Original Article

Chronic Hyperglycemia, Independent of Plasma Lipid Levels, Is Sufficient for the Loss of \( \beta \)-Cell Differentiation and Secretory Function in the \( db/db \) Mouse Model of Diabetes

Cecilie Kjørholt, Mia C. Åkerfeldt, Trevor J. Biden, and D. Ross Laybutt

The \( \beta \)-cell is a highly specialized cell with a unique differentiation that optimizes glucose-induced insulin secretion (GIIS). Here, we evaluated changes in gene expression that accompany \( \beta \)-cell dysfunction in the \( db/db \) mouse model of type 2 diabetes. In \( db/db \) islets, mRNA levels of many genes implicated in \( \beta \)-cell glucose sensing were progressively reduced with time, as were several transcription factors important for the maintenance of \( \beta \)-cell differentiation. Conversely, genes normally suppressed in \( \beta \)-cells, such as a variety of stress response mediators and inhibitor of differentiation/DNA binding 1, a gene capable of inhibiting differentiation, were markedly increased. We assessed whether this global alteration in the pattern of \( \beta \)-cell gene expression was related more to chronic hyperglycemia or hyperlipidemia; \( db/db \) mice were treated with phlorizin, which selectively lowered plasma glucose, or bezafibrate, which selectively lowered plasma lipids. GIIS as well as the majority of the changes in gene expression were completely normalized by lowering glucose but were unaffected by lowering lipids. However, the restoration of GIIS was not accompanied by normalized uncoupling protein 2 or peroxisome proliferator-activated receptor \( \gamma \) mRNA levels, which were upregulated in \( db/db \) islets. These studies demonstrate that hyperglycemia, independent of plasma lipid levels, is sufficient for the loss of \( \beta \)-cell differentiation and secretory function in \( db/db \) mice. Diabetes 54:2755–2763, 2005

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The \( \beta \)-cell possesses a unique metabolic profile that allows it to respond to nutrient secretagogues over their physiological concentration range (1). During the progression to type 2 diabetes, an early observed functional defect is the selective loss of acute glucose-induced insulin secretion (GIIS), whereas secretion in response to other secretagogues is preserved (2,3). The mechanism(s) responsible for the selective loss of GIIS in type 2 diabetes remains unknown. It has been proposed that chronically elevated blood glucose and fatty acid levels, resulting from obesity and insulin resistance, lead to a loss of the unique expression pattern of genes necessary for appropriate GIIS (4,5). This exacerbates hyperglycemia and hyperlipidemia, which causes further \( \beta \)-cell dedifferentiation. However, the relative importance of hyperglycemia and hyperlipidemia as potential causes of \( \beta \)-cell failure in diabetes remains an unresolved issue.

In the present study, we have used the C57BL/KsJ \( db/db \) mouse model to evaluate possible molecular mechanisms that underlie \( \beta \)-cell dysfunction in diabetes. Diabetes arises in \( db/db \) mice because of insufficient \( \beta \)-cell compensation for time-dependent increases in obesity and insulin resistance (6,7). The mice display marked hyperglycemia and hyperlipidemia in association with insulin secretory defects that resemble those found in human diabetes. The deterioration in islet structure and insulin secretory capacity in \( db/db \) mice can be delayed with dietary restrictions (8) and improved with the use of insulin-sensitizing agents that reduce circulating levels of glucose, fatty acid, and triglyceride (9,10), thus demonstrating their detrimental impact. However, the metabolites were not independently varied in these studies.

In this study, we examined the effects of the duration of diabetes exposure on \( \beta \)-cell differentiation. We found that the expression of multiple genes thought to be involved in the specialized function and maturation of the \( \beta \)-cell phenotype were progressively reduced with time in islets of \( db/db \) mice, whereas normally suppressed genes were increased. We also tested the relative influence of hyperglycemia and circulating hyperlipidemia on the altered \( \beta \)-cell phenotype.
LOSS OF β-CELL DIFFERENTIATION IN db/db MICE

**TABLE 1**
Sequences of oligonucleotide primers

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**RESEARCH DESIGN AND METHODS**

