Islet β cell failure in type 2 diabetes

Marc Prentki[1] and Christopher J. Nolan[2]

[1]Molecular Nutrition Unit and Montreal Diabetes Research Center, University of Montreal and Centre Hospitalier de l'Université de Montréal, Montreal, Quebec, Canada. [2]Department of Endocrinology and Diabetes, The Canberra Hospital, and Medical School, Australian National University, Canberra, Australian Capital Territory, Australia.

The major focus of this Review is on the mechanisms of islet β cell failure in the pathogenesis of obesity-associated type 2 diabetes (T2D). As this demise occurs within the context of β cell compensation for insulin resistance, consideration is also given to the mechanisms involved in the compensation process, including mechanisms for expansion of β cell mass and for enhanced β cell performance. The importance of genetic, intrauterine, and environmental factors in the determination of "susceptible" islets and overall risk for T2D is reviewed. The likely mechanisms of β cell failure are discussed within the two broad categories: those with initiation and those with progression roles.

Introduction

Modern lifestyle, with abundant nutrient supply and reduced physical activity, has resulted in dramatic increases in the rates of obesity-associated disease conditions, including type 2 diabetes (T2D) (1). It was estimated that approximately 150 million people worldwide had T2D in the year 2000, with the prediction that this number could double to 300 million by 2025 (1). Numerous studies show that insulin resistance precedes the development of hyperglycemia in subjects that eventually develop T2D (2, S1). However, it is increasingly being realized that T2D only develops in insulin-resistant subjects with the onset of β cell dysfunction (3–6, S2).

The normal pancreatic β cell response to a chronic fuel surfeit and obesity-associated insulin resistance is compensatory insulin hypersecretion in order to maintain normoglycemia. T2D only develops in subjects that are unable to sustain the β cell compensatory response. Longitudinal studies of subjects that develop T2D show a rise in insulin levels in the normoglycemic and prediabetes phases that keep glycemia near normal despite the insulin resistance (β cell compensation), followed by a decline when fasting glycemia surpasses the upper limit of normal of 5.5 mM (β cell failure) (5) (Figure 1). A longitudinal study in Pima Indians showed that β cell dysfunction was the major determinant of progression from normoglycemia to diabetes (7). Furthermore, the natural history of T2D entails progressive deterioration in β cell function (5), associated with loss of β cell mass due to apoptosis (8). Many affected persons that initially have adequate control of their disease with lifestyle changes alone eventually require insulin therapy in the later stage of the disease (Figure 1). Less certain is the time point in T2D development when β cell dysfunction first appears. The recent evidence points to it being early, long before the onset of prediabetes, when glycemia is still classified as normal glucose tolerance (9, S3, S4).

This Review focuses on the mechanisms of islet β cell failure in obesity-associated T2D, taking into account that this failure occurs in islets that are undergoing the processes of compensation for insulin resistance. Compensation involves expansion of β cell mass, enhanced insulin biosynthesis, and increased responsiveness of nutrient-secretion coupling. Compensation fails in subjects that have "susceptible" as opposed to "robust" islets. The genetic and acquired factors, including intrauterine and early life environment, that determine islet susceptibility are discussed. We propose a model of β cell failure in which one or a small number of β cell defects act as the weak link(s) in the processes of β cell compensation that initiate β cell dysfunction. The likely mechanisms of early β cell demise include mitochondrial dysfunction, oxidative stress, ER stress, dysfunctional triglyceride/FFA (TG/FFA) cycling, and glucolipotoxicity. Once hyperglycemia has developed, additional processes linked to glucotoxicity and the diabetic milieu, such as islet inflammation, O-linked glycosylation, and amyloid deposition, accelerate β cell demise, resulting in severe β cell phenotypic alterations and loss of β cell mass by apoptosis.

Compensating for insulin resistance and expanding β cell mass

In insulin-resistant states, pancreatic islets usually respond by increasing insulin secretion to maintain normoglycemia, a process termed β cell compensation. The mechanisms involved are not fully understood, but it is apparent from rodent studies that both expansion of the β cell mass (10, 11, S5) and enhanced β cell function are important (12, 13). Increased β cell mass has been observed in the pancreata of obese compared with lean nondiabetic subjects (8), although the fold increase (about 50%) is less than seen in rodents, especially mice. The stimulating factors implicated in the compensatory islet responses likely include increased nutrient supply (particularly glucose and FFAs), insulin and other growth factor signaling, and increased levels of and sensitivity to incretin hormones such as glucagon-like peptide 1 (GLP-1) (Figure 2).

For compensatory β cell mass expansion, increased nutrient supply in the blood is important as a stimulant, with considerable evidence for roles for both glucose (10, 14, S6) and FFAs (10, 15, S7) (Figure 2). Increased enteric nutrient supply, particularly in the form of fat, may also result in β cell mass expansion through increased GLP-1 production from L cells in the intestine (15, 16). Interestingly, a study in dogs fed a high-fat diet showed β cell compensation even though glucose levels were not elevated, even postprandially, suggesting that glucose is not the primary cause of β cell compensation in that model; increased GLP-1 and FFA signaling were proposed as the stimulants (15).

Nonstandard abbreviations used: AGE, advanced glycation end product; AICAR, 5-aminoimidazole-4-carboxamide riboside; AMPK, AMP-activated protein kinase; ARNT, aryl hydrocarbon receptor nuclear translocator; FA, fatty acid; FAO, FA oxidation; GK, glucokinase; GLP-1, glucagon-like peptide 1; GSIS, glucose-stimulated insulin secretion; IAPP, islet amyloid polypeptide; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IUGR, intrauterine growth retardation; LC-CoA, long-chain acyl-CoA; PC, pyruvate carboxylase; PDX1, pancreas-duodenum homeobox–1; TCF7L2, transcription factor 7–like 2; T2D, type 2 diabetes; TG, triglyceride; UCP2, uncoupling protein 2; ZDF, Zucker diabetic fatty; ZF, Zucker fatty; ZL, Zucker lean.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **116**:1802–1812 (2006). doi:10.1172/JCI29103.

