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Therapeutic opportunities for pancreatic β-cell ER stress in diabetes mellitus

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Abstract

Diabetes mellitus is characterized by the failure of insulin-secreting pancreatic β-cells (or β-cell death) due to either autoimmunity (type 1 diabetes mellitus) or failure to compensate for insulin resistance (type 2 diabetes mellitus; T2DM). In addition, mutations of critical genes cause monogenic diabetes. The endoplasmic reticulum (ER) is the primary site for proinsulin folding; therefore, ER proteostasis is crucial for both β-cell function and survival under physiological and pathophysiological challenges. Importantly, the ER is also the major intracellular Ca^{2+} storage organelle, generating Ca^{2+} signals that contribute to insulin secretion. ER stress is associated with the pathogenesis of diabetes mellitus. In this Review, we summarize the mutations in monogenic diabetes that play causal roles in promoting ER stress in β-cells. Furthermore, we discuss the possible mechanisms responsible for ER proteostasis imbalance with a focus on T2DM, in which both genetics and environment are considered important in promoting ER stress in β-cells. We also suggest that controlled insulin secretion from β-cells might reduce the progression of a key aspect of the metabolic syndrome, namely nonalcoholic fatty liver disease. Finally, we evaluate potential therapeutic approaches to treat T2DM, including the optimization and protection of functional β-cell mass in individuals with T2DM.

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Author contributions

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Introduction

In 2019, diabetes mellitus affected approximately 463 million people of all ages worldwide1 and it is currently the leading cause of macrovascular and microvascular disease, including kidney failure, blindness and heart disease, as well as of lower-limb amputations2. Characterized by elevated blood levels of glucose, diabetes mellitus is divided into multiple categories based on pathogenesis, including type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM) and monogenic diabetes. T1DM is caused by the autoimmune destruction of pancreatic β-cells. By contrast, T2DM is characterized by insulin resistance and hyperinsulinaemia leading to hyperglycaemia3 and this process is accompanied by progressively deficient glucose-stimulated insulin secretion from β-cells, which starts from an early stage of T2DM4. Monogenic diabetes results from single-gene mutations that affect β-cell development, function and/or survival5,6. As all types of diabetes mellitus eventually lead to the loss of functional β-cell mass7, an improved understanding of the mechanisms of β-cell failure is essential. It is also critical to know how surviving β-cells compensate for insulin resistance and loss of β-cell mass when they are forced to increase insulin production beyond their capacity.

The endoplasmic reticulum (ER) is a key intracellular organelle, where proteins enter the secretory pathway and fold into native 3D structures that are required for insertion into the endomembrane system or for release into the extracellular space. Several main functions of the ER include protein folding and processing, intracellular Ca^{2+} storage and regulation, and lipid biosynthesis. The cellular maintenance of ER homeostasis is therefore essential for proper protein folding, assembly and secretion as well as for cell survival8. Notably, a balanced ER environment is important for professional secretory cells such as hepatocytes, B lymphocytes and pancreatic β-cells, which are more susceptible to ER stress than other cells owing to the large amounts of protein entering the secretory pathway9,10,11. In addition, ER stress12,13 due to the perturbation of ER homeostasis, such as a sudden increase in proinsulin synthesis and/or disruption of Ca^{2+} homeostasis in the ER, leads to an accumulation of unfolded and/or misfolded proteins in the ER lumen, which activates the intracellular signalling pathways collectively known as the unfolded protein response (UPR)9,14. Indeed, accumulating evidence suggests that ER stress might play a role in the initiation and progression of T1DM15,16 in addition to the more established roles in T2DM. For an in-depth discussion of β-cell stress in T1DM, we encourage readers to refer to a 2020 review17.

In this Review, we discuss the molecular and cellular mechanisms governing β-cell fate determination in the context of ER stress. We also highlight the UPR signalling pathways involved in the pathogenesis of diabetes mellitus, with a focus on T2DM and monogenic diabetes.

ER protein homeostasis

The ER is the organelle where proteins that will be secreted or that will go to the endomembrane system start their journey after ribosomal translation. Approximately one-third of the metazoan cellular proteome is estimated to translocate across the ER membrane

into the lumen and this ratio is probably even higher for pancreatic β-cells as >50% of the total mRNA in these cells is dedicated to proinsulin synthesis and processing18. In the ER lumen, proteins fold into their proper 3D structures and are edited by post-translational modifications, including glycosylation, hydroxylation, lipidation and disulfide bond formation, in order to attain their biological functions9,11.

The ER has a tight surveillance system for protein quality control to ensure that only properly folded and modified proteins traffic to the Golgi apparatus for further processing. Protein unfolding and/or misfolding in the ER occurs frequently owing to many extracellular and intracellular physiological insults. These include pharmacological intoxication and genetic mutation of client proteins (the proteins to be folded, also known as substrate proteins) or of their counterpart ER chaperone proteins (which assist with folding). Importantly, the elevated expression of client proteins is also associated with protein misfolding such as proinsulin in the context of prediabetes and early-stage T2DM9,12,19. Evidence even suggests that insulin mRNA is increased in people with a genetic predisposition to T1DM20. As a consequence, the accumulation of unfolded and/or misfolded proteins in the ER lumen disturbs ER homeostasis and activates the UPR21.

The unfolded protein response

The UPR is mediated mainly through three ER transmembrane proteins: serine–threonine protein kinase–endoribonuclease IRE1α, protein kinase R-like ER kinase (PERK, also known as EIF2AK3) and cAMP-dependent transcription factor ATF6 (ref.13) (Fig. 1). Under homeostatic conditions, IRE1α, PERK and ATF6 are bound to and inhibited by the ER chaperone BiP (also known as HSPA5), a peptide-dependent ATPase of the HSP70 protein family member localized to the ER lumen.

IRE1α is an ER transmembrane protein with dual serine–threonine kinase and endoribonuclease activities that are attributable to tandem and distinct cytosolic domains. When the IRE1α ER-luminal domain is released from BiP inhibition under conditions of ER stress22, IRE1α dimerizes for transphosphorylation and activation of its endoribonuclease activity, thereby enabling it to cleave the pre-mRNA encoding X-box binding protein 1 (XBP1). This event initiates the removal of an intron to produce a transcriptionally active transcription factor, XBP1s23. XBP1s induces the expression of many target genes that encode proteins with functions in ER protein folding, protein translocation into the ER, trafficking and ER-associated degradation24,25,26. In parallel, upon release from BiP, PERK dimerizes, becomes activated and phosphorylates the α-subunit of the eukaryotic translation initiation factor 2 (known as eIF2α, which is encoded by *EIF2S1)* at Ser51, leading to the rapid and transient attenuation of general mRNA translation27,28,29,30. Paradoxically, the translation of several specific mRNAs is preferentially enhanced in the context of general translational inhibition, including ATF4, which plays an important role in antioxidant response and recovery of protein synthesis31,32. Finally, ATF6 is the third major UPR transducer protein and is a type II transmembrane protein that contains a cytosolic cAMP-responsive element-binding–basic leucine zipper domain. Once released from BiP, ATF6 traffics from the ER to the Golgi complex, where it is cleaved by the sequential action of the membrane-bound transcription factor site 1 and site 2 proteases. This action

liberates an ATF6 p50 cytosolic fragment from the N terminus of ATF6 that acts as a potent transcription factor to induce its target genes33.

