In models of type 2 diabetes the expression of β-cell genes is altered, but these changes have not fully explained the impairment in β-cell function. We hypothesized that changes in β-cell phenotype and global alterations in both carbohydrate and lipid pathways are likely to contribute to secretory abnormalities. Therefore, expression of genes involved in carbohydrate and lipid metabolism were analyzed in islets 4 weeks after 85–95% partial pancreatectomy (Px) when β-cells have impaired glucose-induced insulin secretion and ATP synthesis. Px rats after 1 week developed mild to severe hyperglycemia that was stable for the next 3 weeks, whereas neither plasma triglyceride, non-esterified fatty acid, or islet triglyceride levels were altered. Expression of peroxisome proliferator-activated receptor-α (PPARα), with several target genes, were reciprocally regulated; PPARα was markedly reduced even at low level hyperglycemia, whereas PPARγ was progressively increased with increasing hyperglycemia. Uncoupling protein 2 (UCP-2) was increased as were other genes likely to contribute to secretory abnormalities. Thereafter, the diabetic milieu is associated with insulin secretion dysfunction. We hypothesize that concomitant up-regulation of normally suppressed genes that regulate potentially diversionary pathways of β-cell metabolism contributes to a critical loss of β-cell differentiation and function (2, 8).

In this study, we have extended our previous findings examining the influence of the diabetic milieu on β-cell differentiation in the rat 90% partial pancreatectomy (Px) model of diabetes (8). The Px model is an especially useful model of type 2 and early type 1 diabetes as it avoids the potential artifacts and problems with interpretation that accompany genetic models and the use of β-cell toxins. The model is characterized by active regeneration of β-cells within the first 7–10 days, and thereafter, the diabetic milieu is associated with insulin secretory defects that resemble those found in human diabetes (8, 10). In this model, we examined the expression of transcription factors and enzymes that are important in both carbohydrate and lipid metabolism in β-cells and assessed the relative influence of hyperglycemia and circulating free fatty acids upon the altered β-cell phenotype.

**EXPERIMENTAL PROCEDURES**

**Animals**—Male Sprague-Dawley rats (Taconic Farms, Germantown, NY), weighing 90–100 g, were anesthetized and submitted to 90% Px or sham-Px surgery as previously described (10). Briefly, tissue removal was performed by gentle abrasion with cotton applicators leaving the pancreas within 1–2 mm of the common pancreatic bile duct and extending from the duct to the first part of the duodenum. Sham surgery involved an identical procedure except that the pancreatic tissue was only lightly rubbed instead of being removed. Animals were weighed and blood was obtained in heparinized micropipettes from

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§ The abbreviations used are: GIIS, glucose-induced insulin secretion; Px, pancreatectomy; LPx, low hyperglycemia; MPx, mild hyperglycemia; HPx, high hyperglycemia; Sfx, severe hyperglycemia; MCT, monocarboxylate transporter; RT, reverse-transcribed; TBP, TATA-binding protein; NEFA, non-esterified fatty acid; LCM, laser capture microdissection; PPAR(s), peroxisome proliferator-activated receptor(s); LDH, lactate dehydrogenase; ZDF, Zucker diabetic fatty.
snipped tails of fed rats (9–10 a.m.) weekly for 4 weeks. Whole blood glucose levels were measured with a portable glucose meter (One Touch II glucometer, Lifescan, Milpitas, CA), and plasma insulin was measured by radioimmunoassay (Linco Research, St. Charles, MO). Note that blood glucose levels obtained with glucose meters are about 10% lower than plasma levels found with glucose oxidase techniques (11). Four weeks after surgery, rats were anesthetized, and their islets isolated using collagenase digestion of the pancreatic remnant or sham pancreatectomies followed by further separation with a Histopaque density gradient (Histopaque-1077, Sigma). The islets were prepared using a stereomicroscope to ensure a pure islet preparation, and those of similar size were used for extraction of RNA. Most of the time, the islet yield after Px (about 50 islets) was sufficient for RNA extraction from islets of individual rats. However, in a few cases it was necessary to pool islets from two Px rats with similar glycemic levels. Animals were kept under conventional conditions with free access to water and standard pelleted food. All animal procedures were approved by the Joslin Diabetes Center Animal Care Committee.

Different Degrees of Hyperglycemia Induced by Variable (85–95%) Px—In one set of experiments, different degrees of hyperglycemia were induced by varying the proportion of the pancreas removed to generate 85–95% Px rats. Rats were classified according to their averaged 3- and 4-week-fed blood glucose levels that varied from low to severe hyperglycemia. Low hyperglycemia (LPx) was assigned to rats with blood glucose levels below 100 mg/dl; mild hyperglycemia (MPx) with blood glucose levels of 100–150 mg/dl; and severe hyperglycemia (HPx) within 151–250 mg/dl, and severe hyperglycemia (SPx) above 250 mg/dl.

