Failure of the Adaptive Unfolded Protein Response in Islets of Obese Mice Is Linked With Abnormalities in β-Cell Gene Expression and Progression to Diabetes

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The normal β-cell response to obesity-associated insulin resistance is hypersecretion of insulin. Type 2 diabetes develops in subjects with β-cells that are susceptible to failure. Here, we investigated the time-dependent gene expression changes in islets of diabetes-prone *db/db* and diabetes-resistant *ob/ob* mice. The expressions of adaptive unfolded protein response (UPR) genes were progressively induced in islets of *ob/ob* mice, whereas they declined in diabetic *db/db* mice. Genes important for β-cell function and maintenance of the islet phenotype were reduced with time in *db/db* mice, whereas they were preserved in *ob/ob* mice. Inflammation and antioxidant genes displayed time-dependent upregulation in *db/db* islets but were unchanged in *ob/ob* islets. Treatment of *db/db* mouse islets with the chemical chaperone 4-phenylbutyric acid partially restored the changes in several β-cell function genes and transcription factors but did not affect inflammation or antioxidant gene expression. These data suggest that the maintenance (or suppression) of the adaptive UPR is associated with β-cell compensation (or failure) in obese mice. Inflammation, oxidative stress, and a progressive loss of β-cell differentiation accompany diabetes progression. The ability to maintain the adaptive UPR in islets may protect against the gene expression changes that underlie diabetes development in obese mice. *Diabetes* **62:1557–1568, 2013**

T he critical contribution of deficient insulin secretion to the pathogenesis of type 2 diabetes is beyond doubt (1–3). The normal β-cell response to excess nutrient and obesity-associated insulin resistance is hypersecretion of insulin that maintains blood glucose levels within the normal range. This is associated with both expansion of β-cell mass and enhanced β-cell function such that the amount of insulin secreted per given unit of β-cell mass is increased (2,3). Normoglycemia can be maintained for decades in obese subjects with robust β-cells that cope with this increased demand and sustain the compensatory response. Mechanisms for β-cell compensation have been proposed (2,3), but there has been little exploratory investigation. Type 2 diabetes only develops in subjects unable to sustain the β-cell compensatory response. This is associated with a progressive deterioration of β-cell function, particularly impairment of glucose-stimulated insulin secretion (GSIS), and a loss of β-cell mass through an increased rate of apoptosis (1–5).

Thus, type 2 diabetes arises in subjects with islets that are susceptible to dysfunction and apoptosis under stressful conditions. The current knowledge of the molecular differences between robust and susceptible β-cells is poor.

The *db/db* and *ob/ob* mouse models of obesity have been cornerstones in research of the mechanisms of insulin resistance and β-cell failure (6,7). In these models, the absence of leptin signaling results in similar hyperphagia, obesity, hyperlipidemia, and insulin resistance. However, opposing predisposition to diabetes development is displayed by these mice when bred on the C57BL/6J or C57BL/KsJ background. The *ob/ob* mouse on the C57BL/6J background strain exhibits resistance to diabetes because of successful β-cell compensation, whereas the *db/db* mouse on the C57BL/KsJ background strain displays time-dependent progression to overt diabetes because of the failure of β-cell compensation. The differences in β-cell phenotype (propensity to compensation or failure) are revealed only in the setting of obesity and insulin resistance. We have used these mouse models of obesity with opposing disposition to development of diabetes to study the mechanisms of β-cell compensation (diabetes-resistant *ob/ob* mice) and failure (diabetes-prone *db/db* mice).

Endoplasmic reticulum (ER) stress has been proposed as a mechanism for β-cell dysfunction and death in type 2 diabetes (8–11). ER stress activates a signaling cascade known as the unfolded protein response (UPR), which has roles alleviating the ER stress through the upregulation of ER chaperones and folding enzymes and, paradoxically, activating apoptosis via deleterious UPR signaling if the stress is too severe or prolonged. We found, unexpectedly, that the presence of ER stress in islets was not unique to the model of β-cell failure. Rather, ER stress was indicated in islets of both diabetes-resistant *ob/ob* and diabetes-prone *db/db* mice. However, the models differed in the pattern of the ER stress response, whereas the adaptive UPR was progressively upregulated with β-cell compensation, it declined with β-cell failure. We also tested the influence of improving the chaperone activity of the ER on the gene expression changes that were found exclusively in islets of diabetes-prone *db/db* mice, namely the upregulation of inflammation and oxidative stress gene expression and the progressive loss of β-cell differentiation.

RESEARCH DESIGN AND METHODS

Mice. C57BL/KsJ *db/db* and C57BL/6J *ob/ob* mice and their age-matched lean control mice (C57BL/KsJ or C57BL/6J, respectively) were taken from the Garvan Institute breeding colonies. Animals were kept under conventional conditions with free access to food and water. All procedures were approved by the Garvan Institute/St. Vincent's Hospital Animal Experimentation Ethics Committee and followed guidelines issued by the National Health and Medical Research Council of Australia. To assess the time course changes in islet gene expression, mice were studied at 6 and 16 weeks of age. Blood samples were

taken via tail prick for measurement of glucose levels. Blood collected in EDTA via a terminal heart bleed was used for measurement of plasma insulin, triglyceride, and nonesterified fatty acid levels. Mice were anesthetized and their islets were isolated with liberase RI (Roche) digestion of the pancreas. Islets were further separated with a Ficoll-Paque PLUS gradient (Amersham Biosciences) and handpicked under a stereomicroscope. Immediately after collection, islets were used for extraction of RNA.

Metabolic studies and assays. Intraperitoneal glucose tolerance tests (2 g/kg glucose) were performed in conscious mice after 6 h of fasting. Blood glucose was measured using an Accu-Chek Performa glucose monitor (Roche Diagnostics, Castle Hill, Australia). Plasma insulin was measured using ELISA (Crystal Chem, Downers Grove, IL). Plasma triglyceride was measured using an enzymatic colorimetric method (GPO-PAP reagent; Roche Diagnostics). Plasma nonesterified fatty acid was measured by an acyl-CoA oxidase–based colorimetric method (Wako Pure Chemical Industries, Osaka, Japan).

Insulin secretion assay. Isolated islets were washed in Krebs-Ringer HEPES buffer (containing 5 mmol/L $NaHCO_3$, 1 mmol/L $CaCl_2$, 2.8 mmol/L glucose, 10 mmol/L HEPES, and 0.1% BSA). Groups of five islets, with three to four replicates per animal, were incubated for 1 h at 37°C in Krebs-Ringer HEPES buffer containing 2.8 or 16.7 mmol/L glucose. Insulin was measured in an aliquot of the buffer by radioimmunoassay (Millipore, Billerica, MA).

Treatment of islets. Isolated islets from lean nondiabetic C57BL/KsJ control and obese diabetic *db/db* mice were cultured in RPMI supplemented with 0.2 mmol/L glutamine, 10% heat-inactivated FBS 100 units/mL penicillin, and 100 µg/mL streptomycin at 37°C. Islets were treated with 4-phenylbutyric acid (PBA; 2.5 mmol/L; Sigma, St. Louis, MO) or trimethylamine *N*-oxide (TMAO; 100 mmol/L) for 24 h. Isolated islets from C57BL/6J control and *ob/ob* mice were treated with salubrinal (75 µmol/L; Merck, Kilsyth, Victoria, Australia) for 24 h. Insulin secretion was assessed and RNA was extracted for gene expression analysis.

RNA analysis. Total RNA was extracted using RNeasy Mini Kit (Qiagen, Doncaster, Australia) and cDNA was synthesized using the QuantiTect Reverse Transcription Kit (Qiagen, Victoria, Australia). Real-time PCR was performed using *Power* SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) on a 7900HT Real-Time PCR System (Applied Biosystems). Primer sequences are provided in Supplementary Table 1. The value obtained for each specific product was normalized to a control gene (*cyclophilin A*) and expressed as a fold change of the value in control extracts. *Xbp1* splicing was assessed as previously described (12).