Adaptive UPR prevents β-cell failure

The essential roles of the UPR transducers in pancreatic β-cells are well established by gain-of-function and loss-of-function studies in mouse models as well as by studies from human patients with specific mutations such as those with monogenic diabetes. For example, the deletion of *Perk* (also known as *Eif2ak3)* causes β-cell failure in mice34 and the mutation of *PERK* causes β-cell failure in humans with neonatal diabetes mellitus6. In addition, the mutation of Ser51 to Ala in eIF2α, which is the site of phosphorylation by PERK, also causes β-cell failure similar to *Perk* deletion in a mouse model29,35. indicating that the requirement of PERK for β-cell function is through the phosphorylation of eIF2α. The second UPR transducer, ATF6, is also important for β-cell function. ATF6 deficiency accelerates diabetes in mice fed a high-fat diet36. Furthermore, genetic polymorphisms in *ATF6* are associated with prediabetes in several human populations37,38. Although no *IRE1* mutations have been identified in patients with monogenic diabetes, mice without *Ire1* in β-cells display β-cell failure and develop diabetes owing to defective proinsulin synthesis26. Importantly, the defect in *Ire1* results from a loss of glucose-stimulated proinsulin synthesis as a consequence of the defective induction of gene products required for preproinsulin translocation and processing in the ER26. In addition, downstream targets of the UPR transducers proved essential for β-cell integrity. For example, *Xbp1* deletion in mice decreases the number of insulin granules in β-cells and leads to glucose intolerance as well as to blunted glucose-stimulated insulin secretion39.

As a major suppressor of UPR signalling in the ER lumen, the chaperone activity of BiP also seems essential for β-cell physiology. For example, SIL1, a nucleotide exchange factor for BiP, is essential for the maintenance of β-cell mass in mice and can prevent β-cell apoptosis from occurring owing to increased ER stress40. Furthermore, the expression of a partial loss-of-function variant of ERDJ4, another BiP co-chaperone, causes constitutive ER stress and β-cell dysfunction in mice41. Moreover, mouse and human genetic deficiency of *DNAJC3* (otherwise known as *ERDJ6*), encoding the BiP co-chaperone $p58^{IPK}$, causes early-onset diabetes mellitus in humans and mice42,43. The mutation of *DNAJC3* is a recessive disorder that causes monogenic diabetes in humans44,45. Conversely, the overexpression of BiP protects β-cell function in mouse T2DM models45,46. In addition, physiological ER stress induces protein expression of mesencephalic astrocyte-derived neurotrophic factor (MANF), which in turn enhances the proliferation of β-cells in vitro and in vivo in a low-dose streptozotocin-induced diabetic mouse model47. Lastly and importantly, the ER protein quality control system in β-cells is also considered an essential aspect for the maintenance of optimal β-cell function, by maintaining protein homeostasis in the ER via the ER-associated protein degradation machinery (this topic is comprehensively reviewed in ref.48). Taken together, ample evidence from genetic studies demonstrates that both the functional integrity of ER homeostasis and the UPR are essential for β-cell function and health.

ER stress is linked to human β-cell failure

The failure to maintain ER homeostasis in β-cells leads to their loss of function and cell death, and further causes diabetes mellitus in humans that can be categorized into two main groups. The first is monogenic diabetes, which is a spectrum of disorders that are clinically subdivided based on the age of onset into so-called maturity-onset diabetes of the young (MODY) (Table 1), neonatal diabetes mellitus or early-onset diabetes mellitus. Most loci associated with monogenic diabetes are known to be directly involved in the maintenance of β-cell function, with some genes specifically related to ER stress-mediated β-cell dysfunction; the more severe the defect, the earlier the onset of disease. The second is T2DM, which does not occur during the neonatal period. T2DM is influenced to a much greater degree than monogenic diabetes by β-cell-extrinsic physiological insults (for example, glucolipotoxicity and redox status imbalance) that lead to a decrease in ER protein folding efficiency. Of note, T1DM-associated autoimmune response-derived ER stress in comparison with T2DM has already been summarized17 and will not be discussed here.

Monogenic diabetes

T2DM is polygenic, whereas some rare forms of diabetes result from mutations in a single gene. So-called monogenic diabetes accounts for 1–5% of all patients with diabetes mellitus in Europe49. In this subsection, we discuss how ER stress is involved in the pathogenesis of monogenic diabetes.

*Defects in MODY genes can cause ER stress. Fourteen forms of MODY have been proposed, with mutations occurring in genes encoding transcription factors as well as in genes related to lipid and glucose metabolism and the gene that encodes insulin (*Table 1*). MODY-1 is caused by a loss-of-function mutation in the gene encoding hepatocyte nuclear factor 4α (*HNF4A*). The mechanism by which the loss of HNF4α mediates β-cell dysfunction in MODY-1 is currently unclear. However, a 2012 study suggests that HNF4α transcriptionally activates the BiP-binding protein ankyrin repeat and SAM domain-containing protein 4B (ANKS4b)*50. *Increased expression of* Anks4b *in mice and in mouse cell lines sensitized β-cells to ER stress, and decreased expression of* Anks4b *protected cells from ER stress-induced apoptosis. Given the importance of BiP in ER stress, the HNF4α-mediated induction of ANKS4b might affect ER stress-mediated β-cell dysfunction.*

MODY-2 mutations affect glucokinase, a member of the hexokinase family expressed mainly in hepatocytes and pancreatic β-cells. Genetic defects in glucokinase disrupt β-cell function and cause sustained hyperglycaemia that is sufficiently mild that many patients do not require treatment51. In one French cohort, mutant glucokinase caused insufficient insulin secretion from pancreatic β-cells by increasing the threshold of the circulating concentrations of glucose required to induce insulin secretion, thereby leading to chronic hyperglycaemia52. In mice with the same glucokinase missense mutations as seen in patients with MODY-2, islets display β-cell defects and upregulated expression of CHOP, a pro-apoptotic transcription factor involved in the ER stress response53. By contrast, the chemical activation of glucokinase in mouse islets can activate pathways that promote β-cell survival, including under conditions of ER stress54.

MODY-3 is caused by loss-of-function mutations in the gene encoding hepatocyte nuclear factor 1α (*HNF1A*)55, which lead to a primary defect in β-cell function. Specifically, when a loss-of-function, dominant-negative HNF1α mutant was expressed in an insulin-secreting cell line, cells were sensitized to ER stress owing to the downregulation of XBP1 transcription and BiP protein expression56. Mice with transgenic expression of a loss-of-function mutant HNF1α displayed a diabetic phenotype at 6 weeks of age, recapitulating the MODY-3 phenotype57. Interestingly, in islets from these mice, β-cells were sensitized to ER stress with a highly disorganized and dilated ER structure57. As a consequence, these islets contained less insulin than wild-type mice, suggesting that the UPR is intimately involved in maintaining optimal in vivo insulin function in healthy islets12.

The gene mutated in MODY-4 encodes pancreas–duodenum homeobox protein 1 (PDX1), a transcription factor important for the maintenance of β-cell identity and for the regulation of β-cell function58. Indeed, *Pdx1* deficiency in mice increases β-cell susceptibility to ER stress-associated cell death59. In addition, PDX1 directly regulates genes involved in the stress response60, UPR signalling61 and ER homeostasis, including the gene encoding sarcoplasmic–endoplasmic reticulum calcium ATPase 2 (SERCA2), which pumps Ca^{2+} from the cytosol into the ER62. Together, these findings from studies in mice suggest that β-cell dysfunction associated with *Pdx1* defects is partially due to an inability to manage physiological ER stress. Evidence also points to the translocation of PDX1 from the nucleus to the cytoplasm via ER stress-related mechanisms63, perhaps driven by pro-survival factors like autocrine insulin signalling64.