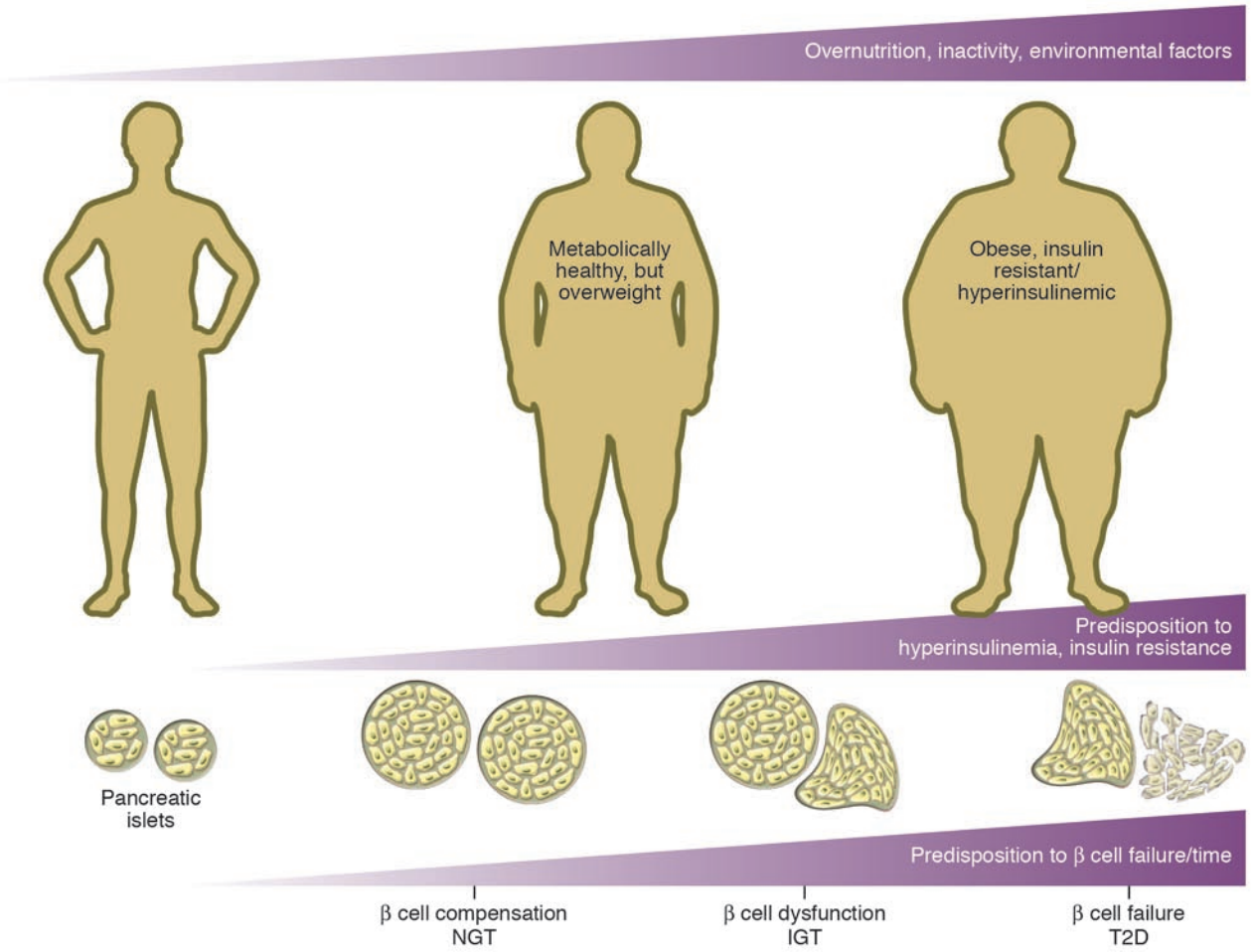

Figure 1
Islet β cell failure and the natural history of T2D. T2D develops in response to overnutriton and lack of physical activity in subjects that have underlying genetic and acquired predispositions to both insulin resistance (and/or hyperinsulinemia) and β cell dysfunction. Over time, islet β cell compensation for the insulin resistance fails, resulting in a progressive decline in β cell function. As a consequence, subjects progress from normal glucose tolerance (NGT) to IGT and finally to established T2D. Even after diagnosis of T2D, β cell function continues to worsen such that subjects progress from needing changes in diet/exercise only to requiring oral hypoglycemic agents and eventually insulin for achievement of adequate glycemic control. Future therapies will be directed not only to achievement of euglycemia, but also changing the course of the disease by reversing the processes of β cell failure.

Evidence points to important roles for various growth factor signaling pathways in compensatory β cell growth. Roles have been proposed for insulin and insulin-like growth factors 1 and 2 acting via insulin receptor substrate–2 (IRS-2) (17). IRS-2 signaling through PKB phosphorylation and inactivation of the forkhead-O transcription factor 1 (FOXO1) increases expression of the homeodomain protein *pancreas-duodenum homeobox–1* (*PDX1*) gene, an important β cell proliferation and survival factor (11, 14, 18, S8–S10). Activated PKB provides protection from apoptosis through phosphorylation and inhibition of proapoptotic proteins such as BAD (11). The major support for this cascade in β cell growth was based on in vitro studies and knockout mouse models. A recent study in Zucker fatty (ZF) rats has provided strong support for PKB activation in the β cell growth response to insulin resistance in this normoglycemic model (11).

GLP-1 enhances β cell proliferation and acts as a survival factor by signaling through multiple pathways. GLP-1 can activate IRS-2 and PKB via the cAMP response element–binding protein (CREB) (19, S11) and transactivation of the EGFR with activation of PKB in a PI3K- and PKC-ζ–dependent manner (20, S12, S13). FFA signaling via GPR40, also known as FFA receptor 1 (FFAR1), a G protein–coupled receptor that acts as a FA receptor and is highly expressed in islet β cells (21, S14), might also have a proliferative effect, as was recently described for oleate in breast cancer cells (22).

There is considerable debate in the literature with respect to the source of cells for islet expansion in adults. The possibilities include proliferation of existing β cells, including cells in close proximity to ductules, and/or neogenesis from pancreatic ductal cells (8, 11, 18, S15). The relevance of this debate to human T2D also needs to be carefully considered, as the capacity for human β cell proliferation and neogenesis, particularly in adults, may be much less than in the rodent models that are mostly studied.

Promoting islet compensation by enhancing β cell function

Studies of insulin-resistant animals clearly show an important role for enhanced β cell function in compensation mechanisms (12, 13, 15). Increased β cell glucose metabolism, FFA signaling, and sensitivity to incretins, as well as increased parasympathetic nervous system activity in islets, have been implicated (Figure 2).

