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Glucose regulation of islet stress responses and β-cell failure in type 2 diabetes

Short title: Glucose regulation of islet stress responses

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Abstract (200 words)

Pancreatic β cells exposed to high glucose concentrations display altered gene expression, function, survival and growth that may contribute to the slow deterioration of the functional β-cell mass in type 2 diabetes. These glucotoxic alterations may result from various types of stress imposed by the hyperglycaemic environment, including oxidative stress, endoplasmic reticulum stress, cytokine-induced apoptosis and hypoxia. The glucose regulation of oxidative stress- and integrated stress-response genes in cultured rat islets follows an asymmetric V-shaped profile parallel to that of β-cell apoptosis, with a large increase at low glucose and a moderate increase at high vs. intermediate glucose concentrations. These observations suggest that both types of stress could play a role in the alteration of the functional β-cell mass under states of prolonged hypo- and hyperglycaemia. In addition, β-cell demise under glucotoxic conditions may also result from β-cell hypoxia and, in vivo, from their exposure to inflammatory cytokines released locally by non-endocrine islet cells. A better understanding of the relative contribution of each type of stress to β-cell glucotoxicity and of their pathophysiological cause in vivo may lead to new therapeutic strategies to prevent the slow deterioration of the functional β-cell mass in glucose intolerant and type 2 diabetic patients.

Keywords
Apoptosis, endoplasmic reticulum stress, gene expression, glucose deprivation, glucotoxicity, hyperglycaemia, hypoglycaemia, hypoxia, insulin secretion, integrated stress response, oxidative stress, phenotypic plasticity, type 2 diabetes, unfolded protein response

Abbreviations

[Ca\(^{2+}\)]\(_c\), cytosolic Ca\(^{2+}\) concentration; ER, endoplasmic reticulum; G10, 10 mmol/l glucose (G2, G5, G20, G30 for 2, 5, 20, 30 mmol/l glucose); GSIS, glucose stimulated insulin secretion; HIF, hypoxia-inducible factor; IGT, impaired glucose tolerance; IL, interleukin; ISR, integrated stress response; ROS, reactive oxygen species; T2D, type 2 diabetes; TG, thapsigargin; UPR, unfolded protein response
Introduction

Type 2 diabetes (T2D) results from the complex interplay of multiple genetic and environmental factors that affect whole-body insulin sensitivity and glucose-stimulated insulin secretion (GSIS) by the endocrine pancreas. While the respective role of each anomaly in the initiation of the disease varies between subgroups of patients, it is well demonstrated that hyperglycaemia only develops if a given decrease in insulin sensitivity is not compensated for by a proportional increase in GSIS [1]. Thus, in most insulin resistant individuals (e.g. as a result of low physical activity and increased visceral obesity), β cells adapt to the higher insulin demand and maintain normoglycaemia at the price of hyperinsulinaemia owing to an increase in pancreatic β-cell function and mass (collectively referred to as the functional β-cell mass) [2,3]. In 25-30% of individuals, however, an insufficient increase in functional β-cell mass (β-cell maladaptation) or a decrease thereof after an initial phase of adequate compensation (β-cell decompensation) leads to the development of impaired glucose tolerance (IGT) and T2D [4]. Thereafter, a slow decline of the functional β-cell mass is responsible for the progressive worsening of T2D over several years [5,6], the relative contribution of changes in β-cell mass in this process remaining a matter of debate [2,3].

Since a large proportion of gene mutations or variants associated with T2D have an impact on β-cell development, function, growth or survival [7], the maladaptation/decompensation of the functional β-cell mass in face of insulin resistance likely has some genetic cause. However, β-cell decompensation may also result from the detrimental effect of various environmental factors in genetically predisposed individuals. Among factors that are present before the onset of hyperglycaemia, hyperlipidaemia (lipotoxicity), low grade systemic inflammation and chronic stimulation of insulin secretion (β-cell overwork/exhaustion) have been implicated in the initiation of glucose intolerance [8-10]. Once established, hyperglycaemia may also contribute to the progressive decrease of the functional β-cell mass, either by exerting its own deleterious effects (glucotoxicity) or by revealing the toxic effects of fatty acids (glucolipotoxicity) [11].

The consequences, mechanisms and pathophysiological role of β-cell glucotoxicity in T2D have been the subject of intense research over the last two decades. As detailed in a number of
comprehensive reviews, β cells exposed to high glucose concentrations in vivo and in vitro display altered gene expression, function, survival and growth that may result from various types of stress imposed by the hyperglycaemic environment (e.g. oxidative stress, endoplasmic reticulum (ER) stress, cytokine-induced apoptosis and hypoxia) (Fig. 1) [8-14]. However, due to the large heterogeneity in experimental models and methods used, studies on the effects and mechanisms of β-cell glucotoxicity have sometimes yielded conflicting results (e.g. on the roles of cytokine production and oxidative stress). There is thus no consensus about the mechanism by which hyperglycaemia alters the functional β-cell mass in human T2D. In this paper, we review recent data on the glucose regulation of islet stress responses, with a particular emphasis on oxidative stress, ER stress, cytokine production and hypoxia, and discuss their contribution to β-cell glucotoxicity and failure in T2D.

**Beneficial effects of physiological glucose stimulation on the functional β-cell mass**

The acute glucose stimulation of insulin secretion by β-cells results from a sequence of events that involves glucose transport across the plasma membrane, acceleration of glycolysis and mitochondrial oxidation of pyruvate by the Krebs cycle, increased ATP production and a rise in the ATP:ADP ratio that closes ATP-sensitive K⁺ channels in the plasma membrane. This is followed by cell depolarization, opening of voltage-dependent Ca²⁺ channels, Ca²⁺ influx down its electrochemical gradient and a rapid increase in cytosolic Ca²⁺ concentration ([Ca²⁺]ₖ) that triggers exocytosis of insulin-containing granules. Glucose also amplifies the efficacy of Ca²⁺ on exocytosis by poorly defined mechanisms that likely depend on increased production and cytosolic export of mitochondria-derived metabolic coupling factors [15,16].