*Fig. 1.* Comparison of mRNA levels in islets of sham (S) and hyperglycemic 90% pancreatectomized (Px) rats. mRNA levels were compared by semi-quantitative multiplex RT-PCR. After normalization of the specific gene to an internal control gene (TBP, α-tubulin, cyclophilin, or 18 S rRNA), mRNA levels in Px islets are expressed as a percent of sham. Values are means ± S.E. determined from six sham and six Px rats. *p < 0.05; **p < 0.01; ***p < 0.001 versus sham for each gene. Average blood glucose: sham, 78 ± 2 mg/dl; Px, 251 ± 24 mg/dl; p < 0.001.

**Lipid metabolism/transport**

|                   | ACC | FAS | HSL | CPT-1 | ACO | MCD |
|-------------------|-----|-----|-----|-------|-----|-----|
| S                 | 156 | 143 | 142 | 142   | 74  | 84  |
| Px (% of sham)    | 152 | 143 | 142 | 142   | 74  | 84  |

**Mitochondrial proton transport/ATP synthesis**

|                   | UCP-2 | ATP synthase α | ATP synthase β |
|-------------------|-------|----------------|----------------|
| S                 | 156   | 95             | 97             |
| Px (% of sham)    | 152   | 143            | 142            |

**Lactate production/transport**

|                   | LDH-A | MCT1 | MCT2 | MCT4 |
|-------------------|-------|------|------|------|
| S                 | 690   | 382  | 160  | 223  |
| Px (% of sham)    | 164   | 50   | 18   | 37   |

**Islet hormones/metabolism enzymes**

|                   | Insulin | Glucagon | GLUT2 | GHD | Glucose-6-Pase | FBPase 1 | FBPase 2 | 12-Lipoxyngease | Cyclooxygenase-2 |
|-------------------|---------|----------|-------|-----|---------------|----------|----------|-----------------|-----------------|
| S                 | 52      | 98       | 56    | 84  | 728           | 214      | Induced   | Induced         | Induced         |
| Px (% of sham)    | 56      | 98       | 56    | 84  | 214           | 52       | Induced   | Induced         | Induced         |