Statistical analysis. All results are presented as means ± SEM. Statistical analyses were performed using unpaired two-tailed Student's *t* test or ANOVA.

RESULTS

Metabolic characteristics at 6 and 16 weeks of age. The *db/db* and *ob/ob* mice displayed increased body weight at both 6 and 16 weeks of age as compared with their respective age-matched control mice (Supplementary Figure 1*A* and *B*). At 6 weeks of age, blood glucose levels were not different among the groups (Supplementary Fig. 1*C*

and *D*). Thus, *db/db* and *ob/ob* mice were nondiabetic at 6 weeks of age, although both groups displayed impaired glucose tolerance compared with their respective controls (Supplementary Fig. 2*A* and *B*). At 16 weeks of age, blood glucose levels were elevated in *db/db* mice but remained unaltered in *ob/ob* mice (Supplementary Fig. 1*C* and *D*). Plasma nonesterified fatty acid levels were unchanged at 6 weeks but tended to be increased at 16 weeks of age in both *db/db* and *ob/ob* mice (Supplementary Fig. 1*E* and *F*). There was a tendency for increased plasma triglyceride levels in *db/db* and *ob/ob* mice at both 6 and 16 weeks of age (Supplementary Fig. 1*G* and *H*). Plasma insulin levels were increased in *db/db* and *ob/ob* mice at both 6 and 16 weeks of age (Supplementary Fig. 1*I* and *J*). In *ob/ob* mice, plasma insulin levels were significantly higher at 16 weeks compared with 6 weeks of age (Supplementary Fig. 1*J*). In islets isolated from *db/db* mice, GSIS was enhanced at 6 weeks but was reduced at 16 weeks compared with islets from age-matched control mice (Fig. 1*A*). In islets isolated from *ob/ob* mice, GSIS was enhanced at 6 weeks but was unaltered at 16 weeks compared with their age-matched controls (Fig. 1*B*). Having established these metabolic indices, we next sought to measure the expression of islet-associated transcription factors, genes involved in β-cell function, as well as stress-response genes, in islets from 6- and 16-week-old obese *db/db* and *ob/ob* mice compared with their respective lean controls.

Changes in levels of islet-associated transcription factor mRNA. After normalization of the gene of interest to a control gene (*cyclophilin A*), mRNA levels in *db/db* islets were quantitated as fold change of their respective age-matched lean C57BL/KsJ control mice. The expression levels of several transcription factors important for islet development and the maintenance of β-cell differentiation were altered in *db/db* islets. *Beta2* and *MafA* mRNA levels were significantly reduced at 6 weeks of age (Fig. 2*A*). Thus, the downregulation of *Beta2* and *MafA* preceded the onset of diabetes in *db/db* mice. *Beta2* and *MafA* mRNA levels were further reduced at 16 weeks of age, showing a significant time-dependent effect (*P* < 0.05 for each gene). *Pdx1* and *Nkx6.1* mRNA levels were unchanged at 6 weeks but were significantly reduced at 16 weeks in *db/db* islets (Fig. 2*A*). Thus, the downregulation of *Pdx1* and *Nkx6.1* was associated with diabetes in *db/db*
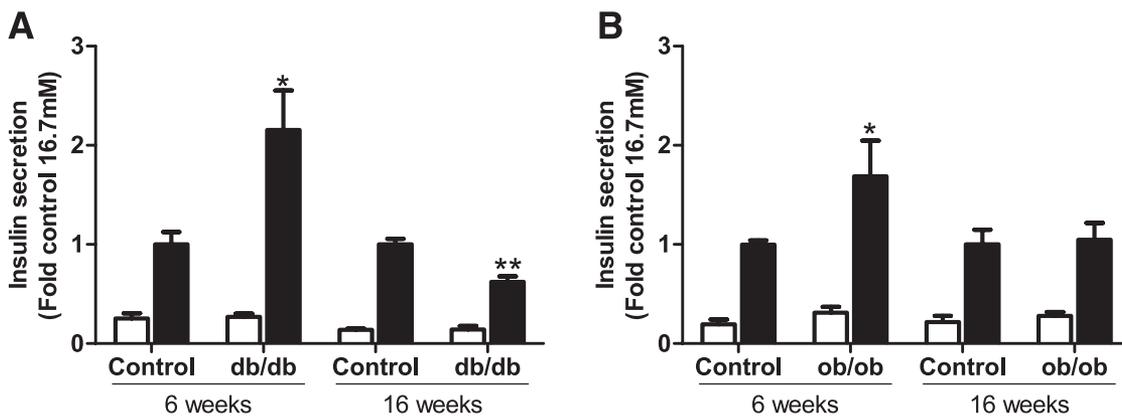

FIG. 1. Glucose-stimulated insulin secretion ex vivo in islets isolated from C57BL/KsJ control and *db/db* mice (*A*), and C57BL/6J control and *ob/ob* mice (*B*) at 6 and 16 weeks of age. Batches of islets were cultured in Krebs-Ringer HEPES buffer containing 0.1% BSA and 2.8 mmol/L (*white bars*) or 16.7 mmol/L glucose (*black bars*) for 1 h. Insulin was measured in an aliquot of the buffer by radioimmunoassay. Insulin secretion was expressed as fold change of the level in age-matched control islets cultured in 16.7 mmol/L glucose. All results are mean ± SEM. *n* ≥ 4 in each group. **P* < 0.05, ***P* < 0.01 genotype effect in each age group.
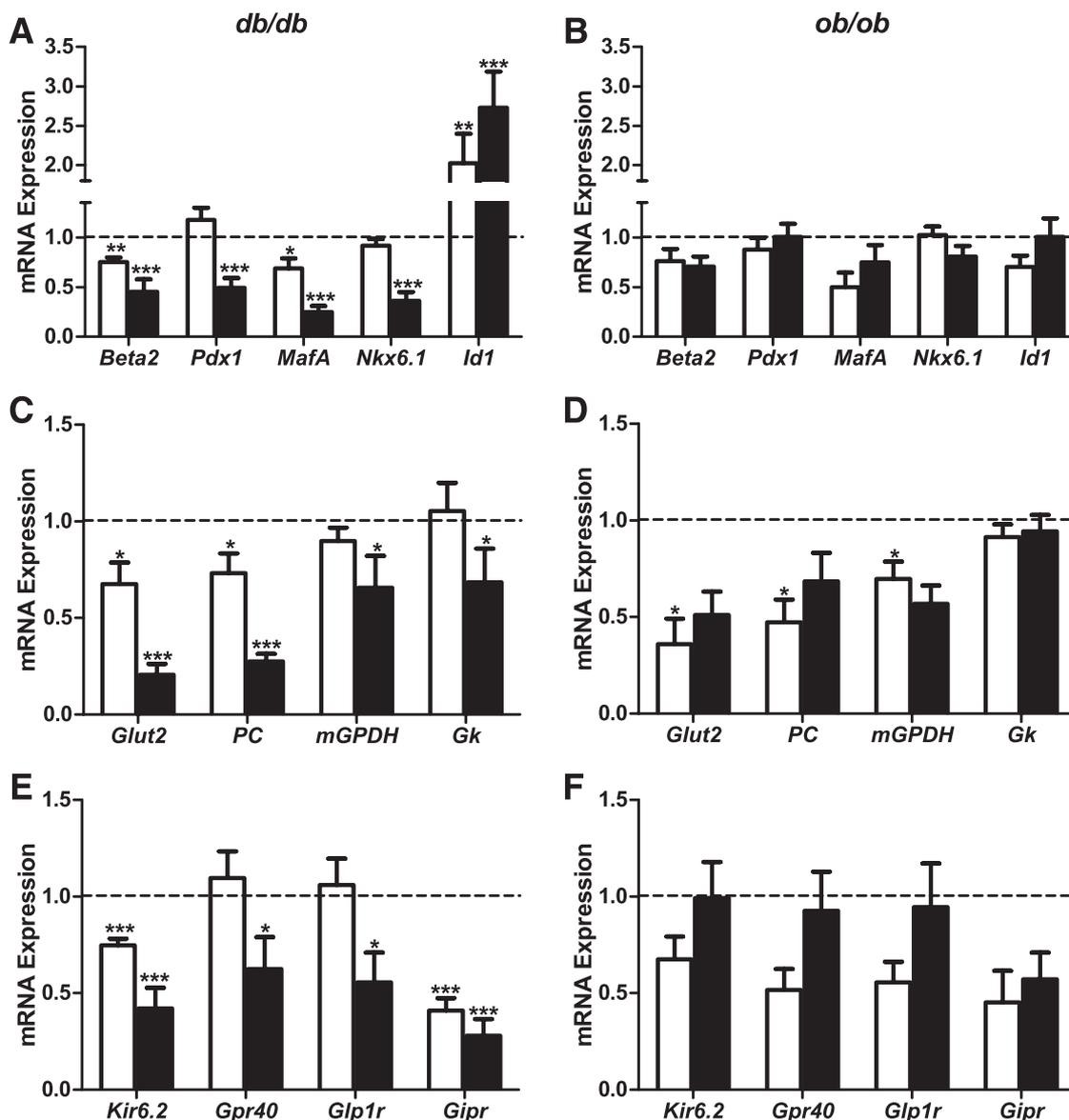
FIG. 2. Changes in mRNA expression of islet-associated transcription factors and genes that optimize β-cell function in islets of *db/db* and *ob/ob* mice at 6 and 16 weeks of age. Islets were isolated from 9 to 10 C57BL/KsJ control and 8 *db/db* mice at 6 weeks of age, 13 to 16 C57BL/KsJ control and 9 to 12 *db/db* mice at 16 weeks of age, 6 C57BL/6J control and 6 *ob/ob* mice at 6 weeks of age, and 4 to 5 C57BL/6J control and 7 *ob/ob* mice at 16 weeks of age. Total RNA was extracted, reverse-transcribed, and analyzed by real-time RT-PCR. mRNA levels were expressed as fold change of the levels in respective age-matched controls (represented by the dashed line). Shown are changes for the indicated genes in islets of *db/db* (*A*, *C*, *E*) and *ob/ob* (*B*, *D*, *F*) mice at 6 (*white bars*) and 16 (*black bars*) weeks of age. All results are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 genotype effect in each age group.

