Chop deletion reduces oxidative stress, improves β cell function, and promotes cell survival in multiple mouse models of diabetes

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The progression from insulin resistance to type 2 diabetes is caused by the failure of pancreatic β cells to produce sufficient levels of insulin to meet the metabolic demand. Recent studies indicate that nutrient fluctuations and insulin resistance increase proinsulin synthesis in β cells beyond the capacity for folding of nascent polypeptides within the endoplasmic reticulum (ER) lumen, thereby disrupting ER homeostasis and triggering the unfolded protein response (UPR). Chronic ER stress promotes apoptosis, at least in part through the UPR-induced transcription factor C/EBP homologous protein (CHOP). We assessed the effect of Chop deletion in multiple mouse models of type 2 diabetes and found that Chop+/− mice had improved glycemic control and expanded β cell mass in all conditions analyzed. In both genetic and diet-induced models of insulin resistance, Chop deficiency improved β cell ultrastructure and promoted cell survival. In addition, we found that isolated islets from Chop+/− mice displayed increased expression of UPR and oxidative stress response genes and reduced levels of oxidative damage. These findings suggest that CHOP is a fundamental factor that links protein misfolding in the ER to oxidative stress and apoptosis in β cells under conditions of increased insulin demand.

Introduction

Type 2 diabetes (T2D) is a world-wide disease of epidemic proportions that is estimated to afflict more than 180 million individuals, with approximately 2.9 million associated deaths per year (1, 2). Because loss of β cell function and mass are central events in the development and progression of T2D, they are key therapeutic targets for treatment of this disease (3, 4). In T2D, β cell toxicity has been linked to stimuli including glucose, lipids, proinflammatory cytokines, glycation products, and islet amyloid (4). Both inflammatory cytokines, glycation products, and islet amyloid (4).

A number of cellular insults disrupt protein folding and cause accumulation of unfolded protein in the ER lumen, including reduction in ER calcium stores, altered protein glycosylation, increased protein expression, unbalanced expression of protein subunits, or expression of difficult-to-fold or mutant inherently misfolded proteins. The UPR is an adaptive response signaled through 3 ER-localized transmembrane sensors, the protein kinases dsRNA-activated protein kinase–like ER kinase (PERK) and inositol-requiring protein 1α (IRE1α) and the transcription factor activating transcription factor 6 (ATF6). This response collectively halts initiation of translation to reduce the load upon the ER; induces expression of protein chaperones, catalysts of folding, and protein degradation machinery; and also engages cellular death signaling (14, 15). PERK-mediated phosphorylation of the eukaryotic translation initiation factor 2 (eIF2) on Ser51 of the α subunit (eIF2α) attenuates general mRNA translation. However, translation of some mRNAs is paradoxically increased, of which the mRNA encoding the transcription factor ATF4 is the most characterized example (16). IRE1α is conserved in all eukaryotic cells and has protein kinase and endoribonuclease activities that, upon activation, mediate unconventional splicing of a 26-base intron from X-box binding protein 1 (Xbp1) mRNA to produce a potent transcription factor (17). ATF6 is a basic leucine zipper-containing transcription factor that, upon accumulation of unfolded protein in the ER lumen, transits to the Golgi compartment, where it is cleaved to yield a cytosolic fragment that migrates to the nucleus to activate gene transcription (18, 19). If the UPR adaptive response is not sufficient to resolve the protein-folding defect, ER dysfunction can aggravate cellular function and lead to apoptotic cell death (20, 21). Growing evidence supports the notion that UPR signaling improves ER homeostasis through attenuation of protein synthesis and increased ER-associated pro-

Nonstandard abbreviations used: ATF4, activating transcription factor 4; CHOP, C/EBP homologous protein; eIF2, eukaryotic translation initiation factor 2; GSIS, glucose-stimulated insulin secretion; HF, high fat; HODE, hydroxyoctadecadienoic acid; IRE1α, inositol-requiring protein 1α; PERK, dsRNA-activated protein kinase-like ER kinase; STZ, streptozotocin; T2D, type 2 diabetes; TEM, transmission electron microscopy; UPR, unfolded protein response; XBP1, X-box binding protein 1.

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tein degradation (ERAD). However, there is less compelling evi-
dence that supports the idea that the UPR can improve the secre-
tion capacity of the cell. The latter conclusion is largely inferred
from gene deletion studies in which defects of ER stress signal-
ing pathways reduce survival and/or differentiation of cells that
secrete large amounts of protein.