**Protein synthesis**

|                   | Translation factors |
|-------------------|---------------------|
| S                 | PPAR α | PPAR γ | PPAR δ |
| Px (% of sham)    | 24 ± 3*** | 298 ± 43*** | 149 ± 36 |
|                   | SREBP-1c |         |        |
| Px (% of sham)    | 61 ± 6*** |         |        |
| Gene name | Size (bp) | 5' oligonucleotide | 3' oligonucleotide | GenBank accession no. | dNTP | Primer | Annealing °C | Cycles | Control gene |
|-----------|-----------|--------------------|--------------------|-----------------------|-------|--------|-------------|--------|--------------|
| PPAR α    | 404       | GGTCCGATTCTTCTCCACTGC | TCCCTCCTCTGAACCTTCTC | M88592                | 160   | 80     | 62          | 28     | TBP          |
| PPAR γ (I and II) | 318  | TTCTGGCCACACCACTTTC | CCCCAGACGCACGACTCTC | Y12882                | 160   | 80     | 62          | 28     | TBP          |
| PPAR β8    | 457       | CATGTTGTTCTACCCGGGCC | AGAGGTACGCGGTCTGCG CGGG | U40064                | 160   | 100    | 60          | 24     | TBP          |
| SREBP-1c   | 315       | TGCGGTTGAGAGGAGGAGAC | GTGCAGACGTCGAGGAGG | AP286470              | 80    | 200    | 55          | 22     | α-tubulin    |
| Acetyl-CoA carboxylase | 246  | CGTGTGTGGAAGTGGGACTG | TCTCGTAGCAACACTGCC | J03808                | 80    | 200    | 55          | 28     | TBP          |
| Acetyl-CoA oxidase | 245  | AGCTCTGGACGCGCGGGAC | GACAGACGACGCAGCAC | J02752                | 160   | 400    | 55          | 23     | α-tubulin    |
| Hormone sensitive lipase | 322  | CTGCTTCTCTTCTCTTGTCTG | TCGAGACACACTGGCGGC | X51415                | 160   | 400    | 60          | 24     | TBP          |
| Malonyl-CoA decarboxylase | 248  | TGTTCTTTCTCTCCACTGGG | AACTCTTTTCTCACTGCTG | AJ0097704              | 160   | 400    | 59          | 22     | Cyclophilin  |
| Uncoupling protein 2 | 345   | ATTGCACGAGGAGGAGGGA | CACAGCAGGAGGAGGAGG | AB006613              | 80    | 200    | 55          | 22     | Cyclophilin  |
| ATP synthase α subunit | 336  | TGCACTTTGAAAGGACTGGCAC | GACGACTACAGACGACG | J05266                | 80    | 200    | 55          | 24     | Cyclophilin  |
| ATP synthase β subunit | 247  | AACATCTGTGCTTCTCACCAC | TAGCAGGACAGGACGAC | M19044                | 80    | 200    | 55          | 24     | Cyclophilin  |
| Lactate dehydrogenase-α | 249  | ACAGTTGATTGGGTTGTTG | CGCTGCTTCTCCTCTTCTG | X01196                | 80    | 200    | 59          | 24     | α-tubulin    |
| Monocarboxylate transporter 1 | 303  | GGTTGCTCTTGACTGATCCG | CTCGCCTTCTTTCTTCTG | D63841                | 80    | 400    | 59          | 24     | TBP          |
| Monocarboxylate transporter 2 | 410  | ATCCGGTCAAGATCCAGC | TCTCCTTCTGTTCTTTCTC | U62136                | 80    | 400    | 59          | 24     | TBP          |
| Monocarboxylate transporter 3 | 441  | GACCCAGCTCCCCATATC | ATGTCCTTCAAGCTTCCC | AF059258              | 80    | 400    | 59          | 24     | TBP          |
| Monocarboxylate transporter 4 | 159  | TGCTACACTCTGCTCTTG | AGTGCACACCTGCCCTCG | U57627                | 80    | 400    | 59          | 24     | TBP          |
| Insulin α and β | 148  | TCTCTACACACACGATGCC | GTGTCACACTGATCCAC | J00747.8              | 80    | 200    | 55          | 15     | 18SrRNA      |
| Glucagon | 245  | ACCATGCTCCTCCGCTG | ATGTCCTTCACTACACG | K02908                | 80    | 200    | 55          | 15     | 18SrRNA      |
| GLUT2  | 183  | TGGTCTCTTCTCTGTCG | AGGCCTCTGGTGTTGATG | J03145                | 80    | 200    | 55          | 20     | Cyclophilin  |
| Glutamate dehydrogenase | 147  | TGGAGCTCTTCTCTTCC | CAGGCTCTCCACTGATCC | X14223                | 160   | 200    | 55          | 23     | α-tubulin    |
| Glucose-6-phosphatase | 338  | ACATCAGCAGGAGGATGGTCA | AAAAGAGTGCAGACGGAGCCAA | U07993                | 80    | 400    | 62          | 28     | TBP          |
| Fructose-1,6-bisphosphatase 1 | 368  | TTCTTGATCAGGAGGATGTC | AGTGTCTCTTTTGTTGCGT | AJ009546              | 80    | 400    | 55          | 32     | TBP          |
| Fructose-1,6-bisphosphatase 2 | 288  | ACACGGGAAGAGGATGTC | TTCTTCTTGTTGCTCTTGTC | AJ009546              | 80    | 400    | 55          | 32     | TBP          |
| 12-Lipoxygenase | 312  | TGCCGAACTGGAAGATGGG | AGTGTCTCTTGACAGCAGTCG | L06040                | 160   | 200    | 59          | 26     | TBP          |
| Cyclooxygenase | 417  | GCTACCACCTGGTGGCTCGG | AACATCCTCTCCACCATG | S67722                | 160   | 400    | 61          | 34     | TBP          |

a Oligonucleotide primer sequences from Ref. 8.
b Ref. 9.
to the internal control gene (ratio of specific product/control gene). These ratios were then used to calculate the percent of sham expression for each Ppx animal in the same RT-PCR. PCR reactions were performed on RT-negative samples to exclude genomic DNA contamination for each cDNA preparation. To observe linear amplification of the multiplex PCR products for each set of primers, control experiments were performed to adjust the PCR conditions such that the number of cycles used was in the exponential phase of amplification for all products and that each PCR product in a multiplex reaction increased linearly with the amount of starting material (from 2.5 to 80 ng of RNA equivalents) as previously described in detail (8).

**Plasma and Islet Lipid Determination**—Plasma lipids were measured from triplicate samples collected in EDTA/paraformaldehyde-coated tubes to avoid activation of lipoprotein lipase by heparin (12). Plasma non-esterified free fatty acid (NEFA) were measured by a colorimetric method (Wako Chemicals, Neuss, Germany). Plasma triglyceride were measured with a triglyceride assay kit (GPO Trinder, Sigma) using glycerol as standard. For islet triglyceride determination, similar size islets isolated from sham and Ppex rats were counted (500 islets) and then suspended in a solution of 2 M NaCl, 2 mM EDTA, and 50 mM sodium phosphate buffer for the extraction of lipid as previously described (13). Triglyceride in the islet extract was measured using the triglyceride assay kit (GPO Trinder, Sigma).