Besides stimulating insulin secretion, glucose also contributes to maintaining a functional β-cell mass through various means. In the short-term (from 15min to a few hours), these effects mainly include stimulation of protein synthesis with a preferential effect on proinsulin and other granule proteins, and stimulation of the transcription of genes important for β-cell function such as preproinsulin and its processing enzymes, Glut2, glycolytic enzymes including glucokinase, ion channels like voltage-dependent Ca²⁺ channel α₁ subunits (these genes are later referred to as “β-cell
enriched genes”). These short-term effects seem essential in replenishing insulin stores and maintaining β-cell glucose responsiveness [17]. Thus, rodent β cells exposed to non-stimulating glucose concentrations for a few days, either in vivo during prolonged fasting or in vitro, display lower mRNA levels of β-cell enriched genes and markedly reduced GSIS, these alterations being rapidly reversed upon re-feeding or in vitro glucose stimulation [18]. In the long-term (several days or weeks), regular glucose stimulation also contributes to maintain the β-cell mass in rodents [14,17]. Thus, although the latter was not detectably affected by prolonged fasting, it was significantly reduced in insulinoma-transplanted mice suffering from sustained hypoglycaemia without plasma free fatty acid elevation [19]. This decrease in β-cell mass has been attributed to an increase in β-cell apoptosis, but reductions in β-cell growth, proliferation and neogenesis cannot be excluded [14]. Indeed, in the focal form of human persistent neonatal hyperinsulinaemic hypoglycaemia, β cells located outside the lesion displayed an atrophied cytoplasm but no morphological sign of apoptosis [20]. In vitro, an increase in β-cell apoptosis has also been reported in rodent islets, purified β-cells and insulin-secreting MIN6 cells cultured for several days in the presence of a low non-stimulating glucose concentration (≤ 5 mmol/l instead of 10 mmol/l glucose) [21-24]. Such an increase does not, however, occur in human islets that are routinely cultured at 5 mmol/l glucose, probably because the threshold glucose concentration for the stimulation of insulin secretion is lower in humans (3 mmol/l) than in rodents (6-7 mmol/l) [16]. Whether prolonged culture at truly non-stimulating glucose concentrations also triggers β-cell apoptosis in humans is not known. There is nevertheless convincing evidence that regular physiological glucose stimulation is critical for optimal preservation of β-cell glucose responsiveness and survival, both in vivo and in vitro.

Deleterious effects of supraphysiological glucose concentrations on the functional β-cell mass

In contrast with regular physiological glucose stimulation, sustained or repeated exposure to high glucose concentrations in vivo and in vitro exerts deleterious effects on β-cell gene expression, function, survival and growth [25]. However, the precise nature of these alterations and their possible cause are still controversial, partly because of important differences between results obtained in in vivo
and in vitro models of β-cell glucotoxicity [8-14]. In this respect, a major difference between in vivo and in vitro studies concerns the glucose concentration at which β-cell glucotoxicity is observed. Thus, in rodents, in vivo glucotoxicity occurs at plasma glucose concentrations (~10 mmol/l glucose) that are still beneficial to the functional β-cell mass in vitro, whereas in vitro glucotoxicity is only induced by very high glucose concentrations (~30-40 mmol/l) that are unlikely to occur in treated T2D patients [21,22,26-30]. This difference does not completely disqualify in vitro studies as it may partly result 1) from the absence, in vitro, of nutrients such as amino acids and of incretin hormones that increase the β-cell sensitivity to glucose [31] and 2) from the inherently shorter duration of exposure to high glucose that can be tested in vitro. It remains, however, possible that the type of stress to which β cells are exposed in in vivo models of hyperglycaemia markedly differs from that induced in vitro, as islet isolation and culture markedly alter the β-cell environment (devascularisation, loss of endothelial cells, disappearance of infiltrating cells, changes in extracellular matrix constituents…) [32].

Glucotoxic alterations of β-cell function - In comparison with normal subjects, insulin secretion in response to an intravenous glucose challenge is altered early on in the development of human IGT and T2D [4]. Thus, a reduction in first-phase insulin secretion and in β-cell sensitivity to glucose is already detected in individuals in which insulin resistance is still associated with an increase in fasting insulin secretion and in total insulin output during an oral glucose tolerance test, a reduction in the latter parameter being only observed in overtly diabetic patients [6]. An early reduction in proinsulin processing with subsequent increase in plasma proinsulin:insulin ratio is also typically observed in IGT and T2D subjects [4]. A similar reduction in GSIS and increase in proinsulin:insulin ratio has been observed in rodent models of T2D and in islets isolated from hyperglycaemic animals [4,11].

In vitro, chronic exposure of human and rat islets or β cells to high glucose concentrations also reduces their maximal rate of GSIS and decreases the processing of proinsulin, the latter effect likely resulting from β-cell over-stimulation [33]. However, whether culture in high glucose decreases or increases the β-cell sensitivity to subsequent acute glucose stimulation remains controversial [13]. Numerous studies, including our own, have shown that rat islets cultured for up to 2 weeks in the
presence of 30 instead of 10 mmol/l glucose are more sensitive to glucose (reduced threshold glucose concentration for the stimulation of insulin secretion) but are less glucose-responsive (lower maximal rate of GSIS) (Fig. 2), the latter effect being partly secondary to β-cell degranulation. The higher sensitivity to glucose, which correlates with a higher ATP:ADP ratio at low glucose concentrations [34], may be due to glucokinase activation or to glycogen accumulation during culture in high glucose and its subsequent degradation when the glucose concentration falls [35]. At first sight, these alterations seem in striking contradiction with the profound defect of GSIS observed in IGT and T2D. However, they are less different if one considers that in vivo GSIS during an oral or intraperitoneal glucose tolerance test corresponds to in vitro GSIS from 6 (rather than 0.5) to 10-20 mmol/l glucose (see legend to Fig. 2). An increase in glucose sensitivity has been reported in various animal models of β-cell over-stimulation, including during the first 2 weeks of hyperglycaemia after a 90% pancreatectomy (Px90) [36]. Such an increase in glucose sensitivity with loss of glucose responsiveness may therefore contribute not only to the initial phase of β-cell adaptation to over-stimulation with or without hyperglycaemia, but also to the reduction of GSIS in IGT and T2D independently from a strong reduction in β-cell mass. We cannot exclude, however, that the concomitant reduction in β-cell glucose sensitivity and responsiveness in T2D rather result from a moderate but prolonged exposure to various types of stress including oxidative stress and ER stress.

