Matrix Metalloproteinases Contribute to Insulin Insufficiency in Zucker Diabetic Fatty Rats

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To assess the molecular changes associated with pancreatic β-cell dysfunction occurring during the onset of type 2 diabetes, we profiled pancreatic islet mRNAs from diabetic male and high-fat–fed female Zucker diabetic fatty (ZDF) rats and their nondiabetic lean counterparts on custom islet-specific oligonucleotide arrays. The most prominent changes in both the male and female models of type 2 diabetes were increases in the mRNAs encoding proteases and extracellular matrix components that are associated with tissue remodeling and fibrosis. The mRNAs for metalloproteinase (MMP)-2, -12, and -14 were sharply increased with the onset of islet dysfunction and diabetes. Zymography of islet extracts revealed a concurrent, >10-fold increase in MMP-2 protease activity in islets from 9-week-old male ZDF rats. Treatment of female ZDF rats receiving a diabeticogenic diet with PD166793, a broad-spectrum MMP inhibitor, substantially prevented diabetes. The effect of this compound was due in part to marked β-cell expansion. These studies indicate that MMPs contribute to islet fibrosis and insulin insufficiency in ZDF rats. Class-targeted protease inhibitors should be explored for their potential therapeutic utility in preservation of β-cell mass in type 2 diabetes. Diabetes 54:2612–2619, 2005

Progressive insulin resistance and loss of β-cell function and mass are primary characteristics of type 2 diabetes. Normally, a decline in the insulin sensitivity of muscle and fat is compensated for by increases in insulin secretion from the β-cell. However, loss of β-cell function and mass results in insulin insufficiency and diabetes (1–5). Hyperglycemia further accelerates the decline in β-cell function (6,7). There is an obvious need for therapeutic agents that are specifically targeted toward preventing β-cell dysfunction.

Normal compensation for insulin resistance involves both the altered regulation of metabolic enzymes that control the sensitivity of β-cells to glucose and increases in β-cell mass. An example of the former type of β-cell compensation is the increase in the activities of one or more hexokinase enzymes that have a high affinity for glucose; this has the consequence of increasing insulin secretion even in low glucose (8,9). Increases in β-cell mass are widely observed as a response to insulin resistance resulting from obesity (9). If these or other compensatory changes are somehow limited, however, impaired glucose tolerance or diabetes may result. In humans with type 2 diabetes, the total β-cell mass is markedly reduced relative to that in nondiabetic individuals (10–13), and this is likely to substantially contribute to hyperglycemia. Although increased rates of apoptosis may account for the decreased β-cell mass in diabetic subjects (12), little is known about the molecular components that contribute to this and other manifestations of β-cell dysfunction.

Certain animal models of type 2 diabetes are also characterized by both insulin resistance and β-cell dysfunction. Male Zucker diabetic fatty (ZDF) rats become insulin resistant within weeks of birth and suffer a subsequent decline in β-cell mass and function, such that diabetes ensues between 9 and 11 weeks of age. This decline has been attributed to a net increase in β-cell apoptosis (14,15). Female ZDF rats also become insulin resistant, but they remain normoglycemic unless they are placed on a specialized high-fat diet (16). Since these models share many similarities with human type 2 diabetes, they present an opportunity to study the molecular details of progressive β-cell failure in the face of insulin resistance.

In the current study, we have used custom oligonucleotide arrays specifically designed to address gene expression changes in rat islets (17) in order to profile gene expression changes in the islets of male and high-fat–fed female ZDF rats before and during the onset of diabetes. By far the most striking changes in both the male and female ZDF models, when compared with identically fed Zucker lean control (ZLC) rats, were increases in the specific proteases and extracellular matrix (ECM) components that are associated with tissue remodeling. In par-
ticular, the mRNAs for metalloproteinase (MMP)-2, -12, and -14 were greatly increased in the ZDF islets, and these increases are contemporaneous with the onset of β-cell dysfunction. Upregulation of these same genes is characteristic of pathological conditions such as fibrosis, metastatic invasion, cirrhosis, and atherogenesis (18–24). To pharmacologically explore whether MMPs contribute to β-cell decline, we treated high-fat–fed female ZDF rats with a broad-spectrum MMP inhibitor, which prevented both loss of β-cell mass and hyperglycemia.