The gene mutated at the MODY-9 locus encodes paired box protein pax 4 (*PAX4*), another transcription factor that is relevant in the development and differentiation of pancreatic β-cells65,66. In addition, *Pax4* overexpression selectively in pancreatic β-cells preserved these cells after streptozotocin-induced cell death in mice; however, the long-term overexpression of *Pax4* reduced insulin secretion and possibly caused the dedifferentiation of mature β-cells67. In addition, in a similar mouse model of *Pax4* overexpression, β-cell death triggered by inducers of ER stress was decreased, accompanied by the upregulation of genes involved in the maintenance of ER integrity68. *Pax4* overexpression also improved Ca^{2+} homeostasis in the ER lumen, decreased sensitivity to thapsigargin-induced ER morphological changes and improved glucose-induced Ca^{2+} oscillations68.

The gene encoding insulin (*INS*) is responsible for MODY-10, also known as mutant insulin gene-induced diabetes of youth (MIDY)5. As the major protein synthesized and processed in β-cells, mutations in *INS* lead to β-cell dysfunction or death through the accumulation of misfolded proinsulin in the ER. The mutations are located either in one of six cysteines that directly affect the disulfide bond formation necessary for proper proinsulin folding in the ER69 or in non-cysteine residues that can also affect the folding efficiency of proinsulin5 and consequently cause ER stress. Intriguingly, although most MIDY mutations arise de novo, the mutated genes are dominantly inherited in mouse models. A mechanism was proposed in which the mutant misfolded proinsulin captures wild-type proinsulin in the ER and prevents its trafficking to the Golgi and maturation. In support of this hypothesis, heterozygous proinsulin mutants interact with wild-type proinsulin and retain it in the ER. For example, more than 50% of wild-type proinsulin was misfolded in islets of Akita mice,

which have a Cys missense mutation in *Ins2* (ref.70). However, another possibility is that the proinsulin mutant disrupts wild-type proinsulin folding by altering the ER protein folding environment9.

Collectively, studies of MIDY mutations as a cause of β-cell failure suggest that both balanced proinsulin synthesis and the strict 3D structural constraint are critically needed for correct proinsulin folding in the ER as well as for proper insulin secretion to exert bioactivity71. Reversely, defects in either parameter (synthesis or coding sequence) predispose to nascent proinsulin misfolding in the ER lumen of β-cells12,71. Interestingly and importantly, mouse and human stem cell studies demonstrated that mutations that reduce proinsulin production but that do not impair the folding of proinsulin molecules reduce ER stress72,73. These findings are consistent with the reduced ER stress observed in β-cells of adult male mice with controlled *Ins* gene ablation12,19. Additionally, any further compensatory and increased proinsulin synthesis will lead to excessive, chronic ER stress in β-cells9.

Collectively, many MODY genes are linked to β-cell ER stress. In addition, other MODY genes that are not mentioned here show some relevance to ER stress in other cell types during diabetes. Therefore, the modulation of ER stress might be beneficial to treat patients with MODY.

Neonatal diabetes mellitus and early-onset diabetes mellitus.—The most well-known neonatal diabetes mellitus genes related to ER stress are *PERK* and *WFS1.* Wolcott–Rallison syndrome is a rare recessive disease characterized by neonatal diabetes mellitus with skeletal dysplasia and growth retardation and caused by mutations in *PERK*6. The mouse model of *Perk* deficiency also shows the same phenotype34 as patients with Wolcott–Rallison syndrome, with the consistent feature of severe insulin-dependent diabetes mellitus developing in infancy. Although the pathophysiological basis for the pancreas phenotype was not characterized in human patients with Wolcott–Rallison syndrome6, the most striking feature in *Perk*-deficient mice was the extensive death of endocrine and exocrine pancreatic cells, especially the pancreatic β-cells, but not the α-cells34. This finding suggests that PERK is particularly important for β-cell survival.

In the UPR, the phosphorylation of eIF2α by PERK serves as a protein synthesis brake that alleviates the protein load of the ER upon ER stress (Fig. 1). Multiple lines of evidence show that defective PERK signalling can trigger proinsulin misfolding. For example, in *Perk*-null mice, ER distention (a morphological indicator suggesting ER stress) was observed by electron microscopy in pancreatic β-cells34. Findings from eIF2α phosphorylation-defective transgenic mice support a model where PERK acts through phosphorylated eIF2α to attenuate the synthesis of proinsulin (and other proteins), which decreases the rate of protein influx into the ER and thereby limits ER stress29,35. In mouse models, the absence of either PERK74 or eIF2α phosphorylation31 is associated with oxidative stress in cells, with the latter playing a causal role in impairing β-cell function. Notably, the antioxidant treatment of mice with defective eIF2α phosphorylation increased β-cell function and survival, indicating that oxidative stress causes β-cell failure35. In addition, the missense mutation of *EIF2B1* (encoding a protein subunit of the eIF2B complex, which acts as a GTP exchange factor to

reactivate eIF2) also causes permanent neonatal diabetes mellitus in humans75. This finding further supports the requirement for the functional phosphorylation of eIF2α by PERK to maintain pancreatic β-cell function and health.

Wolfram syndrome is an autosomal-recessive neurodegenerative disorder characterized by early-onset diabetes mellitus and optic atrophy, with the non-autoimmune loss of β-cells occurring as a result of a mutation in *WFS1*, which encodes Wolframin76. In patients with Wolfram syndrome, overt diabetes develops at a median age of 6 years77. Several molecular mechanisms by which Wolframin deficiency affects β-cell function were proposed, some of which could be explained by constitutive ER stress occurring because of the *WFS1* mutation78. As an ER membrane protein, Wolframin positively regulates ER Ca^{2+} levels by modulating Ca^{2+} channel activity or by interacting with calmodulin, which results in increased Ca^{2+} uptake79,80. Therefore, the deficiency of Wolframin might disturb Ca^{2+} homeostasis in the ER, resulting in ER stress. Of note, inhibitors of ER Ca^{2+} release channels were identified as candidates for Wolfram syndrome drug therapy81,82,83. The expression of *WFS1* is induced by the IRE1α-XBP1s pathway of the UPR, which might account for the requirement for IRE1α in β-cells26. In addition, Wolframin also regulates the UPR by the proteosomal degradation of ATF6 through the E3 ubiquitin-protein ligase synoviolin (*SYVN1*, also known as *HRD1*), the E3 ubiquitin ligase in the ER-associated degradation pathway81. Taken together, these studies suggest that *WFS1* mutations identified in humans cause β-cell dysfunction that leads to diabetes mellitus in an ER stress-dependent mechanism78.

Type 2 diabetes mellitus

T2DM is the most common human metabolic disorder and is characterized by insulin resistance and hyperinsulinaemia, leading to hyperglycaemia. This section discusses the evidence indicating that ER stress contributes to the pathogenesis of T2DM.

Physiological ER stress in T2DM interferes with β-cell function.—Insulin synthesis and basal insulin secretion are both elevated during the early phase of T2DM, which is traditionally considered as compensation for increased peripheral insulin resistance in liver, muscle and adipose tissue. Increased proinsulin synthesis is believed to be a major direct cause of ER stress in β-cells in prediabetes and early-stage T2DM9,84,85. Supporting this notion, the acute reduction of insulin synthesis relieves ER stress in β-cells and promotes β-cell proliferation in mice12,19.

As a principle, both active and inactive subpopulations of the three UPR sensors coexist in healthy β-cells, with the inactive subpopulation sequestered through BiP binding to their ER-luminal domains22. Increased protein synthesis sequesters BiP by recruitment to proinsulin to facilitate protein folding (Fig. 1), thereby releasing the UPR sensors for activation and signalling6,13,19. Of note, the function of the PERK branch of the UPR pathways to fine-tune the rate of new protein synthesis through eIF2α phosphorylation and dephosphorylation6,29,35 is considered especially important in the coordination of proinsulin synthesis17 as well as of proinsulin translocation into the ER for protein folding. CHOP is a downstream transcription factor in the PERK–eIF2α pathway and is considered

an important signal transducer in coordinating insulin synthesis. This effect occurs partly through CHOP modulating the expression of protein phosphatase 1 regulatory subunit 15A (PPP1R15A, also known as GADD34), which encodes a regulatory essential component of the eIF2α phosphatase complex, protein phosphatase 1 (PP1), that dephosphorylates eIF2α.86. In the *db/db* leptin-receptor deficient mouse model of T2DM, chronic ER stress leads to CHOP-dependent β-cell death and *Chop* gene deletion attenuated β-cell death by reducing ER stress and oxidative stress, thereby rescuing the diabetic phenotype of these mice87.