C57BL/KsJ db/db mice and their age-matched lean db/+ littermates (control) were taken from the Garvan Institute breeding colony, which was originally obtained from The Jackson Laboratories (Bar Harbor, ME). Animals were kept under conventional conditions with free access to water and standard pelleted food. All procedures were approved by the Garvan Institute/St. Vincent’s Hospital Animal Experimentation Ethics Committee, following guidelines issued by the National Health and Medical Research Council of Australia. To assess the effects of the duration of diabetes exposure on β-cell differentiation, islets from 2756 DIABETES, VOL. 54, SEPTEMBER 2005 C57BL/KsJ and db/db mice were studied at 10 and 16 weeks of age. Blood was collected from the tail tip of fed mice to determine whole-blood glucose and plasma nonesterified fatty acid (NEFA), triglyceride, and insulin concentrations. Mice were anesthetized, and their islets were isolated with liberase RI (Roche) digestion of the pancreas. Islets were further purified by centrifugal elutriation using a FACs-Paque PLUS gradient (Amersham Biosciences, Uppsala, Sweden) and handpicked under a stereomicroscope. Immediately after collection, islets were used for extraction of RNA.

**RNA extraction and synthesis of cDNA.** Total RNA was extracted from islets using a RNaseasy mini kit (Qiagen) according to manufacturer-suggested protocols. After quantification by spectrophotometry, 200 ng RNA was reverse-transcribed into cDNA in a 20-μl solution containing 10× reverse-transcribed buffer (200 mmol/l Tris-HCl, pH 8.4, and 500 mmol/l KCl), 5 mmol/l MgCl2, 10 mmol/l dithiothreitol, 0.5 mmol/l dNTP, 0.5 μg of Oligo(dT)12–18, 40 units of RNaseOUT recombinant ribonuclease inhibitor, and 50 units of SuperScript II RT (Invitrogen Life Technologies). Reverse-transcribed reactions were incubated for 50 min at 42°C and 15 min at 70°C and then chilled on ice. Residual RNA was digested with 2 units RNase H incubated for 20 min at 37°C. cDNA products were stored at −20°C.

**Real-time PCR.** Real-time PCR was carried out in a volume of 10 μl consisting of 1 μl cDNA, 1× LightCycler enzyme and reaction mix (SYBR Green I dye, TaqDNA polymerase, and dNTP; Roche), 1.5 mmol/l MgCl2, and 600 nmol oligonucleotide primers (Proligo). Oligonucleotide primer sequences (Table 1) were designed with MacVector software (Oxford Molecular). All reactions were performed in a LightCycler (Roche) in which samples underwent 40 cycles of PCR with an annealing temperature of 55°C. Standards for each transcript were prepared in a conventional PCR and purified using High Pure PCR Product Purification kit (Roche). The value obtained for each specific product was expressed relative to the control gene (cyclophilin) for each sample (ratio of specific product to cyclophilin). These ratios were then expressed as a percentage of the ratio in db/+ islet extracts. PCR products were confirmed as the gene of interest on 1% agarose gels.

**Treatment of db/db mice.** Starting at 10 weeks of age, db/db mice were either untreated or treated with phlorizin or bezafibrate for 2 weeks. Phlorizin (Sigma, St. Louis, MO) was dissolved in 1.2 propanediol and injected intraperitoneally twice a day at a dose of 0.4 g/kg. Bezafibrate (Sigma) was added to pulverized diet and thoroughly mixed to a concentration of 200 mg/100 g food (0.2%). After the 2-week treatment period, islets were isolated, and RNA was extracted for gene expression analysis by real-time RT-PCR (LightCycler; Roche) as described above.

**Analytic methods.** Whole-blood glucose levels were measured with a portable glucometer (ACCU-CHEK II; Roche Diagnostics). Plasma insulin concentrations were measured using an ultrasensitive enzyme-linked immunosorbent assay kit (Merckodia AB). Plasma lipid levels were measured from samples collected in EDTA. Plasma NEFA were measured by a acyl-CoA oxidase-based colorimetric method (Wako Pure Chemical Industries). Plasma triglyceride were measured with a triglyceride assay kit (GPO Trinder; Sigma) using glycerol as standard.