It is now evident that conditions associated with high levels of
ER stress can severely compromise β cell function (15). This
is apparent from the β cell failure associated with mutations in
murine and human proinsulin that disrupt disulfide bond pair-
ing and cause misfolding and accumulation of proinsulin in the
ER lumen of β cells (22–26). In addition, mutations that reduce
signaling through the primary sensors of the UPR or interfere with
protein chaperone functions of the UPR impair β cell health (9,
27–31). Finally, stimuli, such as glucose, free fatty acids, cytokines,
and nitric oxide compromise β cell function and induce UPR gene
expression (12, 32–37). Fundamentally, glucose regulates both
PERK-mediated eIF2α phosphorylation and IRE1α-mediated Xbp1
mRNA splicing. Periodic increases in glucose, as well as chronic
hyperglycemia, activate IRE1α in vivo and in isolated rat islets (29,
38). Elevated glucose concentrations increase the rate of mRNA
translation through phosphatase-mediated dephosphorylation of
eIF2α, and the unfolded protein load activates the UPR (38, 39).

The significance of the PERK/eIF2α pathway in supporting β cell
function is underscored by the observation that mice and humans
harboring loss-of-function mutations in PERK develop diabetes
due to loss of β cell mass (13, 27, 40). In addition, homozygous
misense Ser51Ala mutation at the PERK phosphorylation site in
eIF2α causes β cell deficiency in mice (28). Finally, heterozygous
Ser51Ala eIF2α mice fed a high-fat (HF) diet to induce insulin resist-
ance develop β cell failure (9). These genetic alterations of both
PERK and eIF2α increase mRNA translation so that the protein-
folding load exceeds the protein-folding capacity of the ER.

Although increasing evidence indicates that ER stress occurs in
β cells, possibly as a consequence of increased proinsulin synthe-
sis to compensate for insulin resistance, it is unknown whether β
cell failure and loss of glucose homeostasis in vivo result from an
inadequate UPR adaptive response or activation of an apoptotic
response due to chronic, unresolved ER stress. As it is not pos-
ible to address this question in simple cell culture models of β
cell apoptosis, we have studied whether the absence of CHOP, a
key protein implicated in UPR-induced cell death, can prevent loss
of β cell function and mass due to dietary or genetically induced
insulin resistance in vivo.

CHOP was identified as an ER stress–induced transcription factor
that is a significant mediator of apoptosis in response to ER stress
(41, 42). CHOP gene induction is primarily mediated through the
PERK/eIF2α/ATF4 UPR pathway, although IRE1α/XBP1 and ATF6α
pathways also contribute (43–47). CHOP expression is increased in β
cells from diabetic mice and humans (10–12, 48). Although Chop-null
mice do not have a readily detectable phenotype under basal condi-
tions (36, 41), β cells from Chop-null mice are protected from apop-
tosis caused by either nitric oxide (36) or accumulation of a folding-
defective mutant of proinsulin (48). However, as CHOP is not the
only death signal evoked by ER stress, the requirement for CHOP in
β cell failure associated with T2D is unknown. Here, we show that
Chop deletion increases the capacity of islets to produce insulin and
curtails the progression of insulin resistance to diabetes. We dem-
onstrate that deletion of Chop not only prevents β cell apoptosis,
but also improves β cell function by preventing oxidative damage in
response to protein misfolding in the ER. The findings demonstrate
that CHOP is a fundamental factor that links protein misfolding in
the ER to oxidative stress and apoptosis in β cells.

Results
Chop-null mutation increases obesity but prevents glucose intolerance
in HF diet–fed eIF2αS/A mice. Although mice with heterozygous
Ser51Ala mutation at the PERK phosphorylation site in eIF2α
exhibit reduced attenuation of mRNA translation upon ER stress,
they did not exhibit a readily apparent phenotype under standard
conditions of diet. Analysis of glucose-stimulated translation in
islets isolated from HF diet–fed heterozygous Ser51Ala mice,
however, revealed an elevated rate of translation (9). These HF
diet–fed mice develop diabetes and represent what we believe to
be a novel model of β cell failure that results from ER stress due
to elevated proinsulin biosynthesis as a consequence of interac-
tion between genetic (eIF2αS/A allele) and environmental (HF diet)
factors (9). We asked whether β cell survival and/or function are
improved in these heterozygous Ser51Ala mutant mice when the
CHOP-mediated death signal is absent. Deletion of the Chop gene
modestly increased weight gain in HF diet–fed wild-type eIF2αS/S
mice, consistent with recent observations (49). In contrast, Chop
deletion significantly increased obesity in HF diet–fed eIF2αS/A
mice (Figure 1A). The enhanced weight gain of eIF2αS/A/Chop−/−
animals may be caused by accentuation of the metabolic defect
previously reported for the eIF2αS/A mice (9), may be a con-
sequence of the deletion of CHOP action as a negative regulator
of adipogenesis (50, 51), or may be driven by hyperinsulinemia (see
below). Glucose intolerance appeared after 5 weeks of HF
diet in eIF2αS/A/Chop−/− mice compared with HF diet–fed control
eIF2αS/A mice. In contrast, eIF2αS/A mice with the Chop-null
mutation displayed normal glucose tolerance for up to 32 weeks
of HF diet despite their overt obesity (Figure 1B and Supplemen-
tal Figure 1A; supplemental material available online with this
article; doi:10.1172/JCI34587DS1).