**RESEARCH DESIGN AND METHODS**

**Male ZDF rats.** We obtained male ZDF (ZDF/Gumi, fa/fa) and ZLC (ZDF/Gumi, +/–) rats from Charles River Genetic Models (Indianapolis, IN). The animals were shipped 1 week before the experiments to acclimate them to the local environment. Rats were housed three to five per cage under a 12-h light/dark cycle in a pathogen-free environment and with free access to water and Purina 5008 chow (Purina Mills, Richmond, IN). They were studied at 6 (pre-diabetic) and 9 (early diabetic) stage weeks of age. All of the experiments using animals in this study conformed to state and federal guidelines and the published guidelines of the American Association of Laboratory Animal Science. Diet-induced diabetes in female ZDF rats. Female ZDF and ZLC rats were also supplied by Charles River Genetic Models. They were initially fed Purina 5008 but then given either the Gmi-13004 (58% of fat on the basis of kcal %; Research Diet, New Brunswick, NJ) or regular Purina 5008 chow (16.7 kcal % fat) for 5 weeks starting at 8 weeks of age. We monitored body weight, food intake, blood glucose, and serum insulin once a week.

**Treatment with the MMP inhibitor PD166793.** PD166793 [5S-(2′-[(4-bromobiphenyl-4-sulfonylamino)3-methyl-butyric acid], a broad-spectrum MMP inhibitor (25), was kindly supplied by Drs. Walter Soeller, John Thompson, and Ralph Stevenson (Pfizer, Groton, CT). Female ZDF rats were fed with a mixture of the Gmi-13004 diet and PD166793 custom made by Research Diet for 4–5 weeks starting at 8 weeks of age. The mixture was produced by mixing powders of 72.9 mg PD166793 with 1 kg Gmi-13004 and reformatting to standard rodent pellets to administer 5 mg · kg body wt·1·day·1 of the compound. The compound-to-food ratio was based on food intake data obtained in a similar feeding study previously completed at our facility. The treatment is expected to maintain a plasma drug concentration of ~100 μmol/l (27). Nonfasting blood glucose and serum insulin were measured weekly, and the pancreata were isolated at the termination of the feeding study for pancreatic insulin content and islet morphology studies.

**Zymography and MMP activity.** ZDF and ZLC rats (5 and 9 weeks of age (two animals per condition) were isolated by collagenase digestion and Ficoll gradient separation as previously described (27). After a 1-h culture in RPMI-1640 medium (11 mmol/l glucose), islets were homogenized in 50 mmol/l Tris-HCl (pH 7.4), 150 mmol/l NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, and protease inhibitor cocktail (Roche). The homogenates were centrifuged at 10,000 × g for 10 min at 4°C. Protein concentrations in the supernatant were measured with the Bradford assay (Bio-Rad, Hercules, CA). Two micrograms each of the supernatant sample or the gelatinezymography standard (human MMP-2 and -9 mix; Chemicon International, Temecula, CA) were mixed with zymogram sample buffer (Bio-Rad), fractionated by electrophoresis on a gel containing 10% gelatin (Bio-Rad), renatured, and assayed according to the manufacturer’s instruction.

**Metabolex Islet Oligonucleotide Arrays and RNA hybridization.** Custom rat islet Affymetrix Genechips (Metabolex Rat Islet Oligonucleotide Arrays) containing 22,787 probe sets representing at least 90–95% of the mRNAs expressed in rat islets were designed by Metabolex and manufactured by Affymetrix (17). The Affymetrix standard protocol for chip hybridization was used for RNA hybridization experiments (Affymetrix Genome Array Expression Analysis Protocol [PN 701110]). Briefly, 5 μg total RNA was used to synthesize double-stranded cDNA with the Superscript Choice System (Invitrogen, San Diego, CA), followed by an in vitro transcription reaction to yield biotin-labeled cRNA. A total of 10 μg cRNA was used to hybridize each individual chip. The Affymetrix Genechip Analysis Suite (version 3.2) was used to convert the image signal of each probe set to an average difference value; the latter is the mean of fluorescence intensity differences between the 20 pairs of perfect-match and mismatch features (probes). The expression levels of each gene were measured as the mean of the average difference values from the four to five replicate samples for each group and compared with control-cultured islets using the Mann-Whitney test in GraphPad Prism.