**Laser Capture Microdissection (LCM)**—Further gene expression analysis was performed on the β-cells by the central core of islets excised from pancreas sections using the recently developed technique of laser capture microdissection (14). The pancreatic remnants of Ppex and sham rats and the equivalent region of pancreases of Ppex rats were excised and embedded in TissueTek OCT medium (VWR Scientific Products Corporation, San Diego, CA) and frozen in chilled isopentane. Tissue was sectioned at 8–10 μm on a cryostat, mounted on uncoated glass slides, and frozen at −80 °C. The central core of islets with diameter >100 μm was microdissected using LCM as previously described (15). Total RNA from microdissected samples was extracted (15), amplified by T7-based RNA amplification as described (16), and analyzed by radioactive PCR as described above.

**Glucose-induced Insulin Secretion and Islet ATP Assay**—Islets isolated from sham, Ppex, and phlorizin-treated Ppex rats were washed in Krebs Ringer HEPES buffer. Groups of five islets were incubated for 30 min at 37 °C in 1.5 ml of polypropylene tubes (Eppendorf) containing 1 ml of Krebs Ringer HEPES buffer with 2.8 and 16.7 mM glucose. Insulin was measured in an aliquot of the buffer by radioimmunoassay. Tri-chloroacetic acid was added to the tubes containing the islets to a final volume of 5%. After 5 min on ice, the tubes were centrifuged, and the supernatant was mixed with diethylether. The ether phase was discarded, and the extract was diluted in a buffer containing 20 mM HEPES and 3 mM MgCl₂ (pH 7.75). The extract was divided into two parts, one part was used for the extraction of lipid as previously described (13). Triglyceride in the islet extract was measured using the triglyceride assay kit (GPO Trinder, Sigma).

**RESULTS**

**Changes in mRNA Levels 4 Weeks after 90% Ppex**—The expression of transcription factors and enzymes that are important in both carbohydrate and lipid metabolism were measured in the 90% Ppex model. Fig. 1 shows representative gels from RT-PCR analysis comparing mRNAs in islets 4 weeks after sham or 90% Ppex. After normalization of the specific gene to an internal control gene (TBP, α-tubulin, cyclophilin, or 18 S RNA), mRNA levels in Ppex islets were quantitated as a percent of sham. Ppex rats showed clear hyperglycemia; averaged 3- and 4-week blood glucose levels were 78 ± 2 mg/dl for sham rats and 251 ± 24 mg/dl for Ppex rats (n = 6 in each group, p < 0.001).

**Changes in Levels of Transcription Factor mRNA Involved in Lipid Metabolism**—In many tissues, peroxisome proliferator-activated receptors (PPARs) regulate the expression of target genes involved in lipid homeostasis (17). PPARs in the regulation of genes involved in lipid catabolism, and PPARγ in lipidogenesis. The function and target genes of the PPAR δ isoform (also called β, NUC-1 or FAAR) are not known. The expression of PPAR subtypes α, δ, and γ was evaluated in islets 4 weeks after Ppex. PPAR α and γ showed reciprocal regulation in islets after Ppex (Fig. 1); PPARα mRNA levels were markedly reduced, whereas PPARγ mRNA was increased 3-fold. The mRNA levels for PPAR δ tended to be increased in islets after Ppex although not significantly (p = 0.1). Another transcription factor that may act synergistically with the PPARs in the regulation of gene expression is sterol regulatory element binding protein (SREBP)-1c (also called adipocyte differentiation and determination factor 1, ADD-1); mRNA levels for SREBP-1c were significantly decreased in 90% Ppex islets (Fig. 1).

**mRNA Levels of Genes Involved in Lipid Metabolism/Transport**—Since PPAR and SREBP-1c transcription factors were altered in Ppex islets, expression levels of target genes in lipid metabolism were also evaluated (Fig. 1). Consistent with marked reduction in PPARα, a transcriptional target of it, the peroxisomal lipid oxidation enzyme acyl-CoA oxidase, was down-regulated. In contrast, several genes in lipid metabolism were up-regulated after Ppex in association with the induction of PPARγ. mRNA levels for lipogenic genes, acetyl-CoA carboxylase, and fatty acid synthase were increased in Ppex islets. Hormone sensitive lipase, the rate-limiting enzyme of triglyceride lipolysis in adipose tissue, is expressed and active in β-cells (18, 19). Here, hormone-sensitive lipase mRNA levels were increased in Ppex islets (Fig. 1). Expression of the mitochondrial lipid transport enzyme, carnitine palmitoyl transferase 1 (CPT-1), was also increased, whereas, malonyl-CoA deacetylase mRNA levels were unchanged.

