A re-evaluation of the role of the unfolded protein response in islet dysfunction:

maladaptation or a failure to adapt?

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Short running title: The unfolded protein response in islet failure

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Abstract

Endoplasmic reticulum (ER) stress, caused by perturbations in ER homeostasis, activates an adaptive response termed the unfolded protein response (UPR) whose function is to resolve ER stress. If unsuccessful the UPR initiates a pro-apoptotic program to eliminate the malfunctioning cells from the organism. It is the activation of this pro-apoptotic UPR in pancreatic β-cells that has been implicated in the onset of type 2 diabetes and thus, in this context, is considered a maladaptive response. However, there is growing evidence that β-cell death in type 2 diabetes may not be caused by a maladaptive UPR but by the inhibition of the adaptive UPR. In this review, we discuss the evidence for a role of the UPR in β-cell dysfunction and death in the development of type 2 diabetes and ask the question: Is β-cell dysfunction the result of a maladaptive UPR or a failure of the UPR to adequately adapt? The answer to this question is critically important in defining potential therapeutic strategies for the treatment and prevention of type 2 diabetes. In addition, we discuss the potential role of the adaptive UPR in staving off type 2 diabetes by enhancing β-cell mass and function in response to insulin resistance.

Introduction

The endoplasmic reticulum (ER) is an extensive network of tubular membranes within the cytoplasm of the cell that serves as a site for the synthesis of lipids, phospholipids, steroids and almost all secreted and membrane proteins. Thus the maintenance of the ER is essential for preserving cellular function and viability. Disruption in ER homoeostasis caused by, for example, the depletion of ER calcium, perturbations in the ER redox state and/or the accumulation of mis-folded proteins within the ER results in what is commonly referred to as 'ER stress' [Footnote 1]. This stress is sensed by ER transmembrane proteins which activate the unfolded protein response (UPR); an adaptive response whose function is to restore ER

homeostasis and thus alleviate 'ER stress' (Figure 1) (for detailed reviews please see (1–4)). This is achieved by: 1) decreasing ER synthetic load through inhibiting protein synthesis; 2) clearing the ER of misfolded proteins by increasing the expression of components of ER associated degradation (ERAD), which translocates mis-folded proteins out of the ER for subsequent proteosomal degradation and; 3) enhancing the synthesis and folding capacity of the ER by stimulating an increase in both ER mass and function.

The canonical transducers of the UPR are three ER transmembrane proteins: PKR-like ER kinase (PERK), a serine threonine kinase; inositol requiring enzyme 1 (IRE1) which has both serine-threonine kinase and RNA endonuclease activity and; activating transcription factor 6 (ATF6) (Figure 1). PERK phosphorylates the alpha subunit of eukaryotic translation initiation factor 2 (eIF2α) (5,6) and *in vitro* can phosphorylate NF-E2-related transcription factor (Nrf2) (7); although there is limited evidence that Nrf2 can be phosphorylated by PERK *in vivo*. The phosphorylation of eIF2α inhibits protein synthesis thus reducing ER protein folding load. However, it also promotes an increase protein translation from a subset of mRNAs including that encoding activating transcription factor 4 (ATF4) [footnote 2] (8–10), which increase in the expression of mRNAs involved in amino acid metabolism, maintaining redox state and combating oxidative stress (11). There are two isoforms of IRE1 in mammalian cells: IRE1α and IRE1β (for review see (12)) although most research investigating the role of IRE1 in the UPR has focused on IRE1α. IRE1α catalyses the removal of a 26bp sequence from the mRNA encoding the b-zip transcription factor XBP1 (X-box binding protein-1), resulting in a frame shift and the production of a transcriptionally active 'spliced' form of XBP1 (XBP1s) (12,13) . XBP1s enhances the expression of mRNAs encoding proteins that increase folding capacity, such as the ER chaperones BiP and GRP94, and promote ERAD, such as ER-degradation enhancing α-mannosidase-like protein (EDEM) (14). In β-cells IRE1α/XBP1 promotes insulin stimulated proinsulin synthesis (15,16). Although there are also two isoforms of ATF6, ATF6α