mice. We also tested *Id1* (inhibitor of differentiation/DNA binding), which was identified recently as a negative regulator of insulin secretion and β-cell gene expression (13). *Id1* mRNA levels were increased at both 6 and 16 weeks of age in *db/db* islets. We also assessed changes in the expression of islet-associated transcription factors in islets of obese *ob/ob* mice compared with their age-matched lean C57BL/6J control mice (Fig. 2*B*). No significant difference was observed in the mRNA levels of *Beta2*, *MafA*, *Pdx1*, *Nkx6.1*, and *Id1* at both 6 and 16 weeks of age in *ob/ob* islets, although there was a tendency for reduced expression of *Beta2* and *MafA* (Fig. 2*B*). For all genes tested in this study, expression levels in the C57BL/6J and C57BL/KsJ control islets were similar.

mRNA levels of β-cell function genes. Several genes involved in β-cell glucose metabolism were evaluated

(Fig. 2*C* and *D*). The glucose transporter, *Glut2*, the anaplerotic enzyme, pyruvate carboxylase (*PC*), the rate-limiting enzyme of the glycerol-phosphate shuttle, mitochondrial glycerol phosphate dehydrogenase (*mGPDH*), and the enzyme responsible for the majority of β-cell glucose phosphorylation, glucokinase (*Gk*), were downregulated in islets of *db/db* mice. *Glut2* and *PC* were significantly reduced in *db/db* islets at 6 weeks and were reduced further at 16 weeks (Fig. 2*C*). The downregulation of *mGPDH* and *Gk* was observed only at 16 weeks (Fig. 2*C*). Thus, the expression of β-cell glucose metabolism genes showed a tendency for a time-dependent deterioration in *db/db* islets. In *ob/ob* islets, *Glut2*, *PC*, and *mGPDH* mRNA levels were significantly reduced at 6 weeks (Fig. 2*D*). However, in contrast to *db/db* islets, the mRNA levels of glucose metabolism genes did not reduce further with time in *ob/ob*

islets. Moreover, we failed to detect a difference in *Gk* mRNA levels at both ages in *ob/ob* islets (Fig. 2*D*).

The expression of *Kir6.2*, the pore-forming subunit of the ATP-sensitive K⁺ channel also was evaluated (Fig. 2*E* and *F*). The mRNA levels of *Kir6.2* were significantly reduced in *db/db* mice at 6 weeks, and they were reduced further at 16 weeks (Fig. 2*E*). In contrast, *Kir6.2* mRNA levels were not significantly altered in *ob/ob* islets, although there was a tendency for reduced expression at 6 weeks (Fig. 2*F*). The G-protein-coupled receptor GPR40 may play a role in both fatty acid and glucose stimulation of insulin secretion (14–16). In *db/db* islets, *Gpr40* mRNA levels were unchanged at 6 weeks but were significantly reduced at 16 weeks (Fig. 2*E*). In *ob/ob* islets, no significant difference was detected in *Gpr40* mRNA levels, although there was a tendency for reduced expression at 6 weeks (Fig. 2*F*). A similar pattern of expression was observed for the incretin GLP-1 receptor, *Glp1r*; mRNA levels were unchanged at 6 weeks but reduced at 16 weeks in *db/db* islets (Fig. 2*E*), whereas we failed to measure a difference in *ob/ob* islets (Fig. 2*F*). The GIP receptor, *Gipr*, was reduced at both 6 and 16 weeks in *db/db* islets (Fig. 2*E*). There was a nonsignificant tendency for reduced mRNA expression of *Gipr* in *ob/ob* islets (Fig. 2*F*). These results suggest that in contrast to the progressive downregulation of genes important for β-cell function in *db/db* islets, transcript levels are maintained better with time in *ob/ob* islets.

The proportion of β-cells within islets was similar in 16-week-old control and diabetic *db/db* mice (control: 86.4 ± 2.0%; *db/db*: 88.6 ± 0.9%; *n* = 3 in each group). Thus, the changes in gene expression observed in islets of diabetic *db/db* mice are indicative of β-cell dedifferentiation rather than β-cell loss. This is consistent with studies suggesting the importance of β-cell dedifferentiation as a mechanism of diabetic β-cell failure (3,7,17–19).

mRNA levels of UPR 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 (9,20). We previously reported the upregulation of several ER stress genes in islets of diabetic *db/db* mice (10). Here, prediabetic *db/db* mice showed significant upregulation of adaptive (*BiP*, *p58*, *Erp72*, *Fkbp11*, and *Grp94*; Fig. 3*A*) and deleterious (*Atf3*, *Chop*, and *Trib3*; Fig. 3*C*) UPR genes. Interestingly, expression levels of the adaptive UPR genes were higher at 6 weeks than at 16 weeks, showing a reduction with time in *db/db* islets (Fig. 3*A*). In *ob/ob* islets, expression of adaptive UPR genes were increased at 6 weeks and, in contrast to *db/db* islets, they were maintained or increased further at 16 weeks (Fig. 3*B*). Furthermore, the deleterious ER stress genes were not induced in *ob/ob* islets (Fig. 3*D*). The transcription factor XBP1 regulates the expression of many adaptive UPR genes (21,22). In concert with the changes in the adaptive UPR, the level of *Xbp1* splicing (activation) was increased in *ob/ob* and prediabetic *db/db* mouse islets, and it was lowered in diabetic *db/db* mice (Fig. 3*E* and *F*). These differential responses provide the first evidence that the maintenance (or suppression) of the adaptive UPR is associated with β-cell compensation (or failure) in obese mice.