**RESULTS**

**Metabolic state at 10 and 16 weeks of age.** Untreated db/db mice displayed higher blood glucose, plasma NEFA, and plasma triglyceride levels at both 10 and 16 weeks of age compared with age-matched db/+ (control) mice (Fig. 1); and blood glucose levels tended to be higher, although not significantly ($P = 0.087$), at 16 weeks compared with...
10 weeks of age. Plasma NEFA and triglycerides were elevated to similar levels in db/db mice at 10 and 16 weeks of age. Having established these metabolic indexes, we next sought to measure the expression of islet-associated transcription factors, specialized ß-cell metabolism enzymes, ion channels/pumps, and other normally suppressed metabolism enzymes and stress response genes in islets from 10- and 16-week-old obese diabetic db/db and lean nondiabetic db/+ mice.

**Changes in levels of islet-associated transcription factor mRNA.** After normalization of the gene of interest to a control gene (cyclophilin), mRNA levels in db/db islets were quantitated as a percentage of db/+ (Table 2). Note, similar results were obtained comparing expression levels with another housekeeping gene, ß-tubulin (not shown). The expression levels of several transcription factors important for pancreas and islet development and the maintenance of ß-cell differentiation were significantly altered. mRNA levels of Nkx6.1, ß-cell E-box transactivator 2 (B2/NeuroD), pancreatic duodenal homeobox-1 (PDX-1), and Pax6 were significantly reduced in islets from db/db mice. Interestingly, Nkx6.1 mRNA levels were more severely affected than the other transcription factors tested, whereas PDX-1 and Pax6 showed only modest, and in the case of Pax6 delayed, reduction (Table 2). B2/NeuroD mRNA levels were significantly reduced at 10 weeks and were further reduced at 16 weeks of age, showing a significant time-dependent effect \((P < 0.05)\). Reduced Nkx6.1 and PDX-1 in db/db islets showed a similar, although nonsignificant, tendency for a time-dependent deterioration in mRNA levels. Not all transcription factors considered important for ß-cell function were altered in db/db islets. Hepatocyte nuclear factor 1α (HNF1α) mRNA levels were unchanged in db/db mice at both ages. We also tested inhibitor of differentiation/DNA binding 1 (ID-1), a negative regulator of basic helix-loop-helix transcription factors normally expressed at low levels in islets (11) and known to be associated with dedifferentiation in other cell types (12). Through interactions with other non–basic helix-loop-helix proteins, ID-1 can also influence cell proliferation and apoptosis. ID-1 mRNA levels were significantly increased in db/db islets at 10 weeks and were increased further at 16 weeks \((P < 0.01, 10\) vs. 16-week-old db/db mice). Thus, the upregulation of ID-1 in islets of db/db mice was associated with the duration of diabetes. The role of peroxisome proliferator–activated receptor γ (PPARγ) in ß-cells, if any, is poorly understood (13), but it is an important regulator of lipid and glucose homeostasis via well-characterized effects in peripheral tissues (14). The amount of mRNA for PPARγ was significantly increased in diabetic db/db mice at 10 weeks of age. Surprisingly, however, at 16 weeks of age, PPARγ mRNA was normal, which raises questions about its role in the ß-cell dysfunction associated with diabetes.

**mRNA levels of metabolism enzymes.** Several genes involved in ß-cell glucose metabolism were evaluated in db/db islets. The glucose transporter, GLUT2, the anaplerotic enzyme, pyruvate carboxylase; the rate-limiting enzyme of the glycerol-phosphate shuttle, mitochondrial glycerol phosphate dehydrogenase (mGPDH); and the enzyme responsible for the majority of ß-cell glucose phosphorylation, glucokinase, were downregulated in islets of db/db mice. Downregulation of GLUT2 expression has been previously identified in islets of db/db mice (15) and in other models of diabetes (4,16), and here, reduced mRNA levels showed a significant time-dependent deterioration (Table 2). GLUT2, pyruvate carboxylase, and mGPDH were significantly reduced in db/db islets at 10 weeks and were reduced further at 16 weeks. In contrast, glucokinase mRNA levels were only affected with the more prolonged diabetes exposure at 16 weeks of age. Conversely, hexokinase I, an enzyme normally suppressed in ß-cells, was increased in db/db mouse islets. Also increased were the mitochondrial lipid transport enzyme, carnitine palmitoyl transferase 1 (CPT-1), as well as the mitochondrial inner membrane proton carrier, uncoupling protein 2 (UCP-2), which has been shown to be capable of negatively regulating ATP production and GHS (17,18).
levels of Kir6.2, the pore-forming subunit of the ATP-sensitive K+ channel and SERCA2b, a sarco/endoplasmic reticulum Ca2+-ATPase pump, were significantly reduced in db/db mice at 10 weeks and were reduced further at 16 weeks. mRNA levels of a related isoform, SERCA3, were unaltered at 10 weeks but were reduced in db/db islets at 16 weeks of age. This is consistent with findings of reduced SERCA protein and activity in islets of db/db mice (19). In contrast, the mRNA levels of VDCCα1D, the neuro-endocrine isoform of the α-subunit of voltage-dependent Ca2+ channels, were unchanged in db/db mice at 10 and 16 weeks.