Glucotoxic alterations of β-cell survival and growth - There are now good evidence that the β-cell mass is reduced by ~25-50% in T2D patients as compared with weight-matched non diabetic subjects, mainly due to an increase in β-cell apoptosis [2,3]. However, it remains unclear whether this reduction is large enough to contribute significantly to the defect of GSIS. It is also unknown whether this reduction precedes IGT or only develops as a consequence of hyperglycaemia, though the latter hypothesis is supported by the observation that the reduction of the β-cell mass is proportionate to the duration of T2D [3]. That T2D is associated with an increase in β-cell apoptosis has received strong support in some animal models of T2D (Zucker Diabetic Fatty (ZDF) rats, Lepr^{ob/ob} mice, Psammomys obesus) [4]. An increase in β-cell apoptosis was, however, not observed in other models of T2D (e.g.
Goto-Kakizaki (GK) rats and rats submitted to a 90% partial pancreatectomy (Px90)) [10,25]. In vitro, apoptosis of native rat β cells is also increased by culture in high vs. intermediate glucose, but to a minor extent in comparison with the effect of culture in low glucose [21,22,30].

Besides reducing the β-cell mass, hyperglycaemia increases the size of individual rodent β cells (hypertrophy) [26,27]. This adds another level of complexity in evaluating the functional consequence of a 40% decrease in β-cell mass, as it is not known if a double-sized β cell secretes as much insulin in response to glucose stimulation as two normal β cells. Cell hypertrophy may indeed impair O₂ delivery to β cells distant from islet capillaries or modify other aspects of β-cell physiology that may affect the secretory capacity of a given β-cell mass. It is, however, still unknown if β cells are also hypertrophied in human T2D.

**Glucotoxic alterations of β-cell gene expression** - One of the earliest and best characterized alterations of β-cell gene expression in in vivo models of prolonged hyperglycaemia is the reduction in preproinsulin gene expression. This reduction results from reduced expression of transcription factors involved in the glucose regulation of preproinsulin gene transcription, among which PDX1 and MafA play a prominent role [25]. However, islets from T2D rodents and human patients also display reduced expression of many other genes important for β-cell function and of the transcription factors that regulate their expression [25,26,28,37,38]. This almost global reduction in the expression of β-cell enriched genes, which is already observed after prolonged exposure to only moderate hyperglycaemia [27], could contribute to β-cell dysfunction in the early stages of glucose intolerance and T2D. Its normalization by phlorizin but not bezafibrate treatment in several models of T2D attests its dependence on hyperglycaemia rather than hyperlipidaemia [26,28]. In vitro, the reduction in preproinsulin gene expression was initially characterized in HIT-T15 insulin secreting cells while the global reduction in β-cell enriched genes has been confirmed in INS1-derived cell lines [39]. These changes in β-cell gene expression are, however, minor or even absent in rodent islets cultured for 1-2 weeks in high glucose, thereby supporting the idea that culture for a few weeks in high glucose does not fully recapitulate the deleterious effects of in vivo hyperglycaemia. In contrast, these alterations are
induced by \textit{in vitro} exposure to various stressful stimuli, suggesting that they mainly reflect a loss of β-cell differentiation in response to various types of stress [12,13,25].

Besides this apparent loss of differentiation, β cells from hyperglycaemic animals also display increased mRNA levels of genes expressed at low or very low levels in normal β cells, including the glycolytic enzymes hexokinase 1 (\textit{Hk1}) and lactate dehydrogenase A (\textit{LdhA}), the pro- and anti-apoptotic factors \textit{Fas} and \textit{A20}, the antioxidant enzymes haeme-oxygenase 1 (\textit{Hmox1}) and glutathione peroxidase 1 (\textit{Gpx1}), the thioredoxin inhibitor \textit{Txnip} and several transcription factors such as \textit{c-Myc}, Activating transcription factor 3 (\textit{Atf3}) and DNA-damage-inducible transcript 3 (\textit{Ddit3}, also known as \textit{Chop}, \textit{Chop10} or \textit{Gadd153}) [12,26,28,37,40,41]. Most of these genes are typically induced by various types of stress, including oxidative stress, ER stress, cytokine treatment and hypoxia [42-44], but some of them like \textit{Txnip} may be uniquely regulated by glucose-mediated activation of Carbohydrate Response Element Binding Protein [41]. Their impact on β-cell differentiation, function, survival and growth has been extensively studied over the last 10 years. For several genes (including \textit{Ldha}, \textit{c-Myc} and \textit{Txnip}), it has been shown that their forced overexpression induces β-cell dysfunction sometimes associated with β-cell loss of differentiation and apoptosis [41,45-48]. In a few cases, it could also be demonstrated that their deletion protects β cells against glucotoxicity [40,49]. In contrast, a few genes such as \textit{Hmox1} and \textit{Gpx1} are cytoprotective by increasing β-cell defences against oxidative stress [50-52]. As for the loss of β-cell differentiation, the increased expression of these stress-response genes in islets from T2D rodents was largely reduced by phlorizin treatment, confirming the causal role of hyperglycaemia [26,28,52]. However, contrary to the loss of β-cell differentiation, the latter changes in gene expression have also been observed in islets cultured in high glucose for a few days, suggesting that they constitute early sensitive markers of β-cell glucotoxicity (Table 1).

\textbf{Glucose regulation of islet stress responses}

Except for the \textit{in vitro} increase in glucose sensitivity, many glucotoxic alterations of the β-cell phenotype can also be induced by oxidative stress, nitrosative stress, ER stress, and hypoxia that have all been proposed to play a key role in β-cell glucotoxicity [12,13,32,43,53,54]. As the loss of β-cell
differentiation and function and the stimulation of apoptosis do not allow to discriminate between these various types of stress, we postulated that studying the glucose induction of stress-response genes could provide valuable information on the mechanisms of β-cell glucotoxicity and failure in T2D (The reader is referred to Fig.5 for a schematic overview of the concepts discussed in the next sections). We initially characterized the effects of increasing glucose concentrations on the expression of c-Myc and Hmox1 in cultured rat islets. Interestingly, the mRNA levels of both genes, which were almost undetected in fresh islets, were transiently increased by prolonged culture in the presence of 10 mmol/l glucose (peak after 1 day, minimal after 5-7 days), a condition under which β-cell survival and function is optimally preserved [55,56]. These changes in gene expression, which correlate with the activation of the stress-activated protein kinases c-Jun N-terminal kinase (JNK) and Mitogen-Activated-Protein-Kinase of 38 kDa (p38MAPK) [57], were likely due to the stress of isolation, devascularisation and initial adaptation to culture conditions. In contrast, c-Myc and Hmox1 mRNA levels remained constantly elevated during 1 week culture in the presence of 5 or 30 mmol/l glucose, two conditions under which β-cell function and survival are significantly altered [24,55,56]. After 1 week preculture in 10 mmol/l glucose and further 18h culture in 2, 5, 10 or 30 mmol/l glucose, the mRNA levels of both genes were also minimal in 10 mmol/l glucose and significantly increased by either lower or higher glucose concentrations [24,55,56]. These glucose-dependent V-shaped mRNA profiles were parallel to rat islet cell death and inversely related to β-cell glucose responsiveness after prolonged culture under similar conditions [21,22,58], suggesting a possible link between these early changes in gene expression and the late alterations of β-cell function and survival.

