

β -cell failure as a complication of diabetes

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Published online: 6 September 2008
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Abstract Type 2 diabetes mellitus is a complex disease characterized by β -cell failure in the setting of insulin resistance. In early stages of the disease, pancreatic β -cells adapt to insulin resistance by increasing mass and function. As nutrient excess persists, hyperglycemia and elevated free fatty acids negatively impact β -cell function. This happens by numerous mechanisms, including the generation of reactive oxygen species, alterations in metabolic pathways, increases in intracellular calcium and the activation of endoplasmic reticulum stress. These processes adversely affect β -cells by impairing insulin secretion, decreasing insulin gene expression and ultimately causing apoptosis. In this review, we will first discuss the regulation of β -cell mass during normal conditions. Then, we will discuss the mechanisms of β -cell failure, including glucotoxicity, lipotoxicity and endoplasmic reticulum stress. Further research into mechanisms will reveal the key modulators of β -cell failure and thus identify possible novel therapeutic targets. Type 2 diabetes mellitus is a multifactorial disease that has greatly risen in prevalence in part due to the obesity and

inactivity that characterize the modern Western lifestyle. Pancreatic β -cells possess the potential to greatly expand their function and mass in both physiologic and pathologic states of nutrient excess and increased insulin demand. β -cell response to nutrient excess occurs by several mechanisms, including hypertrophy and proliferation of existing β -cells, increased insulin production and secretion, and formation of new β -cells from progenitor cells [1, 2]. Failure of pancreatic β -cells to adequately expand in settings of increased insulin demand results in hyperglycemia and diabetes. In this review, we will first discuss the factors involved in β -cell growth and then discuss the mechanisms by which β -cell expansion fails and leads to β -cell failure and diabetes (Fig. 1).

Keywords β -cell failure · Lipotoxicity · Glucotoxicity · Glucolipotoxicity · Diabetes

1 Importance of proliferation, neogenesis, apoptosis

On the basis of multiple studies performed by several laboratories, it has been shown that the respective contributions of β -cell proliferation, neogenesis and apoptosis to overall β -cell mass varies at different stages of postnatal life as well as in response to stress conditions [1–8]. Maintenance of β -cell mass in adult life results predominantly from proliferation of pre-existing β -cells [9]. However, recent data using lineage tracing demonstrates that neogenesis from ductal progenitors can also contribute to β -cell mass during normal conditions or after β -cell injury [10]. The overall contribution of neogenesis to maintenance of β -cell mass in postnatal life is unclear.

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1.1 Response to increased insulin demand— β -cell proliferation

The regulation of pancreatic β -cell mass occurs via the interplay of multiple proteins. The three major classes of cell cycle proteins—cyclins, cyclin-dependent kinases (CDKs), and cyclin-dependent kinase inhibitors (CKIs) have been extensively studied and have been found to govern cell cycle progression in various mammalian cell types [11]. Their role in β -cell proliferation as demonstrated in various rodent models is briefly summarized here. Cyclin D1 and cyclin D2 are both expressed in β -cells. Cyclin D1 knockout mice exhibit normal islet cell size and number. However, cyclin D1 overexpression in mice has been found to increase β -cell proliferation and mass *in vivo* [12]. Cyclin D2 is essential for regulation of β -cell mass; cyclin D2 knockout mice have decreased β -cell mass and decreased insulin levels [13, 14]. In the class of cyclin-dependent kinases (CDKs), CDK4 and CDK2 are expressed in β -cells. CDK4 knockout mice exhibit normal islets at birth but develop diabetes early in life [15]. The cyclin-dependent kinase inhibitors (CKIs), including the four INK4 proteins and the three members of the Cip/Kip family, are all expressed in pancreatic islets [16]. INK4 proteins complex with CDK4 and prevent the binding of CDK4 or CDK6 to cyclin D1, leading to cell cycle arrest [17]. Mice with a mutant form of CDK4 that is resistant to binding by INK4 proteins exhibit islet hyperplasia [15, 18]. P27Kip1 is particularly interesting because it is regulated by the insulin signaling pathway and is thought to be a major factor in the regulation of β -cell mass. Mice lacking p27 show improved glucose tolerance with increased β -cell mass and proliferation as well as increased serum insulin levels [19, 20]. In contrast, mice overexpressing p27 during the early neonatal period show reduced β -cell mass and glucose intolerance [19]. However, overexpression of p27 in adult mice has no effect on glucose tolerance or β -cell mass. This suggests that p27 functions more in the context of β -cell development or during proliferative conditions and plays a greater role during early postnatal life than during adult life. In addition, β -cells from *db/db* mice exhibit increased p27 levels and deletion of this inhibitor protein rescues the diabetes from mice deficient in IRS2, indicating that p27 is part of the abnormal adaptation of β -cells to insulin resistance [20]. In summary, maintenance of β -cell mass in adult life during normal conditions results predominantly from proliferation of pre-existing β -cells and this process is dependent on the balance between numerous cell cycle proteins [9, 13, 15, 21].