and ATF6β, it is ATF6α which has been implicated in UPR induction. ATF6α activation is initiated by the unmasking of a golgi localisation signal by the dissociation of BiP. This allows ATF6 to translocate to the golgi where it is cleaved by site-1 protease (S1P) and Site-1 protease (S2P) resulting in the release of a 50kD N-terminal fragment (p50) encoding a bZIP transcription factor. P50 has overlapping and compensatory functions to that of XBP1s (17).

If the activation of the UPR is unable to restore ER homeostasis, the UPR switches from an adaptive to a pro-apoptotic program mediated primarily by the chronic activation of IRE1α and/or PERK (3,12). Chronic PERK activation causes the ATF4 dependent increase in the expression of the pro-apoptotic protein C/EBP homologous protein (CHOP), otherwise known as DNA damage-inducible transcript 153 (GADD153) and DDIT3. Chronic IRE1α activation leads to the recruitment of TNF receptor associated factor 2 (TRAF2) (18) and the activation of c-Jun terminal Kinase (JNK) and p38 MAP kinases (18), both of which increases the expression of pro-apoptotic proteins. Prolonged IRE1α activation also promotes apoptosis by degrading mRNAs encoding essential cell-survival proteins through a process called regulated IRE1α-dependent decay or RIDD (19). In addition, both PERK and IRE1α can promote the expression of pro-inflammatory cytokines (20–22). This pro-apoptotic response is important in clearing mal-functioning cells from the organism. However, the death of non-replenishing cells that play a critical function, such as pancreatic β-cells, can have deleterious consequences on the organism and thus in this specific context the UPR is maladaptive *(defn: an adaptation more harmful than helpful to the organism)*[Footnote 3]. It is this feature of the UPR that has gained it a great deal of notoriety in its proposed role in the pathogenesis of type 2 diabetes, the focus of this review.

ER stress and the UPR in the development of β-cell dysfunction in type 2 diabetes.

Obesity is often associated with a decrease in insulin sensitivity in skeletal muscle, liver, and adipose tissues. Yet the majority of people who are obese and insulin resistant do not develop

diabetes. This is due to a compensatory increase in insulin secretion maintained through an increase in both β-cell function and mass (23). This is referred to as β-cell adaptation or β-cell compensation and there is mounting evidence that 'ER stress' and the induction of an adaptive UPR play an important role in this. However, if the β-cells are unable to adequately compensate and/or are unable to sustain a compensatory phenotype, this leads to relative insulin deficiency and ultimately to the onset of diabetes. This 'failure' of the β-cells is initially characterised by the development of β-cell dysfunction exemplified by a loss of first phase insulin secretion and defective proinsulin processing but ultimately by a decrease in β-cell mass primarily due to β-cell death. It has been proposed that this deterioration in β-cell function and loss of viability is caused by the rate of proinsulin synthesis exceeding the processing and folding capacity of the ER, leading to the accumulation of unfolded/unprocessed proinsulin (1,6,24,25) resulting in chronic ER stress and the activation of a proapoptotic UPR. However, it has also been proposed, based on experimental evidence, that ER stress in pancreatic β-cells can be caused by: the formation of islet amyloid, a common feature of human type 2 diabetes; the chronic exposure of pancreatic β-cells to elevated levels of free fatty acids and/or glucose, a hallmark of obesity and insulin resistance, or/and; elevated levels of pro-inflammatory cytokines, another common feature of obesity (26–29).