By contrast, the increased insulin demand that occurs after long-term hyperglycaemia also stimulates β-cell proliferation in *db/db* and Akita mouse islets88. This effect was reported to be mediated through the *ATF6* adaptive branch of the UPR88.

Effects of local inflammation on β-cell ER stress in T2DM.—During the development of T2DM, an inflammatory environment might arise within islets that could have considerable effects on β-cell function and survival, although this hypothesis remains somewhat controversial89,90,91. For example, the histological analysis of pancreas tissue obtained post-mortem from patients with T2DM revealed active inflammation in islets, including the presence of proinflammatory cytokines92,93. immune cell infiltration89,94 and amyloid deposits in β-cells95; such changes could eventually lead to the further deposition of islet amyloid polypeptide as well as to fibrosis and β-cell apoptosis96. From the cellular stress perspective, several cytokines were reported to induce ER stress and to exert detrimental effects on β-cells. For example, in prediabetic db/db male mice, physiological levels of IL-1β and IL-6 reduced ER Ca^{2+} storage, activated UPR signalling and reduced glucose-stimulated insulin secretion from isolated db/db mouse islets97.

In the pathogenesis of T2DM, elevated circulating levels of pro-inflammatory cytokines and immune cell infiltration in the islets are associated with β-cell dysfunction98. Some of this cytokine production originates from islet-resident macrophages99,100; however, chronic hyperglycaemia has been proposed to induce β-cells to produce pro-inflammatory cytokines such as IL-1β92. Moreover, mild ER stress predisposes primary rat β-cells to the pro-apoptotic effects of IL-1β by disrupting the balance between the pro-apoptotic and anti-apoptotic Bcl-2 family of proteins, independent of the XBP1 pathway of the UPR101. In contrast to the established detrimental role of IL-1β in islets, IL-22 and IL-10 were reported to alleviate ER stress initiated either by cytokines or by palmitic acid, both in the MIN6 insulinoma cell line and in mouse and human islets102. The beneficial effect of exogenous IL-22 administration on metabolism was further confirmed in the leptin receptor-deficient *db/db* mouse model103. Therefore, during T2DM-associated inflammation, different cytokines play opposing roles that modify ER stress in β-cells, which in turn correlate with changes in glucose-stimulated insulin secretion.

By contrast, the mechanisms by which ER stress might cause inflammation that leads to β-cell dysfunction are unclear, although one hypothesis has been put forth involving the NLRP3 inflammasome as an executor module to cause β-cell dysfunction 104,105. For example, ER stress in β-cells can induce the expression of thioredoxin-interacting protein (TXNIP) through the activation of both the PERK and IRE1 pathways. Furthermore, TXNIP

was shown to activate the NLRP3 inflammasome and induce IL-1β production104,105. By contrast, the NLRP3 inflammasome is dispensable for ER stress-induced β-cell dysfunction in Akita mice106, a model of proinsulin misfolding-induced β-cell death. The importance of NLRP3 inflammasome activation in T2DM remains to be further explored106. In our opinion, studies are needed that measure Toll-like receptor activation and downstream pathways in response to ER stress.

Glucotoxicity contributes to β-cell ER stress.—Pancreatic β-cells are intrinsically sensitive to blood levels of glucose, in that increased glucose concentration is tightly coupled with increased proinsulin synthesis and insulin secretion9,85. However, chronically elevated blood concentrations of glucose outside the physiological range are detrimental to β-cells. Furthermore, insulin resistance occurs in peripheral tissues during the progression to T2DM. In response, β-cells compensate by increasing insulin production, which exacerbates ER stress35,84,85 (Fig. 2).

Studies published in 2019 demonstrated that the increased rate of proinsulin synthesis occurring in response to insulin resistance and/or high blood concentrations of glucose increases proinsulin misfolding in the ER as detected by the formation of disulfide-linked proinsulin complexes85,107. Proinsulin has three disulfide bonds formed between amino acid positions 6 and 11 on the A-chain (A6–A11), between A7 and B7 on the B-chain (A7–B7), and between A20 and B19 (refs11,108). These oligomers of proinsulin are even observed at low levels in healthy mouse and human islets and an increase in their formation is the first molecular event identified that quantitatively correlates with the progression from prediabetes to overt diabetes in *db/db* mice85. One cause of proinsulin misfolding involves a strong intermolecular disulfide bond between B19 in one proinsulin molecule and B19 in an adjacent proinsulin molecule, which competes with the productive B19–A20 intramolecular disulfide bond85. Increased proinsulin synthesis causes an accumulation of these disulfide-linked multimers in the ER, which seems to correlate with an increased ER stress response. We expect that novel small molecules able to prohibit the B19–B19 linkage85 will prevent the progression from prediabetes to T2DM.

Chronic supra-physiological ER stress in β-cells disrupts intracellular metabolic homeostasis and gene expression109,110, which might skew the flux of metabolites and bioenergetic properties appropriate for islet electrophysiological activity111. These changes might eventually lead to β-cell death, a scenario supported by a 3–10-fold increase in β-cell apoptosis observed in pancreata obtained post-mortem from patients with T2DM compared with those from donors without diabetes96. Several hypotheses were proposed to explain the mechanism for increased β-cell death in patients with T2DM. First, increased proinsulin synthesis is accompanied by the production of reactive oxygen species (ROS)107. As β-cells are intrinsically susceptible to oxidative stress owing to a low level of antioxidant enzyme expression112, the increased generation of ROS is considered one of the main factors responsible for β-cell apoptosis112 and dedifferentiation113,114. Whether the ‘ROS susceptibility’ hypothesis applies to human β-cells is less clear, which warrants further investigation.

The second hypothesis relates to Ca^{2+} concentrations in the ER. It is well established that, upon acute glucose stimulation, accelerated β-cell metabolism occurs, which increases Ca^{2+} pumping into the ER via SERCA2 (refs115,116). Notably, the concentration of Ca^{2+} in the ER ($[Ca^{2+}]_{ER}$) plays a key role in the proper function of several ER-resident molecular chaperones and foldases117,118,119 as well as in the processing of proinsulin120. Consequently, $[Ca^{2+}]_{ER}$ orchestrates chaperone activity in order to adapt the ER folding machinery to accommodate the increased protein synthetic load triggered by glucose stimulation in β-cells121,122,123. Therefore, the chronic hyperglycaemic condition that occurs in T2DM could plausibly disrupt the Ca^{2+} buffering and storage functions of the ER that are required to prevent excessive ER stress in β-cells. Furthermore, studies demonstrated the mechanism by which ATP import into the ER is tightly coupled with the cytosolic concentration of Ca^{2+} (dubbed CaATiER; Ca^{2+}-antagonized ATP transport into the ER)124. These findings further implicate that chronic, supra-physiological ER stress could considerably affect insulin secretion125 and β-cell fate. Indeed, in 2020, an experimental islet model confirmed that pharmacological ER stress induced by tunicamycin promotes Ca^{2+}-dependent basal insulin hypersecretion, possibly causing islet super-activity with even more proinsulin synthesis125, which produces a ‘vicious cycle’ of further ER stress and β-cell dysfunction for T2DM development126 (Fig. 3).