**mRNA Levels of Genes Involved in Mitochondrial Proton Transport/ATP Synthesis**—Glucose-induced hyperpolarization of the mitochondrial membrane and ATP production are thought to play a key role in GSIS (7). Uncoupling protein 2 (UCP-2) has been shown to have a negative effect on ATP production and GSIS by catalyzing a proton leak that dissipates membrane potential associated with mitochondrial respiration (20–22). We found increased UCP-2 mRNA levels in Ppex islets, whereas expression of ATP synthase α and β, part of the complex through which protons enter the mitochondria, were unchanged (Fig. 1).

**mRNA Levels of Genes Involved in Lactate Metabolism/Transport**—Expression of lactate dehydrogenase-A (LDH-A) and monocarboxylate (lactate) transporters in islets is normally low consistent with their low level production of lactate (23). The expression of LDH-A and monocarboxylate transporter (MCT) isoforms 1–4 were evaluated in Ppex islets. LDH-A mRNA levels were markedly increased in Ppex islets (Fig. 1) as previously found (8). In addition, MCT1, MCT2, and MCT4 were increased in Ppex islets, whereas no MCT3 expression was detected.

**mRNA Levels of Islet Hormones/Metabolic Enzymes**—As previously reported (8), chronic hyperglycemia induced by 90% Ppex was associated with decreased mRNA levels of insulin, whereas that of glucagon was unchanged (Fig. 1). Glucose transporter 2 (GLUT2) was reduced to a similar extent as insulin as previously reported (8). In contrast, the amino acid metabolism gene, glutamate dehydrogenase, was unchanged (Fig. 1). Interestingly, glucose-6-phosphatase mRNA levels, which in sham islets were only about 5% of that found in the liver (not shown), were dramatically increased in Ppex islets. Similarly, there was increased mRNA levels for fructose-1,6-bisphosphatase, 12-lipoxygenase, and inductive cyclooxygenase (COX-2). No expression of fructose-1,6-bisphosphatase was detected in sham islets (n = 6), whereas PCR products for the liver (FBPase-1) and muscle (FBPase-2) isoforms were detected in Ppex islets (n = 6). Similarly, COX-2 was induced in Ppex islets compared with the minimal levels found in control islets.

**Changes in Gene Expression in Rats with Different Degrees of Hyperglycemia 4 Weeks after Ppex**—By varying the proportion of the pancreas removed (85–95%), rats were generated that had...
fed blood glucose levels ranging from low (LPx, <100 mg/dl), mild (MPx, 100–150 mg/dl), high (HPx, 151–250 mg/dl), and severe (SPx, >250 mg/dl) hyperglycemia. The time course changes in body weight and fed blood glucose in these animals are shown in Fig. 2. As previously described (8, 10) weight gain was slightly decreased during the first few days after surgery; Px rats had a significantly lower body weight at 1 week post-surgery. After 1 week, Px rats gained weight at the same rate as sham-operated rats. The blood glucose levels of all groups of Px rats were significantly increased compared with sham at all time points. Averaged 3- and 4-week fed blood glucose levels varied from 74 to 87 mg/dl in sham rats, 87 to 93 mg/dl in LPx rats, 112 to 124 mg/dl in MPx rats, 164 to 240 mg/dl in HPx rats, and 278 to 323 mg/dl in SPx rats. Changes in plasma insulin, NEFA, and triglyceride levels at 4 weeks after surgery in these groups are shown in Fig. 3. Plasma insulin levels tended to be decreased in all groups of Px rats but were only significantly reduced in rats with high and severe hyperglycemia. Plasma NEFA and triglyceride levels at 4 weeks after surgery in these groups are shown in Fig. 3. Plasma insulin levels tended to be decreased in all groups of Px rats but were only significantly reduced in rats with high and severe hyperglycemia. Plasma NEFA and triglyceride levels were unchanged in Px rats regardless of the level of hyperglycemia. Similarly, islet triglyceride levels were unchanged in Px rats (Fig. 3).