**Real-time PCR.** Islets were isolated from 9-week-old male ZDF and ZLC rats as above. Total RNA was extracted, and cDNA was synthesized from 1 μg islet RNA using Superscript II (Invitrogen). Gene-specific PCR primers and FAM-labeled probes (Assay on Demand) were purchased from Applied Biosystems (ABI, Foster City, CA; ABI probe/primer assay ID nos.: MMP-2 Rn01538167_m1, MMP-12 Rn00588640_m1, MMP-14 Rn00579172_m1, CTSS Rn05680723_m1, CTSS Rn05680723_m1, β-Actin Rn00667889_m1). Samples were run on an ABI Prism 7700 Sequence Detector, and the results were analyzed using ABI Prism 7700 software. Changes in gene expression were calculated by using the comparative threshold cycle method (available at http://docs.appliedbiosystems.com/pebiодocs/00105622.pdf) and compared for statistical significance with Student’s t test.

**Pancreatic β-cell mass.** Three sections of the whole pancreas from each rat were imaged after staining with insulin antibody. The relative cross-sectional area of β-cells in these images was determined by quantification of the cross-sectional area occupied by β-cells and the total pancreatic cross-sectional area with Metamorph (Universal Imaging, Downingtown, PA) image analysis software.

**Histochemical staining of pancreatic sections.** Animals were anesthetized with pentobarbital (50 mg/kg i.p.), and the pancreas was quickly separated from adjacent tissues (fat and lymph nodes, etc.) and placed in Bouin’s fixative solution. After 4 h fixation, tissue samples were washed with running tap water, placed in 50% ethanol, and subsequently embedded in paraffin. Pancreas sections (5 μm thick) were cut using a Surgipath microtome (Surgipath Medical Industries, Richmond, IL). All primary antibodies were used at the dilution and incubation time optimized individually in our laboratory. Polyclonal anti-insulin and -glucagon antibodies were from Zymed Laboratories (San Francisco, CA). Pancreatic staining was visualized with the alkaline phosphatase-conjugated secondary antibodies (Vector Laboratories, Burlingame, CA). Sections were visualized with the Vector blue-substrate solution (Vector Laboratories) and mounted in Entellan (Merck) before viewing with a Leica microscope fitted with a digital camera. Pancreatic islet area and insulin staining were quantified by using Image-Pro Plus software.

**RESULTS**

**Development of diabetes in male and female ZDF rats.** The male ZDF rats used in this study spontaneously developed overt hyperglycemia at 8 weeks, whereas their heterozygous lean littermates (ZLC) remained normoglycemic throughout the study (Fig. 1A). Since we studied the male ZDF and ZLC rats at ages of 6 and 9 weeks, the ZDF rats were in either a pre-diabetic (6-week) or early diabetic (9-week) stage of the disease, respectively.

The chow-fed female ZDF rats were normoglycemic throughout the observation period in the study (Fig. 1B), yet they were severely obese and had elevated serum insulin levels compared with their heterozygous (ZLC) female littermates (Fig. 1C and D). The high-fat (Gmi-13004) feeding of these rats was initiated at 8 weeks of age and led to hyperglycemia by 2–3 weeks (Fig. 1B). The plasma insulin levels of the female ZDF rats increased in the first 2 weeks of the high-fat feeding but declined sharply after the 3rd week of feeding (Fig. 1D). Similar high-fat feeding in female ZLC rats only induced a slight increase in serum insulin levels and did not affect blood glucose (Fig. 1E).

