented as a ratio of insulin:β-actin mRNA, with the ratio for the heterozygous ZDF-lean group set at 100%. ■, ZDF-lean heterozygous group (L/AN × ZDF); ▨, ZDF-lean homozygous group. Each data point represents the mean of at least nine independent samples ± SE. *$P < 0.05$.

(RIPE3) shows a highly significant reduction in the islets of the homozygous ZDF-lean rats (Fig. 4*E*), implicating this region in the β-cell transcriptional defect in the ZDF rat. We were unable to demonstrate a similar decrease in the activity of an isolated A2-C1 element, but this element has very little activity on its own (data not shown).

Interestingly, another piece of evidence does implicate the A elements in the defect in the full-length promoter. Mutation of the A3/4 element in the context of the intact promoter reduces the severity of the defect in the ZDF-lean homozygous islets (Fig. 4*F*). This effect could result from interactions with the proximal A2-C1-E1 region. On the other hand, the E elements do not appear to be essential for the defect, because combined mutation of the E2 and E1 elements in the –249-bp promoter does not decrease the defect (Fig. 4*G*). We interpret these data to mean that the ZDF-lean rat islets have a defect in insulin promoter activity that involves the A2-C1-E1 region but requires intact upstream A elements to be fully apparent in the context of the intact promoter.

To test for alterations in the nuclear complexes that bind to these promoter elements, we compared the binding of nuclear extracts from heterozygous and homozygous ZDF-lean fetal islets by electromobility shift assay with DNA probes spanning the A2-C1, E, and A elements. No significant difference in complex formation could be detected (data not shown). Because the transcription factors that activate the C1 element have not been identified, we cannot be certain that this assay tests for changes in binding of the functionally important C1 binding proteins. In addition, the electromobility shift assay cannot rule out alterations in the transcriptional activation capacity of DNA-binding proteins or detect defects in non-DNA-binding cofactors that impact transcriptional activity.

DISCUSSION

Type 2 diabetes results from the failure of the β-cell to adequately increase insulin production to compensate for insulin resistance. However, the relationship between insulin resistance and the β-cell dysfunction remains controversial. The ZDF rat provides a useful model for testing the independent