β-cell compensation: a positive role for the UPR. With increased demand for insulin there is a need to increase secretory capacity by increasing both the mass of β-cells and the processing capacity of individual β-cells to synthesise and secrete insulin. Importantly, there is evidence that the UPR plays a positive role in these important compensatory adaptations. Transgenic animal models in which the UPR is compromised provide evidence that the UPR is important in β-cell compensation. For example, β-cell and hypothalamic IRE1α knock-out (KO) mice when placed on a high fat diet (HFD) to promote obesity and insulin resistance have reduced β-cell mass due to a reduced rate of β-cell replication, possibly caused by a decrease in XBP1s

dependent expression of cyclin-D1, a critical regulator of cell cycle progression (30). However, these results are yet to be confirmed using a β-cell specific IRE1α knock-out (KO) mice.

As IRE1α/XBP1 is also required for glucose-stimulated insulin synthesis (15,16) increased activation through this pathway may be important in increasing β-cell function. ATF6α may also be important in β-cell compensation as ATF6α null mice have exacerbated glucose intolerance when placed on a HFD due to a reduction in insulin secretion compared to their wild type HFD fed controls (31). Moreover, *in vitro* studies on dispersed mouse or human islets indicate that increased β-cell proliferation in response to an increase in insulin demand is mediated by the activation of ATF6 (32). However, β-cell specific knock-out of ATF6α in mice has no discernible effect on β-cell development or function (33) and human carriers of Atf6α 'hypomorphic' mutations have only been characterized to have achromatopsia, a cone photoreceptor defect (34). Thus the role of ATF6α in β-cell function is unclear.

β-cell specific ablation of PERK in mice results in the development of diabetes (6,35), likely due to a reduction in β-cell proliferation and neonatal β-cell expansion (35), whereas the conditional deletion of PERK in adult mice has been reported to cause increased β-cell death (32). However, PERK's role in β-cell compensation in these transgenic mouse models has not been explored, although mice carrying a non-phosphorylatable mutant of eIF2α, PERK's primary and perhaps only substrate, in β-cells develop glucose intolerance due to β-cell failure likely caused by an inability to mount an effective UPR (25).

Studies of rodent models of obesity and insulin resistance also provide evidence that ER stress and the activation of an adaptive UPR are important in β-cell compensation (Table 1). *ob/ob* mice are leptin deficient and consequently rapidly become obese and severely insulin resistant but do not develop diabetes due to successful β-cell compensation sustained through an increase in both β-cell function and mass. In islets isolated from these mice, the expression of markers of an adaptive UPR increase between 6 and 16 weeks of age (36) and this is

concomitant with an increase in β-cell mass and function. Likewise, the islets isolated from Zucker and female Zucker Diabetic Fatty (ZDF) rats or pre-diabetic *db/db* mice, genetic models of obesity and β-cell compensation, have increased expression of markers of the adaptive UPR compared to their lean controls (36,37). The story is similar with high fat diet (HFD) fed mice, considered a more physiologically relevant model of insulin resistance-associated β-cell compensation (38), as islets isolated from HFD-fed mice also have increased expression of markers of an adaptive UPR compared to their lean controls (29,39). Interestingly, increased CHOP expression is observed in many of these models of β-cell   adaptation (Table 1), indicating that levels of CHOP expression *per se* is a poor marker of a maladaptive UPR [Footnote 2].

The activation of the UPR in all these animal models is presumably in response to an increase in insulin resistance and the demand for insulin. Congruent with this presumption hyperglycemia in Wistar rats, induced by glucose infusion, activates an adaptive UPR in islets as determined by increased expression of XBP1s and the ER chaperones BiP and GRP94 (40). Similarly, mild hyperglycemia imposed on human islets when transplanted into mouse recipients, also results in the activation of an adaptive UPR (41). These effects on the UPR are likely due to an increased demand for insulin rather than hyperglycemia *per se* (32,42). Indeed, a reduction in insulin synthesis has been shown to reduce ER stress in mice (42).

The activation of an adaptive UPR increases insulin processing and secretory capacity and there is good evidence to support the notion that this protects β-cells from the detrimental effects of ER stress. For example, the overexpression of the ER chaperone BiP in β-cells protects mice against high-fat-diet-induced diabetes (39). Conversely a reduction in BiP expression as a result of CEBPβ-mediated down-regulation of ATF6 is associated with diabetes (43). Moreover, administration of pharmacological chaperones such as TUCDA and PBA and, the more recently discovered, azoramide can restore rodent islet function both *in vitro* and *in*

vivo (32,36,40,44–46). In humans, PBA has also been shown to partially alleviate lipid-induced β-cell dysfunction (47).