**mRNA levels of β-cell hormone/processing genes/exocytosis pathway components.** At the time points studied, the proportion of β-cells to non-β-cells was reported as equal or even increased in db/db compared with normal islets due to β-cell hyperplasia (20). Thus, the observed changes in gene expression are indicative of dedifferentiation of the β-cells. However, despite the downregulation of several important islet-associated transcription factors, insulin mRNA levels were preserved in diabetic db/db mice at 10 and 16 weeks of age (Table 2), consistent with results in 12-week-old db/db mice (21). mRNA levels for islet amyloid polypeptide (IAPP) and the central processing enzyme prohormone convertase 2 were significantly increased in 10- and 16-week-old db/db mice. Also increased in db/db islets were mRNA levels for genes implicated in the control of distal secretory processes that are commonly used by all secretagogues: Calcyclin, a Ca2+-binding protein, has been implicated in Ca2+-induced release of insulin (22); and synaptosomal-associated protein of 25 kDa (SNAP25), a t-SNARE protein, is thought to be an essential component of the exocytotic machinery (23), and its altered expression will likely impact insulin granule movement (24). Thus, in contrast to the downregulation of genes that confer the “glucose-sensing” ability of the β-cell, transcript levels of β-cell hormones and genes implicated in distal secretory mechanisms are better maintained or even increased.

**mRNA levels of stress genes.** The role of cellular stress and stress response mediators in the failure of β-cells in diabetes has been the subject of much recent attention (25–27). Activating transcription factor 3 (ATF-3) is a stress-inducible gene normally expressed at low levels in β-cells (28). mRNA levels of ATF-3 were markedly induced in islets of db/db mice. Similarly, mRNA levels of the

![Table 2](image-url)
antioxidant enzymes heme oxygenase-1 and glutathione peroxidase were increased in islets of db/db mice. Also increased was the cell-surface protein, Fas, which in β-cells can promote both apoptosis and proliferation (29). These results suggest that induction of stress gene expression is part of the adaptive response of β-cells exposed to hyperglycemia.

**Effect of selectively reversing hyperglycemia or hyperlipidemia on changes in gene expression in db/db mice.** We next tested whether the changes in mRNA levels in db/db mice were more related to chronic hyperglycemia or hyperlipidemia. Hyperglycemia was selectively normalized in db/db 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. Circulating hyperlipidemia was selectively normalized in db/db mice by using bezafibrate. The actions of bezafibrate to lower plasma lipids involve activation of PPARα and enhanced expression of genes involved in fatty acid uptake and oxidation in the liver (30).

As shown in Fig. 2, hyperglycemia was normalized in phlorizin-treated db/db mice (blood glucose levels were not different from db/+ mice); whereas, plasma lipid levels remained elevated (compared with db/+ mice) and were not different from untreated db/db mice. In bezafibrate-treated db/db mice, hyperlipidemia was reversed (plasma NEFA and triglyceride levels were not different from db/+ mice), whereas hyperglycemia was unaffected (blood glucose levels in bezafibrate-treated db/db mice were not significantly different compared with untreated db/db mice; Fig. 2). db/db mice displayed marked hyperinsulinemia (Fig. 2) as previously described (7). Interestingly, plasma insulin concentrations did not decrease in phlorizin-treated db/db mice despite the normalization of blood glucose (Fig. 2). Similarly, plasma insulin concentrations were not affected by lowering lipids (bezafibrate treatment).