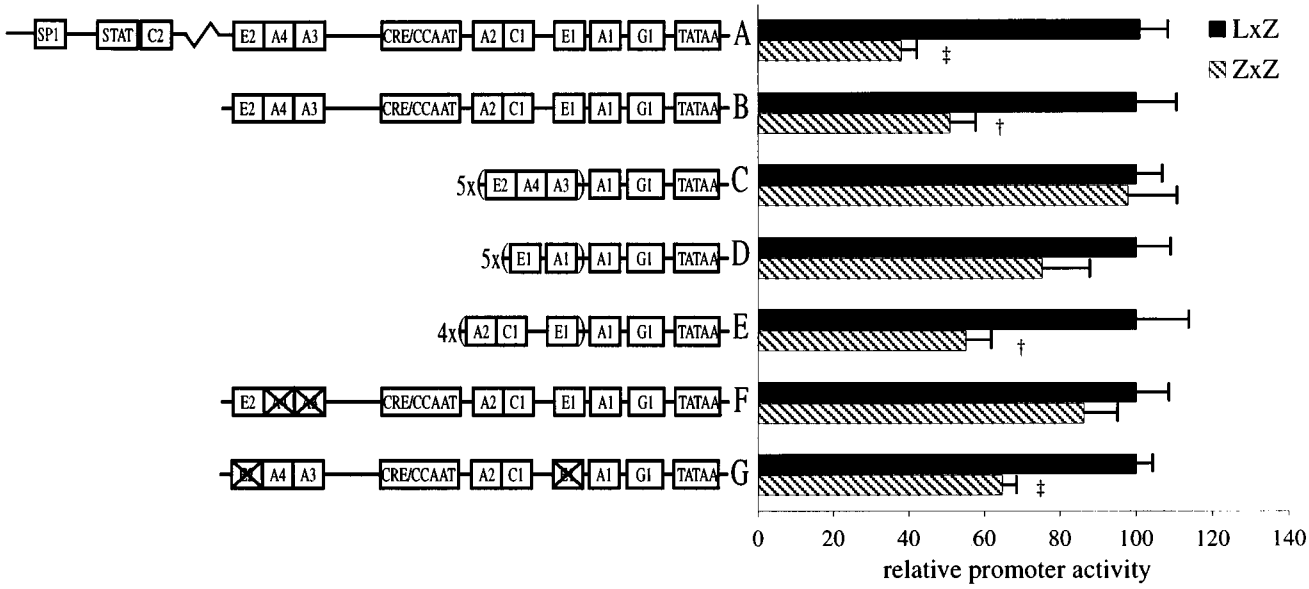

FIG. 4. Mapping the insulin promoter defect. To identify the critical regions of the insulin promoter involved in the transcriptional defect, islets were transfected with either a truncated portion of the promoter or key elements of the rat insulin 1 promoter in tandem repeats upstream of the –85-bp minimal rat insulin 1 promoter driving the expression of CAT and incubated in 16 mmol/l glucose. ■, ZDF-lean heterozygous group (L/AN × ZDF); ▨, ZDF-lean homozygous group. CAT activity in the ZDF-lean heterozygous islets grown at 16 mmol/l glucose was set at 100% for each promoter. Promoter constructs are diagrammed to the left and are designated as follows: *A*: –410-bp rat insulin 1 promoter. *B*: –249-bp rat insulin 1 promoter. *C*: five tandem repeats of the E2-A3/4 elements upstream of the –85-bp minimal RIP. *D*: five tandem repeats of the E1-A1 elements upstream of the –85-bp minimal RIP. *E*: four tandem repeats of the A2-C1-E1 elements upstream of the –85-bp minimal RIP. *F*: –249 RIPΔS20—mutated A3/4 element in the context of the –249-bp RIP. *G*: –249 RIPΔS22ΔS10—mutated E1 and E2 elements in the context of the –249-bp RIP. Each data point represents the mean of at least six independent transfections ± SE. †$P < 0.02$ vs. ZDF-lean heterozygous group; ‡$P < 0.01$ vs. ZDF-lean heterozygous group.

contributions of insulin resistance and β-cell dysfunction to the etiology of type 2 diabetes. The present study demonstrates that the ZDF-lean +/+ rat, derived from the ZDF rat, has a defect in β-cell function manifested as a decrease in insulin gene transcription. This defect is present in fetal animals in an eumetabolic intrauterine environment and is not related to leptin signaling, as ZDF-lean animals have normal leptin signaling (wild-type for the *fa* gene mutation). Therefore, our data clearly demonstrate that the ZDF rats have a β-cell defect that is inherited independently from the leptin receptor mutation.

Defects in β-cell gene transcription also have been implicated in human diabetes. Four of the five known genetic forms of maturity-onset diabetes of the young (MODY) are caused by mutations in β-cell transcription factors (24–27). The phenotype of these mutations in humans, however, is distinct from the ZDF-lean phenotype. The MODY mutations cause an autosomal dominant form of diabetes in the absence of insulin resistance. On the other hand, the β-cell transcription defect in ZDF animals is autosomal recessive. Furthermore, the ZDF transcriptional defect by itself is not sufficient to cause overt diabetes, because we know that ZDF-lean rats do not spontaneously develop diabetes. Likewise, the Zucker fatty rat does not have diabetes, demonstrating that the leptin-signaling defect alone is also not sufficient to cause diabetes. Only the combination of the β-cell defect and obesity with its resultant insulin resistance results in frank diabetes. In this regard, the ZDF rat model is similar to classic human type 2 diabetes.