mRNA levels of antioxidant genes. Oxidative stress has been proposed as a mechanism for gluco-lipotoxicity of β-cells (20,23). We assessed the expression of oxidative stress–inducible genes. mRNA levels of the antioxidant enzymes heme oxygenase-1 (*HO-1*), glutathione peroxidase (*GPx*), catalase, and superoxide dismutase 1 (*Sod1*) were upregulated in islets of *db/db* mice; time-dependent increases were displayed for *HO-1*, *GPx*, and *catalase* (Fig. 4*A*). In contrast, we failed to detect differences in the antioxidant genes tested in *ob/ob* islets (Fig. 4*B*).

mRNA levels of inflammation genes. Emerging evidence implicates systemic and tissue inflammation with obesity and type 2 diabetes (24,25). Here, time-dependent increases in the expression of chemokines and cytokines were observed in *db/db* islets (Fig. 4*C* and *E*). At 6 weeks, the expressions of *IL-6*, *Cxcl1*, and *Ccl2* were significantly increased. Minor nonsignificant increases were observed for *IL-1β* (*P* = 0.09) and *TNF-α* (*P* = 0.17), but these are worth highlighting because of the interest of low-grade inflammation in type 2 diabetes islets (24). At 16 weeks, the expression of all the tested chemokines and cytokines were markedly increased in *db/db* islets (Fig. 4*C* and *E*), as were the mRNA levels for *CD68*, a commonly used marker for macrophages (Fig. 4*E*). In complete contrast, the expressions of these inflammatory genes were unaltered in *ob/ob* islets at 6 and 16 weeks of age (Fig. 4*D* and *F*). These data provide the first demonstration of an escalating inflammatory response in association with β-cell failure in diabetes-prone *db/db* mice, juxtaposed with an absence of islet inflammation in association with successful β-cell compensation in obese diabetes-resistant *ob/ob* mice. Furthermore, mRNA levels of proapoptotic genes, *Bax* and *Bak1*, were selectively increased in islets of *db/db* mice (Fig. 4*E* and *F*).

Changes in gene expression in islets of C57BL/6J *db/db* mice. We also evaluated gene expression in islets of 6-week-old C57BL/6J *db/db* mice compared with control C57BL/6J (Supplementary Figs. 3–5). In islets of C57BL/6J *db/db* mice, the adaptive UPR genes were upregulated (Supplementary Fig. 4), whereas differences in oxidative stress and inflammatory gene expression were not detected (Supplementary Fig. 5). These results are broadly consistent with the findings in *ob/ob* mice, demonstrating that the different background strains, rather than the *ob* (*Lep*) and *db* (*Lepr*) mutations, are critically important for the β-cell response to obesity in these models.

Enhanced signaling downstream of PERK/eIF2α does not affect the changes in gene expression in islets of *ob/ob* mice. We next examined the effects of enhancing signaling downstream of PERK/eIF2α on the mRNA changes in islets of obese *ob/ob* mice. Isolated islets from *ob/ob* mice were treated with salubrinal, an inhibitor of eIF2α dephosphorylation (26). As shown in Fig. 5*A*, salubrinal treatment led to the expected increase in *Chop* expression in *ob/ob* islets. However, salubrinal treatment had no effect on the changes in the expression of islet-associated transcription factors and genes that optimize β-cell function in *ob/ob* islets (Fig. 5*A*). Moreover, salubrinal treatment did not affect GSIS, which was increased in *ob/ob* islets (Fig. 5*B*). These findings suggest that the induction of signaling downstream of PERK/eIF2α is insufficient for the loss of β-cell phenotype that accompanies β-cell decompensation.

Effect of chemical chaperone treatment on the changes in gene expression in islets of *db/db* mice. We next examined the effects of chemical chaperone treatment on the mRNA changes in islets of diabetic *db/db* mice. PBA acts as a chemical chaperone in the ER by improving folding capacity and trafficking (12,27). As shown in Fig. 6*A*, PBA treatment of *db/db* islets partially restored the abundance of islet-associated transcription
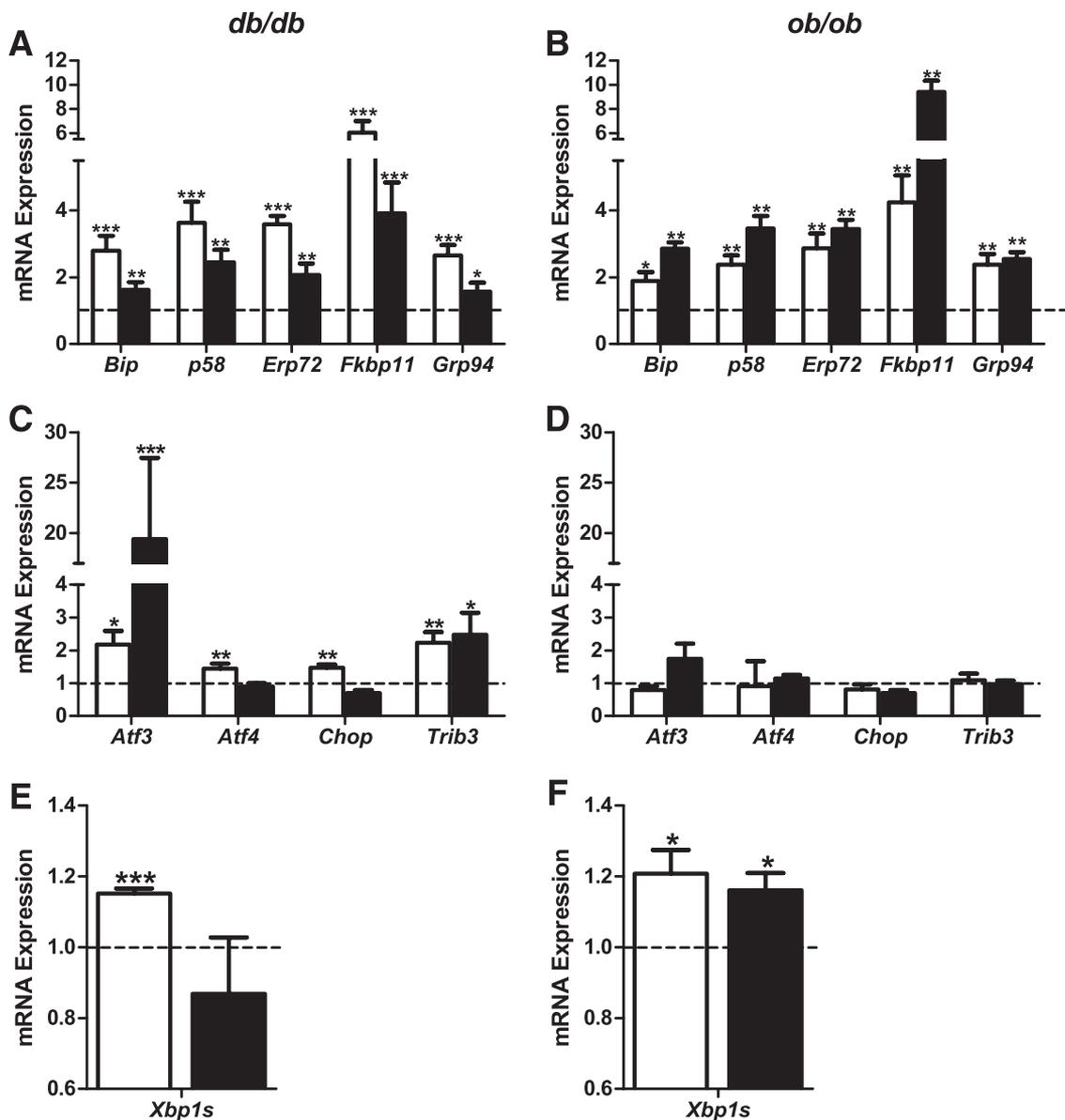
FIG. 3. Changes in mRNA expression of UPR genes in islets of *db/db* and *ob/ob* mice at 6 and 16 weeks of age. Islets were isolated from 9 to 10 C57BL/KsJ control and 8 *db/db* mice at 6 weeks of age, 13 to 16 C57BL/KsJ control and 9 to 12 *db/db* mice at 16 weeks of age, 6 C57BL/6J control and 6 *ob/ob* mice at 6 weeks of age, and 4 to 5 C57BL/6J control and 7 *ob/ob* mice at 16 weeks of age. Shown are changes for the indicated genes in islets of *db/db* (*A, C, E*) and *ob/ob* (*B, D, F*) mice at 6 (*white bars*) and 16 (*black bars*) weeks of age. *A–D*: Total RNA was extracted, reverse-transcribed, and analyzed by real-time RT-PCR. mRNA levels were expressed as fold change of the levels in respective age-matched controls (represented by the *dashed line*). *E* and *F*: *Xbp1* cDNA was amplified by PCR and digested with *Pst*I, which cuts unprocessed *Xbp1* into fragments. Processed (activated) *Xbp1* lacks the restriction site and remains intact. Processed (intact) and unprocessed (cut) *Xbp1* were quantified by densitometry. The value obtained for processed *Xbp1* was expressed as a ratio of the total (processed + unprocessed) *Xbp1* mRNA level for each sample. These ratios are expressed as fold change of the ratio in respective age-matched controls. All results are mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ genotype effect in each age group.