At the end of the 2-week treatment period, mRNA levels were analyzed in islets isolated from db/+; untreated db/db, phlorizin-treated db/db, and bezafibrate-treated db/db mice. Strikingly, the normalization of blood glucose in phlorizin-treated db/db mice restored the abundance of the islet-associated transcript factor levels to the levels apparent in islets from control db/+ mice (Fig. 3); islet mRNA levels for Nkx6.1 and B2/NeuroD were reduced in untreated db/db mice and were completely restored after phlorizin treatment. In contrast, lowering hyperlipidemia without affecting hyperglycemia (bezafibrate treatment) had no effect on islet transcription factor mRNA levels; mRNA levels for Nkx6.1 and B2/NeuroD in bezafibrate-treated db/db mice were reduced compared with db/+ mice and not significantly different from untreated db/db mice (Fig. 3). ID-1 upregulation in db/db islets also correlated with the presence of hyperglycemia and was not related to lipidemia; ID-1 mRNA levels were normalized in phlorizin-treated db/db mice to control db/+ values, whereas levels in bezafibrate-treated db/db mice were increased to values similar to untreated db/db mice (Fig. 3). In contrast, the upregulation of PPARγ in db/db islets did not correlate with glycemia or lipidemia; mRNA levels were increased in the untreated, phlorizin-treated, and bezafibrate-treated groups of db/db mice (Fig. 3).

As shown in Fig. 4, the normalization of blood glucose in phlorizin-treated db/db mice reversed the changes in expression of glucose metabolism enzymes, whereas the normalization of plasma lipids was without affect; reduced mRNA levels for GLUT2, pyruvate carboxylase, and mGPDH in untreated db/db, phlorizin-treated db/db, and bezafibrate-treated db/db mice in the fed state and after an overnight fast. db/db mice were treated with phlorizin (0.8 g·kg⁻¹·day⁻¹) or bezafibrate (0.2% of food) for 2 weeks. Values are means ± SE for 15 db/+; 8 untreated db/db, 9 phlorizin-treated db/db (db/dbP), and 4 bezafibrate-treated db/db (db/dbB) mice in the fed state and 10 db/+; 4 untreated db/db, 4 db/dbP, and 4 db/dbB mice in the fasted state. *P < 0.05, **P < 0.01, ***P < 0.001 vs. db/+ mice in the fed state. ‡‡‡P < 0.001 vs. db/+ mice in the fasted state.
glucose (phlorizin) but were unaffected by lowering lipids (bezafibrate). Increased mRNA levels of the Ca\(^{2+}\)-binding protein calcyclin were also normalized by lowering glucose but were unaffected by lowering lipids. Of all the genes tested, only CPT-1, the rate-limiting mitochondrial lipid transport enzyme, was regulated by both glycemia and lipidemia in islets of db/db mice; the higher levels of islet CPT-1 mRNA in untreated db/db mice were significantly reduced toward normal in both phlorizin- and bezafibrate-treated db/db mice. In contrast, the higher levels of UCP-2 mRNA in db/db mice were not affected by the lowering of either glucose or lipid (Fig. 4). Thus, our data in Fig. 6), in conflict with evidence supporting a role in insulin secretion (see insulin secretory assay

**FIG. 3. Reversibility of changes in transcription factor mRNA levels in db/db mice.** mRNA levels were compared by real-time RT-PCR in islet preparations of 8–13 db/+ , 3–7 untreated db/db, 3–8 phlorizin-treated db/db (db/dbP), and 3 bezafibrate-treated db/db (db/dbB) mice. Shown are mRNA levels for the islet-associated transcription factors Nkx6.1 and B2/NeuroD and the normally suppressed ID-1 and PPARγ. After normalization of the specific gene to a control gene (cyclophilin), mRNA levels are expressed as a percentage of db/+ . Values are means ± SE. **P < 0.01, ††P < 0.001 vs. db/+ for each gene; †P < 0.05, ††P < 0.01, †††P < 0.001 vs. untreated db/db mice for each gene.