Increased islet glucose metabolism. Islet β cell glucose metabolism is essential for coupling glucose sensing to insulin release (23, 24, S16). It is well accepted that the metabolism of glucose through pyruvate to acetyl-CoA with subsequent mitochondrial oxidation increases the ATP/ADP ratio, which results in closure of ATP-sensitive K^+ (K^+_{ATP}) channels, depolarization of the plasma membrane, opening of voltage-dependent Ca^{2+} channels, and Ca^{2+} triggering of insulin granule exocytosis (23, S17). This pathway, often termed the K^+_{ATP} channel–dependent pathway, is considered to be the major triggering pathway for glucose-stimulated insulin secretion (GSIS) (23, S17). In addition, pyruvate from glucose can be metabolized via pyruvate carboxylase (PC) into the anaplerosis/cataplerosis pathway, which can impact on insulin secretion by increasing levels of cataplerosis-derived signaling molecules. Anaplerosis refers to the processes by which Krebs cycle intermediates in the mitochondrion are replenished/increased, whereas cataplerosis refers to their egress from the cycle. Cataplerosis-derived signals include NADPH from the malate-pyruvate shuttle and additional pyruvate cycling processes (25, 26, S18), citrate (26, 27, S19), glutamate (28), and lipid signaling molecules from the malonyl-CoA/long-chain acyl-CoA (malonyl-CoA/LC-CoA) pathway (4, 12, 29, 30).

Studies of islet β cell compensation within models that maintain normoglycemia show upregulation of glucose metabolism pathways (12, 13, 31). The activity of glucokinase (GK), the high-K_m enzyme that phosphorylates glucose on its entry into the β cell (24), was increased in the short-term glucose infusion rat model (31), in the insulin-resistant (but normoglycemic) spontaneously hypertensive rat model (12), and in both ZF and Zucker diabetic fatty (ZDF) rats compared with Zucker lean (ZL) control rats (S20). It is important to note that the expression of GK, along with the glucose transporter GLUT2, are strongly and positively related to the state of differentiation of β cells, and both are under regulatory control of PDX1 (32). Thus, PDX1 provides a link between factors and pathways that promote β cell growth and those that enhance β cell function.

Detailed studies of glucose metabolism in islets of the ZF rat have been performed (12). The ZF rat has a leptin receptor mutation and is obese, hyperlipidemic, and severely insulin resistant, but unlike the ZDF rat, it remains normoglycemic (12, S5, S20). For this reason the ZF rat is an excellent model for the study of β cell compensation mechanisms. In the ZF compared with the ZL rat, it was found that total glucose utilization was increased by about 1.5- to 2-fold and that both glucose oxidation and glucose flux via PC and anaplerosis were increased (12). With respect to the latter, increases were observed in enzymes and metabolites involved in anaplerosis and the malate-pyruvate and citrate-pyruvate shuttles of ZF islets (12). Inhibition of these pathways by phenylacetic acid, which inhibits PC, markedly impeded GSIS (12). Hence, the increased glucose oxidation and anaplerosis likely contribute to compensatory hyperinsulinemia by increasing the activity of signaling pathways of GSIS coupling (12, 26, 29).

Lipid signaling, lipolysis, and TG/FFA cycling. While it is well established that a supply of FFAs to the β cell is essential for normal GSIS (33), FFAs also amplify GSIS (4, 29) as well as insulin secretion to other nutrient and non-nutrient secretagogues (29). The mechanisms by which FFAs do this are not fully elucidated, but it is clear that there are at least 2 pathways involved. The first is through the binding of FFA to FFAR1/GPR40 on the cell membrane, which activates intracellular signaling, including an increase in intracellular Ca^{2+} (21, 34). The second involves interaction between anaplerosis that generates malonyl-CoA and intracellular partitioning of LC-CoA, the malonyl-CoA/LC-CoA pathway (4, 12, 25, 29). Essentially, malonyl-CoA inhibits fatty acid oxidation (FAO) such that LC-CoAs accumulate in the cytosol, favoring the production of lipid signaling molecules including fatty acid (FA) esterification products such as diacylglycerol (DAG) (4, 29, 30).

We recently studied the effects of FFAs on GSIS in isolated islets from ZF and ZL rats. Exogenous FFA markedly potentiated GSIS in ZF islets at elevated glucose levels (16 mM) but also allowed for very robust secretion at physiological glucose levels (5–8 mM) that was not evident in ZL islets (35). In investigating the underlying mechanisms, we found increased glucose-responsive FFA esterification and lipolysis pathways (both increased 2- to 3-fold at 16 mM glucose) in ZF islets, indicative of enhanced TG/FFA cycling. Importantly, FAO was not impaired and there was no steatosis in the ZF islets. Interruption of TG/FFA cycling by the lipase inhibitor orlistat blunted FFA augmentation of GSIS, suggesting a role for this cycle in compensation processes (Figure 2).

GLP-1. In addition to its role of promoting β cell growth, the incretin hormone GLP-1 is likely to be involved in enhancing the function of individual β cells in compensation processes (15, 16) (Figure 2). Of considerable interest in our studies of isolated ZF rat islets was the finding of heightened sensitivity of these compensating islets to the incretin effect of GLP-1. Furthermore, GLP-1 and FFAs had synergistic actions in their augmentation of GSIS (35). Thus, it is attractive to hypothesize that the "on" signal for β cell compensation entails a synergistic interaction of fuels (FFAs and postprandial hyperglycemia) and incretin hormones.

Brain, autonomic nervous system, and islet compensation. Pancreatic islets are richly innervated with parasympathetic and sympathetic neurons (36). The former activate, while the latter suppress, insulin secretion (36). There is some evidence (Figure 2) that the brain and increased parasympathetic nervous system activity are involved in compensatory growth and function of islets. For example, lesions of the ventromedial hypothalamus in rats caused islet β cell proliferation beginning at day 1, and this involved vagal hyperactivity, as vagotomy or atropine administration completely inhibited the effect (37). Furthermore, vagotomy reduces the hyperinsulinemia in the *ob/ob* mouse (S21), and cholinergic agonists enhance insulin secretion more effectively in insulin-resistant mice (38) and obese humans (39) compared with their insulin-sensitive counterparts. Changes in sympathetic activity in islet tissue are also likely to be involved in islet compensation. Thus, lipid infusion in rats lowers sympathetic α2-adrenergic nervous activity and leads to increased β cell responsiveness to glucose (40).