Chop-null mutation preserves β cell morphology and function in HF
diet–fed eIF2αS/A mice. The improved glucose tolerance observed
in the eIF2αS/A/Chop−/− mice was not due to increased insulin
sensitivity (Supplemental Figure 2A), but rather was associated
with a 6-fold increase in islet mass and pancreas insulin content
(Figure 1C and Supplemental Figure 1, B and C). Ultrastructural
analysis was performed to monitor the distension of ER cisternae
and reduction of secretory granule content characteristic of ER
stress and β cell failure. Compared with β cells from HF diet–fed
eIF2αS/S mice, β cells from HF diet–fed eIF2αS/A/Chop−/− mice dis-
played a significantly distended ER and reduced insulin granule
number, as previously reported (9). However, strikingly, the num-
ber of dense-core insulin granules in eIF2αS/A/Chop−/− mice was
not significantly reduced compared with those in eIF2αS/A/Chop−/
mice or eIF2αS/S/Chop−/− mice, although ER distension was still
detectable (Figure 1, D and E).

The improved glucose tolerance and preserved granule content
suggested that Chop deletion preserves β cell function by maintain-
ing an adequate pool of secretory granules that were responsive
to nutrient stimuli. Consistent with this theory, after 35 weeks of
HF diet, the serum insulin levels were increased 2- to 3-fold in
eIF2αS/S/Chop−/− mice compared with eIF2αS/A/Chop−/−, eIF2αS/S/Chop−/
and eIF2αS/A/Chop−/− mice (Figure 1F). Glucose-stimulated insulin
secretion (GSIS) was significantly reduced in islets isolated from
eIF2αS/S/Chop−/− mice compared with wild-type eIF2αS/S/Chop−/− mice.
In contrast, islets from HF diet–fed eIF2αS/AChop−/− mice remained glucose responsive for insulin secretion (Figure 1G). As the GSIS studies were performed with selected islets of similar size and the secretion of insulin was expressed as a percentage of total insulin content, the improved GSIS observed in eIF2αS/AChop−/− islets was not due to increased β cell mass, but rather reflected a genuine improvement in β cell function. These findings show that, despite the HF diet and overt obesity, glucose homeostasis was maintained in eIF2αS/AChop−/− mice because there was an increase in the number of functional β cells as measured by insulin granule content and GSIS. The results suggest that β cell failure in this eIF2αS/A mutant mouse model is mediated through the UPR-inducible gene Chop. This conclusion is also supported by the observation that Chop deletion reduced apoptosis and increased β cell mass in pancreata from homozygous eIF2αSer51Ala embryos (Supplemental Figure 3).

Chop-null mutation prevents loss of β cell mass and diabetes in a HF diet/streptozotocin model of T2D. The previous data indicated that CHOP plays a negative role in β cell function when ER stress signaling is compromised in conjunction with a biosynthetic burden of enhanced proinsulin translation due to insulin resistance. We next evaluated the role of CHOP under conditions that combine the primary determinants of T2D, insulin resistance, and inadequate β cell function and mass in the absence of a genetic predisposition to β cell failure. Wild-type and Chop-null mice were fed a 60% HF diet for 5–6 weeks, and β cell mass was reduced by administration of a moderate dose of streptozotocin (STZ). This treatment increases the proinsulin biosynthetic burden upon the remaining β cells and challenges their ability to survive and function. This approach has been successful in evaluating therapeutic strategies that alter insulin resistance or improve β cell function (52–54).
the absence of any additional determinant of obesity, the Chop–null mice exhibited a slight increase in weight that was not statistically different from wild-type animals (Figure 2A). However, the fed glucose levels of Chop-null mice were significantly lower than those of wild-type mice after 5–6 weeks of HF diet feeding. In addition, the overt hyperglycemia that developed in wild-type mice 4 days after administration of STZ was averted in Chop-null mice (Figure 2B).