**FIG. 4. Reversibility of changes in metabolism enzymes and ion channels/pumps in db/db mice.** mRNA levels were compared by real-time RT-PCR in islet preparations of 8–13 db/+ , 3–7 untreated db/db, 3–8 phlorizin-treated db/db (db/dbP), and 3 bezafibrate-treated db/db (db/dbB) mice. Shown are mRNA levels for glucose metabolism enzymes (GLUT2, pyruvate carboxylase, and mGPDH), the K\(^+\) channel subunit (Kir6.2), an isoform of Ca\(^{2+}\)-ATPase pump (SERCA2b), the Ca\(^{2+}\)-binding protein (calcyclin), the mitochondrial proton carrier (UCP-2), and the mitochondrial lipid transport enzyme (CPT-1). After normalization of the specific gene to a control gene (cyclophilin), mRNA levels are expressed as a percentage of db/+ . Values are means ± SE. **P < 0.01, ††P < 0.001 vs. untreated db/db mice for each gene. 

Thus, the secretory dysfunction in islets of db/db mice was similar to that found in humans with type 2 diabetes. Strikingly, insulin secretion at 25 mmol/l glucose was restored in islets isolated from phlorizin-treated db/db mice but remained severely blunted in bezafibrate-treated db/db mice (Fig. 6). These data imply a causal relationship between chronic hyperglycemia and the loss of GIIS in db/db mice.

**DISCUSSION**

Here, we show using the db/db mouse model of type 2 diabetes that β-cell dysfunction is associated with a widespread disruption of gene expression with the induction of several normally suppressed genes and decreased expres-
an aliquot of the buffer by radioimmunoassay. Values are means of 2.8 mmol/l (H11550 and three bezafibrate-treated glucose in each group.

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FIG. 5. Reversibility of changes in stress gene mRNA levels in db/db mice. mRNA levels were compared by real-time RT-PCR in islet preparations of 8–13 db/+ , 3–7 untreated db/db, 3–8 phlorizin-treated db/db (db/dbP), and 3 bezafibrate-treated db/db (db/dbB) mice. Shown are mRNA levels for ATF-3, heme oxygenase-1 (HO-1), glutathione peroxidase (GPX), and Fas. After normalization of the specific gene to a control gene (cyclophilin), mRNA levels are expressed as a percentage of db/+ . Values are means ± SE. *P < 0.05, **P < 0.01, ***P < 0.001 vs. db/+ for each gene. †P < 0.05 vs. untreated db/db mice.

FIG. 6. Effect of selectively reversing hyperglycemia or hyperlipidemia on changes in GIIS in db/db mice. Groups of five islets isolated from nine db/+ and three untreated db/db, three phlorizin-treated db/dbB, and three bezafibrate-treated db/dbB mice were cultured in the presence of 2.8 mmol/l (□□) and 25 mmol/l (□□) glucose. Insulin was measured in an aliquot of the buffer by radioimmunoassay. Values are means ± SE. **P < 0.01, ***P < 0.001 for insulin secretion at 25 vs. 2.8 mmol/l glucose in each group.

estingly, expression of Nkx6.1, the most severely affected, lies downstream in the hierarchy of transcription factor control of β-cell differentiation (34). Normal expression is restricted to β-cells of the adult pancreas, and its absence leads to profound inhibition of β-cell formation (34). The downregulation of Nkx6.1 and other potentially important islet transcription factors could contribute to the altered expression of genes essential for GIIS (35). The expression of multiple genes that confer the glucose-sensing ability of the β-cell, namely the glucose metabolism genes (GLUT2, pyruvate carboxylase, mGPDH, and glucokinase), and ion channels/pumps (Kir6.2, SERCA2b, and SERCA3) were downregulated. Furthermore, parallel increases were observed for a gene capable of inhibiting differentiation, ID-1, as well as other normally suppressed genes. In normal islets, ID-1 expression is low, but it is induced in isolated islets and β-cell lines cultured under high glucose conditions, with little effect in other cell types (11), and its forced expression can inhibit insulin promoter activity (36,37). These findings raise the possibility that increased ID-1 plays a role linking hyperglycemia to β-cell dedifferentiation in db/db mice.