1.2 Response to increased insulin demand—neogenesis

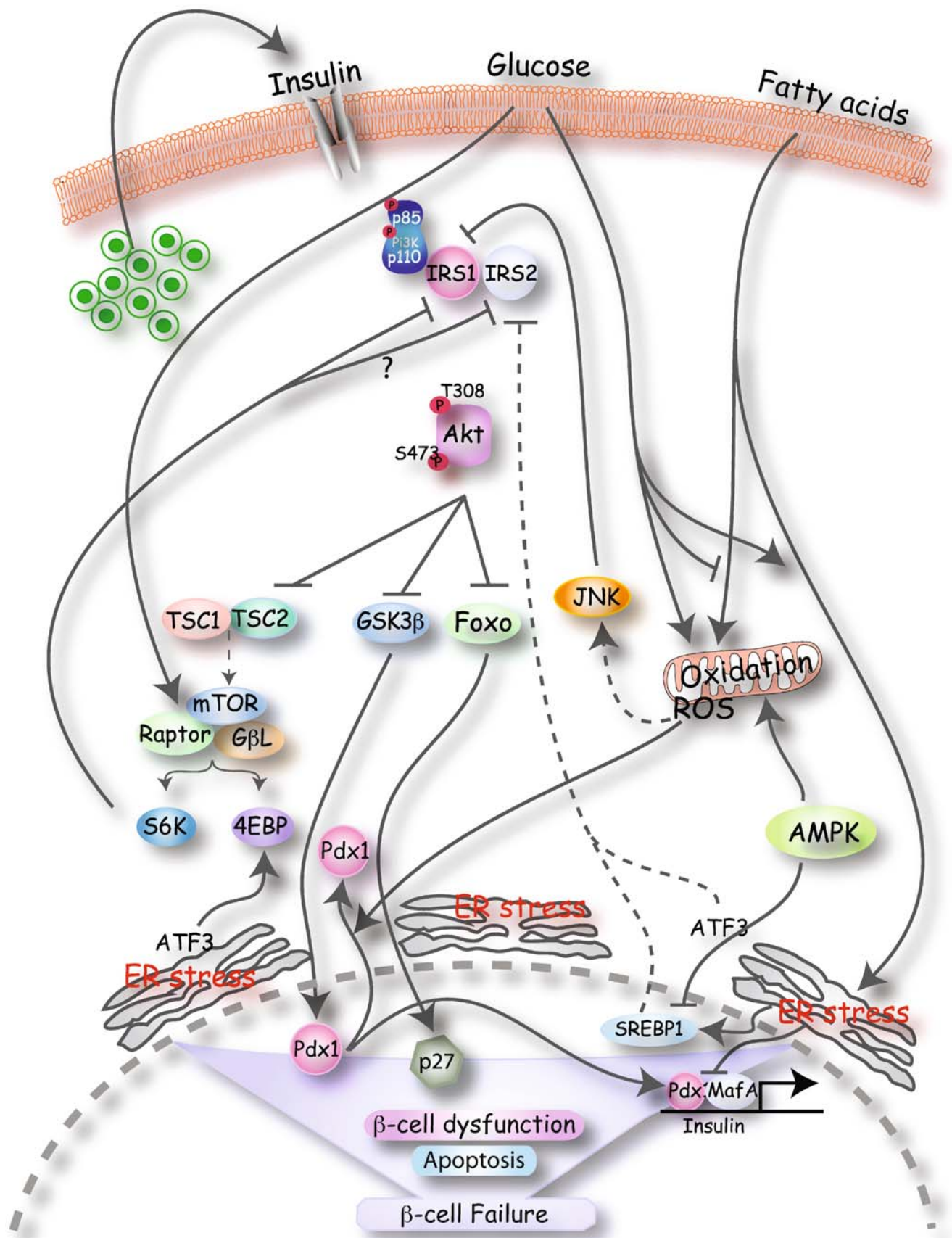
The contribution of neogenesis to the maintenance of β -cell mass in both normal conditions and during

Fig. 1 The mechanisms by which β -cell failure and apoptosis occur are complex, not completely unraveled and involve the interplay of numerous factors and conditions. These factors are summarized in this figure. Glucotoxicity and lipotoxicity lead to the production of ROS, which activate JNK. JNK activity leads to a decrease in IRS signaling and may directly be involved in decreased Pdx-1 activity by translocation from the nucleus to the cytoplasm [183]. In addition, glucose and FA have both been found to induce ER stress. Chronic glucose elevation inhibits FA oxidation and favors the generation of ceramide and lipid partitioning, which ultimately results in β -cell dysfunction and apoptosis. AMPK activation promotes fatty acid oxidation by phosphorylation and inhibition of acetyl-CoA carboxylase or via down regulation of the transcription factor sterol-regulatory-element-binding-protein-1c (SREBP1c) and subsequent decreases in acetyl-CoA carboxylase. Glucose and FA also activate the UPR response and induce ER stress. The ER stress response and its effectors are activated in response to misfolded proteins in order to protect β -cells from apoptosis; however, activation of these processes under conditions of long-term elevation of FFA and glucose can lead to β -cell dysfunction and ultimately apoptosis. Activation of ER stress leads to inhibition of insulin mRNA and protein expression and may also be pro-apoptotic. The mechanisms for induction of apoptosis by ER stress are not completely known, but induction of CHOP is an important component. In addition, induction of ATF3 and SREBP can down-regulate IRS signaling by repressing IRS2 transcription. One interesting finding is that inhibition of IRS signaling seems to be a common pathway induced by the majority of the mechanisms described for β -cell failure. An additional event is the increase in mTOR signaling by nutrient excess (glucose). This results in negative feedback inhibition on IRS1 and possibly IRS2 by activation of S6K signaling. The decrease in IRS signaling induces GSK3 β and Foxo1 function. Activation of these molecules ultimately reduces Pdx1 levels and increases the levels of the cell cycle inhibitor p27