The improved glycemic control of Chop-null HF diet-fed, STZ-treated animals was investigated by analysis of glucose tolerance, insulin tolerance, insulin secretion, insulin content, and islet morphology (Figure 2, C–J). In the absence of HF diet or with 45% HF feeding, glucose tolerance and β cell function were similar between wild-type and Chop−/− mice (Figure 1, Supplemental Figure 1, Figure 3, and Supplemental Figure 4). However, under conditions of 60% HF diet, glucose intolerance was more severe in wild-type mice compared with Chop−/− mice (Figure 2C). One week after STZ administration, the wild-type mice were severely hyperglycemic and glucose intolerant, while the Chop−/− mice were only mildly hyperglycemic and glucose intolerant (Figure 2, B and D). Blood glucose levels were 50% lower, and insulin secretion upon fasting and refeeding was signifi-

**Figure 2**

Chop-null mutation prevents hyperglycemia and glucose intolerance by maintaining insulin content and secretion in a HF diet–fed, STZ-treated nongenetic model of T2D. Chop+/+ and Chop−/− mice were fed a 60% HF diet (HFD) for 5.5 weeks prior to administration of a dosage of 150 mg/kg STZ as described in Methods, and measurements were performed for up to 16 days after STZ with continued HF feeding. (A) Body weight. (B) Fed blood glucose levels. (C and D) Glucose tolerance measurements. Glucose tolerance was tested after HF diet alone for 5.5 weeks (C) and 4 days after STZ treatment and continued HF diet (D). (E and F) Fasting and refed blood glucose and serum insulin levels. Glucose and insulin measurements were taken 13 days after STZ treatment from mice that were fasted overnight and refed for 3.5 hour. (G–I) Serum was collected for measurement and mice were sacrificed for determination of pancreatic insulin content and histology 16 days after STZ administration. (G) Fed serum insulin levels, (H) pancreatic insulin content, and (I) islet morphology stained with H&E. Scale bars: 500 μm (top), 100 μm (bottom). (J) Insulin tolerance measurements. Insulin tolerance was tested 15 days after STZ treatment. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 3
Chop-null mutation increases obesity and maintains glucose tolerance in Lepr<sup>db/db</sup> mice through expanded β cell mass and improved cell function. Analysis was performed on samples collected from mice at 9–10 (B–E) or 6 (F and G) months of age. (A) Body mass. Representative mice at 20 wk of age are shown. (B) Glucose tolerance tests; n = 3–5 mice per condition. Significant differences between Lepr<sup>β<sub>0</sub></sup>Chop<sup>+/+</sup> and Lepr<sup>β<sub>0</sub></sup>Chop<sup>−/−</sup> are indicated. (C) Islet morphology from H&E and immunofluorescence staining. Scale bars: 400 μm (top), 50 μm (bottom). (D) Serum insulin levels; n = 7–17 mice per condition. (E) GSIS analysis; islets from 2 mice per condition were analyzed in triplicate. (F and G) TEM images of β cells and insulin granule quantitation from similar total areas from 2 mice per condition. Scale bar: 1 μm. *P < 0.05, **P < 0.01, ***P < 0.001.

Cantly elevated in Chop-null mice versus wild-type mice (Figure 2, E and F). Analysis of fed insulin levels, pancreatic insulin content, and islet morphology confirmed that the improved glucose homeostasis in the Chop-null mice was coincident with elevated basal insulin levels and a larger islet/β cell mass (Figure 2, G–I). There was no significant difference in insulin tolerance (Figure 2J). GSIS was not measured in islets isolated from these mice due to the low β cell mass of the wild-type mice in this model. However, in vitro STZ treatment of islets isolated from wild-type and Chop-null mice did not reveal any differences in the acute toxicity of STZ, as GSIS was fully inhibited in both populations of islets (data not shown), suggesting that Chop deletion influences subsequent β cell recovery and/or function.