Other studies have also sought to evaluate gene expression in the ZDF rat. Seufert et al. (28) demonstrated an upregulation of C/EBPβ and a downregulation of pancreatic duodenal homeobox transcription factor 1 (PDX-1) in ZDF (*fa/fa*) rats relative to Wistar or ZDF-lean *fa*/+ controls. In these studies, they examined animals at 7 weeks, when the ZDF rats are obese, and at 12 weeks, when the ZDF rats are obese and diabetic. Indeed, the nonobese ZDF-lean *fa*/+ controls showed no difference in either C/EBPβ or PDX-1 levels compared with the Wistar rat controls. In addition, Harmon et al. (29) demonstrated a reduction in PDX-1 expression in ZDF (*fa/fa*) rat islets compared with ZDF-lean controls (a mixture of ZDF-lean *fa*/+ and ZDF-lean +/+ animals) when the animals are obese, as well as a decrease in insulin mRNA when the animals are overtly diabetic. These defects were ameliorated by treatment with troglitazone, which markedly improved both glucose control and hyperlipidemia.

We interpret these data to mean that the alteration in the expression of C/EBPβ, PDX-1, and insulin result either from the metabolic complications of obesity or from homozygosity for the leptin receptor defect. Both of these studies demonstrate the development of altered β-cell gene expression in the setting of obesity and its metabolic complications. Indeed, it is for this reason that we studied β-cell function in ZDF-lean +/+ animals that have normal leptin receptors and do not develop obesity. Thus, a key point of our study is that it demonstrates an intrinsic defect in β-cell gene expression in the ZDF-lean +/+ rat, in the absence of leptin signaling defects.

The β-cell defect in the ZDF-lean rats is relatively specific for the insulin gene promoter. This defect results in a reduction in insulin mRNA levels but does not markedly reduce insulin protein levels within the fetal pancreas, suggesting that there is posttranscriptional compensation for the defect. This compensation provides an adequate insulin supply under normal conditions, but when peripheral resistance increases insulin demand, the decreased insulin transcription rate may become limiting and result in relative insulin deficiency and hyperglycemia. In addition, the defect in insulin gene transcription may extend to other β-cell genes and impair the β-cell's capacity to respond to insulin resistance by increasing β-cell mass and insulin production. Furthermore, the insulin-resistant state causes other metabolic changes, such as increases in circulating free fatty acid levels; and the defect in gene transcription may impair the ability of the β-cell to tolerate these changes (30–32). Because the ZDF-lean rat has two wild-type alleles for the leptin receptor, it does not develop obesity and insulin resistance and is therefore capable of maintaining normoglycemia despite the defect in β-cell gene transcription. However, in the obese ZDF rat, the β-cells eventually fail because the inherited β-cell defect impairs the ability to tolerate the metabolic alterations characteristic of insulin resistance.

The defect in insulin gene transcription can be mapped to a small functional element within the insulin promoter, the A2-C1-E1 mini-enhancer, although other portions of the promoter appear to be affected, as well. The evidence for involvement of several regions in the promoter could reflect the cooperative interactions within the promoter, such that a defect at any one site affects the function of interacting sites. Alternatively, defects in a transcription factor or regulatory protein that controls either the abundance or function of several distinct factors binding at different promoter sites could give a similar picture. On the other hand, the unaffected activity of the IAPP promoter along with the two viral promoters demonstrates that there is not a generalized defect in β-cell transcription.

Interestingly, the activity of the potent glucose-responsive E2-A3/4 element (13) in isolation is not affected in the ZDF-lean fetuses. Together with the absence of any decrease in the activity of the glucose-responsive IAPP promoter, these data suggest that a simple impairment of glucose sensing cannot explain the defect in insulin gene transcription.

These studies demonstrate how a modest β-cell defect(s) could contribute to the development of classic type 2 diabetes in humans. Unlike the much rarer MODY form of diabetes, classic type 2 diabetes results from a combination of defects, and it probably requires the inheritance of more than one impaired gene. This report is the first demonstration of an autosomal recessive inheritance pattern for a spontaneous genetic defect in the β-cell that contributes to the development of type 2 diabetes. Further studies of the factors involved in the ZDF rat β-cell defect may help us recognize genetic defects in humans as well as identify potential therapeutic targets for treating this disease.

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