adaptation to stress has been debated. The formation of new β -cells from precursor cells is a process that normally halts after birth. Numerous studies have implicated the pancreatic ducts as a source of new β -cells under regenerative conditions or after exposure to exendin-4 or beta-cellulin [7, 8, 22–25]. Most recently, Bonner-Weir et al. [10] genetically labeled pancreatic ductal cells with carbonic anhydrase II to serve as a duct cell-specific promoter to drive Cre recombinase. This technique allowed for the identification of ductal cells as the source of new islets via lineage tracing experiments.

1.3 Response to increased insulin demand—apoptosis

Apoptosis in β -cells is a highly complex process, governed by pro- and anti-apoptotic genes, extracellular signals and intracellular ATP levels [26]. β -cell apoptosis is a major contributor to the development of type 1 diabetes, with a reduction in β -cell mass of approximately 70% at the time of diagnosis. In contrast, there is a 25–50% reduction in β -cell mass seen at the time of diagnosis of type 2 diabetes, suggesting that β -cell dysfunction also contributes to the initial disease process [27, 28]. However, β -cell apoptosis could be a major contributor to the



pathogenesis of β -cell failure in late stages of the development of Type 2 Diabetes [29, 30].

2 Factors that govern β -cell mass

2.1 Glucose and nutrients

Evidence implicating the role of glucose in β -cell mass and proliferation comes from *in vitro* and *in vivo* experiments. Glucose induces β -cell replication in fetal islets and adult rat islets [31, 32]. *In vivo* experiments have shown that rats and mice subjected to glucose infusion demonstrate increased β -cell mass [33–35]. These results have also been demonstrated using insulinoma cells [36]. Some evidence suggests that glucose also increases β -cell mass via anti-apoptotic effects in rats treated with glucose infusion and in insulinoma cells [34]. The contribution of proliferation, cell size and apoptosis in response to glucose infusion is unclear, but the early changes appear to be mediated by increases in proliferation and cell size.

The signaling pathways and mechanism by which glucose stimulates β -cell mass and proliferation are not entirely understood. Several pathways have been implicated: (1) Autocrine effects of secreted insulin. (2) Induction of calcium signaling. (3) Activation of the TSC2/mTOR signaling pathway. Glucose activation of the insulin receptor activates Akt signaling, via autocrine effects of secreted insulin [37, 38]. Activation of the insulin receptor and other receptor tyrosine kinases activates Akt signaling, a major pathway for β -cell proliferation [39, 40]. In addition to activation of Akt signaling, glucose can induce the mTOR signaling pathway directly or indirectly, by increasing the concentration of ATP and subsequent inactivation of AMP kinase. Recent data suggests that the mTOR signaling pathway is an important regulator of β -cell mass and proliferation [41–45]. Finally, an increase in intracellular calcium could also play an important role in growth signals induced by glucose as demonstrated by recent studies addressing the importance of calcineurin, the only calcium-regulated phosphatase. β -cell-specific deletion of the calcineurin phosphatase regulatory subunit, calcineurin b1 (Cnb1), induces age-dependent diabetes characterized by decreased β -cell proliferation and mass. This phenotype was rescued by overexpression of active NFATc1, indicating the importance of this pathway in regulation of β -cell mass by calcium signals [41]. Activation of other transcription factors like CREB and SRF could also participate in growth responses induced by glucose/calcium signaling [42, 43]. It is important to note that while moderate increases in glucose levels induce β -cell proliferation and survival, prolonged exposure of β -cells

to significant elevations in blood glucose levels causes impaired proliferation and increased β -cell failure and apoptosis (see glucotoxicity below).

2.2 Growth factors

2.2.1 Prolactin, placental lactogen, growth hormone, PTHrP and HGF

Numerous growth factors have been shown to regulate β -cell proliferation. The lactogens prolactin, placental lactogen (PL) and growth hormone (GH) increase β -cell mass in response to increased insulin demand. Prolactin receptors are expressed by β -cells and their expression is upregulated during pregnancy [44]. *In vitro* experiments in islets showed that incubation with prolactin, placental lactogen and growth hormone leads to increased β -cell proliferation [45, 46]. Transgenic mice overexpressing PL in β -cells exhibit increased β -cell mass and hyperinsulinemia [47]. Moreover, mice lacking the prolactin receptor or the growth hormone receptor have reduced β -cell mass, decreased insulin secretion and glucose intolerance [48, 49]. Another factor that has been shown to augment β -cell proliferation in *in vitro* and *in vivo* studies is parathyroid hormone-related protein (PTHrP) [50, 51]. However, it is notable that PTHrP knockout mice have normal β -cell mass as compared to controls, suggesting the PTHrP may not be crucial for normal β -cell mass *in vivo* [52].