Chop-null mutation prevents glucose intolerance and improves β cell function in Lepr<sup>db/db</sup> mice. We proceeded to investigate whether Chop-null mutation could prevent β cell loss in the leptin receptor-deficient Lepr<sup>β<sub>0</sub></sup> mouse, a model of diabetes that encompasses obesity, insulin resistance, and β cell failure. The β cell defects in the Lepr<sup>β<sub>0</sub></sup> mouse model depend on the C57BL/KsJ strain background. In this model, we were able to study the Chop contribution to β cell failure by analyzing first-generation littersmates from crosses between double heterozygous Chop<sup>−/−</sup> and Lepr<sup>β<sub>0</sub></sup> mice in a mixed C57BL/KsJ and C57BL/6J background. All homozygous Lepr<sup>β<sub>0</sub></sup> progeny displayed hyperglycemia in the presence of the wild-type Chop allele, indicating there was sufficient C57BL/KsJ genetic contribution to elicit the diabetic phenotype.

Although the obesity of Lepr<sup>β<sub>0</sub></sup> mice was further increased by Chop-null mutation (Figure 3A), development of fasting hyperglycemia and glucose intolerance was dramatically prevented (Figure 3B and Supplemental Figure 4, A and B), similar to the effect of Chop-null mutation in the HF diet-fed eIF2α<sup>−/−</sup> mice and HF diet-fed, STZ-treated mice. The improved glucose tolerance was not a consequence of increased sensitivity to insulin (Supplemental Figure 2B). Histological examination indicated that Chop deletion was associated with a 6-fold increase in islet mass in the Lepr<sup>β<sub>0</sub></sup> mice, and this correlated with serum hyperinsulinemia and glucose-responsive insulin secretion in vitro (Figure 3, C–E, and Supplemental Figure 4C). Ultrastructural analysis demonstrated that islets from Lepr<sup>β<sub>0</sub></sup> mice contained significantly fewer insulin granules than islets from Lepr<sup>β<sub>0</sub></sup> mice (Figure 3, F and G). In contrast, granule depletion was significantly attenuated in Lepr<sup>β<sub>0</sub></sup> mice that harbor Chop deletion (Figure 3, F and G). In summary, these studies suggest that Chop deletion improves β cell function in the Lepr<sup>β<sub>0</sub></sup> mouse.

Chop-null mutation causes β cell proliferation and reduces β cell apoptosis in the islets of Lepr<sup>β<sub>0</sub></sup> mice. To elucidate how Chop deletion may affect β cell mass, we studied β cell replication and antioxidative stress responses suggested that Chop deletion improved the capacity of β cells to accommodate oxidative stress. Therefore, we measured products of protein oxidation (carboxyls) and lipid peroxidation (hydroxyoctadecadienoic acid [HOME]) in...
isolated islets. Islets from Lepr<sup>db/db</sup> diabetic mice displayed a 3-fold increase in protein carbonyls and a 2-fold increase in HODEs compared with those from control Lepr<sup>db/+</sup> mice (Figure 6A). Therefore, insulin resistance in the Lepr<sup>db/db</sup> mice was associated with oxidative stress in the islets, an observation consistent with literature that indicates antioxidant molecules can improve glucose homeostasis, restore β cell function, and reduce oxidative stress markers in the islets of Lepr<sup>db/db</sup> mice (55, 60–63). In contrast, Chop deletion significantly reduced both products of protein oxidation and lipid peroxidation in islets from these obese Lepr<sup>db/db</sup> mice.

The reduction in oxidative damage observed upon Chop deletion may be result from protection of the islets from oxidative damage caused by ROS or it may be an indirect consequence of improved glycemia (8, 64). To discriminate these possibilities, we analyzed islets in the absence of hyperglycemia and insulin resistance contributed by the Lepr<sup>db/db</sup> mutation. Islets were isolated from wild-type mice and Chop<sup>–/–</sup> mice and treated with tunicamycin to inhibit N-linked glycosylation and induce unfolded protein accumulation in vitro. Tunicamycin treatment increased protein oxidation and lipid peroxidation products 2.5- to 3-fold in wild-type islets (Figure 6B). In contrast, islets from Chop<sup>–/–</sup> mice displayed significantly reduced levels of carbonyls and HODEs after tunicamycin treatment. In addition, H<sub>2</sub>O<sub>2</sub> treatment increased levels of carbonyls and HODEs to similar extents in islets isolated from Chop<sup>+/+</sup> and Chop<sup>–/–</sup> mice (Figure 6C). Importantly, these results show that the Chop-null mutation protects β cells from oxidative damage that occurs in response to ER stress.