The expression of genes found altered in 90% Px rats (Fig. 1) were tested in islets from rats with different degrees of hyperglycemia (Fig. 4). Expression of transcription factors and enzymes involved in lipid metabolism were altered in association with changes in levels of extracellular glucose rather than lipid. A progressive increase in mRNA levels for PPARγ corresponded to the degree of hyperglycemia in Px rats, whereas PPARα was markedly reduced even at low level hyperglycemia. Similarly, SREBP-1c mRNA levels were reduced in all Px groups with some suggestion of a decreasing trend with increasing blood glucose. Acyl-CoA oxidase levels were not significantly altered in LPx rats but were decreased to an equivalent degree in MPx, HPx, and SPx rats. Acetyl-CoA carboxylase mRNA levels were not significantly altered in LPx or MPx rats but were increased in HPx rats and further increased in SPx rats. With UCP-2 mRNA levels a similar association with increasing blood glucose was observed. LDH-A and MCT1 mRNAs were significantly increased in each group of Px rats with a tendency for increasing levels with increasing blood glucose. Glucose-6-phosphatase (liver type) mRNA levels were not significantly altered in LPx or MPx rats but were increased in HPx and SPx rats.

Reversibility of Changes in Gene Expression after Px—We tested whether the changes in mRNA levels were due to hyperglycemia by using phlorizin, an inhibitor of glucose reabsorption in the kidney. Phlorizin blocks the Na+/glucose cotransporter in the proximal tubules of the kidney causing glucosuria and the normalization of circulating glucose levels. As shown in Fig. 5, phlorizin treatment lead to reduced body weight gain in Px rats. A similar reduction in body weight gain was observed in vehicle-treated sham rats. Hyperglycemia in Px rats was normalized for the 2-week period of phlorizin treatment, whereas blood glucose levels of vehicle-treated sham rats were not significantly changed (Fig. 5). Plasma lipid levels were not changed after phlorizin treatment (NEFA: Px untreated 0.40 ± 0.05, Px phlorizin-treated 0.40 ± 0.05 mEq/l; triglyceride: Px untreated 1.2 ± 0.3, Px phlorizin-treated 1.0 ± 0.2 mg/l). As shown in Fig. 6 (left panels), the reduction in PPARα and the increase in PPARγ, UCP-2, MCT1, and glucose-6-phosphatase were already apparent in islets by 2 weeks after Px. The normalization of blood glucose in Px rats with phlorizin reversed the changes in gene expression to levels that were not significantly different from sham (Fig. 5B, Fig. 6, right panels). In contrast, the vehicle had no effect on islet gene expression in sham-treated rats (Fig. 6, right panels). Similarly results were observed for the other genes altered in Fig. 1 (not shown).

LCM—Changes parallel to those in whole islets were observed in β-cell-enriched tissue excised by laser capture microdissection with targeting of the central core of islets. As expected, insulin mRNA levels were clearly decreased in the islet core tissue excised from Px rats (Fig. 7). In contrast, a clear increase was observed in PPARγ, glucose-6-phosphatase, fructose-1,6-bisphosphatase, LDH-A, and 12-lipoxygenase mRNA in the islet core tissue excised from Px rats (n = 3) compared with no or weak detection in the islet core of sham pancreas (n = 3) (Fig. 7). Constitutive mRNA for UCP-2 was detected in islet core tissue from sham rats and was increased 2-fold after Px.

Glucose-induced ATP Production and Insulin Secretion—In islets isolated from sham rats, ATP levels were significantly increased with changes from 2.8 to 16.7 mM glucose (Fig. 8A) in association with increased insulin release (Fig. 8B). In contrast, ATP levels showed no change from 2.8 to 16.7 mM glucose in Px islets (Fig. 8A). Basal insulin release was higher in Px islets compared with sham as expected from previous studies (24). However, there was a nearly complete loss of glucose-induced insulin secretion in Px islets (Fig. 8B). The glucose-induced increase in ATP production (Fig. 8A) and insulin secretion (Fig. 8B) were restored toward normal in islets isolated from phlorizin-treated Px rats.

DISCUSSION

The development of β-cell dysfunction is fundamental to the pathogenesis of type 2 diabetes and is important during the early stages of type 1 diabetes and for islet transplantation. The β-cell abnormalities include a loss of GHS even in the earliest stages of progression to diabetes (5). Here we show in the Px model that β-cell dysfunction is associated with a global disruption of gene expression with the induction of several normally suppressed genes and decreased expression of genes that optimize β-cell function. This change in β-cell phenotype is associated with reduced glucose-induced ATP synthesis and loss of GHS providing a molecular basis for the β-cell dysfunction found in diabetes.