To better understand the mechanisms of β-cell dysfunction and apoptosis at low and high vs. intermediate glucose concentrations, we recently measured the effect of glucose (2, 5, 10 and 30 mmol/l) on the transcriptome of rat islets at an early time point when β-cell dysfunction is present but cell apoptosis is still undetected [30]. By cluster analysis of the genes that were significantly up- or downregulated by glucose, we identified ten mRNA profiles with unidirectional up- or downregulation between the various glucose concentrations tested and eight complex V-shaped or inverse V-shaped profiles with a nadir or peak level of expression in 5 or 10 mmol/l glucose. In our preparations of
almost pure endocrine islet cells, the expression of NFκB target genes that are strongly induced by cytokines, like iNos, IkBa and prointerleukin-1β (proIL1β), were not affected by low or high vs. intermediate glucose concentrations, thereby supporting earlier reports that excluded the role of IL1β production and autocrine effects in β-cell glucotoxicity under our culture conditions (Table 1)[13,30,59]. In contrast, several oxidative stress- and ER stress-response genes including c-Myc and Hmox1 had an asymmetric V-shaped profile similar to that of apoptosis (Fig. 3 and Table 1), suggesting that both types of stress may play a role in β-cell demise after culture at extreme low and high glucose concentrations. However, the results also pointed to pathways that may contribute to β-cell demise under only one of these conditions. For instance, the mRNA levels of many hypoxia-inducible factor (HIF)-target genes increased exponentially between 5 and 30 mmol/l glucose, suggesting a role for hypoxia, at least in vitro, in β-cell glucotoxicity (Table 1) [30,60].

Glucose regulation of oxidative stress – It is usually accepted that high glucose concentrations trigger oxidative stress by increasing the production of reactive oxygen species (ROS) in a variety of cell types including β cells. The mechanisms involved comprise increased glucose flux through the polyol and hexosamine pathways, glucose autoxidation, increased protein glycation, activation of Advanced Glycation End products (AGES) receptors with subsequent increase in NFκB-target gene expression, activation of cytosolic NADPH oxidase, and incomplete O₂ reduction at the level of complex I and III of the mitochondrial electron transport chain [43]. The latter mechanism may be particularly important in β cells exposed to high glucose concentrations in vitro as they have a high NADH content, a highly hyperpolarized mitochondrial membrane and a high ATP:ADP ratio under these conditions [34]. Because β cells express mitochondrial Mn-superoxide dismutase (MnSOD) that converts superoxide to hydrogen peroxide (H₂O₂) but very little H₂O₂-detoxifying enzymes such as catalase and GPx [61], they are deemed particularly vulnerable to oxidative stress. In agreement with a role of the latter in β-cell glucotoxicity, high glucose rapidly increased β-cell ROS production as revealed with the fluorescent probes dihydrofluorescein and hydroethidine [62], by the accumulation of nitrotyrosine and 8-OH-deoxyguanosine, a DNA-derived oxidation product [63], or by direct
measurement of islet H$_2$O$_2$ concentrations [29]. In vivo, an increase in the islet content in lipid peroxidation product 4-OH nonenal and in 8-OH-deoxyguanosine has also been documented in human and rodent T2D [64,65]. However, using the same fluorescent probes as Bindokas and coll., Martens and coll. reported that β-cell ROS production is maximal in the absence of glucose when NADPH production and β-cell antioxidant defences are low, while it is markedly reduced upon stimulation of NADPH production by glucose and other nutrient insulin-secretagogues [66].

Although both types of studies may seem diametrically opposed at first sight, they are compatible with our hypothesis (based on the observation that c-Myc, Hmox1 and Metallothionein 1a (Mt1a) mRNA levels are V-shaped with a minimum after culture in 10 mmol/l glucose) that both low and high vs. intermediate glucose concentrations trigger β-cell oxidative stress. c-Myc, Hmox1 and Mt1a are indeed typically induced by various types of stress following a cascade of events that include activation of MAPKs (JNK, p38MAPK…) and of several transcription factors (TFs) such as Nuclear Factor Erythroid-derived 2 Like 2 (NFE2L2), AP-1, NFκB and Heat shock factors [67]. As a further support to this hypothesis, various strategies that increase β-cell antioxidant defences have been shown to reduce oxidative stress after culture in both low and high glucose concentrations. During culture at low glucose, these strategies or the inhibition of JNK and other MAPKs significantly reduced β-cell apoptosis but their effect on GSIS has not been reported [66,68]. In hyperglycaemic animals and in islets cultured in high glucose, they could normalize β-cell preproinsulin and oxidative stress-response gene expression to some extent, but their ability to improve β-cell function and oxidative stress-response were highly dependent on the type and concentration of antioxidants (free radical scavengers, catalytic antioxidants, over-expression of antioxidant enzymes) as well as on the model used, preventing easy extrapolation of these conclusions to human T2D [56,59,69-72]. Until now, conclusive evidence that antioxidants improve β-cell function and survival in human T2D are still lacking. A careful evaluation of non-classical antioxidant systems expressed in various β-cell subcellular compartments and a better characterization of how, where and which ROS are produced in low and high vs. intermediate glucose concentrations may, in the future, improve the choice of antioxidant treatment and, hence, its
therapeutic efficacy on the dysfunction and death of β cells exposed to extreme glucose concentrations.

Glucose regulation of ER stress – Synthesis and folding of proteins in the ER critically depends on adequate production and function of a variety of ER chaperones. When ER chaperone function decreases (following ER Ca\(^{2+}\) emptying, ATP depletion… ) or ER client protein load increases (increased protein synthesis, expression of mutated proteins…), activation of the unfolded protein response (UPR) transiently reduces protein synthesis and triggers a transcriptional program aimed at restoring ER homeostasis and increasing cell defences against various types of stress. However, if unsuccessful, continuous activation of the UPR eventually triggers cell apoptosis. [54]. The ER stress response involves the unconventional splicing of X-box binding protein 1 (Xbp1) pre-mRNA by the ER stress sensor “ER to nucleus signalling 1” (ERN1, previously known as Inositol-requiring 1α) with subsequent increase in active XBP1, the proteolytic activation of ATF6, and the phosphorylation of the eukaryotic translation initiation factor 2 α (EIF2A) by its kinase EIF2AK3 (also known as PERK). While both XBP1 and ATF6 stimulate the expression of ER chaperones and of components of the ER-associated degradation pathway, EIF2A phosphorylation by EIF2AK3 induces a general translational pause with selective increase in Atf4 mRNA translation and subsequent stimulation of expression of ATF4-target genes like Atf3 and Ddit3. The latter part of the UPR, which we later refer to as the Integrated Stress Response (ISR), can also be activated independently of the UPR following EIF2A phosphorylation by EIF2AK1, 2 and 4 [73]. Therefore, an increase in Atf3 or Ddit3 mRNA levels should not be considered as a sign of ER stress unless it is associated with an increase in ERN1 phosphorylation, Xbp1 mRNA splicing, ATF6 activation or EIF2AK3 phosphorylation [74].