factors toward the levels apparent in islets from non-diabetic control mice. The lowered mRNA levels for *Pdx1*, *MafA*, and *Nkx6.1* in untreated *db/db* islets were significantly reversed toward normal in PBA-treated *db/db* islets (Fig. 6*A*). *Beta2* mRNA levels displayed a tendency for restoration toward normal in PBA-treated *db/db* islets. PBA treatment of *db/db* islets also led to the partial restoration toward normal of several genes involved in β-cell glucose metabolism and function. The reduced mRNA levels of *PC* and *mGPDH* in untreated *db/db* islets were significantly restored toward normal in PBA-treated *db/db* islets (Fig. 6*A*). In contrast, PBA treatment had no effect on the reduced expression of *Kir6.2* or *Gpr40* in *db/db*

islets (Fig. 6*A*). However, *Glp1r* mRNA levels were normalized in PBA-treated *db/db* islets to levels equivalent to nondiabetic control mouse islets (Fig. 6*A*). These changes in gene expression were associated with increased insulin secretion in PBA-treated *db/db* islets (Fig. 6*B*). Furthermore, these changes were associated with upregulation of the adaptive UPR and XBP1 splicing in PBA-treated *db/db* islets (Fig. 7). The data demonstrate that improving the adaptive UPR gene expression in *db/db* islets leads to the partial recovery of β-cell gene expression and insulin secretion.

We examined the effects of another chemical chaperone, TMAO. No significant differences were observed in
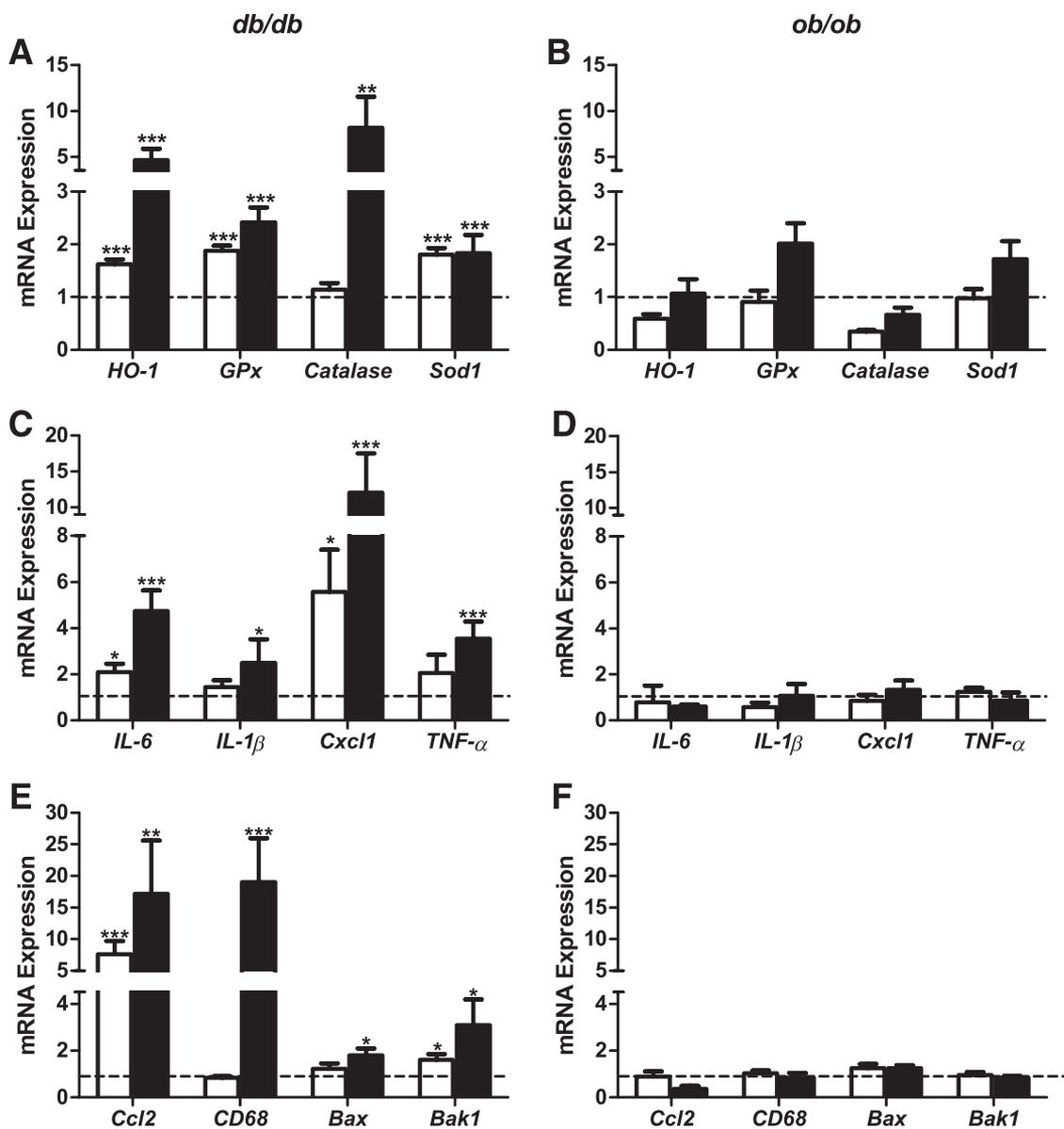
FIG. 4. Changes in mRNA expression of antioxidant and inflammation genes in islets of *db/db* and *ob/ob* mice at 6 and 16 weeks of age. Islets were isolated from 9 to 10 C57BL/KsJ control and 8 *db/db* mice at 6 weeks of age, 13 to 16 C57BL/KsJ control and 9 to 12 *db/db* mice at 16 weeks of age, 6 C57BL/6J control and 6 *ob/ob* mice at 6 weeks of age, and 4 to 5 C57BL/6J control and 7 *ob/ob* mice at 16 weeks of age. Total RNA was extracted, reverse-transcribed, and analyzed by real-time RT-PCR. mRNA levels were expressed as fold change of the levels in respective age-matched controls (represented by the *dashed line*). Shown are changes for the indicated genes in islets of *db/db* (*A, C, E*) and *ob/ob* (*B, D, F*) mice at 6 (*white bars*) and 16 (*black bars*) weeks of age. All results are mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ genotype effect in each age group.

islet-associated gene expression or insulin secretion in TMAO-treated *db/db* islets compared with untreated *db/db* islets (Supplementary Fig. 6). In association with this, TMAO treatment had no effect on adaptive UPR gene expression in *db/db* islets (Supplementary Fig. 7). These findings suggest that the attenuation of ER stress alone is insufficient to induce improvements in gene expression and insulin secretion in *db/db* islets. Taken together, the data suggest that suppression of the adaptive UPR makes a necessary contribution to abnormalities of the β-cell phenotype in diabetes-prone mice.