β-cell dysfunction and death in type 2 diabetes: Maladaptation or a failure to adapt?
Pharmacological induction of ER stress in both clonal pancreatic β-cell lines and human or rodent islets of Langerhans, using agents such as thapsigargin or tunicamycin, results in β-cell death (48–50). Similarly, incubation of clonal β-cell lines or isolated islets with the long-chain saturated free fatty acid palmitate also causes ER stress, UPR activation and ultimately cell death (50–53). *In vivo*, the expression of mis-folding mutants of insulin that cause chronic ER stress also cause β-cell death in mice and in humans resulting in permanent neonatal diabetes (54). CHOP clearly plays an important role in ER stress induced β-cell death (26,55,56). For example, in the Akita mouse, a model of diabetes that expresses a mis-folding mutant of insulin resulting in chronic ER stress, the ablation of CHOP delays diabetes onset (56,57). In summary chronic unresolvable ER stress *in vitro* or *in vivo* can cause β-cell dysfunction and death through the activation of a proapoptotic UPR. Yet, there is limited evidence that, in the development of type 2 diabetes, β-cell dysfunction and death *is caused by* chronic ER stress and the induction of a proapoptotic UPR. *db/db* mice, a well characterised model of type 2 diabetes, are defective in leptin signalling and as a consequence rapidly develop obesity and insulin resistance. Despite initial β-cell compensation these mice develop diabetes due to a decline in β-cell function and mass. Surprisingly the expression of XBP1s and ATF4, proximal markers of IRE1 and PERK activation respectively and surrogate markers of ER stress are reduced in islets isolated from diabetic 16 week old *db/db* mice compared to their pre-diabetic 6 week old controls (36). Similarly, in islets isolated from diabetic HFD-fed obese female Zucker Diabetic Fatty rat (HFD-fZDF), another well characterised rodent model of type 2 diabetes, there is no detectable increase in the phosphorylation status of eIF2α and IRE1, compared to their age-matched obese pre-diabetic fZDF rats (37). In addition, the expression

of markers of an adaptive UPR are also either significantly decreased or show a tendency towards a decrease in animal models of diabetes and surprisingly this is invariably associated with no change or a decrease in CHOP expression (an indicator of the activation of a proapoptotic UPR) (36,37) (Table 1).

Tellingly, and in line with what was observed in studies using rodents, the expression of 691 genes out of 692 ER- associated genes, many of which are markers of ER stress and the UPR, were unchanged in β-cell enriched samples isolated from diabetic human subjects compared to BMI matched non-diabetic controls (58). Although, ER distension, a morphological indicator of ER stress, was detected in β-cells isolated from diabetic subjects (58). Interestingly, the expression of BiP and XBP1s as well as the expression of CHOP is lower in cultured islets isolated from type 2 diabetics compared to those isolated from non-diabetics (58). Similarly, in a separate study, the expression of XBP1s, ATF6 and the phosphorylation of eIF2α were all found to be decreased in islets within pancreata isolated from type 2 diabetics compared to non-BMI matched non-diabetic controls (59). However, increased nuclear localisation of CHOP has been reported in islets from type 2 diabetics compared to BMI matched non-diabetic subjects (60). Given the challenges associated with these types of studies using human tissue it is difficult to interpret the data and thus reach a conclusion with any confidence. However, evidence for chronic ER stress and/or activation of a proapoptotic UPR in islets from diabetic subjects is clearly limited.