Discussion

Studies in cultured cells indicate that acute UPR activation is an adaptive response to ER stress, whereas sustained UPR activation is associated with cell death (20). Recent studies have demonstrated that insulin resistance is associated with markers of UPR activation, including CHOP induction, in murine and human islets (10–12). However, it is not known whether UPR signaling is an adaptive mechanism that sustains β cell function and survival or whether UPR signaling contributes to β cell failure and death. To study this problem, we analyzed the role of the UPR in protein secretion and cell survival in vivo under conditions that pressure the β cell to produce elevated levels of insulin. Through deletion of the CHOP-mediated death signal, we have uncovered an important adaptive function of the UPR to limit oxidative stress in response to elevated protein secretion. Surprisingly, Chop deletion not only prevented UPR-induced death, but also improved the capacity of the β cell to produce insulin in 3 models of insulin resistance–induced β cell failure: heterozygous Ser51Ala eIF2α mutant mice fed a HF diet, mice fed a HF diet in conjunction with STZ treatment, and Lepr<sup>db/db</sup> mice as a genetic model of insulin resistance. In all models, Chop deletion preserved the β cell mass and improved β cell function, monitored by glucose homeostasis and GSIS. Finally, Chop deletion also attenuated the loss in β cell mass and apoptosis in embryonic homozygous Ser51Ala eIF2α mutant mice, a model of β cell demise that occurs in the absence of insulin resistance (Supplemental Figure 3). The sum of our findings support the idea that CHOP is a fundamental factor...
causing β cell failure and apoptosis in response to the chronic ER stress that coincides with β cell compensation for insulin resistance. Our findings indicate that in the absence of a death signal, UPR signaling can improve protein secretory capacity and preserve the functional integrity of the ER in β cells.

Our results support the hypothesis that Chop deletion improves ER function and protects against oxidative stress in response to ER stress in β cells. However, as our studies were performed in mice with Chop deletion in all tissues, the possibility exists that Chop deletion affects ER function in other tissues to alter organismal metabolism and, therefore, β cell function. Indeed, our findings as well as previously published results (49) demonstrate that Chop deletion can increase obesity. There are several possible mechanisms by which Chop deletion might increase obesity. First, Chop deletion in the absence of CHOP could increase signaling through the C/EBPs and islets (48) and in β cells that are exposed to nitric oxide (36). However, as our studies were performed in mice with Chop deletion upon ER stress was observed in isolated β cells (36). Therefore, we can rule out potential effects of Chop deletion in other cell types, the protective effect of Chop deletion upon ER stress was observed in isolated islets (Figure 6B), indicating that the improved β cell function does not require other tissues.

Previous studies demonstrated that Chop deletion reduces apoptosis and delays glucose intolerance in heterozygous, but not homozygous, Akita mice that express Cys96Tyr misfolded proinsulin (48) and in β cells that are exposed to nitric oxide (36).
the molecular mechanism by which CHOP mediates β cell apoptosis under these conditions is not understood, and it is also not known whether protein misfolding in the ER contributes to death of β cells that produce wild-type proinsulin. Our findings demonstrate that CHOP deletion prevents glucose intolerance by improving the β cell functional capacity of the ER to produce folded proinsulin and limit oxidative stress. This conclusion is supported by mRNA expression analysis that demonstrated CHOP deletion was associated with increased expression of genes encoding antioxidative stress function, i.e., Sod1, Sod2, Gpx1, Pparg, and Ucp2.

Presently, most data support the idea that CHOP is induced by the PERK/eIF2α/ATF4 as well as the IRE1/XBP1 and ATF6 UPR subpathways to activate proapoptotic gene expression, restore transcription initiation, and increase the oxidizing potential in the ER lumen (42, 72). CHOP induces expression of GADD34, a subunit of type 1 protein phosphatase that directs eIF2α dephosphorylation to increase mRNA translation as homeostasis in the ER is restored (42, 73). CHOP is also implicated in the induction of ERO1α, a molecule that oxidizes protein disulfide isomerase (PDI) so it can function to rearrange improperly formed disulfide bonds within unfolded proteins. Disulfide bond formation during oxidative protein folding in the ER generates oxidative stress as a consequence of electron transfer from cysteine residues through PDI and ERO1 to molecular oxygen to form hydrogen peroxide (74, 75). Future studies should elucidate whether CHOP deletion protects β cells from oxidative damage through the reduced expression of GADD34 and/or ERO1.