In the third hypothesis, the modification of cellular proteins by advanced glycation end-products (AGEs) was proposed to constitute another potential death mechanism for ER stress-mediated β-cell death127. The formation of AGEs is increased in cultured islets exposed to long-term high glucose conditions128. However, we consider this mechanism less likely to be relevant for in vivo ER stress-mediated β-cell death than other mechanisms. Of note, the deletion of fructosamine-3-kinase (FN3K) in mice, a ubiquitous enzyme that is important for intracellular protein deglycation, did not exacerbate β-cell glucotoxicity129. By contrast, systemic administration of AGEs can exert deleterious effects by promoting ER stress in β-cells in mice130. A direct causal role for AGEs in ER stress-mediated β-cell death has yet to be established in vivo.

Therapeutic manipulation of β-cell stress

Adult human pancreatic islets exhibit very low or no β-cell turnover7,96,131. In the context of prediabetes and early-stage T2DM, the adaptation of islets to secrete more insulin to meet the needs of the body from unchanged β-cell mass indicates that insulin synthesis in mature β-cells is upregulated by metabolic demand. As discussed earlier, the compensatory increase in proinsulin synthesis during the development of insulin resistance is accompanied by increased proinsulin misfolding in the ER (Fig. 2), further aggravating ER stress and eventually leading to β-cell death. Supporting this notion, increased β-cell apoptosis was observed in islets from patients with late-stage insulin-dependent T2DM96, whereas others found no change in β-cell mass of patients with early-stage T2DM132. Interventions aimed at reducing proinsulin synthesis-associated ER stress to preserve β-cell mass12 and insulin secretion function (namely ‘functional β-cell mass’) are currently under investigation to provide a novel T2DM therapy.

One of the hallmarks of individuals with metabolically unhealthy obesity and/or prediabetes or early T2DM is chronic fasting hyperinsulinaemia. Hyperinsulinaemia might contribute to the development of nonalcoholic fatty liver disease (NAFLD) directly via actions on hepatocyte lipid homeostasis and indirectly via adipocyte expansion and lipid spill-over. The causal role of hyperinsulinaemia in hepatic steatosis and subsequently in T2DM is being increasingly accepted126 (Fig. 3). Correcting this early β-cell functional defect could be a promising first step to treat metabolic syndrome. Insulin-deficient mouse models confirmed that targeting pancreatic β-cells to pre-emptively correct hyperinsulinaemia is beneficial133. Specifically, mice with a reduced insulin gene dosage, which are incapable of diet-induced hyperinsulinaemia, were shown to be protected from systemic insulin resistance, obesity and hepatic steatosis as well as to have extended longevity and a reduced risk of cancer133,134,135. Similarly, inducible partial insulin gene deletion was found to cause a loss of adipose tissue mass in already obese adult mice136. Supporting the notion established from mouse models with insulin gene dosage modulation, a 2021 meta-analysis of 60 studies with 112 cohorts containing a total of 5,603 participants found statistically significant associations between period 1 (earlier period) fasting insulin level and period 2 (later period) BMI, indicating that changes in fasting insulin levels precede changes in weight in humans137.

Complementary results were shown using a mouse model of *Chop* gene deletion in β-cells, as germline *Chop* deletion reduces ER stress and prevents β-cell death; however, a cell-autonomous role of *Chop* was not established87. Preliminary findings (published in a pre-print) from a β-cell-specific *Chop* gene depletion mouse model show that β-cell-specific *Chop* gene depletion corrected the hepatic steatosis induced by either high-fat diet feeding or by ageing138. These animals also display increased glucose tolerance under a hyperglycaemic clamp condition138 with improved diurnal rhythm in metabolism (J.Y. and R.J.K., unpublished observations). Notably, the β-cell-specific *Chop* gene depletion mouse model produced similar results to mice with reduced insulin synthesis, in that the pancreatic β-cells not only show reduced ER stress but also had reduced insulin mRNA expression and delayed insulin secretion under physiological glucose levels138. Importantly, GLP1-conjugated *Chop* antisense oligonucleotides (GLP1–*Chop* ASO) demonstrated good efficacy in reducing *Chop* expression in islets when administered in vivo138, with desirable tissue selectivity and no obvious toxicity139. Therefore, *CHOP* might serve as an attractive target for translational T2DM therapeutic development to reduce steatosis and correct NALFD (Fig. 4)138.

The foregoing observations made in preclinical models might help us to re-think past views favouring the early implementation of insulin therapy to decrease fasting glucose levels140. Indeed, clinical studies published in 2020 demonstrate that hyperinsulinaemia drives hepatic de novo lipogenesis in NALFD141,142,143. These findings highlight that correcting hyperinsulinaemia might be needed as a therapeutic approach for NALFD treatment in T2DM. Specifically, evidence is mounting that hyperinsulinaemia plays an indispensable role in promoting NAFLD through de novo lipogenesis in patients with obesity and NAFLD141,143. For example, in a group of patients with NAFLD, hepatic de novo lipogenesis was discovered to statistically significantly contribute to steatosis: de novo lipogenesis was estimated to contribute to 38% of intrahepatic triglyceride (palmitate)

in patients with NAFLD versus 19% in patients with obesity without NAFLD141. The same group also pinpointed that, in human NAFLD, an increase in insulin delivery to the liver and subsequently to the extrahepatic tissues was causal for impaired glucose homeostasis142. Echoing this study, another group also reported that de novo lipogenesis is elevated in patients with NALFD and T2DM, and further showed that the hepatic level of saturated fatty acids shows a strong negative correlation with hepatic insulin sensitivity143. By contrast, evidence from rodent models argues against a causal role for physiological levels of insulin resistance in promoting de novo lipogenesis91,144.

Whether insulin resistance drives compensatory hyperinsulinaemia or vice versa has long been debated. Unsurprisingly, human patients with T2DM are a heterogeneous population, with the causal relationship differing widely between individuals and between ethnic groups. For example, in a 2020 epidemiological study in Chinese adults, insulin resistance showed a stronger association with diabetes mellitus than did β-cell dysfunction when measured by HOMA indexes145. In our opinion, the exacerbated hyperinsulinaemia versus insulin resistance feedback loop in patients with prediabetes or early-stage T2DM can be reasonably reconciled as a ‘vicious cycle’ (Fig. 4). A theoretical consideration of typical insulin action predicts that hyperinsulinaemia inevitably drives up tissue insulin resistance as a necessary outcome, perhaps through direct insulin receptor desensitization146,147,148. Targeting ER stress in β-cells could be a promising therapeutic strategy to address dysregulated insulin hypersecretion and thus potentially improve insulin resistance in T2DM and NAFLD125,138, which is reasonably expected to drive further hyperinsulinaemia (Fig. 4).

Conclusions

It is now evident that ER stress, which occurs at least in part as a consequence of increased proinsulin synthesis, contributes to β-cell failure in both T1DM and T2DM in humans. Many factors influence insulin protein synthesis and ER health, including compensatory efforts of β-cells in the face of insulin resistance. The increased proinsulin expression promotes aberrant proinsulin aggregation, including aberrant intermolecular disulfide bonding between neighbouring proinsulin molecules. This is the earliest molecular defect that has been observed during the prediabetic stage of T2DM development. This propagative misfolding results in complexes with an increased molecular weight that might further disrupt ER function and impair β-cell health, thereby leading to a downward spiral causing β-cell death during T2DM progression.