There is considerable evidence that a failure to mount an effective UPR has marked deleterious consequences to both β-cell function and viability. For example, Wollcott-Rallison syndrome, a rare human autosomal recessive genetic disorder caused by the impairment or loss of function mutations in PERK, is characterised by early onset diabetes due to pancreatic β-cell failure (61). Similarly, the ablation of PERK in mice results in the selective death of β-cells and the development of diabetes (24). Likewise, conditional deletion of IRE1α from the β-cells of mice

results in glucose intolerance due to a reduction in insulin content(15,30,62) and β-cell failure, primarily due to reduced anti-oxidative capacity (15). Increased unspliced XBP1 (XBP1s) protein may also play a role as it has been reported to inhibit ER function (63) and autophagy (64).

β-cells from autophagy-deficient mice also have a compromised UPR and, when crossed onto *ob/ob* mice, develop diabetes due to ineffective β-cell compensation (65). This correlates with a reduction in the adaptive UPR and surprisingly a decrease in the expression of pro-apoptotic CHOP (65), a classical although non-specific marker of chronic ER stress [Footnote 2]. A decrease in ER folding capacity by the genetic ablation or reduction of the expression of ER chaperones such as p58[ipk] and BiP in β-cells can also lead to β-cell dysfunction and death (39,66). Interestingly, β-cells of mice carrying a non-phosphorylatable form of eIF2α and hence have a defective UPR develop ER stress and death coincident with the induction of oxidative stress (20) as do mice deleted for the ER co-chaperone P58[IPK] (66). Deletion of IRE1α in β-cells also leads to increased oxidative stress and the development of β-cell dysfunction (15). Notably the administration of anti-oxidants reduces ER stress *in vivo* and preserves β-cell function in mouse models of diabetes (40,66,67). Thus a decrease in the UPR activation decreases β-cell resistance to oxidative stress. In addition, β-cell damage and death caused by, for example, increased oxidative stress would inevitably lead to inflammation which can itself induce both oxidative and ER stress (29) and thus exacerbate the development of β-cell dysfunction and death (Figure 2).

The decrease in the adaptive UPR observed in β-cells from diabetic animals could be a consequence of hyperglycemia as the expression of many adaptive UPR genes including the ER chaperones BiP and Grp94 are down-regulated in mouse islets transplanted into diabetic mice compared to those transplanted in non-diabetic control animals (68). Furthermore, normalisation of glycaemia in these diabetic mice restores the expression of markers of an adaptive UPR in the transplanted islets. Thus it is possible that chronic hyperglycemia not only

increases the demand for insulin resulting in the activation of the UPR but ultimately compromises the adaptive UPR. There is evidence from studies of *db/db* mice that the activation of JNK may play an important role in switching off an adaptive UPR (69). Decreased expression of markers of an adaptive UPR are coincident with increased JNK activation in islets from *db/db* mice and the inhibition of JNK in islets isolated from *db/db* mice improves adaptive UPR gene expression and reduces cell death (69). CHOP may also play a role in inhibiting the adaptive response as mice deleted of *Chop* display improved glycaemic control and expanded β-cell mass in genetic and diet-induced models of insulin resistance and this is associated with increased expression of adaptive UPR genes (67). Thus, the balance between the adaptive UPR and CHOP may be critically important in the regulation of β-cell function and survival during ER stress (Figure 2).

Genetic evidence that a defective UPR may be an important predisposing factor for the development type 2 diabetes in humans is somewhat limited although genome wide association studies (GWAS) have implicated polymorphisms within the Atf6α gene with type 2 diabetes in both Pima Indians and Dutch Caucasians (70,71). Moreover, polymorphisms within the Wolfram syndrome 1 (*WFS1*) gene, a negative regulator of ER stress (72), has also been associated with increased risk of type 2 diabetes (73).