Hepatocyte growth factor (HGF) is a mesenchyme-derived growth factor that is involved in cell proliferation, migration and differentiation in a variety of tissues [53]. Numerous studies have demonstrated the role of HGF in the function and proliferation of β -cells [54]. Activated HGF exerts its actions via the transmembrane c-met receptor [55]. HGF and c-met are expressed at high levels during early pancreatic development and are subsequently maintained at low levels in adult rats [56]. Transgenic mice with overexpression of HGF have increased β -cell mass and proliferation and display improved glucose tolerance; isolated β -cells of these animals show increased glucose-stimulated insulin secretion (GSIS) and glucose utilization [54]. Furthermore, mice injected with exogenous HGF gene prior to receiving streptozotocin showed a protective effect on β -cell death and an increase in β -cell proliferation relative to controls [57]. In contrast, mice with deletion of c-met in β -cells are glucose-intolerant and demonstrate impaired GSIS [58]. These glucose intolerant mice have normal β -cell mass and proliferation, suggesting that HGF is essential for normal insulin secretion, but is not crucial for β -cell development. Studies thus far suggest that HGF upregulates β -cell proliferation, decreases apoptosis, and increases β -cell function, making it an attractive potential target for therapy [59].

These growth factors impact β -cell proliferation via diverse signaling pathways. Prolactin and placental lactogen both bind to the prolactin receptor. The prolactin receptor and growth hormone receptor belong to the cytokine family of receptors which act via the Janus Kinase (JAK)/Signal Transduction and Activators of Transcription (STAT) pathway [60]. Activation of this pathway results in upregulation of Cyclin D2 [61]. Cyclin D2, as discussed above, is essential for β -cell proliferation. PTHrP exerts its effects on β -cell proliferation via the adenylate cyclase/PKA and MAP kinase pathways [11, 62]. This leads to inactivation of the JNK/c-Jun pathway by dephosphorylation, leading to upregulation of insulin gene expression [62]. HGF binds to the c-met receptor and causes activation of the MAPK and PI3K/Akt pathways, leading to β -cell proliferation [54]. In addition, *in vitro* studies in HGF-treated INS-1 cells demonstrated increased expression of Protein Kinase C, suggesting another pathway by which HGF increases β -cell proliferation [63].

2.2.2 Insulin growth factors and insulin

The insulin-like growth factors I (IGF-I) and II (IGF-II) and their receptors are expressed at different stages during pancreatic development. Insulin and IGF-I bind to the insulin and IGF-I receptor respectively, but can each also cross-react with the other receptor. This makes it difficult to separate the specific effect of these peptides in β -cell growth. IGF-I and IGF-II increase β -cell proliferation in rat islets and insulinoma cell lines *in vitro* [64–66]. Glucose enhances IGF-I mediated proliferation of insulinoma cells in culture and this process is phosphoinositide 3-kinase (PI3K) dependent [36]. Interestingly, overexpression of IGF-I in β -cells is associated with increased β -cell proliferation, but not mass [67]. In contrast, transgenic mice overexpressing IGF-II exhibit an increase in β -cell mass due to augmented proliferation [68]. Taken together, there is clear evidence that the IGF molecules are important factors in β -cell proliferation and mass.

The effect of insulin receptor signaling on the β -cell has been assessed in both *in vivo* and *in vitro* models. Insulin infusion in mice induces β -cell proliferation and increases β -cell mass and this effect is augmented by concomitant glucose infusion [33]. Mice deficient in the insulin receptor in β -cells exhibit hyperglycemia and reduced β -cell mass with age [37]. MIN6 cells with 80% knockdown of the insulin receptor exhibit decreased proliferation, suggesting that insulin is a growth factor at least for this insulinoma cell line [69]. It is important to mention that the effects of the insulin receptor in β -cell function are less clear.

The abnormalities demonstrated in animal models deficient in insulin receptor substrate 2 (IRS2) suggest that events downstream of the insulin and IGF receptors are

critically important for β -cell mass [70]. IRS2 signaling leads to stimulation of the PI3 kinase/Akt and ERK1/2 pathways [71]. Akt, also known as protein kinase B (PKB), has been proposed to be one of the critical mediators of many IRS2 signals in β -cells. Mice deficient in AKT2 develop insulin resistance and diabetes due to impaired adaptation of β -cells, providing evidence for the importance of Akt signaling in β -cells [72]. Moreover, overexpression of Akt in β -cells induces β -cell mass by augmented proliferation, cell size and resistance to apoptosis [39, 40]. Akt exerts several biological functions by modulation of multiple downstream targets. The current evidence suggests that Foxo1, GSK3 β and the mTOR signaling pathway could be critical downstream effectors of Akt signaling [73–77]. The events linking GSK3, Foxo1 and mTOR to regulation of β -cell proliferation and apoptosis are ill defined.

2.3 Incretins

The two most studied incretin hormones are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). The incretin hormones have been shown to increase β -cell proliferation and decrease β -cell apoptosis [78]. Glucose-intolerant rats demonstrate increased β -cell mass after infusion with GLP-1 [79]. Mice treated with GLP-1 similarly show increased insulin secretion, β -cell size and neogenesis [80]. GLP-1 has also been shown to cause enhanced β -cell regeneration after partial pancreatectomy and streptozotocin administration [81, 82]. In addition to its effects on β -cell proliferation, β -cell regeneration and insulin secretion, GLP-1 has also been shown to have an anti-apoptotic effect [83]. GIP has been less studied, but also has been shown to induce proliferation in cultured β -cells [84].