Over the last few years, it has become increasingly evident that β-cell function and survival critically depend on the maintenance of ER homeostasis by the UPR to cope with their high rate of proinsulin synthesis. It has, in contrast, been shown that excessive activation of a normal or only partially defective UPR also triggers β-cell apoptosis and diabetes, indicating that too high UPR activation is also deleterious to the functional β-cell mass. It has thus been proposed that sustained
activation of proinsulin (and maybe proIAPP) synthesis with possible alterations of ER Ca\(^{2+}\) homeostasis and chaperone function in IGT and T2D induces an ER stress, thereby contributing to β-cell apoptosis and dysfunction [54]. In support of this hypothesis, several \textit{in vitro} studies have demonstrated that high glucose concentrations moderately increase ERN1 phosphorylation, \textit{Xbp1} mRNA splicing and ATF4-target gene expression in β cells, suggesting that ER stress may play a role in β-cell glucotoxicity [74,75]. Furthermore, \textit{Atf3} and \textit{Ddit3} are expressed at higher levels and seem to contribute to β-cell apoptosis in several animal models of T2D [40,76]. In humans, it was initially reported that islets isolated from T2D patients do not display any increase in the expression of genes characteristic of UPR activation unless they are cultured for a few days in high glucose concentrations [77]. However, two recent studies showed increased expression of various UPR markers, including ER chaperones and DDIT3, on pancreatic sections from T2D patients [78,79]. There is thus good evidence that an increase in β-cell ER stress occurs in human and animal models of T2D and may contribute to β-cell apoptosis.

It should be noted, however, that, \textit{in vitro}, the maximal glucose stimulation of \textit{Xbp1} mRNA splicing was moderate in comparison with the effect of the ER stress-inducing agents thapsigargin (TG) and cyclopiazonic acid. This glucose effect was maximal within 2h of stimulation, was rapidly reversed by a drop in glucose concentration, and was inhibited by low concentrations of cycloheximide that suppress the glucose stimulation of protein synthesis without inhibiting its basal level [74]. It therefore seems that, depending on its level and duration of activation, the UPR initially favours the adaptation of β-cells to the higher insulin synthetic load before triggering their apoptosis. Therefore, one may predict that inhibiting the UPR in T2D could be detrimental to β-cell function unless one can exclusively inhibit its pro-apoptotic components such as DDIT3 and ATF3 expression as well as JNK activation. In contrast, strategies that increase β-cell ER chaperone expression and function may be beneficial to the maintenance of the functional β-cell mass in IGT and T2D [80].

In parallel with what was observed for the glucose regulation of oxidative stress, we and others have recently shown in MIN6 cells and cultured rat islets that the expression of ATF4-target genes characteristic of ISR activation, such as \textit{Ddit3} and \textit{Atf3}, are minimal after culture in 10 mmol/l
glucose, strongly increased by culture in low glucose, and only slightly increased by high glucose concentrations (Fig. 3) [74,81]. That the ISR is strongly activated by glucose deprivation was further supported by the observation that EIF2A phosphorylation is high in MIN6 cells cultured at low glucose and markedly reduced by nutrient stimulation. It is, however, unclear whether EIF2A phosphorylation resulted from the activation of EIF2AK3 by ER stress or from the activation of another EIF2A kinase independently from UPR activation [81,82]. Nevertheless, as Ddit3 and Atf3 have been shown to favour β-cell apoptosis, it is tempting to speculate that the ISR may, in addition to oxidative stress, also play a role in β-cell apoptosis after culture in low glucose concentrations.

Mechanisms of oxidative stress, ER stress and ISR activation by extreme glucose concentrations

Until now, the available evidence point to the role of oxidative stress and ISR activation in β-cell apoptosis after culture in either low or high vs. intermediate glucose concentrations. However, their cause likely differs under both conditions.

Low glucose-induced oxidative stress and ISR activation - Although one study has suggested that the low influx of Ca^{2+} and the low rate of insulin secretion play a role in β-cell apoptosis during culture in low glucose due to a lack of autocrine activation of PI3K and PKB [23], we have shown that the inhibition of Ca^{2+} influx and insulin secretion by diazoxide in the presence of 10 mmol/l glucose does not increase β-cell apoptosis, oxidative stress and ISR gene expression [24,74]. Thus, β-cell oxidative stress, ISR and apoptosis at low glucose rather seem to result from low ATP and NADH production with consequent activation of AMPK, decreased ROS detoxification, activation of JNK, and triggering of the intrinsic mitochondrial apoptosis pathway [83]. It has indeed been demonstrated that nutrients that are efficiently metabolized by the β cell (combination of leucine and glutamine, α-ketoisocaproate, monomethyl-succinate…) can suppress β-cell apoptosis, oxidative stress, and the mRNA levels of oxidative stress and ISR genes (including the pro-apoptotic genes c-Myc, Ddit3 and Atf3) in the presence of low glucose concentrations [24,30,74,83].
To test whether the ISR and oxidative stress are causally related, we recently compared the effect of TG and H$_2$O$_2$ on stress-response gene mRNA levels in cultured rat islets. As shown in Table 1, H$_2$O$_2$ only moderately affected \textit{Atf3} and \textit{Ddit3} expression while dramatically increasing \textit{c-Myc}, \textit{Hmox1} and \textit{Mt1a} mRNA levels. In contrast, TG markedly increased both ISR and oxidative stress-response genes. These results may suggest that oxidative stress results from ISR activation by low glucose, but a word of caution is necessary as TG induces a full ER stress-response whereas low glucose only triggers the ISR without increasing \textit{Xbp1} mRNA splicing [74]. On the other hand, it seems rather safe to assume that ISR activation at low glucose does not result from the increase in oxidative stress. Future research should aim at defining the mechanism of low glucose induction of β-cell ISR and oxidative stress and their possible role in the atrophy and apoptosis of β cells exposed to sustained \textit{in vivo} hypoglycaemia.