Upregulation of insulin gene expression. In addition to upregulation of nutrient-secretion coupling, a concomitant increase in insulin biosynthesis is necessary to maintain enhanced β cell function required for compensation (41) (Figure 2). The regulation of insulin gene transcription involves complex interaction between second messenger signals derived from nutrient metabolism and hormonal stimuli (e.g., PI3K and p38 mitogen-activated protein kinase), transcription factors (e.g., PDX1, MafA, and Beta2/NeuroD), and various transcrip-

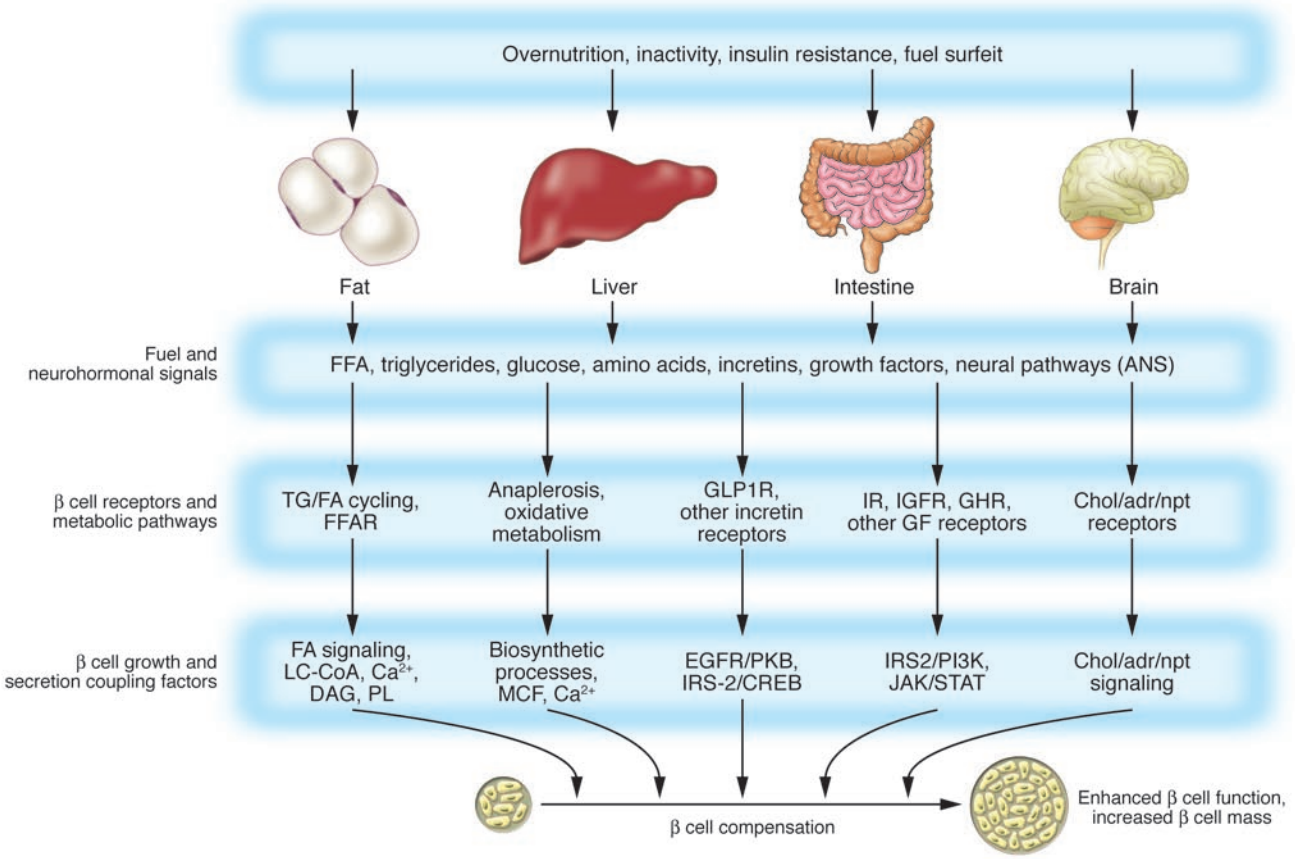

Figure 2
Mechanisms of β cell compensation for insulin resistance. Normally islet β cells respond to insulin resistance by increased secretion through the processes of compensation. These include an expansion of β cell mass, increased insulin biosynthesis, and enhanced nutrient secretion coupling processes with increased sensitivity to glucose, FFAs, and GLP-1 stimuli. Enhanced glucose utilization, glucose oxidation, anaplerosis/cataplerosis, and TG/FFA cycling result in increased production of coupling signals necessary for insulin exocytosis. For expansion of β cell mass, roles are evident for increased activity of growth factor signaling pathways, postprandial glucose, and GLP-1 signaling that promote β cell proliferation and neogenesis and prevent apoptosis. Furthermore, signaling for growth may occur in response to FFAs, via the FFA receptors (FFAR) and via lipid signaling molecules derived from TG/FFA cycling. adr, adrenergic; ANS, autonomic nervous system; chol, cholinergic; CREB, cAMP response element–binding protein; DAG, diacylglycerol; GF, growth factor; GLP1R, GLP-1 receptor; GHR, growth hormone receptor; IGFR, insulin-like growth factor receptor; IR, insulin receptor, IRS-2, insulin receptor substrate 2; MCF, metabolic coupling factors; npt, neuropeptide; PKB, phosphokinase B; PL, phospholipids.

tion factor DNA binding sites (e.g., A3, C1, and E1 sites) on the insulin promoter (41, 42, S22, S23). Both glucose and GLP-1 are major stimuli for activation of insulin gene transcription and are involved in this aspect of β cell compensation (41, S23). The role for FFAs are less clear, with some studies showing that elevated concentrations of saturated FFAs are inhibitory in the context of hyperglycemia (41, S22). The upregulation of insulin biosynthesis in compensation could also be at the level of translation and posttranslational processing (43, 44, S24, S25). Insulin biosynthesis is known to be highly regulatable by acute changes in nutrient availability (43, 25), but less is known about longer-term adaptations in transcriptional/translational regulation of insulin production to situations such as chronic overnutrition (S24). An interesting proposal is that increased expression of an insulin splice variant with increased translation efficiency may be a factor involved in β cell compensation (44). Overall, there has been little exploratory investigation into the mechanisms of stimulation of insulin biosynthesis in compensation processes, with the role of FFAs, in particular, being uncertain at this stage.