The key question is how to resolve β-cell ER stress. There is already evidence that many drugs used to treat T2DM have direct and indirect ER stress-relieving actions on β-cells, including GLP1 agonists149,150,151,152 and thiazolidinediones153. Small molecules that improve proper proinsulin folding would be most desirable as they prevent the root cause of ER stress and might be tissue specific. Subsequently, two key questions need to be answered regarding the physiological role of ER stress in pancreatic β-cells: first, how does ER stress alter Ca^{2+} signalling in β-cells in response to nutrients? Second, how does ER stress alter metabolic flux in β-cells in response to nutrients? The complex interplay between cytosolic Ca^{2+} and the ATP import process into the ER, that is, the CaATiER mechanism124, might

constitute a key regulatory mechanism. This mechanism could explain the crosstalk between metabolic cues and insulin secretion triggered by cytosolic Ca^{2+} signalling, the latter of which is heavily influenced by ER Ca^{2+} efflux or 'leak' from Ca^{2+} channels, for example, inositol trisphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs)154. Hypothetically, under long-term ER stress, the increased Ca^{2+} leak from the ER lumen of β-cells inhibits the process of ATP-for-ADP exchange across the ER membrane via the SLC35B1 transporter (also known as AXER)155. This Ca^{2+} leak could therefore interfere with nutrient sensing to reduce the intracellular ATP levels required for insulin secretion in healthy β-cells. Experimental models with knockout or knockdown of key components in this pathway are crucially needed to validate this hypothesis.

Paradoxically, reducing the insulin production burden and suppressing inappropriate hyperinsulinaemia87,134,136,138 might reduce insulin resistance and ameliorate the symptoms of metabolic syndrome. The favourable systemic physiological outcome could be explained by a 'β-cell to hepatocyte' signalling axis (Figs 3,4) mediated, in large part if not exclusively, via direct and indirect insulin action on hepatocytes. Thus, the successful application of approaches to reduce ER stress in β-cells in humans in the future could both decrease the burden of hyperinsulinaemia and dyslipidaemia in T2DM and considerably improve the comprehensive metabolic status of these patients for a desirable clinical outcome.

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Key points

- Physiological and chronic endoplasmic reticulum (ER) ‘stress’ exists in healthy β-cells.
- Adaptive unfolded protein response signalling via chaperones maintains ER protein folding homeostasis in healthy β-cells.
- Gene mutations in maturity-onset diabetes of the young exacerbate physiological ER stress, which causes β-cell loss.
- Proinsulin is prone to misfolding and increased insulin production can exacerbate physiological ER stress on the path to type 2 diabetes mellitus.
- The therapeutic inhibition of genes that promote ER stress in β-cells (for example, *CHOP*) might reduce the disease burden for patients with type 2 diabetes mellitus and is worthy of further exploration.

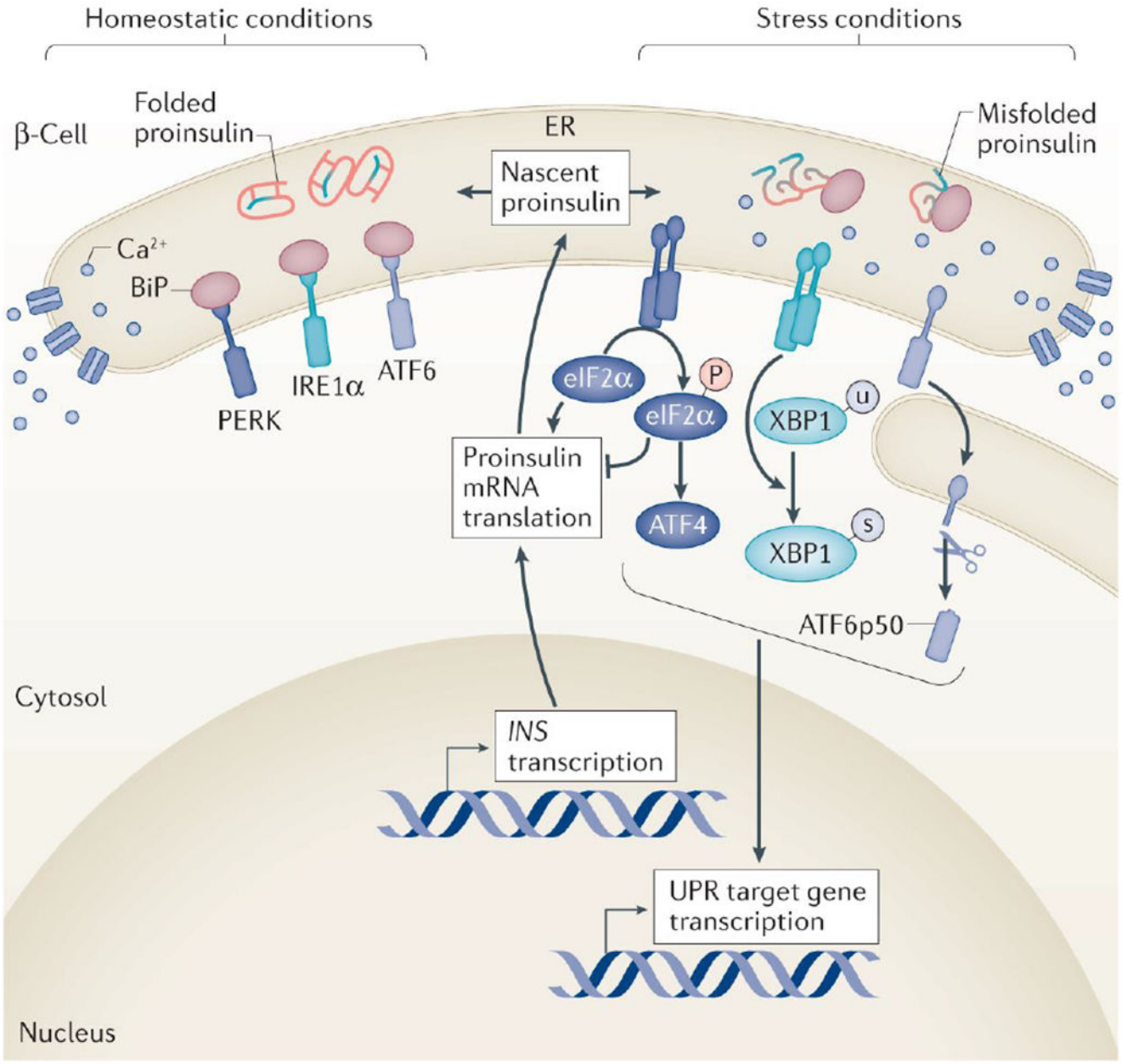

Fig. 1: ER stress and the UPR pathways in β-cells.

Increased proinsulin synthesis is a major direct cause of physiological endoplasmic reticulum (ER) stress in β-cells. Misfolded proinsulin in the ER binds to and titrates away the molecular chaperone BiP from the unfolded protein response (UPR) sensors. These UPR sensors are three ER transmembrane proteins, serine–threonine protein kinase–endoribonuclease IRE1α, protein kinase R-like ER kinase (PERK) and cyclic AMP-dependent transcription factor ATF6. Activated PERK phosphorylates the eukaryotic translation initiation factor 2 on a serine residue (Ser52 for human protein EIF2S1), which generally inhibits all translation. UPR signalling also activates multiple transcription factors, for example, XBP1 splicing and translation, ATF4 translation and ATF6 translocation to Golgi apparatus for proteolytic processing. The translocation of the transcription factors into

the nucleus activates stress response genes that play important roles in mitigating the ER stress initially but could lead to cell death with chronic ER stress. P, phosphorylation; s, spliced; u, unspliced.