\textit{High glucose-induced oxidative stress and ER stress activation} - The stimulation of β-cell apoptosis and of \textit{c-Myc}, \textit{Hmox1} and \textit{Mt1a} expression by culture in 30 instead of 10 mmol/l glucose was significantly reduced by diazoxide and nimodipine that inhibit the glucose stimulation of Ca$^{2+}$ influx and insulin secretion, and significantly enhanced by sulphonylureas that exert the opposite effects, suggesting that β-cell over-work may play a role in these glucotoxic effects [22,55,56]. In contrast, these pharmacological agents did not affect the stimulation of \textit{Xbp1} mRNA splicing and the increase in ISR gene expression by culture in 30 mmol/l glucose [74]. It therefore seems that the \textit{in vitro} glucotoxic alterations of β-cell function and survival are better correlated with oxidative stress than ER stress. A further argument supporting the latter conclusion comes from the comparison of the effects of glucose on cell survival and stress-response gene expression in mouse vs. rat islets. As in rat islets, the rate of apoptosis and the mRNA levels of oxidative stress and ISR response genes in C57BL6 mouse islets were markedly increased by culture in low vs. intermediate glucose concentrations (Fig. 4). However, in contrast with rat islets, culture in high vs. intermediate glucose concentrations did not significantly increase mouse islet cell apoptosis and the mRNA levels of oxidative stress and ISR genes such as \textit{Mt1a}, \textit{c-Myc}, \textit{Hmox1} and \textit{Atf3} despite similar increases in \textit{Xbp1}
pre-mRNA splicing (compare Fig. 4 and Fig. 3). These results, which are in good agreement with earlier studies showing that β-cell glucotoxicity is not observed in mouse islets except in a few strains like DBA/2 mice [29,48], strongly suggest that oxidative stress rather than ER stress is the culprit of in vitro β-cell glucotoxicity (Fig. 5). However, this conclusion may not be valid in IGT and T2D where β-cells are exposed to other types of oxidative stress- and ER stress-inducing conditions, including hyperlipidaemia, moderate systemic inflammation and, in humans but not rodents, accumulation of amyloid fibrils [4].

**High glucose-induced cytokine production and NFκB activation** - It is well established that, in vitro, inflammatory cytokines (IL1β, IFNγ, TNFα…) alter β-cell survival and function by inducing nitrosative stress, oxidative stress and, to a lesser extent, ER stress and the ISR [13,84]. It is thus not surprising that cytokines induce changes in stress-response gene expression that are very close to those induced by H₂O₂, TG, low glucose and high glucose concentrations (Table 1). The provocative proposal that the β-cell pathophysiology is similar in T2D and T1D was initially build on these similarities and on the observations that in vivo and in vitro exposure to high glucose concentrations increases NFκB DNA binding activity and expression of NFκB-target genes, either as a result of β-cell oxidative stress [52] or following the glucose stimulation of IL1β production and autocrine activation of apoptosis in β cells [12,85]. These results are, however, highly controversial. Thus, as illustrated in Table 1, we and others have shown that high glucose and H₂O₂ do not increase the mRNA levels of the NFκB-target genes iNOS, IkBa and Pro-IL1β in cultured rat and human islets, in agreement with a lack of increase in NFκB DNA-binding activity [13,59]. Despite these negative studies, the beneficial effect of the recombinant IL1-receptor antagonist anakinra on glucose tolerance in human T2D indicates that, in some patients at least, circulating or locally produced IL1β contributes to glucose intolerance [32,86] (Fig. 5).

**High glucose-induced hypoxia** – Adequate supply of O₂ is crucial to mitochondrial ATP production. Under normoxic conditions, the regulatory subunit of Hypoxia-Inducible Factors 1 and 2
(HIFα) are hydroxylated on prolyl residues in an O2, Fe2+ and 2-oxoglutarate-dependent manner. Hydroxylated HIFα is then recognized by the Von Hippel Lindau factor (encoded by the \textit{Vhlh} gene), poly-ubiquitylated and degraded by the proteasome. Under hypoxic conditions (O2~0.3-5% or pO2~2.3-38 mmHg), HIFα prolyl-hydroxylation and degradation is reduced, allowing HIFα to translocate to the nucleus where it dimerizes with its partner HIF1β and increases the transcription of genes that contain a hypoxia-response element in their promoter (\textit{Glut1}, most glycolytic enzymes, pyruvate dehydrogenase kinase 1 (\textit{Pdk1}), adrenomedullin (\textit{Adm}), \textit{Vegfs}, erythropoietin, etc.) [87]. This response triggers a switch from aerobic mitochondrial ATP production to anaerobic glycolytic ATP production at the cellular level, an increase in blood flow and capillary growth at the organ level, and an increase in O2 transport capacity at the organism level.

In β cells, the response to hypoxia may not only favour cell survival and resistance to subsequent hypoxic episodes but also impair β-cell function. It has indeed been demonstrated that prolonged HIF activation (by \textit{Vhlh} gene inactivation) in β cells in the absence of hypoxia increases their basal rate of insulin secretion and reduces their maximal GSIS, thereby inducing glucose intolerance [88]. These alterations, which resemble those induced by prolonged culture in high glucose concentrations [34], likely resulted from 1) increased anaerobic glycolytic production of ATP at low glucose following the increased expression of GLUT1 and of most glycolytic enzymes except glucokinase, and 2) reduced pyruvate utilization and ATP production by mitochondria at high glucose following the inhibition of pyruvate dehydrogenase by PDK1 and the increased conversion of pyruvate to lactate by LDH-A [88].