**Increased expression of ECM proteins and proteases in islets of ZDF rats.** To elucidate the molecular mechanism of β-cell failure in type 2 diabetes, we obtained expression profiles of pancreatic islets before and after the development of diabetes in both male and female ZDF rats using custom rat islet oligonucleotide arrays (17). Of the 12,000 genes or their splicing variants profiled in these studies, the most striking change we observed in the islets of diabetic ZDF rats was the large increase in expression of a set of genes known to be involved in tissue remodeling.
The expression of several ECM-degrading proteases, including MMP-2, -12, and -14 and cathepsin-S and -K, were increased by 3.5- to 55-fold in the diabetic islets. MMP-23 mRNA was elevated 10-fold in both male and female ZDF models, and the change was significant in the fat-fed females (\( P / H 0.000277 \)) but not in the males (\( P / H 0.064 \)). The other matrix-degrading proteases that are represented on the custom rat islet arrays (i.e., MMP-7, -9, -11, -13, -16, and -24 and cathepsin-B, -D, -H, and -L) did not display significant changes of at least twofold in either diabetes model. The mRNAs for specific protein inhibitors of MMPs (TIMPs [tissue inhibitor of MMPs]), which are often increased in parallel with the proteases in activated tissues, were also significantly upregulated in the islets of male and female ZDF rats relative to islets from ZLC rats (Table 1).

The large increases in mRNA levels of the proteases observed to increase by oligonucleotide arrays in ZDF rat islets were also observed by quantitative RT-PCR (Table 2). In this experiment, the mRNA levels of MMP-2 and cathepsin-K and -S were increased by 300–500% in 9-week-old ZDF rat islets relative to age-matched islets from ZLC rats. MMP-12 and -14 were increased 4,200 and 9,700%, respectively, in the ZDF islets relative to the ZLC islets.

Tissue remodeling is often associated with angiogenesis and fibrosis. These phenomena were also detected by our expression profiling in islets from both male and female ZDF rats. Many fibrillar collagens and other ECM species (type I, II, III, and XI collagen, fibronectin, etc.) were profoundly increased in islets from 9-week male ZDF and high-fat-fed female ZDF rats relative to their nondiabetic controls (Table 1). In addition, molecular markers of angiogenesis (VEGFR2 [vascular endothelial growth factor receptor 2], VCAM [vascular cell adhesion molecule 1b], etc.) were also significantly increased in the diabetic islets. The induction of the ECM components and proteases in ZDF islets was progressive in both the male and female ZDF rats, starting before the onset of hyperglycemia, but escalating substantially afterward (Fig. 2). The increased collagen mRNA is consistent with the collagen fibers found within diabetic ZDF islets (30) (Fig. 5).

To determine whether MMP activity is increased in the islets in parallel with MMP mRNAs (Fig. 3A), we performed gelatin zymography on islets isolated from male ZDF and ZLC rats at 6 and 9 weeks of age. Gelatinase activity was detected in 6- and 9-week ZLC and 6-week ZDF islets, but the activity migrates much more slowly than either MMP-2 or -9; there was no detectable MMP-2 in these samples (Fig. 3B). In contrast, the 9-week ZDF islet samples display a large amount of gelatinase activity at the mobility of MMP-2, which parallels the large increase in MMP-2 mRNA we observed at this time point in islets from ZDF male rats.

In contrast to the large changes in the expression of the remodeling genes described above, there was little or no change in genes that are known to be selectively expressed in the β-cell (Table 1). The mRNAs for the β-cell transcription factors pancreatic duodenal homeobox-1 (Table 1).
and NKKX6.1, and the glucose transporter GLUT2 were not substantially different between the ZLF and ZDF islets in both males and high-fat–fed females. Thus, the \( \beta \)-cell dysfunction observed in ZDF islets is probably not due to an overall loss of the \( \beta \)-cell character, either by dedifferentiation of \( \beta \)-cells or by loss of \( \beta \)-cells relative to other islet cell types.