Loss of β-Cell Differentiation in Px Rats—We have previously shown that islets from Px rats display decreased expression of genes important for glucose-stimulated insulin secretion, β-cell development, and the regulation of β-cell gene expression (8). Thus, genes that are highly expressed in β-cells and important for normal β-cell function such as insulin, GLUT2, glucokinase, mitochondrial glycerol phosphate dehydrogenase, pyruvate carboxylase, ion channels (potassium channel Kir6.2, voltage-de-
dependent calcium channel α1D, SERCA3), and several islet-associated transcription factors (PDX-1, Nkx6.1, Pax6, BETA2/NeuroD, HNFs) are decreased in Px rats. The present study extends these findings and shows that increased expression of normally suppressed genes may be an integral part of this change in β-cell phenotype. The increased expression of genes such as LDH-A, MCT isoforms, UCP-2, glucose-6-phosphatase, and fructose-1,6-bisphosphatase could, in theory, impair glucose-induced ATP synthesis and insulin secretion because these enzymes regulate pathways that could be diversionary to those normally operative in β-cells. Indeed, islets from Px rats display a specific defect in GIIS. The increased expression of hexokinase (8) may contribute to the lowered threshold for basal insulin release in Px rats (24).

**Role of Hyperglycemia in the Loss of β-Cell Differentiation in Px Rats**—Our results show that graded levels of chronic hyperglycemia lead to the progressive loss of β-cell differentiation. These changes in gene expression were reversible with normalization of blood glucose levels by phlorizin. Furthermore, they were unrelated to circulating NEFA and triglyceride levels or islet triglyceride content, which showed no change in Px rats. Therefore, these data suggest that chronic hyperglycemia, rather than plasma fatty acids, trigger the changes in β-cell gene expression and the altered secretory function in Px rats. However, the data do not discount the possibility that alterations in intracellular lipid mediators, such as diacylglycerol, phospholipid, or long-chain acyl CoAs, may play a role in hyperglycemia-induced β-cell dysfunction.
Various studies have investigated the hypothesis that an increase in free fatty acids participate in the pathogenesis of \(\beta\)-cell dysfunction and death in diabetes (25–30). Other studies have shown that fatty acids can lead to altered \(\beta\)-cell gene expression; islets exposed in vitro for 2 days to high levels of fatty acids result in decreased expression of insulin as well as other genes (31, 32). However, studies in our laboratory utilizing long-term (4 day) lipid infusion in normal rats showed that, in vivo, increased fatty acids have little influence on a broad spectrum of islet-associated genes (33). Interpretation of these discrepancies is not straightforward. One must be concerned about whether the use of fatty acids in in vitro experiments recreates the in vivo milieu. Moreover, it is important to recognize that the effects of free fatty acids on \(\beta\)-cells may differ for fatty acid subtypes and duration of exposure (26, 28).

Hyperglycemia and elevated extracellular free fatty acids have been implicated as causes of \(\beta\)-cell death (29, 34), although some have found hyperglycemia to have a protective influence (35). Px rats display a specific defect in GIIS but no measurable increase in \(\beta\)-cell apoptosis or replication at the 4-week time point (36). This lack of increased apoptosis may be related to activation of several stress genes (heme-oxygenase, glutathione peroxidase, A20, superoxide dismutase) and other genes that confer protection against cell death (37). Instead of lipids exerting a toxic effect on Px islets, we hypothesize that the up-regulation of lipid anabolic pathways may be related to the trophic actions of glucose and the induction of \(\beta\)-cell hypertrophy found in the Px model. Consistent with this hypothesis, the transcription factor c-Myc is induced in islets after Px (8).
and a c-Myc recognition site exists in the promoter region of the acetyl-CoA carboxylase gene (38).

Role of PPARs in β-Cells—The role of PPARs in β-cells has been the subject of much recent attention. A functional peroxisomal proliferator response element has been identified in the GLUT2 gene (39), and PPARα agonists lead to increased GLUT2 in normal islets but not in ZDF islets in which PPARα expression is reduced (40). The PPARγ isoform is expressed in all human islet endocrine cell types, which is consistent with its being functionally important in β-cells (41). Furthermore, troglitazone, believed to be a ligand for PPARγ, leads to changes in β-cell gene expression and improved insulin secretion in ZDF rats although this may be secondary to its metabolic improvement (42). This is an important point because any maneuver that lowers glucose levels in diabetes appears to be associated with improved β-cell function (43). Recent studies in mice with targeted ablation of PPARγ showed no alteration in glucose homeostasis but an increase in islet size (44), which does not fit with the concept of anabolic pathways of lipid metabolism having trophic effects on β-cells.

Although the endogenous ligands that bind to and activate PPARγ are not known, the prostaglandin compound derived from the cyclooxygenase pathway, 15-deoxy-Δ12,14-PGJ2, is a potent PPARγ activator. Therefore, the up-regulation of COX-2 in islets raises the possibility that endogenous ligands are produced for PPARγ activation. Consistent with this possibility, PPAR target genes were altered in Px rats. COX-2 expression in Px islets also has the potential to lead to the production of prostaglandins (PGE2) that have been shown to inhibit of prostaglandins (PGE2) that have been shown to inhibit of glycolysis in isolated islets and INS(832/13) cells (46) and that UCP-2 expression is reduced in Px rats although this may be secondary to its metabolic improvement (42). This is an important point because any maneuver that lowers glucose levels in diabetes appears to be associated with improved β-cell function (43). Recent studies in mice with targeted ablation of PPARγ showed no alteration in glucose homeostasis but an increase in islet size (44), which does not fit with the concept of anabolic pathways of lipid metabolism having trophic effects on β-cells.