GLP-1 binds to the GLP-1 receptor (GLP1R), a G protein-coupled receptor expressed in many tissues, including β -cells [78]. Activation of the GLP1R stimulates cyclic AMP formation and activation of downstream targets such as protein kinase A and cAMP-dependent guanine nucleotide exchange factors [85]. GLP1R activation also leads to an increase in intracellular calcium, which triggers insulin release [86, 87]. An important downstream target of GLP-1 is the transcription factor pancreatic duodenal homeobox-1 (Pdx-1) [88]. Indeed, many of the effects of GLP1R activation seem to be due to effects on Pdx-1 [89]. GLP1R activation has also been shown to activate PI3K/Akt; human islets and MIN-6 cells treated with the GLP-1 agonist exendin-4 demonstrated increased IRS2 expression and stimulated Akt phosphorylation [90]. GLP1R activation causes activation of the PI3K/Akt pathway via the epidermal growth factor receptor (EGFR) [91]. Binding of GLP-1 to its receptor can also transactivate the EGF receptor

via production of EGF-like ligands. This is caused by c-SRC activation; inhibitors of c-SRC and the EGFR both cause suppression of PI3K activation by GLP-1 in INS-1 cells [92]. The GIP receptor is another G protein-coupled receptor. Like GLP-1, it also acts via adenylyl cyclase to upregulate cyclic AMP. GIP leads to stimulation of the MAP kinase pathway and the PI3K/Akt pathway [78, 84].

3 Mechanisms of β -cell failure

3.1 Glucotoxicity

Glucose concentration is the major determinant for regulation of β -cell mass and function, as discussed above. Transient increases in glucose levels within physiological range induce insulin secretion and potentially beneficial signals. In contrast, glucotoxicity induced by prolonged hyperglycemia causes β -cell dysfunction and altered β -cell mass [93]. The effects of chronic hyperglycemia in β -cells have been assessed in animal models and *in vitro* using insulinoma cells and isolated islets. In the setting of chronic exposure to hyperglycemia, rat islets exhibit basal insulin hypersecretion and defective GSIS [94]. In animal models and humans, chronic hyperglycemia is associated with alterations in β -cell mass and function [29]. The β -cell has an incredible ability to adapt and compensate for chronic hyperglycemia, as seen in the Zucker diabetic fatty (ZDF) rat, but ultimately, obesity, chronic hyperglycemia, and worsening insulin resistance lead to increased β -cell apoptosis [95]. Similarly, postmortem studies in human type 2 diabetic patients reveal low frequency of replication and reduced β -cell mass, mainly by increased apoptosis [96].

Proposed mechanisms by which glucotoxicity acts include mitochondrial dysfunction with production of reactive oxygen species (ROS), endoplasmic reticulum (ER) stress and increased levels of intracellular calcium. Although high glucose has been shown to induce these pathways and impair insulin secretion, these processes do not appear to consistently result in β -cell apoptosis [93]. Initial studies showed that exposure of β -cell lines to conditions of prolonged hyperglycemia led to decreased β -cell function with decreased insulin mRNA levels, insulin content, and insulin release [97]. Chronically increased glucose concentrations cause increased glucose metabolism through oxidative phosphorylation. This causes mitochondrial dysfunction and the production of reactive oxygen species (ROS) [98]. Several lines of evidence suggest that this is an important mechanism for the induction of β -cell dysfunction. β -cells have a limited defense against excess ROS production due to low levels of ROS-detoxifying enzymes [99]. Markers of oxidative stress are significantly higher in the islets of type 2

diabetics than of controls, and the levels of these markers correlate with the degree of impairment of GSIS [100]. Overexpression of antioxidant enzymes in isolated islets resulting in decreased levels of ROS prevents islet dysfunction in conditions that mimic prolonged hyperglycemia [100]. Also, improved β -cell function in *db/db* mice and ZDF rats treated with antioxidant agents such as n-acetylcysteine or aminoguanidine provide further evidence for the role of oxidative stress in the deleterious effects of chronic hyperglycemia [98, 101]. A similar improvement in β -cell function was observed in isolated islets from diabetic patients treated with antioxidant agents [102]. Increases in oxidative stress lead to decreased transcription of the insulin gene by decreasing Pdx1 and Maf A binding [97, 103]. The mechanisms by which ROS decrease β -cell mass and function are not completely understood. However, it is known that the generation of ROS will ultimately activate stress-induced pathways, including nuclear factor κ B (NF- κ B), c-Jun N-terminal kinase (JNK), and hexosamines [104, 105]. The activation of the JNK signaling pathway after induction of oxidative stress inhibits IRS1 signaling by phosphorylation of IRS-1 on Ser³⁰⁷ [106, 107].