**Effect of MMP inhibitor PD166793 on islet dysfunction and development of diet-induced diabetes in the female ZDF rat.** To further evaluate the role of MMPs in \( \beta \)-cell dysfunction, we studied the effect of a broad-spectrum MMP inhibitor on the development of diet-induced diabetes in high-fat–fed female ZDF rats. Eight-week-old female ZDF rats were fed either a high-fat diet (Gmi-13040, 48% kcal of fat) or the same high-fat diet mixed with PD166793 (25,26) for 4 weeks. The mixture was designed to deliver \(-5\) mg/kg of the compound daily.

The female ZDF rats were normoglycemic and hyperinsulinemic before the high-fat feeding started. The high-fat feeding induced overt hyperglycemia and a progressive decline in serum insulin levels in 3 weeks. Adding PD166793 to the high-fat diet substantially reduced the rise in blood glucose (Fig. 4A) and the decline of plasma insulin levels (Fig. 4B) induced by the diabetogenic diet. This occurred without affecting food intake and body weight (Fig. 4C and D). In further support of the improvement of glucose homeostasis by the MMP inhibitor, the blood glucose excursion (or glucose area under the curve values) during an intraperitoneal glucose tolerance test (IPGTT) in high-fat + PD166793–fed female ZDF rats was significantly lower than those fed with high-fat diet alone (Fig. 4E).

The improvement of glucose homeostasis in PD166793–treated ZDF rats was a result of the enhancement of \( \beta \)-cell function. The MMP inhibitor–treated animals had significantly greater insulin secretory response during an IPGTT (Fig. 4F), higher pancreatic insulin content (Fig. 4G), and substantially greater \( \beta \)-cell mass (Fig. 4H) relative to the female ZDF rats on high-fat diet alone or chow-fed female ZDF rats (Fig. 4E and F).

**TABLE 1**

<table>
<thead>
<tr>
<th>Accession no.</th>
<th>ZLC AD (n = 5)</th>
<th>ZDF AD (n = 5)</th>
<th>Fold increase (diabetic/nondiabetic)</th>
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<tr>
<td><strong>Proteases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-2</td>
<td>391 ± 61</td>
<td>5,692 ± 1,164</td>
<td>15*</td>
</tr>
<tr>
<td>MMP-12</td>
<td>22 ± 62</td>
<td>1,848 ± 1,043</td>
<td>84*</td>
</tr>
<tr>
<td>MMP-14</td>
<td>120 ± 105</td>
<td>4,640 ± 1,378</td>
<td>39*</td>
</tr>
<tr>
<td>Cathepsin-K</td>
<td>1,688 ± 546</td>
<td>ND</td>
<td>&gt;30*</td>
</tr>
<tr>
<td>Cathepsin-S</td>
<td>1,639 ± 558</td>
<td>ND</td>
<td>&gt;70*</td>
</tr>
<tr>
<td>TIMPs</td>
<td></td>
<td></td>
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<tr>
<td>TIMP-1</td>
<td>3,399 ± 3,161</td>
<td>6,703 ± 1,270</td>
<td>2.0*</td>
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<tr>
<td>TIMP-2</td>
<td>192 ± 45</td>
<td>1,547 ± 447</td>
<td>8.0*</td>
</tr>
<tr>
<td>TIMP-3</td>
<td>723 ± 208</td>
<td>2,151 ± 749</td>
<td>3.0*</td>
</tr>
<tr>
<td><strong>ECM components</strong></td>
<td></td>
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<tr>
<td>Biglycan</td>
<td>1,023 ± 89</td>
<td>12,996 ± 2,130</td>
<td>13*</td>
</tr>
<tr>
<td>Collagen type I α1</td>
<td>ND</td>
<td>4,391 ± 2,115</td>
<td>&gt;220*</td>
</tr>
<tr>
<td>Collagen type III α1</td>
<td>ND</td>
<td>9,812 ± 3,247</td>
<td>32*</td>
</tr>
<tr>
<td>Fibrillin-I</td>
<td>115 ± 42</td>
<td>2,775 ± 1,201</td>
<td>24*</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>537 ± 171</td>
<td>8,384 ± 2,694</td>
<td>16*</td>
</tr>
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<td><strong>Growth factors</strong></td>
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<tr>
<td>CTGF</td>
<td>734 ± 257</td>
<td>4,635 ± 1,595</td>
<td>6.3*</td>
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<tr>
<td>IGF-1</td>
<td>414 ± 203</td>
<td>1,089 ± 192</td>
<td>&gt;21*</td>
</tr>
<tr>
<td>TGF-β3</td>
<td>281 ± 49</td>
<td>1,089 ± 192</td>
<td>3.9*</td>
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<td><strong>Angiogenesis markers</strong></td>
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<td>VCAM-1b</td>
<td>680 ± 295</td>
<td>4,092 ± 764</td>
<td>6*</td>
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<tr>
<td>VEGF-R2</td>
<td>104 ± 34</td>
<td>442 ± 749</td>
<td>4.2*</td>
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<td><strong>β-Cell proteins</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PDX-1</td>
<td>952 ± 287</td>
<td>531 ± 84</td>
<td>0.6*</td>
</tr>
<tr>
<td>GLUT2</td>
<td>7,378 ± 804</td>
<td>5,437 ± 1,163</td>
<td>0.7*</td>
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<tr>
<td>NKKX6.1</td>
<td>1,076 ± 313</td>
<td>1,122 ± 37</td>
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</table>