We also tested the effects of PBA treatment on the changes in antioxidant and inflammatory stress gene expression in *db/db* islets (Supplementary Fig. 8). The higher levels of *HO-1* and *GPx* mRNA in *db/db* islets were not affected by PBA treatment. Similarly, the improvement in

chaperone activity was without effect on inflammatory cytokines or chemokines expression; the higher mRNA levels of *IL-6*, *IL-1β*, *Cxcl1*, and *TNF-α* were not different between untreated and PBA-treated *db/db* islets (Supplementary Fig. 8).

DISCUSSION

Here, we have used mouse models of obesity with opposing disposition to develop diabetes to study the mechanisms of β-cell compensation and failure. The data demonstrate that "robust" β-cells of diabetes-resistant *ob/ob* mice and "susceptible" β-cells of diabetes-prone *db/db* mice display several striking differences with time. Robust β-cells display a sustained adaptive UPR, a preserved β-cell phenotype, and an absence of apoptotic, inflammatory, or oxidative
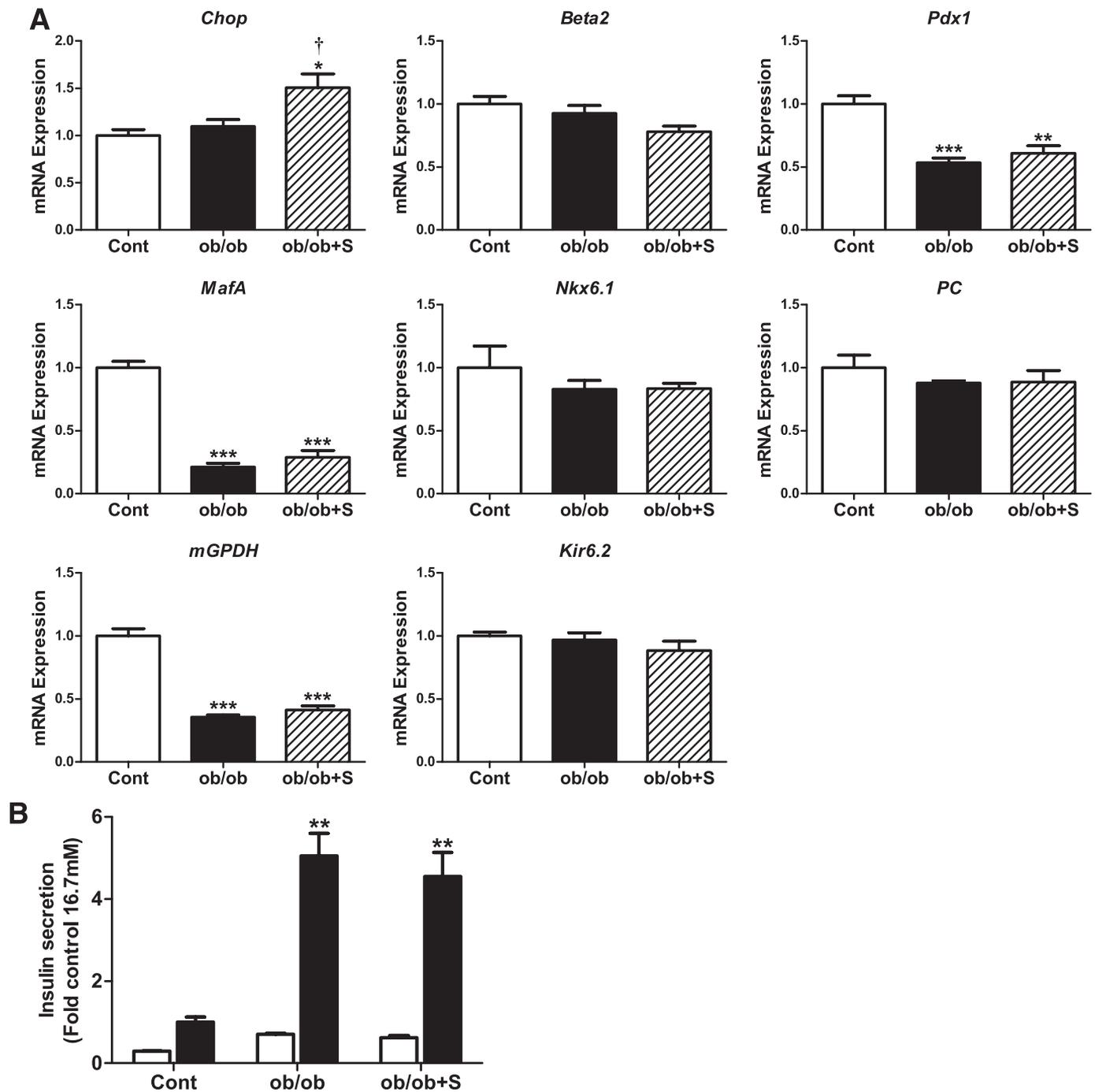
FIG. 5. Effect of enhancing signaling downstream of PERK/eIF2α on the changes in gene expression and insulin secretion in islets of *ob/ob* mice. *A*: Islets isolated from *ob/ob* and age-matched control mice were cultured in the absence (control [cont], *white bars*; *ob/ob*, *black bars*) or presence (*ob/ob+S*, *striped bars*) of salubrinal (75 μmol/L) for 24 h. Total RNA was extracted, reverse-transcribed, and analyzed by real-time RT-PCR. mRNA levels were expressed as fold change of the level in control islets. *n* = 3–6 in each group. *B*: Batches of islets were cultured in Krebs-Ringer HEPES buffer containing 0.1% BSA and 2.8 mmol/L (*white bars*) or 16.7 mmol/L glucose (*black bars*) for 1 h. Insulin was measured in an aliquot of the buffer by radioimmunoassay. Insulin secretion was expressed as fold change of the level in control islets cultured in 16.7 mmol/L glucose. *n* = 3–6 in each group. All results are mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ genotype effect; †$P < 0.05$ salubrinal treatment effect in *ob/ob* mouse islets.

stress gene expression. In contrast, susceptible β-cells display a decline in the adaptive UPR, a progressive loss of β-cell differentiation, and the presence of apoptotic, inflammatory, and oxidative stress gene expression. The change in phenotype of susceptible β-cells is partially reversed by PBA treatment, suggesting that suppression of the adaptive UPR contributes to the loss of β-cell differentiation. These findings raise the possibility that the maintenance of

the adaptive UPR provides a molecular link between obesity-associated insulin resistance and β-cell compensation and, conversely, that suppression of the adaptive UPR, together with inflammation, oxidative stress, and the loss of β-cell differentiation, underlies β-cell failure and progression to diabetes (Fig. 8).