We have shown that increased protein misfolding in the ER increases oxidative damage in wild-type islets. Oxidative stress may further accentuate protein misfolding by directly modifying protein-folding intermediates, by disturbing protein chaperone functions, or by perturbing ER Ca2+ homeostasis (76). Previous studies demonstrated that islets from Lep(+/−) mice exhibit oxidative stress and neutralizing this stress can improve β cell function and prevent progression of T2D (62, 63). Indeed, antioxidant therapy has proven beneficial in diabetic animal models and possibly in humans with T2D (8, 60). Because they express low levels of antioxidant enzymes, β cells may be particularly sensitive to oxidative stress (58, 59). In addition, other stresses, such as proinflammatory cytokines, nitric oxide, hyperlipidemia, and hyperglycemia, may also produce ROS that could further disrupt protein folding in the ER lumen. We propose that oxidative damage that is caused by ER stress may be fundamental in the etiology of the β cell failure associated with both T1D and T2D.

Although the signaling pathways that are activated in response to misfolded protein accumulation in the ER have been identified, there is no evidence to support the idea that manipulation of these pathways can increase the protein folding and/or secretion potential of the ER. We believe our results, which show that deletion of the UPR-induced gene Chop improves the function of the secretory pathway, are the first example in which modulation of UPR signaling was demonstrated to preserve ER function. The improved ER function prevented β cell failure and the development of diabetes caused by insulin resistance and obesity. Although Chop was identified as an ER stress–induced transcription factor that mediates apoptosis, it is possible that changes in gene expression due to Chop deletion improve the functional capacity of the ER to reduce both protein misfolding and ER stress–mediated cell death signaling (Figure 7). Alternatively, in the absence of the CHOP-mediated death signal, UPR adaptive transcription may continue to improve the functional capacity of the ER. Further studies are required to elucidate how Chop deletion influences the transcriptional profile of the cell to preserve the functional capacity of the ER and reduce accumulation of ROS. Our findings should encourage the search for specific modulators of ER stress signaling that have the potential to improve the functional capacity of the ER for the treatment of numerous diseases associated with protein misfolding within this organelle.

**Methods**

**Animal husbandry.** Ser51Ala eIF2α mice (9) and Chop−/− mice (41) were backcrossed with C57BL/6J mice (The Jackson Laboratory). The eIF2αA/ and Lep(+/−) mice (C57BKS.Cg-mLEpr+/−; Lep6); JAX mice) were bred with Chop−/−.
Total RNA was extracted from freshly isolated Lepr\textsuperscript{−/−} mice (Crystal Chem Inc.). Mouse studies) to overnight-fasted animals. Blood glucose was measured tolerance was measured after i.p. injection of 2 g glucose/kg body weight between 9 and 11 am.

All procedures were conducted according to the protocols and guidelines approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA).

Glucose tolerance tests and blood glucose and serum insulin analysis. Glucose tolerance was measured after i.p. injection of 2 g glucose/kg body weight (elF2α and HF diet-fed, STZ-treated mouse studies) or 1 g/kg (Lepr\textsuperscript{−/−} mouse studies) to overnight-fasted animals. Blood glucose was measured using a OneTouch Ultra glucometer (LifeScan Inc.) with a sensitivity of 10 mg/dl. Serum insulin was measured with an ultrasensitive ELISA kit (Crystal Chem Inc.).

**Figure 7**

Model depicting interrelationships between protein folding, UPR, CHOP, ROS, and apoptosis in β cells. The UPR induces genes to improve ER protein folding and reduce oxidative stress and also induces the proapoptotic gene Chop. CHOP enhances ROS formation, possibly through induction of GADD34 or ERO1. Chop-null mutation reduces proapoptotic gene expression to permit increased expression of UPR protective genes and antioxidative stress response genes to minimize ER stress and oxidative stress, thereby improving protein folding to support insulin production (depicted in blue). CHOP may also act, directly or indirectly, to repress transcription of some UPR protective genes or antioxidative stress response genes. Deletion of Chop in combination with insulin resistance increases β cell mass, reduces oxidative stress in islets, and preserves insulin secretion and glucose tolerance.

**Islet morphology and immunohistochemistry.** Pancreata were isolated, fixed with 10% buffered formalin, embedded in paraffin, sectioned, and stained with H&E for visualization by light microscopy. Insulin- and glucagon-containing cells were identified by immunofluorescence staining using guinea pig anti-human insulin antibody (Linco) with donkey anti–guinea pig Texas Red secondary antibody (Jackson ImmunoResearch) and rabbit anti-glucagon antibody (Linco) with goat anti-rabbit Alexa Fluor 488 secondary antibody (Invitrogen). Confocal images were recorded digitally by camera, and β cell mass was measured using Image-Pro Plus software (Media Cybernetics).