In addition to oxidative stress, chronic hyperglycemia can disrupt β -cell mass and function by inducing ER stress (see below), increasing intracellular calcium and increasing nutrient signaling. Chronic hyperglycemia leads to long-term increases in cytosolic Ca²⁺ that may be proapoptotic and induce β -cell dysfunction [94, 108]. Another potential mechanism of apoptosis by glucose is the production of interleukin-1 beta [104]. Finally, recent publications support the concept that the nutrient-regulated mTOR/S6K signaling pathway exerts negative feedback to regulate IRS proteins by Ser/Thr phosphorylation [109–111]. Experiments in INS1 cells showed that IGF1 signaling downregulates IRS2 protein levels by activation of mTOR signaling, suggesting that a similar mechanism is operating in β -cells. The biological and physiological effects of this feedback regulation are not clear, but modulation of IRS signaling by mTOR/S6K could be implicated in the adaptive responses of β -cells to nutrient excess. However there is no direct evidence suggesting that this mechanism is part of signaling events induced by glucotoxicity. In summary, there is some knowledge of the signaling pathways induced by chronic hyperglycemia. However, the downstream events and targets governing the effects of glucotoxicity have not been completely elucidated.

3.2 Lipotoxicity

Dyslipidemia characterized by an increase in circulating free fatty acids (FFA) is one of the major abnormalities in

the lipid profile of diabetics. Experiments in humans suggest that elevation of FFA in healthy individuals has stimulatory effects on insulin secretion, but may contribute to progressive β -cell failure in some individuals with a genetic predisposition to diabetes [112–114]. *In vitro* experiments using isolated islets demonstrated toxic effects of FFA on insulin secretion and apoptosis [115]. It is important to note that several *in vitro* experiments have been performed using concomitant high glucose concentrations. The current evidence suggests that the deleterious effects of lipids are observed predominantly in the presence of high glucose. Therefore, we will expand on the different mechanisms in the next section.

3.3 Glucolipototoxicity

In the process of glucolipototoxicity, toxic actions of FFA on tissues become apparent in the context of hyperglycemia as described by Prentki et al [116]. Studies in humans reveal that lipid infusion in type 2 diabetic patients causes impaired insulin secretion [117]. Long-term exposure of islets or insulin-secreting cells to increased levels of fatty acids is associated with inhibition of GSIS *in vitro* [118, 121], impairment of insulin gene expression [122–127], and induction of cell death by apoptosis [115, 117, 128–135]. Notably, reducing plasma FFA concentrations in type 2 diabetics with the niacin derivative acipimox was associated with enhanced insulin sensitivity and improvement in oral glucose tolerance tests [93]. These and other experiments support the concept that FFA alters β -cell function and survival.

Several mechanisms by which glucolipototoxicity impairs GSIS have been postulated. One of the proposed mechanisms for glucolipototoxicity is the inhibition of FFA oxidation by elevated glucose [116]. In the setting of hyperglycemia and elevated FFA, glucose metabolism results in elevated levels of malonyl CoA, a known inhibitor of carnitine palmitoyl transferase-1 (CPT1). The inhibition of CPT1 decreases fatty acid oxidation, which causes accumulation of elevated cytosolic long-chain acyl-CoA esters, generation of ceramide and lipid partitioning [93]. Previous experiments have shown that long-chain acyl-CoA esters result in β -cell dysfunction [136]. Studies also suggest that AMP-activated protein kinase (AMPK) activity may play a role in glucolipototoxicity. AMPK activation promotes fatty acid oxidation by phosphorylation and inhibition of acetyl-CoA carboxylase or via down regulation of the transcription factor sterol-regulatory-element-binding-protein-1c (SREBP1c) [99, 137]. In addition to affecting insulin secretion, glucolipototoxicity can decrease insulin gene expression by alterations in Pdx1 and MafA binding to the insulin promoter [127, 138]. Ceramide generation and activation of JNK with subsequent decrease

in IRS signaling have been postulated to relate the signals on the insulin promoter [93, 126]. In summary, high glucose inhibits detoxification of fat and promotes partitioning of FFA to toxic complex lipids, which in turn induces β -cell dysfunction, inhibits insulin gene expression and causes apoptosis. This concept was supported by the findings that treatment with an inhibitor of fat oxidation promoted β -cell death, while treatment with an AMPK activator, which leads to increased FFA oxidation, protected β -cells from glucolipototoxicity [117]. In addition, alteration of the mitochondrial pathway of pyruvate metabolism, with a reduction in the glucose-induced exchange of pyruvate with intermediates of the Krebs cycle has also been proposed as a mechanism by which an increased lipid supply blunts normal GSIS [139].

Another possible mechanism by which FFA may impair β -cell function involves the expression of uncoupling protein-2 (UCP2), part of the UCP family of proteins, which act to regulate cellular ATP production. Previous studies have shown that chronic exposure of islets or insulinoma lines to elevated FFA cause increased UCP2 expression and UCP^{-/-} mice are protected from impaired β -cell function [140]. The mechanisms by which UCP2 may play a role in β -cell failure are unclear. Some studies have suggested increased UCP2 expression leading to increased ROS production as a possible mechanism, but this has not been reproduced nor have antioxidants been shown to cause any benefit in restoring impaired GSIS in lipid-exposed islets [141]. Recently, the ATP-binding cassette transporter subfamily A member 1 (ABCA1), a mediator of reverse cholesterol efflux, was shown to be an important mediator of the effects of FFA on insulin secretion. Conditional deletion of ABCA1 results in increased cellular cholesterol content and impaired insulin secretion at the level of exocytosis [142].