Data are means ± SD of average differences (AD) from Affymetrix software analysis of rat islet microarrays. *Significant difference (\( P < 0.05 \)) between ZLF and ZDF rats by the Mann-Whitney test. CTGF, connective tissue growth factor; ND, not detectable (average difference values below the background level of 20); PDX-1, pancreatic duodenal homeobox-1; TGF, transforming growth factor; TIMP, tissue inhibitor of MMP; VCAM-1b, vascular cell adhesion molecule 1b; VEGF-R2, vascular endothelial growth factor receptor 2.

**TABLE 2**

<table>
<thead>
<tr>
<th>mRNA</th>
<th>ZLC (%)</th>
<th>ZDF (%)</th>
<th>( P )</th>
</tr>
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<tbody>
<tr>
<td>MMP-2</td>
<td>100 ± 70</td>
<td>512 ± 62</td>
<td>0.0005</td>
</tr>
<tr>
<td>MMP-12</td>
<td>100 ± 43</td>
<td>4,186 ± 976</td>
<td>0.0004</td>
</tr>
<tr>
<td>MMP-14</td>
<td>100 ± 49</td>
<td>9,683 ± 5,038</td>
<td>0.0110</td>
</tr>
<tr>
<td>Cathepsin-K</td>
<td>100 ± 92</td>
<td>310 ± 57</td>
<td>0.0184</td>
</tr>
<tr>
<td>Cathepsin-S</td>
<td>100 ± 26</td>
<td>444 ± 186</td>
<td>0.0129</td>
</tr>
</tbody>
</table>

Data are % of ZLC ± SD (\( n = 3 \) for each strain). Student's \( t \) test was used to obtain a \( P \) value for each comparison.
Islet morphology in male and female ZDF rats. Consistent with previous reports (20–23) and in contrast to 9-week ZLC islets, islets in 9-week ZDF rats have lost the normal spherical shape and display finger-like projection into the exocrine pancreas. Also, α-cells were often present in the center of islets, and β-cells were separated irregularly by fibrotic material (Fig. 5). High-fat feeding of female ZDF rats caused increased islet disruption that was similar to that seen in 9-week-old male ZDF rat pancreata, but concomitant treatment with PD166793 with the high-fat diet resulted in islets that appeared more like the islets of the chow-fed rats (Fig. 5).