Comparison of results with db/db mice with other models of diabetes. Insulin mRNA levels were preserved in db/db islets, indicating that adaptation of β-cells is accompanied by abnormalities in the glucose-sensing machinery, whereas transcript levels of the β-cell hormone are better maintained. A similar phenomenon was observed in a rat partial pancreatectomy model with mild β-cell deficiency in which several glucose-sensing genes and islet transcription factors were significantly reduced, whereas insulin levels were only affected with more severe β-cell depletion (32). In the db/db mouse, hyperglycemia and hyperlipidemia appear as a result of the failure of their β-cells to meet the secretory demand generated by increasing obesity and insulin resistance. In the partial pancreatectomy rat, increased secretory demand is imposed by β-cell deficiency in the absence of obesity or hyperlipidemia. In both models, the persistent hyperglycemia leads to a gradual change in β-cell phenotype, with secretory defects and changes in islet gene expression that are remarkably similar with a few notable exceptions. Thus, IAPP was increased in islets of db/db mice, whereas in partial pancreatectomy rats, it was increased in animals with low-level hyperglycemia and reduced in animals with more severe diabetes. LDH-A (lactate dehydrogenase A subunit) was not significantly altered in db/db islets, unlike in partial pancreatectomy islets, which showed a severalfold induction (32). Species differences in islet susceptibility to stress will likely influence the severity of β-cell dedifferentiation under conditions of increased secretory demand (38), with constitutive differences in defense mechanisms likely to play an influential role (39,40). It is also important to recognize that leptin signaling, which may impair insulin secretion (41) and induce β-cell apoptosis (42), is defective in db/db mice. The progression to diabetes in ZDF rats is associated with a similar global alteration in β-cell gene expression (4), even though the ZDF rat carries an inherited defect in insulin gene transcription (43), which is presumably not carried in the db/db mice. Taken together, these studies in animal models highlight the importance of the severity and duration of
hyperglycemia as critical factors in the deterioration of the β-cell phenotype. Importantly, similar gene expression alterations have been recently identified in islets from human type 2 diabetic patients (44).

Role of hyperglycemia in the loss of β-cell differentiation in db/db mice. The loss of β-cell function in humans has been linked to increases in fasting plasma glucose levels, even in the nondiabetic range (45). Because of its similarities with human type 2 diabetes, the C57BL/KsJ db/db mouse model seems particularly suitable to address the question of the relative influence of hyperglycemia versus hyperlipidemia in the progressive loss of β-cell function. Using independent variation of circulating glucose on the one hand and fatty acids and triglyceride on the other, we show here that hyperglycemia independent of hyperlipidemia plays a critical role in the loss of β-cell differentiation in db/db mice. This is in accordance with results from a study in ZDF rats, in which high glucose levels, and not high plasma triglycerides, appear to be associated with the decreased insulin gene expression and increased islet triglyceride content in this model (46). However, other studies in ZDF rats have proposed a causative role of high fatty acids in the development of β-cell dysfunction (47), and others have shown that fatty acids can directly lead to alterations in β-cell gene expression broadly consistent with the dedifferentiation observed here (48–51). Given the likelihood that intracellular lipid pathways play an important role in the β-cell dysfunction of diabetes (52), it is possible that hyperglycemia in vivo leads to alterations in β-cell lipid metabolism that recapitulate the effects on gene expression of fatty acid addition in vitro. Additionally, circulating fatty acids might exert synergistic toxic effects in concert with hyperglycemia (53,54).

In conclusion, the finding in C57BL/KsJ db/db mice of time-dependent alterations in the pattern of islet gene expression is consistent with the hypothesis that a gradual loss of differentiation contributes to the dysfunction of β-cells in diabetes. The validity of this relationship is further strengthened by studies in phlorizin-treated db/db mice in which the restoration of G6CS occurs in parallel with the normalization of the majority of the changes in islet gene expression. Phlorizin treatment normalized blood glucose without affecting plasma NEFA or triglyceride levels, strongly suggesting that hyperglycemia, independent of hyperlipidemia, plays a causal role in the progressive loss of β-cell differentiation and secretion in db/db mice. Taken with other studies (4,32,55,56), it is apparent that there are similarities in the abnormal patterns of gene expression that accompany β-cell dysfunction in several rodent models of diabetes, suggestive of common mechanisms. Similar mechanisms may be responsible for β-cell dysfunction in human type 2 diabetes (44).

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