It is well established that glucose stimulates the expression of most glycolytic enzymes in cultured rat islets and insulin-secreting cell lines as well as in animal models of T2D [28,37,53,55,89,90]. As discussed before, the increase in HK1 and LDH-A that are not expressed in normal β-cells may contribute to the higher glucose sensitivity and the lower glucose oxidation:utilization ratio observed in islets from hyperglycaemic animals [89,91]. In our recent study on the effects of glucose on the transcriptome of cultured rat islets, we confirmed that the mRNA levels of most glycolytic enzymes (except glucokinase) increase exponentially between 5 and 30 mmol/l glucose [30]. We also observed that other HIF-target genes, such as \textit{Adm} and hypoxia-upregulated 1 (also known as \textit{Orp150}), have a
similar mRNA profile, suggesting that high glucose induces β-cell hypoxia in cultured rat islets. Such an effect would not be really surprising, for it is well demonstrated that the acceleration of β-cell mitochondrial metabolism in response to glucose stimulation increases their O$_2$ consumption rate, thereby decreasing islet pO$_2$ [92]. In agreement with this hypothesis, we recently showed that glucose activates HIF1 and HIF2 and increases the mRNA levels of glycolytic enzymes and Adm in a pO$_2$-sensitive manner in INS1 cells as well as in whole rat islets [60]. Furthermore, glucose concentration-dependently induced β-cell hypoxia in cultured rat islets, as shown with the hypoxia marker pimonidazole (MB and JCJ, manuscript in preparation). As shown in Table 1, these effects were much lower than those induced by 18h culture in the presence of 5% O$_2$, indicating that high glucose-induced hypoxia is only moderate. Table 1 also shows that Adm mRNA levels were markedly induced by short-term cytokine treatment, but this effect was not accompanied by significant changes in LdhA and Gapdh mRNA levels, strongly suggesting that it did not result from cytokine-induced HIF activation.

There is thus good evidence that hypoxia may contribute to β-cell glucotoxicity in vitro (Fig. 5). The in vivo situation is, however, more complex. It has indeed been shown that, under normal conditions, rodent islet blood flow (IBF) increases upon acute glucose stimulation independently from changes in islet pO$_2$, the latter only slightly decreasing from 37 to 32 mmHg [93]. An increase in basal IBF is also observed under various conditions of increased insulin demand with or without hyperglycaemia (e.g. in diabetic OLETF rats, young diabetic GK rats and Lepr$^{ob/ob}$ mice) [94,95]. In that case, however, this higher basal IBF could not be further increased upon acute glucose stimulation, suggesting that, in prediabetic states or in early stages of T2D, β cells may suffer from hypoxia or intermittent hypoxia during post-prandial glucose excursions. Over time, progressive reduction of IBF and β-cell hypertrophy in diabetic animals may further exacerbate β-cell hypoxia [26,94,96]. This hypothesis is strongly supported by the observation that hypoxia-response gene expression is increased in islets from diabetic Lepr$^{ob/ob}$ mice (D.R. Laybutt, personal communication) and from 12 week-old ZDF rats in which the islet microvasculature is markedly altered [53]. Other factors that may impede IBF and contribute to β-cell hypoxia in T2D include islet fibrosis, amyloid
deposits, and islet microangiopathy [32]. If our hypothesis is correct, cycles of hypoxia/reoxygenation may contribute to β-cell oxidative stress in IGT and T2D [97], thereby contributing to the slow deterioration of the functional β-cell mass over the years.

**Conclusion**

The glucose regulation of islet oxidative and integrated stress responses follow an asymmetric V-shaped profile parallel to that of β-cell apoptosis, with a large increase at low glucose and a moderate increase at high vs. intermediate glucose concentrations, suggesting that both types of stress play a role in the alteration of the functional β-cell mass under states of prolonged hypo- and hyperglycaemia. In addition, β-cell glucotoxicity may also result from hypoxia and, at least *in vivo*, from exposure to inflammatory cytokines released by non-endocrine islet cells. A better understanding of the relative contribution of each type of stress to β-cell glucotoxicity and of their pathophysiological cause *in vivo* may lead to new therapeutic strategies to prevent the slow deterioration of the functional β-cell mass in IGT and T2D patients.
Animal experiments

All experiments were approved by the local ethics committee for animal experimentation and performed according to the Principles of Laboratory Animal Care.

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Figure legends

**Figure 1 Possible mechanisms of β-cell glucotoxicity** - Sustained or repeated exposure to high glucose concentrations rapidly alters β-cell gene expression and function while slowly affecting the β-cell mass through changes in cell survival and growth (proliferation and hypertrophy). These alterations, which may influence each other in many ways (dotted arrows), seem to result from β-cell exposure to various types of stress.

**Figure 2 Typical alterations of GSIS in rat islets cultured in high vs. intermediate glucose concentrations:** 1) increased glucose sensitivity; 2) decreased glucose responsiveness – After 1 week culture in RPMI medium containing 10 or 30 mmol/l glucose, rat islets were perfused with Krebs medium containing 0.5, 6, 10 and 20 mmol/l glucose in the eventual presence of 250 µmol/l diazoxide (Dz) and 30 mmol/l extracellular K⁺ (K30), as indicated on top of the figure. Insulin secretion was measured in 2 min effluent collections (means of 3 experiments). Although the rate of insulin secretion in G20 was only reduced by 56% after culture in high glucose (arrow 2), its combination with a 12-fold higher rate of secretion in G6 (arrow 1) lead to a 27-fold reduction in GSIS between G6 and G20. Please note that the response to high K⁺ stimulation was not affected by culture in high glucose. Figure adapted from [34].

**Figure 3 Effects of low, intermediate and high glucose concentrations on insulin secretion (A), cell apoptosis (B-D), and the mRNA levels of oxidative stress- and ER stress-response genes (E-J) in cultured rat islets** - Islets were isolated from 200g male Wistar rats. They were then precultured for 1 week in serum-free RPMI medium containing 10 mmol/l glucose and supplemented with 5 g/l BSA. Batches of 50-100 islets were further cultured in the presence of 2, 5, 10 and 30 mmol/l glucose for 18h (A, E-J) or 7 days (B-D). A, insulin secretion during culture was measured by RIA. B, islet cell caspase activation was measured with the fluorescent inhibitor of caspases FAM-VAD-fluoromethylketone. C, Cytoplasmic histone-associated DNA fragments were quantified with the kit
Cell Death Detection ELISA PLUS (Roche Diagnostics, Mannheim, Germany). D, The percentage of apoptotic cells (TUNEL-positive:DAPI-positive nuclei) was determined with the In Situ Cell Death Detection Kit (Roche Diagnostics) on sections of islets fixed in paraformaldehyde 4% and embedded in paraffin. E-J, Xbp1 mRNA splicing and islet Gene:Tbp mRNA ratios were measured by RT-PCR as described [74]. These ratios are expressed relative to the ratio measured after culture in 10 mmol/l glucose. Shown are means of 2 experiments (C), means ± s.e.m. (open circles) and individual data (black dots) for all islets from a single culture (D) or means ± s.e.m. for 3-7 experiments (A, B, E-J). *, p<0.05 vs. islets cultured in 10 mmol/l glucose by two-way ANOVA followed by a test of Bonferroni. Except for data shown in panel D, data were adapted from [30,74].