Determinants of "susceptible" islets in T2D

Lessons from studies in ZF and ZDF rats. Although the ZDF rat has the same leptin receptor mutation and similar levels of hyperphagia, obesity, and hyperlipidemia as the ZF rat (S20), males spontaneously develop diabetes (12, S5, S20). The ZDF rat has been a cornerstone for research on the role of lipotoxicity in β cell failure (45–47, S26). By approximately 8 weeks of age, male ZDF rats develop mild hyperglycemia and by 10 weeks profound diabetes (45). The islets show increased β cell apoptosis (S5, S27, S28) and moderate accumulation of intracellular TGs by 8 weeks and enormous TG overload by 10 weeks (45, 48) in concordance with markedly increased expression of enzymes responsible for lipogenesis and lipid esterification (48, S26, S29, S30). Islet steatosis in ZDF rats so far has been documented using biochemical determination of total islet TG content. However, β cell steatosis needs to be confirmed by electron microscopy or other methods, as ZDF islets at 10 weeks of age may contain fibroblasts loaded with TGs. The major pathogenic factors that have been implicated in causing islet

steatosis and β cell failure are the presence of hyperlipidemia and the absence of leptin signaling (47, S29). However, the fact that the ZF rat is capable of long-term β cell compensation with the maintenance of normoglycemia without evidence of islet steatosis, despite the presence of these identical pathogenic factors, suggests that additional factor(s) are involved in the β cell demise in the ZDF rat. A mild defect mapped to the A2-C1-E1 minienhancer of the insulin promoter in ZDF rats is inherited independently of the leptin receptor mutation (49).

We favor the view that inadequate β cell compensation due to the defective insulin promoter (49) results in onset of mild hyperglycemia at 8–9 weeks of age in the ZDF rat and that it is this early hyperglycemia that causes pathogenic handling of FFAs by the ZDF islets, resulting in islet steatosis and severe β cell failure. This is due to the effect of glucose to alter the AMP-activated protein kinase/malonyl-CoA/LC-CoA (AMPK/malonyl-CoA/LC-CoA) metabolic signaling network (50), causing inhibition of FAO and the accumulation of toxic lipids (glucolipotoxicity) (4). Consistent with this view, exercise and 5-aminoimidazole-4-carboxamide riboside (AICAR), which both activate AMPK, prevent or delay T2D in ZDF rats (51, S31), although these interventions may also modulate insulin sensitivity. The ZDF rat is an example in which islets are susceptible to β cell compensation failure because of an inherited defect, in contrast to ZF islets, which are remarkably robust.

Species differences. The susceptibility of islets to dysfunction and apoptosis is very dependent on species and genetic background. For example, islets from the gerbil *Psammomys obesus* and human islets in culture are highly susceptible to dysfunction and apoptosis when exposed to elevated glucose (52–54, S32, S33) compared with rodent islets that are relatively resistant (55, S34). *Psammomys obesus* is normoglycemic on its natural low-calorie diet but becomes diabetic when fed a high-calorie chow diet (54). In this model, failure of the β cell to synthesize enough insulin to maintain normoglycemia in response to high calorie intake, rather than increased apoptosis, appears to be instrumental in the development of hyperglycemia (54). Furthermore, the effects of dietary modification or manipulation of metabolic genes is highly dependent on the strain of mice (56, 57, S35). For example, expression of either the leptin gene (*ob/ob*) or the leptin receptor gene (*db/db*) mutations on the C57BL/6J background causes massive obesity and insulin resistance with only transient diabetes, while the same mutations on the C57BL/KsJ strain cause severe diabetes (58, S36). Other studies have shown that high-fat diet–fed C56BL/6J mice are the diabetes-prone strain, while the A/J mouse strain is resistant (S35, S37). Another example is DBA2 mice, which are prone to islet β cell failure when stressed. On a standard chow diet, DBA2 mice (normally insulin sensitive) hypersecrete insulin in response to glucose in comparison to the C57BL/6J strain (diabetes resistant in these studies) (59). Interestingly, isolated islets from DBA2 mice have increased glucose utilization and glucose oxidation (59), suggesting that there may be a link between increased glucose oxidation, enhanced insulin biosynthesis and secretion, and risk of islet β cell failure.

Genetic factors and human T2D. While several rare monogenic forms of obesity and diabetes, including maturity-onset diabetes of the young (MODY), have been described (60, S38, S39), the genetic basis of T2D is clearly much more complex. T2D is believed to be a polygenic disease in which variations within multiple genes, each adding some risk, contribute to clinically significant predisposition (61). Presumably some of these genes will convey risk for systemic insulin resistance and others for the susceptible islet. Through various genetic approaches, polymorphisms within numerous genes relating to adipocyte, hepatic, and skeletal muscle metabolism, insulin action, and pancreatic islet function have been discovered that are associated with decreased/increased risk of T2D. Of particular note are polymorphisms of *PPARγ* (62), *PPARγ, coactivator 1-α* (*PGC1α*) (63), transcription factor *Kruppel-like factor 11* (*KLF11*) (64), *transcription factor 7–like 2* (*TCF7L2*) (65), *calpain 10* (*CAP10*) (66, S40), *KCNJ11* (*Kir6.2*) (67, S41, S42), and allele III of a variable number tandem repeat 0.5 kb upstream of the insulin genes (*INS-VNTR*) (68). For the majority of these polymorphisms, it is uncommon for a significantly increased T2D risk to be shown in more than one or a few population cohorts, consistent with enormous heterogeneity in heritability for this condition (61). Apparent exceptions are the described variants in the transcription factor *TCF7L2* (65) and of *KCNJ11* (*Kir6.2*) (67, S41, S42). Polymorphisms of *TCF7L2* are common in Icelandic, Danish, and US cohorts (overall 38% and 7% of the populations are heterozygous and homozygous, respectively) and, irrespective of the population cohort studied, are associated with increased T2D risk: overall relative risk of 1.45 and 2.41 for heterozygous and homozygous inheritance, respectively (65). Interestingly, TCF7L2 is thought to act by regulating proglucagon gene expression (65, S43) and could therefore affect production of GLP-1, which has important roles in β cell compensation mechanisms, as discussed above. Variants in *KCNJ11* (*Kir6.2*), which encodes a component of the K^+_{ATP} channel critical to nutrient-secretion coupling, are relatively common, as indicated by population-based studies and meta-analyses (67, S41, S42). The E23K polymorphism of *KCNJ11* (*Kir6.2*) was present with homozygosity in 27% of T2D subjects compared with only 14% in nondiabetic subjects within a French cohort study (S41). Also of direct relevance to the β cell are polymorphisms of *KLF11*, the product of which is a transcription factor that binds the insulin promoter and increases its activity (64).