Conclusions and future perspectives. There is mounting evidence that β-cell dysfunction and death in type 2 diabetes is caused by the inactivation of the UPR and, as a consequence, the failure of the β-cell to adequately adapt rather than the more commonly proposed model of chronic ER stress and the activation of a proapoptotic UPR. Although chronic unresolvable ER stress can lead to β-cell death through activation of a proapoptotic UPR, which may be beneficial under certain physiological or pathological conditions, it is unclear as to whether β-cells are exposed to such severe stress in type 2 diabetes. During the development of type 2

diabetes, islets are subjected to a slow and gradual increase in the demand for insulin with obesity and decreasing insulin sensitivity and this occurs over several years. It is likely that this translates into cycles of UPR activation and adaptation with relatively small changes in demand provoking a transient and subtle activation of an adaptive UPR. This would result in small but effective increases in ER folding and processing capacity thus alleviating ER stress.

Despite clear evidence for the activation of an adaptive UPR in both obese insulin resistant rodents and humans, the evidence that β-cell failure and death is mediated by the activation of a proapoptotic UPR is limited. However, there is evidence for a decrease in the expression of markers of an adaptive UPR in islets undergoing β-cell failure in rodent models of type 2 diabetes and in human subjects with type 2 diabetes. Moreover, UPR dysfunction is known to lead to β-cell dysfunction and/or the inability of β-cells to adequately compensate in the face of an increase in the demand for insulin. Thus β-cell dysfunction and death in type 2 diabetes may well be caused by a failure of the UPR to adequately adapt rather than the activation of a proapoptotic, and in the context, maladaptive UPR [Footnote 3]. However, further investigation is required to establish this.

As much of the work on the role of ER stress in β-cell failure in type 2 diabetes has been conducted in rodent islets it is important to consider whether there are significant differences between how rodent and human islets respond to ER stress. Based on several *in vitro* studies it is unlikely that there are fundamental differences in the mechanism of UPR activation or in their resilience to ER stress (26,50,74). However, one important difference, that may well impact on islet survival, are their ability to adapt to increased demand (for reviews see (75,76)). Rodent β-cell mass readily increases in response to increased demand primarily through proliferation, thus decreasing insulin secretory demand per β-cell and presumably relieving ER

stress. Indeed, decreased insulin production relieves ER stress and interestingly promotes β-cell replication in mice (42). In contrast, although human islet mass has been shown to increase in adults who are obese these changes are comparatively small compared to the changes observed in mice and this is primarily mediated by an increase β-cell size rather than number (77). Thus one may predict that human β-cells are more likely to be subjected to a comparatively greater demand for insulin and thus be more susceptible to ER stress, which in turn may lead to a greater propensity to develop β-cell dysfunction and death.

Other important unresolved questions are: what leads to the failure of the adaptive UPR and: If β-cell death is not caused by the activation of a proapoptotic UPR then what is it caused by? There is evidence that 'UPR failure' may be caused by the activation of stress-activated signalling pathways, whereas β-cell death appears to be mediated by a culmination of stresses of which oxidative stress plays a particularly critical role. As there have been a number of reports demonstrating the importance of the UPR in limiting oxidative stress (e.g.(11,15,25)) it is not surprising that one consequence of UPR failure is an increase in oxidative stress. Notably, the administration of anti-oxidants reduce ER stress and preserve β-cell function (40,66,67) and thus have therapeutic potential. Another important question is: Is it possible to intervene therapeutically to promote further adaptation? Clearly increasing ER folding capacity through the administration of pharmacological chaperones in rodent models of diabetes has beneficial effects (32,36,40,44–46,78) and there is some evidence that it is also beneficial in humans (47). Thus the identification and an evaluation of the efficacy of drugs that increase ER folding capacity and/or reduce oxidation stress is an important avenue of pharmacological exploration.

Footnotes.

1. Experimentally ER stress is most often defined by its consequences. That is the morphological distension of the ER and/or the activation of the unfolded protein response (described below). Thus this is not a measurement of ER stress *per se* but that the cells have encountered or are encountering ER stress. Unfortunately 'ER stress' infers a pathophysiological perturbation of the ER despite the fact that physiological changes in ER homeostasis also activate the UPR. This causes much confusion as experimental evidence of UPR activation is often provided as evidence of pathology.