Recent studies have shown that high glucose levels lead to down-regulation of PPARα in isolated islets and INS(832/13) cells (46), which is consistent with the finding of down-regulation of PPARα in our Px model and in ZDF rats (47), which is associated in both cases with an increase in PPARγ. The differences between the Px and ZDF models are complicated by the finding that ZDF islets have a high content of triglycerides. Importantly, it has yet to be shown that this lipid is actually in β-cells; it may be in fibroblasts or other cell types, which are typically found in the islets of mature ZDF rats (1). Although the islets of the Px model and ZDF rats are different in many ways, the β-cell dysfunction may be similar.

Role of UCP-2 in β-Cells—Our findings suggest that UCP-2 has a negative influence upon β-cell function since increased UCP-2 mRNA levels in Px rats are associated with reduced glucose-induced ATP production and impaired GIIS. Mechanically, it has been proposed that UCP-2 uncouples mitochondrial oxidative respiration by catalyzing a mitochondrial inner-membrane H+ leak that bypasses ATP synthase leading to decreased glucose-induced ATP production and a decreased ability of glucose to inhibit KATP channels (20). On the other hand, evidence also suggests that UCP-2 participates in cellular defense against oxidative stress in β-cells (48), a function that could represent an adaptive response of β-cells to chronic hyperglycemia. In contrast with our results, it has been reported that glucose down-regulates UCP-2 expression in INS(832/13) cells (46) and that UCP-2 expression is reduced in diabetic ZDF rats (49). However, up-regulation of UCP-2 has been observed in islets after chronic glucose infusion in rats (50) and after culturing islets in high glucose (51). Fatty acids have been shown to lead to an increase in UCP-2 in islets (52). UCP-2 is potentially a target gene of PPARγ in β-cells since troglitazone leads to an increase in UCP-2 expression in isolated islets (49).
Data about the effects of altered UCP-2 expression on insulin secretion function is also contradictory. In normal islets, UCP-2 overexpression leads to reduced ATP production and inhibition of GSIS (20, 21), whereas in ZDF rats, an increase in ATP and enhanced insulin secretion was observed (53). Important support for a negative role of UCP-2 on insulin secretion comes from studies showing that the ablation of the UCP-2 gene leads to hyperinsulinemia and hypoglycemia (22). Islets from these UCP-2-deficient mice had higher ATP levels and increased GSIS.

Role of Lactate Production/Transport Genes in β-Cells—The expression of LDH-A and of the MCT isofoms is suppressed in normal β-cells but not in clonal β-cell lines that are less differentiated (23, 54). Increased MCT expression in the β-cells of the Px model seems likely to be linked to the induction of LDH-A. Increased MCT and LDH-A could be deleterious to insulin secretion because carbon molecules might be diverted from oxidative phosphorylation and ATP production. The overexpression of LDH-A has been shown to perturb mitochondrial metabolism and GSIS in MIN6 β-cells (54), but overexpression of rabbit LDH-A in INS-1 cells had no adverse effect on insulin secretion (55).

Other genes normally suppressed in β-cells and induced in Px rats may contribute to impairment of the glucose sensing system. The increase in glucose-6-phosphatase may account for the increase in glucose futile cycling reported in diabetic animals (56). Similarly, induction of fructose-1,6-bisphosphatase may contribute to impairment of the glucose sensing system. Secondary to the diabetic state, islet dysfunction is not caused by a single component of the secretory machinery but rather by global disruption of several pathways.

In conclusion, the global nature of the changes in gene expression of metabolic pathways in Px rats supports our hypothesis that a critical change in β-cell phenotype participates in the adaptive response of β-cells to chronic hyperglycemia. Changes correlated with the degree of hyperglycemia were reversed with phlorizin treatment and not related to plasma lipids or islet triglycerides, implicating the importance of hyperglycemia rather than circulating fatty acids in the loss of β-cell differentiation. However, an altered expression of PPARs and lipid metabolism genes suggests that lipid mediators may still play a role in hyperglycemia-induced β-cell dysfunction. These findings suggest that the β-cell dysfunction, which is secondary to the diabetic state, is not caused by a single component of the secretory machinery but rather by global disruption of several pathways.

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