Gene transcript profiling and human T2D risk. Microarray analysis is difficult in human islets due to variations among donors as well as problems accessing pancreatic tissue and in the preservation and yield of islets. A recent study, however, reported gene profiling in isolated islets from 7 normal and 5 T2D subjects (69). Major decreases in expression of genes of the insulin signaling pathway (*insulin receptor*, *IRS2*, and *Akt2*), of the MODY gene *hepatic nuclear factor 4α* (*HNF4α*), and several glucose metabolism genes were reported (69). A novel finding from this study was a 90% reduction in the expression of the transcription factor *aryl hydrocarbon receptor nuclear translocator* (*ARNT*) (also called *hypoxia-inducible factor 1β*, or *HIF1β*) (69, S44). Inhibition of *ARNT* expression in MIN-6 mouse insulinoma cells by RNAi caused impaired GSIS (69). Furthermore, β cell–specific knockout of *ARNT* in mice caused impaired glucose tolerance (IGT) and changes in islet gene expression similar to those found in human T2D (69). Clearly, further examination of the role of ARNT in islet β cell failure is warranted, with particular focus on whether the reduced ARNT expression in T2D islets is an early defect in pathogenesis or a consequence of upstream events.

Microarray technology has also been used to examine muscle biopsy samples from subjects with normal glucose tolerance, at risk of T2D, and with T2D (70, S45). Both studies showed evidence for downregulation of key mitochondrial and oxidative phosphorylation metabolism genes; these genes are regulated by the nuclear respiratory factor (NRF) transcription factor and its coactivator PGC1α (70, S45). Impaired oxidative phosphorylation in muscle

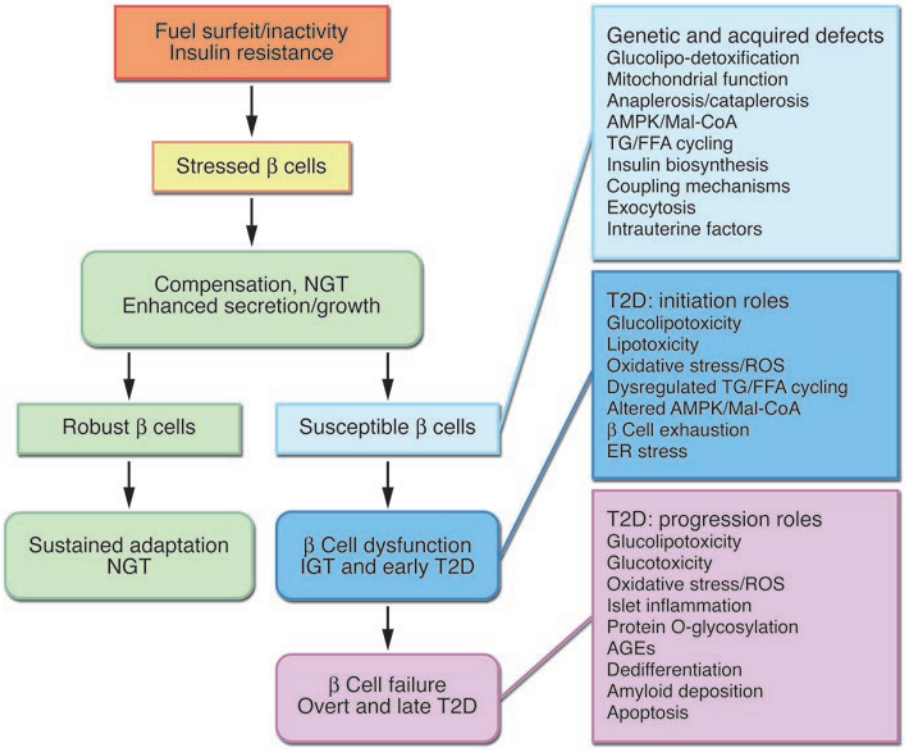

Figure 3
Mechanisms of β cell failure in T2D. Islet β cell compensation for insulin resistance is sustained provided β cells are robust, resulting in long-term maintenance of NGT. Compensation processes, however, fail if there are genetic or acquired factors that result in susceptible β cells. The defect(s) create weak link(s) in the compensation process that promote β cell dysfunction by mechanisms with initiator roles that result in IGT and early T2D. Hyperglycemia, once established, promotes a further series of mechanisms, under the umbrella of glucotoxicity, that cause severe β cell failure and overt and late T2D. AMPK/Mal-CoA, AMPK/malonyl-CoA signaling network.

has been linked to intracellular lipid accumulation and insulin resistance (71, S46, S47); a similar defect has been implicated in islet β cell failure in T2D (71, 72).

Intrauterine environmental factors. Epidemiological evidence points to a role for low birth weight or intrauterine growth retardation (IUGR) in the pathogenesis of metabolic syndrome and T2D (73, S48–S51). There is also evidence, although not quite as clear, that maternal hyperglycemia in diabetic pregnancy is also an early life environmental risk factor that adds to an already increased genetic risk for T2D for the infant (74, S52, S53). This human data is strongly supported by experiments in rodents, in which dietary or surgical approaches that cause IUGR or maternal hyperglycemia in pregnancy invariably result in abnormal metabolic phenotypes in the offspring that are characteristic of metabolic syndrome with high risk of T2D (72, 75, 76, S54, S55). The effect of the intrauterine metabolic environment on adult metabolic health has been called metabolic imprinting (S56). IUGR rats have been shown to have mitochondrial dysfunction in the β cell, which is associated with increased production of ROS and impaired insulin secretion (72). Mitochondrial dysfunction in the IUGR model is also progressive, with accumulation of mitochondrial DNA mutations (72). The main findings in islets of IUGR offspring in a murine model were a 2.5-fold increase in islet hexokinase activity, basal insulin hypersecretion, and complete loss of GSIS (75).