As discussed above, multiple studies have shown that fatty acids can induce β -cell death by apoptosis and that this effect is potentiated by glucose [117, 130, 134, 143]. Several mechanisms have been proposed to mediate fatty acid induced apoptosis in β -cells, including ceramide formation leading to altered lipid partitioning, and the generation of ROS (for review [144]). More recently, ER stress and the unfolded protein response have received experimental support [138, 145, 146]. In addition to these processes, apoptosis after fatty acid administration can result from the activation of the JNK pathway and decreased Akt signaling with subsequent activation of Foxo1-dependent gene expression [132, 143, 145]. While the exact mechanisms are not delineated, it is clear that elevated FFA play a role in impaired β -cell function and apoptosis. It is conceivable that the combination of elevated FFA and chronic hyperglycemia synergize to create a milieu conducive to β -cell dysfunction and failure.

3.4 ER stress

Recent evidence suggests that ER stress links obesity with insulin resistance (reviewed in [147, 148]). Studies in humans and rodent models also implicate this mechanism in β -cell adaptation to the diabetic milieu. Evidence for ER stress in islets from type 2 diabetics has been demonstrated by increased staining for ER chaperones and CHOP along with increased ER size [146, 149, 150]. In rodent models, increased ER stress markers have been demonstrated in mouse islets from *db/db* mice [146]. Insulin-2 mutations in Akita mice induce accumulation of misfolded insulin and progressive β -cell loss caused by ER stress, demonstrating the importance of this pathway for β -cell survival [151]. Similarly, mice with deletion of *wfs1*, the affected gene in Wolfram Syndrome, which is characterized by juvenile-onset diabetes mellitus, also exhibit decreased β -cell mass and impaired GSIS [151, 152]. Taken together, these data suggest that ER stress is present in human β -cells and that this could be a common mechanism for the two major pathophysiological events in type 2 diabetes, insulin resistance and β -cell failure.

ER stress has been postulated to result from increased biosynthetic demand induced by chronic hyperglycemia, elevated FFA, and chronic over-nutrition in the β -cell. This pathway is best understood in the context of the Unfolded Protein Response (UPR). The UPR alleviates ER stress, restores homeostasis, and prevents cell death by inducing a number of downstream responses that: 1) decrease new protein arrival to the ER; 2) increase the amount of ER chaperones to improve folding capacity; and 3) increase the cell's capacity to dispose of misfolded proteins. If unable to successfully perform these tasks, the UPR will trigger the apoptosis cascade [152]. The three primary modulators of the UPR are: inositol requiring protein 1-alpha (IRE1-alpha), activating transcription factor 6 (ATF6), and protein kinase RNA (PRK)-like ER associated kinase (PERK) [153]. These sensors remain inactive via interaction with the ER chaperone BiP until activated by increased ER stress [154].

IRE1-alpha possesses critical endoribonuclease activity that induces the splicing of X-box binding protein 1 (XBP1). The spliced form of XBP1, XBP1s, regulates the transcription of genes involved in ER expansion as well as protein maturation, folding and export. In addition, XBP1 modulates the expression of genes that regulate the degradation of misfolded proteins and ER-targeted mRNAs in order to decrease protein synthesis [152]. These actions may be critical in β -cell adaptation to ER stress. Previous evaluation has shown that short-term glucose exposure of isolated islets induces IRE1-alpha signaling but not XBP1 splicing and this was found to be important for pro-insulin biosynthesis [155]. In contrast, long-term exposure of islets

to hyperglycemia is associated with XBP1 splicing and progressive inhibition of insulin mRNA and protein expression [155]. Recent work demonstrates that activation of IRE1-alpha caused by chronic high glucose leads to insulin mRNA degradation [156, 157]. In addition to causing the degradation of mRNA, IRE1-alpha may also signal apoptosis via activation of the JNK signaling pathway by interaction with TNF receptor-associated factor (TRAF) 2 and the activation of procaspase 12 [158, 159]. Palmitate has also been shown to activate IRE1-alpha signaling, suggesting that this pathway could be relating some of the ER stress signals induced by fatty acids (for review [152]). The potentiation of ER stress markers by glucose has not always been reproduced in primary β -cells and human islets [160].

ATF6, another essential modulator of the UPR, is released from BiP in response to accumulation of unfolded proteins in the ER lumen. ATF6 acts as a co-activator of the UPR by binding to the promoters of UPR-responsive genes, including those controlling ER chaperones [161–163]. ATF6 augments the expression of XBP1 mRNA, providing more substrate for IRE1-induced generation of XBP1s [164]. Combined null mutations in both isoforms of ATF6 in mice result in an early developmental lethal phenotype, but loss of function of ATF6-alpha alone in mice results in impaired recovery from acute stress and inability to tolerate chronic stress [165]. Studies of Dutch and Pima Indians reveal that missense mutations and polymorphisms within ATF6-alpha may be linked to type 2 diabetes [166, 167]. Further evidence that this signaling pathway is important is demonstrated by induction of ATF6 signaling by palmitate [145]. Recent evidence showed that ATF6 levels were increased in the pancreatic islets of diabetic OLETF rats [168]. In the same study, induction of ATF6 by ER stress was associated with repression of the insulin gene via the up regulation of SHP. This suggests that this pathway could also be implicated in ER stress induced β -cell dysfunction.