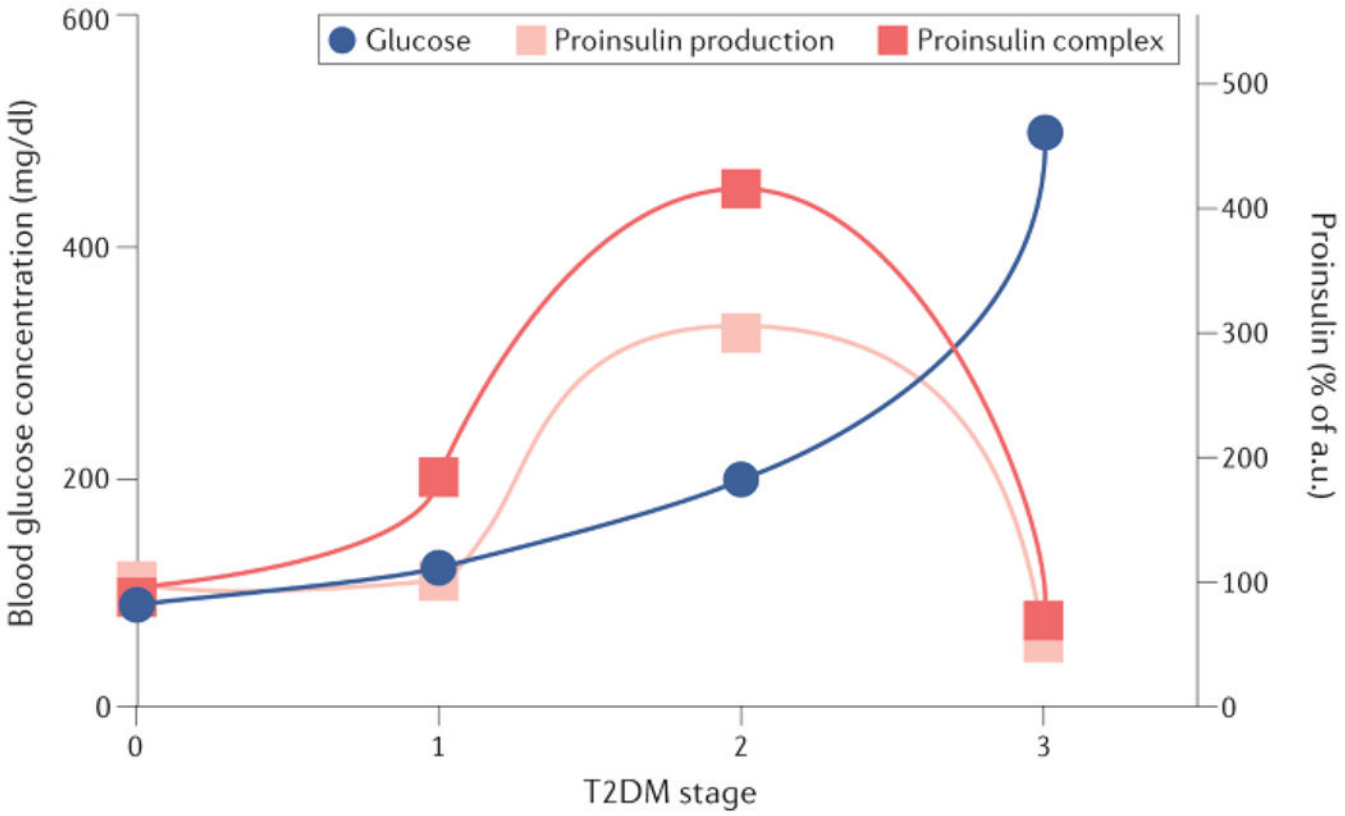

Fig. 2: Islet dysfunction during the development of T2DM.

Our working model presents multiple stages of β-cell failure during different stages of type 2 diabetes mellitus (T2DM) and predicts that proinsulin misfolding in the endoplasmic reticulum is a key early feature of pancreatic β-cell demise in animal models and humans during the development of T2DM. Stage 0, normal blood glucose, with normal pancreatic insulin and proinsulin content; stage 1, normal blood glucose, with high pancreatic insulin and proinsulin content; stage 2, abnormal blood glucose, with lower pancreatic insulin, in combination with high pancreatic proinsulin-to-insulin ratio; stage 3, high blood glucose, with low pancreatic insulin and proinsulin content. Proinsulin complex, high molecular weight, intermolecular disulfide cross-linked proinsulin molecules ($n \geq 2$), with or without other covalently linked non-proinsulin molecules which contain at least a reactive cysteine. Right axis, 100% refers to 'proinsulin complexes' found in healthy islets, using a specific proteomic assay. a.u., arbitrary unit.

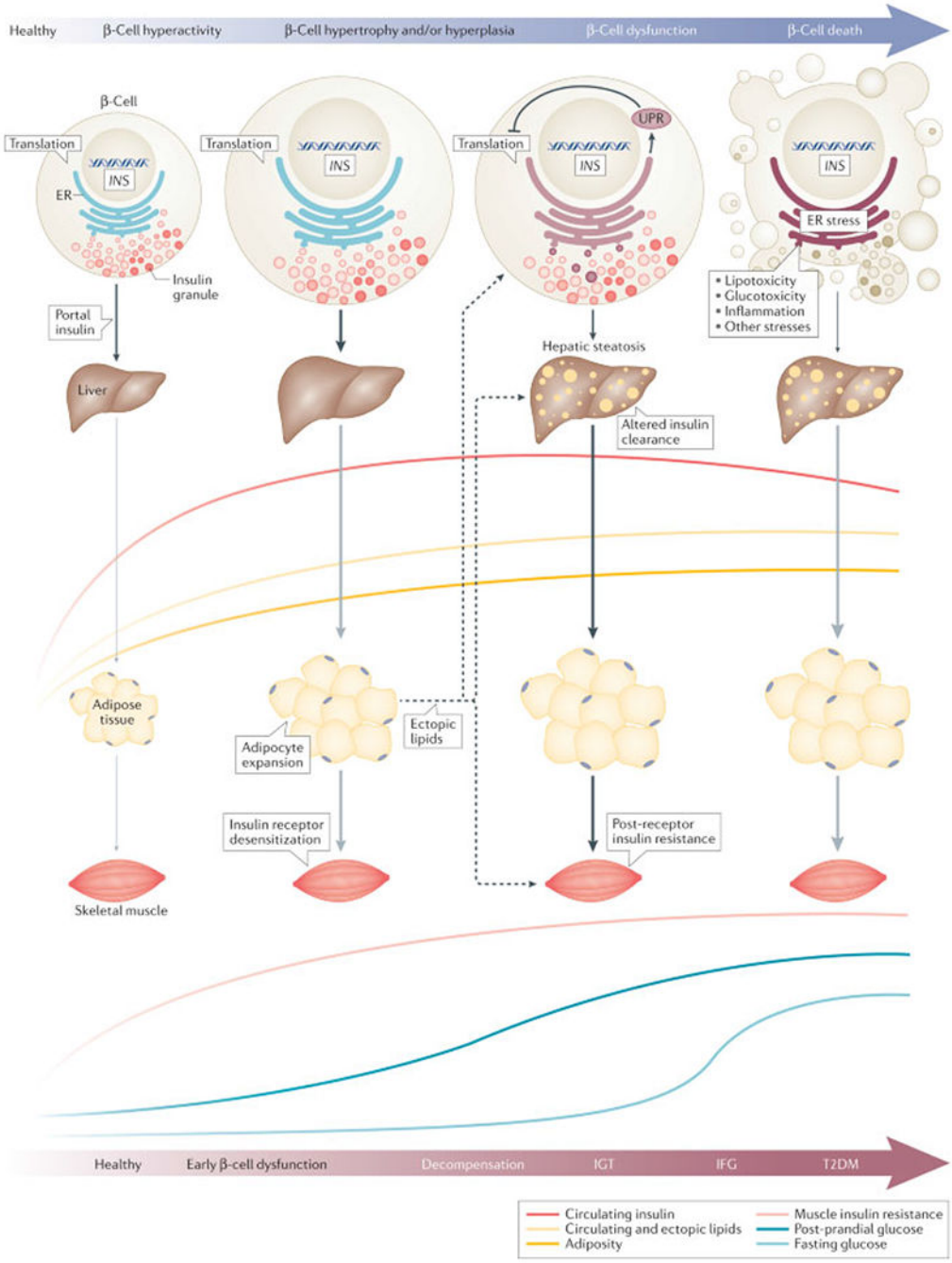

Fig. 3. The association of β-cell ER stress with insulin hypersecretion, hepatic steatosis, adiposity and insulin resistance.