Tissue remodeling in islets of pre-diabetic and diabetic ZDF rats. Consistent with the finding of increased remodeling and fibrosis-associated mRNAs during the development of spontaneous diabetes in male ZDF rats and the diet-induced diabetes of female ZDF rats, we observed deposition of collagen fibers within islets of both pre-diabetic and diabetic ZDF rats. In ZLC islets, collagen fibers were restricted to the islet capsule (Fig. 5). In contrast, a network of collagen fibers infiltrated the majority of the islets in pre-diabetic, chow-fed female ZDF rats, and the nested collagen fibers became denser in the diabetic islets and often occupied over one-third to one-half of the total islet area; PD166973 treatment of high-fat–fed female ZDF rats appeared to prevent this increased density of fibers (Fig. 5).

DISCUSSION

ZDF rats have a leptin receptor signaling defect that leads to obesity and insulin resistance. These animals also have islet dysfunction, which precipitates diabetes in both males and high-fat–fed females. It has been demonstrated previously that ZDF islets become progressively star shaped, fibrotic, and disorganized during the onset of diabetes (28–31). Our comprehensive profiling studies have now uncovered several molecular components that are substantially increased in ZDF islets contemporaneously with the decline in islet function and morphology. In these studies, we examined >12,000 rat islet–expressed genes for expression changes, but the largest changes occurred in only a few mRNAs, and almost all of these fit in the category of genes involved in tissue remodeling.

ZDF islets displayed very large increases in the cysteine proteases cathepsin-K and -S; the metalloproteases MMP-2 (gelatinase A), MMP-12 (macrophage metalloelastase), and MMP-14 (MT1-MMP); and ECM components such as collagens, fibronectin, and fibrillin at the same time these islets are showing evidence of pervasive intraislet collagen deposition.

FIG. 2. mRNA levels of tissue remodeling genes detected by custom Genechips in islets of male and female ZDF rats. Islets were isolated from male ZLC (9-week [mZLC-9w]), male ZDF (6-week [mZDF-6w] and 9-week [mZDF-9w]), female ZLC on chow (fZLC), and female ZDF rats before (fZDF-0w) and 1 (fZDF-1w), 3 (fZDF-3w), and 5 (fZDF-5w) weeks after high-fat feeding. The total RNA samples isolated from the islets from each rat were individually hybridized with Metabolex Rat Islet Genechips. The mRNA levels for each gene are expressed as means ± SE of the average difference (AD) values of three to five replicates in each group. Each panel shows the mRNA level of a representative-remodeling gene in islets of the ZDF rats. In most cases, the mRNA levels of these genes increase progressively with age or as the feeding proceeds.

FIG. 3. Comparison of mRNA expression levels and gelatin zymographic detection of MMP-2 in male ZDF and ZLC islets at 6 and 9 weeks of age. A: Comparison of MMP-2 mRNA abundance detected on oligonucleotide arrays. Values are the mean average difference (n = 5 for each) ± SD. B: Gelatin zymography of islet homogenates (2 μg each). Areas of protease activity appear as clear bands against a dark background, where the protease has digested the gelatin substrate. The migration of the activated MMP-2 (62-kDa) standard is indicated on the right, and the positions of the molecular weight markers (in kDa) are indicated on the left.
A large increase in MMP-2 in 9-week-old ZDF males was confirmed by gelatin zymography. MMPs are translated as zymogens, and zymography of homogenates does not indicate whether the proteases are active in the islet. However, since MMP-2 is activated by MMP-14, which is also induced in parallel, and since these MMPs are being induced in parallel with other proteins that normally participate in tissue remodeling, it is reasonable to assume that these large increases in mRNAs for MMP-2, -12, and -14 and, at least, MMP-2 protein result in an increase in MMP activity within ZDF islets. The remodeling genes whose expression is increased in ZDF islets undoubtedly underlie a major part of changes in overall MMP activity within ZDF islets.

The remodeling genes whose expression is increased in ZDF islets undoubtedly underlie a major part of changes in islet morphology observed in these models. Further, the ability of a class-selective MMP inhibitor to prevent islet dysfunction and diabetes in the fat-fed female ZDF rats suggests that MMPs contribute directly to islet dysfunction in these animals. Since the mRNAs for the β-cell transcription factors pancreatic duodenal homeobox-1 and NKK6.1 and the β-cell glucose transporter were not substantially changed in abundance relative to the total mRNA content of the islet between ZDF and ZLC during the onset of diabetes, the fraction of the islet that is occupied by β-cells is probably also little changed (Table 1).