2. Stresses other than ER stress caused by, for example, nutrient limitation, infection, inflammation, increased reactive oxygen species and/or DNA damage can also increase ATF4 expression through increased phosphorylation of eIF2α mediated by one of three alternative eIF2α kinases namely GCN2, HRI, and PKR. Thus it is worth noting that the phosphorylation of eIF2α or an increase in the expression of its downstream effectors such as ATF4 and CHOP is not, in and of itself, evidence for UPR activation or indeed 'ER stress'.

3. It is conceivable that if cells were irreversibly dysfunctional then their elimination would be considered beneficial. This may be the case with some β-cells in the latter stage of type 2 diabetes but there is no clear evidence for this. On the other hand, many studies have demonstrated the reversibility of β-cell dysfunction in type 2 diabetes, including with bariatric surgery (79) and the normalisation of glycemia with intensive insulin treatment (80,81) or pharmacotherapy (82) . Thus, rather than terminally dysfunctional, β-cells in type 2 diabetes likely represent a functional reserve. Therefore in this context, the elimination of dysfunctional β-cells under chronic ER stress conditions is defined as maladaptive.

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Table 1. Studies in which changes in ER stress / UPR activation were determined in animal models of insulin resistance and type 2 diabetes

ANIMAL MODELS OF INSULIN RESISTANCE AND β-CELL COMPENSATION

Model	Evidence for ER stress/on-going UPR activation	Markers of an Adaptive UPR	Maladaptive UPR (CHOP expression)
Prediabetic *db/db* mouse (6 wks) (36)	↕ XBP1s	↕ BiP, p58, Erp72, Fkbp11, Grp94	↕
Zucker fatty Rat (37)		↕ BiP,HYOU1, FKBP12	↕
fZDF rat (37)		↕ BiP	↕
HFD-fed mouse (29,39,83)	↕ XBP1s (29,83) ↔ P-eIF2 (39)	↕ BiP (29,39) ND (83)	ND (29) ↕ (83) ↔ (39)
ob/ob mouse (36)	↕ XBP1s	↕ BiP, p58, Erp72, Fkbp11, Grp94	ND
HFD-fed Rats (84)	↕ P-PERK	↕ BiP	ND

ANIMAL MODELS OF β-CELL FAILURE AND TYPE 2 DIABETES

Model	ER stress/UPR activation	Markers of an Adaptive UPR	Maladaptive UPR (CHOP expression)
db/db mouse (16 wks) (36)	↓ XBP1s	↓ BiP, Grp94, Erp72, Fkbp11 compared to levels in pre-diabetic mice	↓ compared to levels in pre-diabetic mice
HFD-fZDF rat (37)	↔ P-eIF2a, P-IRE1	↔ / ↓	↔

ND = No data

Figure Legends

Figure 1. A simplified overview of the adaptive UPR. PERK, IRE1 and ATF6 signal to the nucleus through the action of the transcription factors ATF4, XBP1s and ATF6 which bind to response elements (e.g. the antioxidant response elements (ARE), amino acid response element (AARE), unfolded protein response element (UPRE), ER stress response element (ERSE)) within promoters to induce transcription of mRNAs whose products are important in increasing ER folding capacity, increasing ERAD and reducing oxidative stress.

Figure 2. **Schematic showing the role of the UPR in β-cell compensation or failure in type 2 diabetes.** An increase in the demand for insulin can causes ER and oxidative stress. This activates an adaptive UPR which, if effective, relieves stress and promotes β-cell adaptation. However, failure to mount a successful UPR can result in increased ER and oxidative stress which can lead to inflammatory response, all of which can promote the development of β-cell dysfunction and death leading to hyperglycemia. Hyperglycemia promotes oxidative stress, inflammation and ER stress. ER stress can promote oxidative stress and inflammation, inflammation can promote ER stress and oxidative stress and oxidative stress can promote ER stress and inflammation. Thus a viscous cycle ensures ultimately resulting in reduced β-cell mass and the onset of diabetes.