In this working model of endoplasmic reticulum (ER) stress during the development of prediabetes and type 2 diabetes mellitus (T2DM), insulin hypersecretion is a key functional defect, both causing and being caused by increased ER stress levels in β-cells. Our model also illustrates the effects of hyperinsulinaemia on nonalcoholic fatty liver disease, adipocyte expansion and peripheral insulin resistance in multiple tissues. Eventually, unresolved β-cell ER stress, which triggers the unfolded protein response (UPR), in the face of glucolipotoxicity, inflammation and other stresses, can result in β-cell apoptosis.

Hyperinsulinaemia, driven by β-cell hyperactivity and increased functional β-cell mass, causes adipocyte expansion and, combined with additional metabolic stresses, leads to ectopic lipid spill-over into liver and muscle and systemic insulin resistance. Insulin receptor desensitization (that is, insulin resistance) causes further hyperinsulinaemia by overwhelming the insulin clearance capacity. Eventually, β-cells lose their ability to respond effectively to glucose through dysfunction and sometimes death, marking the transition from prediabetes to T2DM. IFG, isolated fasting glucose; IGT, impaired glucose tolerance; post-receptor insulin resistance, defect in the insulin signalling cascade irrespective of insulin receptor abnormality.

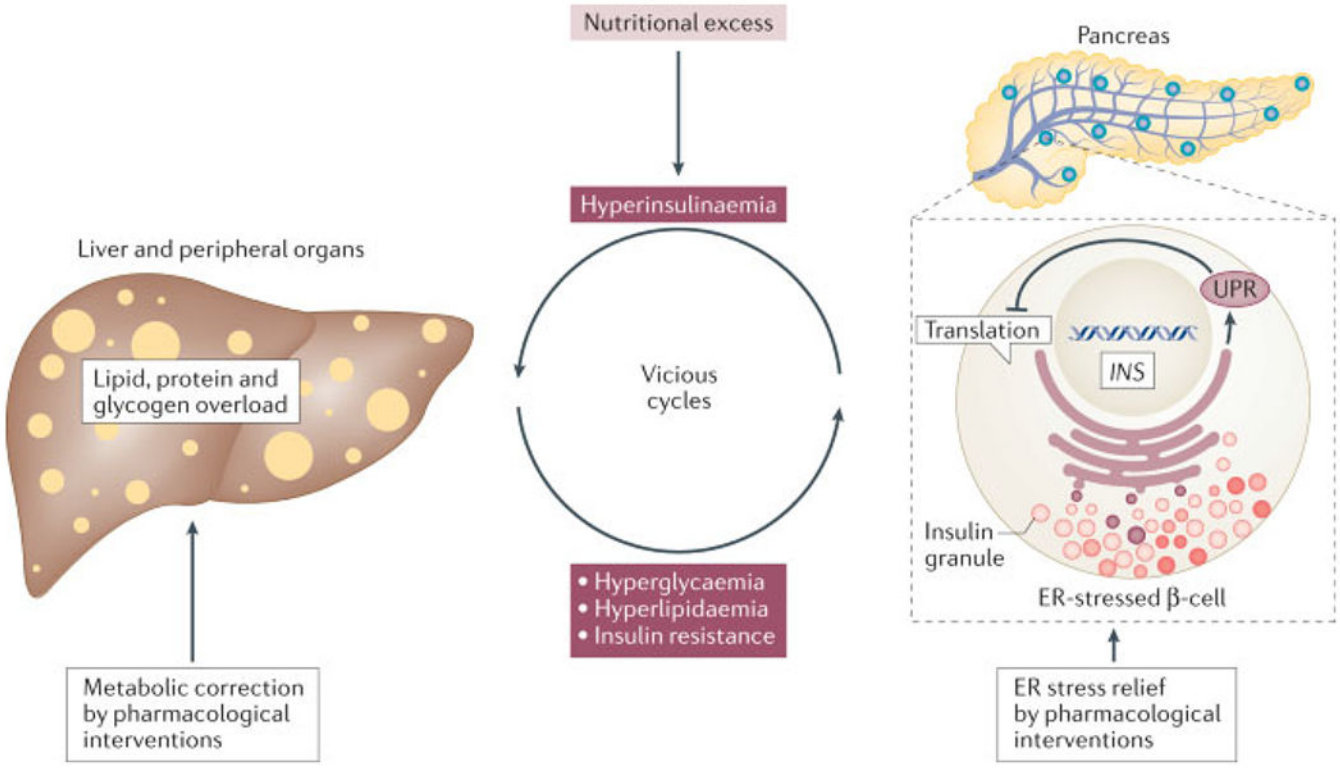

Fig. 4. Therapeutic intervention to correct vicious cycles in the metabolic syndrome.

The traditional view holds that insulin resistance in peripheral organs (for example, liver, muscle and adipose tissue) drives up the demand for insulin production from pancreatic β-cells. In response, the current therapeutic strategies almost exclusively focus on reducing insulin resistance in peripheral tissues, with limited success. However, observations derived from experimental models suggest that targeting endoplasmic reticulum (ER) stress in β-cells could be a promising therapeutic strategy for both type 2 diabetes mellitus and nonalcoholic fatty liver disease. Owing to the causal relationship between increased ER stress in β-cells and uncontrolled insulin hypersecretion, a reasonable therapeutic strategy might be to reduce insulin production in β-cells, which would reduce hyperinsulinaemia and insulin resistance in peripheral tissues. Therefore, we propose that ER stress relief in β-cells by pharmacological interventions should be explored as an alternative strategy and could provide a powerful therapeutic approach. In the ER-stressed β-cell, the different ‘coloured’ insulin granules represent insulin granules with a low amount of or no insulin crystals, which is often used as morphological evidence for ‘ER stress’ in β-cells. UPR, unfolded protein response.

Table 1 |

Evidence of ER stress caused by MODY gene mutations

MODY subtype[a]	Gene symbol	Gene class	References demonstrating a link to ER stress in β-cells
MODY-1	*HNF4A*	Transcription factor	Sato et al., 2012 (ref.[50])
MODY-2	*GCK*	Glucose-sensing enzyme	Matschinsky et al., 1993 (ref.[51]), van Buerck et al., 2012 (ref.[53]), Shirakawa et al., 2013 (ref.[54])
MODY-3	*HNF1A*	Transcription factor	Yamagata et al., 1996 (ref.[55]), Kirkpatrick et al., 2011 (ref.[56])
MODY-4	*PDX1*	Transcription factor	Sachdeva et al., 2009 (ref.[61])
MODY-5	*HNF1B*	Transcription factor	None reported
MODY-6	*NEUROD1*	Transcription factor	None reported
MODY-7	*KLF11*	Transcription factor	None reported
MODY-8	*CEL*	Fat metabolism	None reported
MODY-9	*PAX4*	Transcription factor	Mellado-Gil et al., 2016 (ref.[68])
MODY-10	*INS*	Hormone	Liu et al., 2010 (ref.[70])
MODY-11	*BLK*	Kinase	None reported
MODY-12	*ABCC8*	ABC transporter	None reported
MODY-13	*KCNJ11*	Ion channel	None reported
MODY-14	*APPL1*	Adaptor protein	None reported

ER, endoplasmic reticulum; MODY, maturity-onset diabetes of the young.

[a]The list of MODY sub-types from www.OMIM.org was accessed in March 2021.