**Apoptosis assays and β cell proliferation.** BrDU incorporation was used to analyze β cell proliferation as previously described (56). To continuously label mice with BrDU for 23 days, we substituted drinking water containing 1 mg/ml BrDU dissolved in 0.007 N NaOH. BrDU water bottles were wrapped with aluminum foil to prevent light exposure, and freshly prepared solution was provided every other day. Pancreatic tissue was harvested and processed as described above for immunohistochemistry. Sections were stained with BrDU In-Situ Detection Kit (BD Biosciences — Pharmingen), and replicating cells were counted from digital photographs obtained using a phase contrast microscope. The islet areas were quantified using Image-Pro Plus software (Media Cybernetics).

**TUNEL assays were performed using the ApoAlert DNA Fragmentation Assay Kit (BD Biosciences — Clontech). Tissue sections from pancreata were first labeled for detection of insulin as described above, followed by TUNEL assay. Confocal images were recorded and TUNEL-positive cells were counted manually from the images. The islet areas for adult pancreatic sections were quantified using Image-Pro Plus software (Media Cybernetics).**

**Pancreatic insulin content.** Pancreata were extracted by homogenization in a cold solution of acid/ethanol containing 80% ethanol and 0.19 M hydrochloric acid, sonicated on ice, and incubated at 4°C overnight (about 16–20 h). The extracts were then centrifuged, and supernatants were diluted into sample buffer. Insulin and glucagon contents were measured by ELISA (Crystal Chem Inc.) and radioimmunoassay (Glucagon RIA GL-32K; Linco Research Inc.), respectively.

**β Cell ultrastructure.** Transmission electron microscopy (TEM) was performed on pancreas tissue (Figure 1D) and isolated islets (Figure 3F) as previously described (9). The number of insulin granules per islet area was determined using Image-Pro Plus software (Media Cybernetics).

**Islet isolation.** Islets of Langerhans were manually isolated after collage nase P (Roche) digestion and Ficoll gradient centrifugation (77, 78).

**GSIS.** Five islets of similar size were pre-incubated in basal glucose (3.3 mM) for 1 h at 37°C in Krebs-Ringer Bicarbonate HEPES buffer containing 129 mM NaCl, 5 mM NaHCO\textsubscript{3}, 4.8 mM KCl, 1.2 mM MgSO\textsubscript{4}, 1.2 mM KH\textsubscript{2}PO\textsubscript{4}, 1.0 mM CaCl\textsubscript{2}, and 10 mM HEPES at pH 7.4, plus 0.1% RIA-grade BSA (Sigma-Aldrich). Sequential static incubations were performed in low glucose (3.3 mM) for 15 or 30 min, followed by stimulatory incubation in high glucose (16.7 mM) for 30 min. Insulin release data are expressed as a percentage of total insulin content determined by a ratio of cold acid/ethanol lyss and ELISA.

**Gene expression analysis.** Total RNA was extracted from freshly isolated islets using RNaseasy mini kit (Qiagen) for reverse-transcription into cDNA in a 20-μl reaction using iScript cDNA Synthesis Kit (BioRad). Reverse transcription reactions were incubated sequentially for 5 min at 25°C, 30 min at 42°C, and then 5 min at 85°C. CDNA products were stored at –20°C. iQ SYBR Green Supermix kit (BioRad) was used for quantitative real-time PCR (20 μl) using the iCycler iQ Real-Time PCR detection system (Bio-Rad). The thermal cycling parameters were as follows: step 1, 95°C for 10 min; step 2, 95°C for 15 s; step 3, 59°C for 1 min. Step 2 was repeated for 40 cycles. Reactions were terminated by incubation at 4°C. The relative amounts of mRNA were calculated from the Ct values using 18s rRNA for normalization. Primer sequences are presented in Supplemental Table 1.
Quantitation of oxidation products. Protein carbonyls were measured in islet extracts by ELISA (Biocell Corp.). Lipid peroxidation was quantified by detection of HODEs as previously described (79, 80). The islet protein content was determined by Bradford assay.

Statistics. Data are represented as mean ± SEM. Statistical significance of differences between groups was evaluated using the Student t test or 1-way ANOVA test (Tukey’s test). P < 0.05 was considered statistically significant.

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