β cell failure in T2D: human islet studies

A few studies utilizing postmortem and surgical pancreas specimens have provided valuable information on the pathogenesis of islet β cell failure in T2D (8, 69, 77, 78). Among these is a landmark study of β cell volume together with markers of β cell proliferation and apoptosis in autopsy pancreata (124 in total) from unaffected subjects and those with impaired fasting glucose (IFG) and T2D (8). The study reported a 40% and 63% loss of islet β cell volume in IFG and T2D obese subjects, respectively, compared with weight-matched controls. Lean T2D subjects had a 41% loss of β cell volume compared with lean controls. Furthermore, the decreased volume was not a consequence of reduced β cell proliferation but was associated with increased β cell death by apoptosis (8). The fact that loss of β cell mass was evident in subjects with IFG suggests that β cell mass changes are not necessarily confined to late-stage T2D (8). A study of Korean pancreas specimens provided similar data to support a role for selective loss of islet β cells in the pathogenesis of T2D (78). Another study showed that islets obtained from T2D cadaveric donors were smaller, secreted insulin poorly in response to glucose, and did not reverse diabetes upon transplantation into nude mice (77). These findings underscore the structural and functional abnormalities in islet tissue of T2D subjects.

Molecular basis of β cell failure: mechanisms with initiation roles

Mitochondrial dysfunction with production of ROS. Increased metabolism of glucose and FFAs through mitochondrial oxidation will result in an increased mitochondrial membrane potential and superoxide production (71, 79, 80). Increased superoxide production causes increased exposure of the cell to ROS and activation of uncoupling protein 2 (UCP2) (71, 81). In addition, chronically elevated levels of FFAs and glucose induce the UCP2 gene in β cells (82, S57). Increased UCP2 helps to safely dissipate the elevated mitochondrial membrane potential and promotes fuel detoxification because oxidation of these fuels becomes increasingly coupled to heat rather than ATP production. However, this occurs at the expense of ATP synthesis efficiency and consequently insulin secretion (71, 81). In other words, uncoupling of oxidative phosphorylation, resulting in impaired insulin-secretory capac-

ity but reduced ROS production, is the price the β cell pays for its survival in the presence of fuel surfeit. Islets that attempt to compensate for insulin resistance with abnormally high rates of glucose oxidation (e.g., the DBA2 mouse; ref. 59) or have an inherited or acquired deficiency in mitochondrial function (e.g., IUGR offspring; ref. 72) will be at risk of functional failure and oxidative damage. While it is important to realize that the production of ROS can have important regulatory functions on intermediary metabolism by altering the cellular redox state (83), the β cell has limited defense against excess ROS production because the expression levels of ROS-detoxifying enzymes in the β cell are particularly low in comparison with those in other cells (23). Overall, these considerations are compatible with the view that ROS may contribute to both early and late phases of β cell failure (84) (Figure 3). A note of caution with respect to the role of ROS in β cell failure is found in a study showing that ROS production is actually reduced by elevated glucose in purified rat β cells (85). However, it is difficult to extend this in vitro situation to chronic fuel surfeit exposure in vivo and to situations in which β cells are already stressed or have preexisting defects in mitochondrial function. Thus, we showed that elevated FFAs markedly enhance apoptosis and ROS production in β cells stressed by incubation in the absence of serum and are much less toxic in healthy cells cultured in the presence of serum (86).

Impaired anaplerosis and cataplerosis. The anaplerosis/cataplerosis pathways provide metabolic coupling factors important for insulin secretion, and the activity of these pathways is increased in healthy compensating islets. Evidence indicates that disruption of this pathway causes loss of GSIS (Figure 3). For example, inhibition of PC (the first step in anaplerosis from glucose-derived pyruvate) by phenylacetic acid inhibited GSIS in both ZL and ZF islets (12). Furthermore, exposure of MIN-6 cells to palmitate caused reduction in PC activity and was associated with impaired GSIS (87). In a similar study, exposure of another insulinoma cell line, INS 832/13, to elevated FFAs (oleate and palmitate) caused impairment in pyruvate cycling as measured by ^{13}C NMR (88). In the latter study, provision of a membrane-permeant ester of malate (used to reestablish anaplerosis) to FFA-treated INS 832/13 cells or lipid-laden ZDF islets caused at least partial recovery of GSIS (88). At variance from the cell line studies, exposure of rat islets in vitro to elevated oleate caused increased pyruvate cycling and enhanced basal insulin release and GSIS (89). It may be that saturated FFAs (such as palmitate) are inhibitory, whereas monounsaturated FFAs like oleate activate the anaplerosis/cataplerosis pathways. Although the role for defects in β cell anaplerosis/cataplerosis in human T2D is unknown, there is defective expression of PC in 2 diabetic rodent models, Goto-Kakizaki and ZDF rats (90, 91). Furthermore, improved GSIS following insulin treatment in the Goto-Kakizaki rat was paralleled by recovery of β cell PC expression (91).

Dysregulation of TG/FFA cycling and lipolysis. The islet β cell TG/FFA cycle provides a lipid signaling pathway by which the glucose oxidation K^+_{ATP} channel pathway of nutrient-secretion coupling can be amplified (35). In compensating islets this not only allows for greater insulin secretion without a need for very high rates of glucose oxidation, but also provides a mechanism by which ATP produced by glucose oxidation can be consumed via a "futile" cycle that transforms excessive energy from fuels (FFAs and glucose) into heat. The ATP consumption occurs in the acyl-CoA synthase step of the cycle, in which FFA is activated to LC-CoA (92). Thus, TG/FFA cycling could potentially protect the β cell from fuel surfeit and excessive increases in mitochondrial membrane potential and ROS production. In support for a role of dysfunctional TG/FFA cycling in β cell failure are studies in which enzymes of the cycle are disrupted. Pharmacological inhibition of the lipolysis arm is known to inhibit GSIS (93, S58), whereas overexpression of hormone-sensitive lipase (HSL), which prevents accumulation of TGs, also impairs secretion (94). We showed impaired GSIS in fasted male HSL-knockout mice, and this was reversed by provision of exogenous FFAs (95). One study showed that a higher capacity of islets to accumulate TGs is associated with reduced islet FFA-induced cytotoxicity (96). Further, MIN-6 cells that express a high level of stearoyl-CoA desaturase 1 (SCD1) (an enzyme that desaturates FAs and favors TG synthesis) are resistant to lipid-induced toxicity compared with low expressers (97). TG/FFA cycling, particularly in response to monounsaturated FFAs, may also divert FFA metabolism away from toxic lipids such as ceramide (98, S28, S59). To summarize, while TG/FFA cycling in the β cell has only recently been appreciated, it has potentially important roles in β cell compensation processes and in the protection of islets from damage during compensation. Disruption of this cycle could contribute to β cell failure in T2D (Figure 3) such that this new aspect of islet biology warrants further investigation.