**Figure 4** Effects of low, intermediate and high glucose concentrations on insulin secretion (A), cell apoptosis (B-D), and the mRNA levels of oxidative stress- and ER stress-response genes (E-J) in cultured mouse islets – Islets were isolated from 3-9 month-old males and females C57BL6J mice. Methods were as described in legend to Fig. 3. Shown are means ± s.e.m. (open circles) and individual data (black dots) for all islets from a single culture representative for 2 experiments (D) or means ± s.e.m. for 3 experiments. * p<0.05 vs. islets cultured in 10 mmol/l glucose by two-way ANOVA followed by a test of Bonferroni.

**Figure 5** High glucose induction of islet stress-response gene expression – Data from the literature and Table 1 suggest that, *in vitro*, high glucose predominantly increases the expression of oxidative stress-response genes in an NFκB-independent manner, probably via oxidative stress-mediated activation of NFE2L2 and of the stress-activated protein kinases JNK and p38MAPK (SAPK) (*in vitro* exposure to H$_2$O$_2$ slightly increases the expression of NFκB-target genes, as indicated by the thin dotted arrow, but this does not occur with the level of oxidative stress induced by high glucose). High glucose also stimulates the expression of ER stress-response genes via ERN1-mediated activation of XBP1 and EIF2AK3-mediated activation of ATF4. However, the effect on XBP1-target genes that improve β-cell folding capacity predominates over that on ATF4-target genes that play a pro-apoptotic
role in β cells (in vitro treatment with TG also increases oxidative stress-response gene expression, but it is not clear if this occurs with the moderate level of ER stress induced by high glucose). Finally, high glucose increases hypoxia-response gene expression via HIF1 and HIF2 activation, likely reflecting a state of moderate hypoxia in β cells (culture under hypoxic conditions also triggers oxidative stress-response gene expression, but it is not clear if this occurs with the moderate level of hypoxia induced by high glucose). In comparison with these in vitro effects of high glucose concentrations, in vivo hyperglycaemia similarly affects islet stress-response gene expression, except for the additional stimulation, in some but not all studies, of the expression of inflammation stress-response genes via NFκB activation, likely as a result of IL1β release by non-endocrine cells (endothelial cells, peri-islet inflammation…) in the vicinity of β cells. Arrow thickness is grossly proportional to the importance of the pathway in β-cell glucotoxicity according to the authors. Broken arrows, events that only take place in vivo in some patients or animal models of T2D. ↑, activation or increased expression.
Table 1  Effects of various types of stress on islet gene mRNA levels

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<td><strong>ATF4-target genes (ISR marker, not specific of ER stress)</strong></td>
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The effect of in vivo hyperglycaemia was compiled from published (except &) studies carried out in animal models of T2D [28,40,52,55,76,78]. The effects of cytokines were measured in 1 week precultured rat islets further cultured for 6h in the presence of 10 mmol/l glucose + 50 IU/ml recombinant human IL1β, as described [59]. The effects of other types of stress were measured in precultured rat islets further cultured 18h in the presence of 2-5 mmol/l glucose (Low glucose)[30], 30 mmol/l glucose (High glucose)[30], 10 mmol/l glucose + 50 µmol/l H₂O₂ (Oxidative stress)[59,70], 10 mmol/l glucose + 1 µmol/l TG (ER stress)[74], or 10 mmol/l glucose in the presence of 5 instead of 20% O₂ (Hypoxia) (MB and JCJ, unpublished results). The effects of a 3 day-culture in 30 mmol/l glucose (late) are only mentioned if different from that at 18h. Changes in gene mRNA levels were expressed relative to that measured in their proper control (see corresponding references) before being converted in +, = or – signs according to the following rules: =, no significant change; +, ++, ++++, +++++ and ++++++, significant increase up to 2, 5, 10 and 1000-fold respectively; – and --, significant decrease down to 75 and 50% of controls respectively. # denotes discordant data in the literature regarding the effects of in vivo hyperglycaemia and in vitro culture in high glucose concentrations. & D.R. Laybutt, personal communication. ND, not determined.
Sustained / repeated exposure to high glucose concentrations

Oxidative stress
ER stress
IL1β production
Hypoxia
...

Altered β-cell gene expression

Altered β-cell survival / growth

β-cell dysfunction
Figure 2

Jonas et al. Glucose regulation of islet stress responses
Figure 3

A) Insulin secretion (ng islet⁻¹ h⁻¹)

B) Caspase activation (relative to G10)

C) DNA fragmentation (relative to G10)

D) Islet cell apoptosis (% TUNEL-positive nuclei)

E) Mitf/Tbp mRNA ratio

F) Spliced/total Xbp1 mRNA ratio

G) Ddit3/Tbp mRNA ratio

H) Atf3/Tbp mRNA ratio

I) Hmox1/Tbp mRNA ratio

J) Mt1a/Tbp mRNA ratio

K) Myc/Tbp mRNA ratio

L) Caspase activation (relative to G10)

M) Spliced/total Xbp1 mRNA ratio

N) Ddit3/Tbp mRNA ratio

O) Atf3/Tbp mRNA ratio

P) Hmox1/Tbp mRNA ratio

Q) Mt1a/Tbp mRNA ratio

R) Myc/Tbp mRNA ratio

Glucose (mmol/l)
Figure 4

A) Insulin secretion (ng/islet' h')

B) Caspase activation (relative to G10)

C) DNA fragmentation (relative to G10)

D) Islet cell apoptosis (% TUNEL-positive nuclei)

E) Mt1a/Tbp mRNA ratio

F) Spliced/total Xbp1 mRNA ratio

G) Ddit3/Tbp mRNA ratio

H) ATF3/Tbp mRNA ratio

I) Hmox1/Tbp mRNA ratio

J) Mt1a/Tbp mRNA ratio

Glucose (mmol/l)
Figure 5

**Glucose regulation of islet stress responses**

1. **IL1β**
   - In vivo?
2. **High glucose**
3. **Oxidative stress**
   - NFκB↑
   - iNos↑
   - IL1β↑
   - Lkα↑
   - Fas↑
   - proIL1β↑
   - NFE2L2↑
   - SAPK↑
4. **ERN1↑**
5. **ER stress**
   - BiP↑
   - Foldases↑
   - ATF4↑
6. **HIF1-2↑**
7. **Hypoxia**
   - Ddit3↑
   - Atf3↑
   - Trib3↑
8. **Adm↑**
   - Hk1↑
   - Ldha↑
   - Pdk1↑