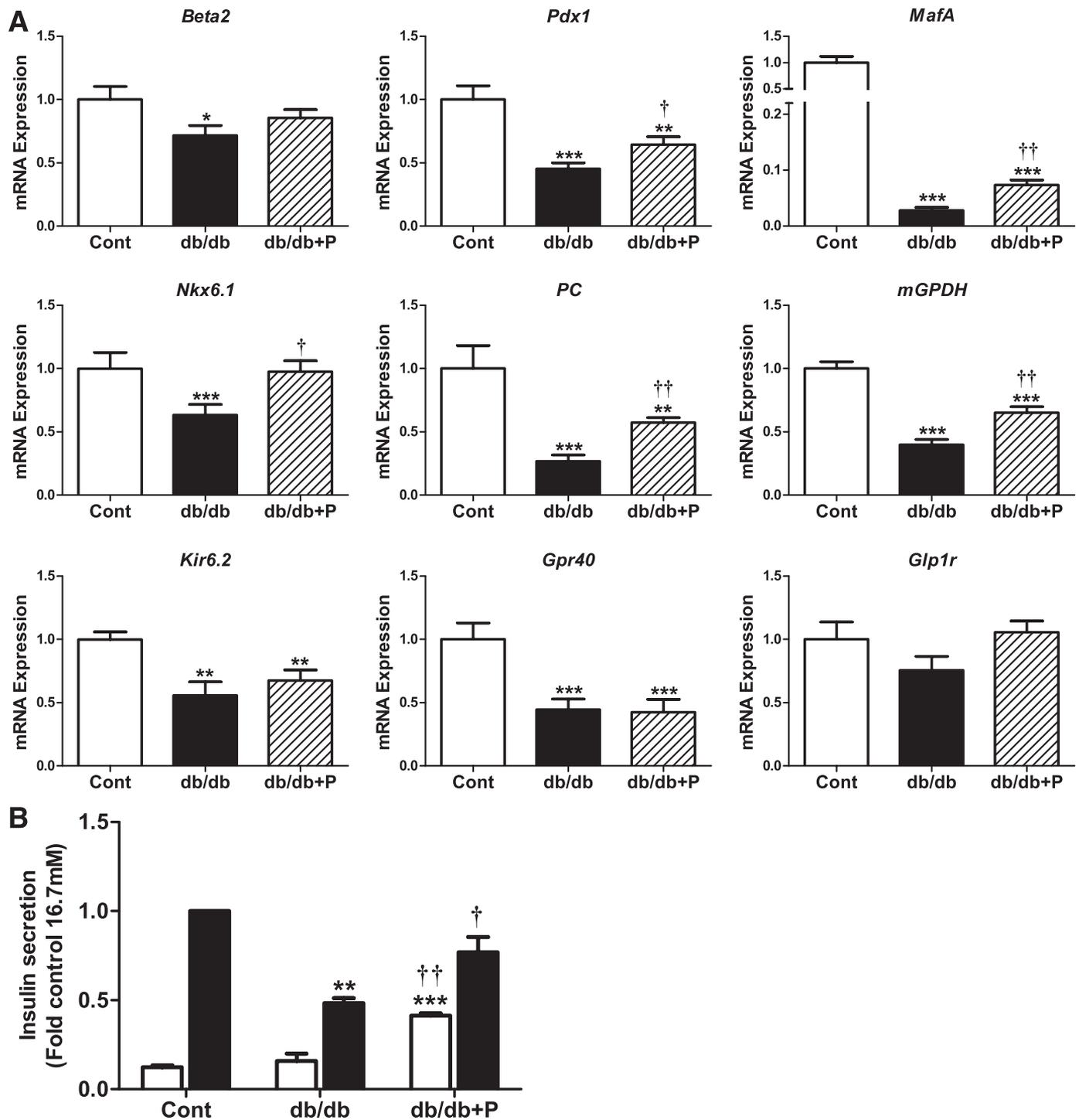**Mechanisms of β-cell compensation.** β-cell compensation for obesity-induced insulin resistance likely involves

FIG. 6. Effect of chemical chaperone treatment on the changes in gene expression and insulin secretion in islets of *db/db* mice. *A*: Islets isolated from diabetic *db/db* and age-matched nondiabetic control [cont] mice (12–14 weeks of age) were cultured in the absence (control, *white bars*; *db/db*, *black bars*) or presence (*db/db+P*, *striped bars*) of the chemical chaperone PBA (2.5 mmol/L) for 24 h. Total RNA was extracted, reverse-transcribed, and analyzed by real-time RT-PCR. mRNA levels were expressed as fold change of the level in control islets. *n* = 7 in each group. *B*: Batches of islets were cultured in Krebs-Ringer HEPES buffer containing 0.1% BSA and 2.8 mmol/L (*white bars*) or 16.7 mmol/L glucose (*black bars*) for 1 h. Insulin was measured in an aliquot of the buffer by radioimmunoassay. Insulin secretion was expressed as fold change of the level in control islets cultured in 16.7 mmol/L glucose. *n* = 3 in each group. All results are mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ genotype effect; †$P < 0.05$, ††$P < 0.01$ PBA treatment effect in *db/db* mouse islets.

both increased β-cell mass and enhanced insulin secretion per β-cell (2,3). Interestingly, a partial loss of β-cell differentiation was observed in islets from 6-week-old *ob/ob* mice as well as in prediabetic *db/db* mice. This may be reflective of increased proliferation and its inverse relationship with differentiation (28,29). However, the β-cell phenotype in *ob/ob* islets recovers rather than deteriorates with time. Perhaps β-cell mass eventually equilibrates with increased insulin demand as a result of enhanced proliferation in the early stages combined with an absence of apoptotic signaling. GSIS was enhanced in *ob/ob* and *db/db* mice at 6 weeks of age, and this was unexpectedly
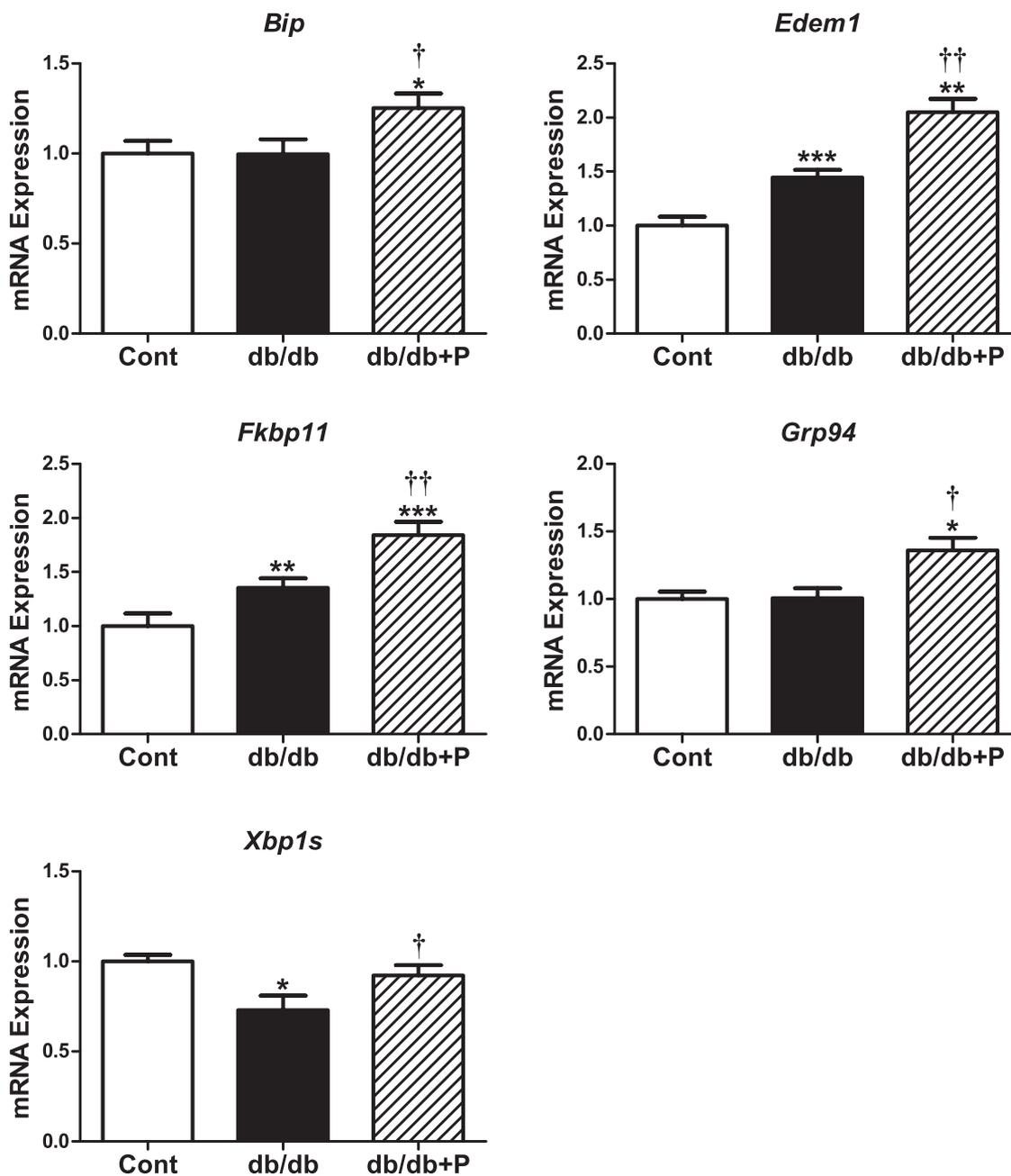
FIG. 7. Effect of chemical chaperone treatment on the expression of adaptive UPR genes in islets of *db/db* mice. Islets isolated from diabetic *db/db* and age-matched nondiabetic control (cont) mice (12–14 weeks of age) were cultured in the absence (control, *white bars*; *db/db*, *black bars*) or presence (*db/db*, *striped bars*) of the chemical chaperone PBA (2.5 mmol/L) for 24 h. Total RNA was extracted, reverse-transcribed, and analyzed by real-time RT-PCR. mRNA levels were expressed as fold-change of the level in control islets. *Xbp1* splicing was analyzed and expressed as described in Fig. 3. All results are mean ± SEM. $n = 7$ in each group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ genotype effect; †$P < 0.05$, ††$P < 0.01$ PBA treatment effect in *db/db* mouse islets.