MMPs are normally expressed in islets, and they may be involved in islet development (31–34). The expression of MMPs and other remodeling genes in islets of these insulin-resistant animals might be triggered as a requisite for islet expansion that normally occurs in response to an increase in insulin demand. MMP activity has also been implicated in pathologies associated with cancer, fibrosis, arthritis, and atherosclerosis, and inhibitors of MMP activity represent a potential therapeutic approach for modification of these pathologies. MMPs have not previously been implicated in islet dysfunction or as etiologic factors in diabetes.

PD166973 prevents β-cell dysfunction and diabetes in female ZDF rats on a high-fat diet. PD166793 is a broad...
MMPs AND ISLET REMODELING IN ZDF ISLETS

Insulin + glucagon  Collagen

FIG. 5. Morphological evidence of islet remodeling in male and female ZDF rats. Pancreas (tail) sections from 9-week male ZLC and ZDF rats and female ZDF rats fed for 5 weeks with chow, a high-fat (Gmi-13004) diet, or a high-fat diet plus 5 mg/kg PD166973 were either double stained for insulin (green) and glucagon (red) or stained for collagen fibers with Picosirius Red.

spectrum, but class-selective, MMP inhibitor that has been shown to block left ventricular remodeling and dysfunction in a rat model of heart failure (26). Preservation of normoglycemia and normal glucose tolerance in compound PD166793-treated animals is accompanied by preservation of the high serum insulin levels that are a consequence of the insulin resistance present in these animals. In fact, the high serum insulin levels that are maintained over several weeks of the study in the PD166793-treated animals indicates that the compound is not reversing insulin resistance in these animals. The most striking effect of the compound is on pancreatic insulin content and β-cell volume. High-fat–fed rats treated with the MMP inhibitor were also more morphologically normal than those of untreated high-fat–fed animals. Others have shown that insulin-resistant, but nondiabetic, ZDF rats show a greater than threefold expansion of β-cell mass relative to control lean (and non–insulin-resistant) animals (8). The sharply contrasting insulin insufficiency observed in the ZDF models is probably the result of both a failure to expand β-cell mass and an increase in β-cell apoptosis in the face of insulin resistance and increased insulin demand. Since the MMP inhibitor allows for a marked increase in both pancreatic insulin content and β-cell volume, MMPs appear to contribute to the failure of β-cell expansion in ZDF islets. MMP inhibition increased islet area well above that of even the chow-fed animals but did not completely restore pancreatic insulin content to that found in the chow-fed rats. This difference between effects on β-cell area and insulin content in PD166973-treated animals could be due to the increased demand for insulin in high-fat–fed ZDF female rats.

How might MMPs limit expansion of β-cell mass? MMPs avidly cleave matrix proteins, and this might interfere with critical cell-matrix interactions. MMPs may also degrade growth factors or receptors that are required for β-cell survival and expansion. MMPs appear to play a role in neuronal damage after trauma or ischemia, and in analogy to our studies on islet dysfunction, treatment of mice with a broad-spectrum MMP inhibitor (BB-94) reduced hippocampal neuronal damage after global cerebral ischemia (35). The angiogenesis marker genes VCAM-1b and VEGFR2 are upregulated three- to sixfold in ZDF islets relative to ZLC islets (Table 1), and this may reflect a response to transient ischemia in the islets brought on by initial expansion in the face of an increased demand for insulin.

Our studies demonstrate that islet MMPs contribute to the development of diabetes in ZDF rats. Since human islets also express high levels of MMPs and other remodeling protease mRNAs (J.D.J., A.S., unpublished observations), these studies also suggest that selective protease inhibition may represent a novel approach to preserving islet function in the face of insulin resistance and thereby prevent or reverse the progression to diabetes.

REFERENCES
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