Finally, the activation of PERK causes phosphorylation of eukaryotic translation initiation factor-2a (eIF2a), resulting in an overall decrease in mRNA translation but increased translation of select proteins such as ATF4 [169–171]. ATF4 induces the transcription of genes involved in amino acid import, glutathione biosynthesis, protein synthesis (inducing of 4EBP) and resistance to oxidative stress as well as the proapoptotic gene CHOP [172–176]. Palmitate has been shown to activate PERK and eIF2a phosphorylation, leading to inhibition of protein synthesis and induction of ATF4 and CHOP in islets and insulinoma cells [138, 143, 177]. CHOP induction by FFA is mediated by ATF4 binding to the C/EBP-ATF binding site in the CHOP promoter, as well as by c-Fos and Jun-B dimer binding to the activator protein-1 (AP-1) binding site and possibly Foxo1 [145, 178]. The importance of the

PERK pathway in β -cell survival has been assessed in genetically modified animals. Deficiency in the PERK-eIF2a pathway leads to progressive loss of β -cell mass and severe diabetes in both humans and mice [153, 179]. It would then follow that activation of the PERK-eIF2a pathway would provide “a respite” for the ER and prevent further damage. However, further study into the function of the PERK/eIF2a pathway reveals that chronic induction of the PERK-eIF2a pathway may also lead to cell apoptosis at least in part by upregulation of CHOP [174]. Indication that CHOP is a critical component in apoptosis induced by this pathway was demonstrated by delayed onset of diabetes and ameliorated β -cell apoptosis in Akita mice with targeted disruption of CHOP [180].

Current evidence suggests that ER stress is an important contributor to β -cell failure in Type 2 Diabetes. The molecular mechanisms by which FFA- and glucolipotoxicity-induced ER stress cause β -cell apoptosis are not well understood. In addition to induction of CHOP, ER stress can induce apoptosis by JNK, ATF-3 and inhibition of Bcl-2 and/or activation of proapoptotic members of the Bcl-2 family (for review [152]). In particular, activation of JNK could lead to suppression of IRS/Akt signaling through serine phosphorylation of IRS-1 in liver and β -cells [143, 181]. Inhibition of IRS/Akt signaling reduces survival signals and ultimately leads to apoptosis. Inhibition of Foxo1 using a dominant-negative mutant reduces ER stress markers and promotes β -cell survival at least in part by modulation of CHOP [143]. Recently, a potential important link between ER stress and IRS2 signaling was demonstrated by transcriptional repression of the IRS2 promoter by ATF3 [182]. Inhibition of IRS2 signaling was also observed by ER stress-induced activation of SREBP in insulinoma cells treated with high levels of glucose, suggesting that this pathway could also play a role [183]. While the exact mechanisms of ER stress mediated apoptosis are not completely understood, there is no question that chronic hyperglycemia and nutrient excess lead to activation of the UPR and its cascade of downstream responses. It is likely that cumulative damage from hyperglycemia, over-nutrition, and elevated FFA levels overwhelm the ER of the β -cell, resulting in activation of the UPR and eventual apoptosis and β -cell failure.

4 Concluding remarks

Type 2 diabetes mellitus is a disease with devastating complications that is increasing in prevalence at an alarming rate, at great cost to the lives of patients and to society as a whole. The inherent defect in this disease is β -cell failure in the setting of insulin resistance. Pancreatic β -cells possess the ability to greatly increase their mass in response to

stress conditions such as insulin resistance. Elegant studies have identified some of the cell cycle machinery governing β -cell proliferation. The proliferation of β -cells is regulated by a multitude of nutrient signals and growth factors. Further research into the machinery of β -cell proliferation may identify potential therapeutic strategies in the treatment of diabetes. The findings that certain factors, such as incretins, can upregulate β -cell mass is an exciting prospect for possible methods of increasing β -cell mass or improving the adaptation of β -cells to insulin resistance. Furthermore, recent lineage-tracing experiments provide insight into the origins of new β -cells. Delineation of the processes and conditions required for β -cell neogenesis are of great relevance to the understanding and possible treatment of diabetes. Understanding the mechanisms involved in β -cell dysfunction and failure observed in late stages of diabetes is also a topic of major importance. Research has begun to unravel how excess glucose and lipids lead to impaired β -cell function and apoptosis. The generation of reactive oxygen species, ER stress, alterations in β -cell metabolism, decreased IRS signaling and induction of pro-apoptotic signals have been found to be key players in β -cell failure. Further research into the process of ER stress may reveal how a response designed to protect the β -cell can ultimately lead to its demise. As the pathways of β -cell expansion and β -cell failure are further clarified, the essential modulators of these processes will be identified, providing potential novel therapeutic targets to investigate. For now, it is conceivable that pharmacological agents that decrease oxidative stress, modulate ER stress or sensitize β -cells to the action of insulin could have major implications to delay or prevent the development of diabetes. Finally, it is important to note that current evidence indicates that diet and exercise are the most effective interventions to prevent or delay type 2 diabetes.

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