associated with increased expression of adaptive UPR genes. This suggests that ER stress is an early response to obesity, but also that some features of the UPR are beneficial, helping with β-cell adaptation in the compensation for obesity-associated insulin resistance. This is generally consistent with findings of UPR activation in obese nondiabetic human pancreata (30) and of the requirement of an intact UPR for normal β-cell function and glucose homeostasis in humans and mice (11,31). The maintenance of the adaptive UPR under conditions of obesity may facilitate the ongoing requirement for enhanced rates of insulin processing and secretion. Our findings suggest that XBP1

activation may play a role in the regulation of the adaptive UPR under these conditions, although coordination with the other arms of the ER stress response is likely. Alteration of XBP1 activation alone has profound effects on β-cell gene expression, insulin secretion, and apoptosis (32,33).

Mechanisms of β-cell failure. ER stress appears sufficient to cause β-cell failure (11,31,34–36), and its potential role in the pathogenesis of type 2 diabetes is well-supported (9,10,30,37). However, our findings suggest that the presence of ER stress activation is, in itself, not an indicator of β-cell failure; rather, the nature of the subsequent response is
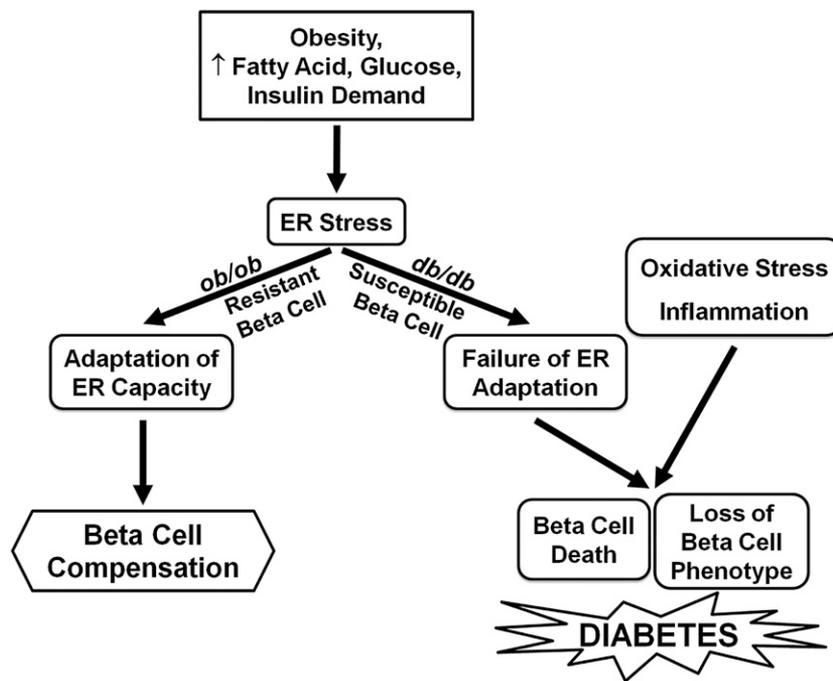
FIG. 8. Proposed mechanisms contributing to β-cell compensation and failure during progression to type 2 diabetes. Obesity and its associated metabolic changes including hyperlipidemia, glucose intolerance, and increased insulin demand lead to ER stress in pancreatic β-cells. In normal β-cells, upregulation of the adaptive UPR facilitates the enhancement of ER capacity, maintenance of β-cell compensation, and prevention of diabetes. In genetically susceptible β-cells, suppression of ER adaptation together with oxidative stress and inflammation leads to the loss of β-cell phenotype and increased β-cell death that ultimately results in type 2 diabetes.

critical in the regulation of β-cell function and survival. The expression of several proapoptotic ER stress genes, including *Chop* (12,37,38), *Atf3* (39,40), and *Trib3* (41), have been implicated in β-cell dysfunction and death, whereas upregulation of the adaptive UPR may confer protection from β-cell failure and diabetes (11,42). Importantly, the regulation of this process is not solely dependent on obesity, hyperlipidemia, or increased insulin demand because these parameters were similar in *db/db* and *ob/ob* mice. Therefore, the factors leading to the decline in XBP1 activation and suppression of the adaptive UPR in diabetic *db/db* mice remain unknown. Hyperglycemia may play a role (43–47), although previous studies suggest that elevated glucose levels increase rather than reduce XBP1 splicing and adaptive UPR gene expression. Interestingly, our recent studies demonstrate that inflammatory cytokines exert an inhibitory effect on XBP1 and the adaptive UPR in β-cells (48). Therefore, the low-grade inflammation observed in islets of type 2 diabetes models may influence β-cell function and survival via regulation of the pattern of downstream UPR signaling. Recent studies also demonstrate complex interactions between the UPR and oxidative stress in β-cells (37,46,49).

Our studies support and extend the case for increasing chaperone capacity of the ER as a therapeutic approach for type 2 diabetes (11,50). Chaperone treatment in vivo protects against insulin resistance (27) and β-cell dysfunction (46,51). Our studies demonstrate that part of the benefit of improving the chaperone activity of the ER in diabetes includes partial recovery in the expression of genes important for the maintenance of β-cell differentiation and function. Because our studies were performed ex vivo, the effects are likely direct and not secondary to improvements in glucose tolerance (27,51).

Our studies also support the roles of inflammation and oxidative stress as potential contributors to β-cell failure.

Both features were completely absent in the robust islets of diabetes-resistant mice but were progressively exacerbated in susceptible islets. Their precise contributions remain to be clarified, and complex interactions between inflammation, oxidative stress, and ER stress are probable and of likely importance (37,40,46,48,49).

Interestingly, many genes found to be regulated by the UPR previously were reported to be dependent on hyperglycemia in *db/db* islets (7). In particular, the islet-associated transcription factors and selective genes important for β-cell function commonly are regulated by the UPR and hyperglycemia. The divergent regulation of other genes may have been influenced by the different time course of analyses, 2-week (7) and 24-h treatments for hyperglycemia and the UPR, respectively.

In conclusion, our study demonstrates for the first time the markedly different patterns of time-dependent changes in islet gene expression in obese mouse models of β-cell compensation and β-cell failure. The unique gene expression patterns of adaptive UPR, inflammation, oxidative stress, and β-cell differentiation in these models provide important insight into the regulation of β-cell function and survival under conditions of obesity. The role of the adaptive UPR in the protection against diabetes has been largely overlooked in studies investigating the association between deleterious UPR signaling with β-cell dysfunction and apoptosis. Thus, our study is the first to link failure of the adaptive UPR in islets of obese mice with abnormalities in β-cell gene expression and progression to diabetes.

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