Altered AMPK/malonyl-CoA signaling and FAO. It is important to note that both ZF rat islets (which have high rates of TG/FFA cycling; ref. 35) and FFA-resistant MIN-6 cells (which have high expression of SCD1; ref. 97) maintain normal or elevated FAO and do not develop lipoapoptosis. Oxidative clearance of FFAs is likely to a be a major factor in preventing steatosis in the islet, as it is in other tissues such as liver (99). The AMPK/malonyl-CoA signaling network has a major role in regulating FAO (50, S60). AMPK senses cellular energy status and is activated by an increase in the AMP/ATP ratio brought on by fasting or exercise. AMPK activates cellular energy production (e.g., glucose oxidation and FAO) and reduces energy consumption (e.g., FA synthesis and esterification) (50, S60). Malonyl-CoA, on the other hand, promotes nutrient storage (4, 29, 50). Importantly, AMPK phosphorylates acetyl-CoA carboxylase and malonyl-CoA decarboxylase, the enzymes that regulate malonyl-CoA synthesis and degradation, respectively, with the effect of lowering malonyl-CoA (50, S60). In addition to conditions of food deprivation and exercise, AMPK can be activated by the adipokines, including adiponectin (100, S61) and leptin (101), and pharmacological agents such as metformin and the thiazolidinediones (101, 102). Chronically reduced activity of β cell AMPK and increased malonyl-CoA levels, due to consistent overnutrition and reduced physical activity, together with the hypoadiponectinemia and leptin resistance of the metabolic syndrome, will result in downregulation of β cell FAO pathways and therefore place β cells at risk of lipoapoptosis (50). Altered AMPK/malonyl-CoA signaling underpins the mechanism of glucolipotoxicity (50), as discussed below.

Lipotoxicity and/or lipoadaptation. There is much written about lipotoxicity and the β cell; however, in our experience elevated lipids appear to be relatively benign to β cells, provided that they are not dramatically elevated in vitro and that glucose is not simultaneously elevated. In fact, the emerging evidence indicates that elevated FFAs and hyperlipidemia are major signals that permit β cell adaptation to insulin resistance, as exemplified by the obese ZF rat (35). In our view, β cell lipotoxicity studies in vitro and in vivo have investigated either the adaptive process of the β cell to FFAs or glucolipotoxicity as described in the next section. In other words, β cell

studies made with reasonable concentrations of FFAs (both in vivo and in vitro), in the absence of hyperglycemia, have investigated what may be termed "lipoadaptation" rather than "lipotoxicity." It is certainly possible that islets may be more susceptible to lipotoxic damage at normal glucose levels if the islets have preexisting defects in FA detoxification processes, such as disorders in FAO. Acquired defects in islet FAO, due for example to reduced adiponectin levels associated with central adiposity (S62), could also lower the hyperglycemic threshold at which lipotoxicity occurs.

Glucolipotoxicity. According to the glucolipotoxicity hypothesis, toxic actions of FFAs on tissues will become apparent in the context of hyperglycemia (4, 30). This derives from the fact that elevated glucose, via the AMPK/malonyl-CoA signaling network, curtails fat oxidation and consequently the detoxification of fat, while at the same time promoting partitioning of FFAs into complex lipids, some of which are cytotoxic (4, 30). In support of this hypothesis, we demonstrated a marked synergistic effect of high glucose and saturated FFAs in inducing apoptosis in both rat INS 832/13 and human islet β cells (103). Clearly, cell death was markedly increased in the presence of elevated glucose and FFAs, thereby confirming the occurrence of islet cell glucolipotoxicity. In order to show that AMPK/malonyl-CoA signaling was involved in the mechanism, we studied glucolipotoxicity in INS 832/13 β cells cultured with and without the pharmacological agents AICAR and metformin (activators of AMPK that promote FAO), triacsin C (inhibitor of the activation of FFAs to LC-CoA such that FAO and esterification processes are inhibited), and etomoxir (inhibitor of carnitine palmitoyltransferase–1 activity such that FAO alone is inhibited) (103). AICAR, metformin, and triacsin C all reduced glucolipotoxicity, whereas etomoxir increased it (103). This hypothesis is also consistent with the finding that hyperglycemic ZDF rats develop steatosis, whereas normoglycemic ZF rats are resistant to steatosis. It is also consistent with another study in ZDF rats that showed that antecedent hyperglycemia, not hyperlipidemia, led to increased islet TG content and reduced insulin gene expression (46). Also supportive is a study of subjects with elevated FFAs and/or IGT (104). Deterioration in acute insulin response over time was greatest in the subjects that had both elevated FFAs and IGT (104).

While it seems likely that glucolipotoxicity is involved in progressive β cell damage once hyperglycemia is established (Figure 3), does it have a role in early β cell damage? How high do glucose concentrations need to be for this mechanism to be activated? Considering that the key determinant in this mechanism is the status of the AMPK/malonyl-CoA network and its effect on partitioning of lipids, it could be envisaged that overnutrition with inactivity, together with hypoadiponectinemia and elevated FFAs related to visceral obesity, would place an individual at high risk of glucolipotoxicity (50). In such circumstances only minor degrees of postprandial hyperglycemia may be needed to tip the balance toward β cell damage. The emerging evidence indicates that exaggerated postprandial glucose excursions in IGT subjects is a predictive factor of diabetes and cardiovascular disease (105). Thus, the Study to Prevent NIDDM (STOP-NIDDM) trial has shown that acarbose treatment of IGT subjects to reduce elevated postprandial glucose levels decreased the risk of progression to diabetes by 36% (106).

In our in vitro studies of glucolipotoxicity, saturated FAs were cytotoxic, whereas monounsaturated FAs (oleate) were protective (103). This is certainly not unique to our work on β cells (98, S59) or to this cell type (107, S63). In considering why oleate is protective, it is of interest that monounsaturated FAs are more readily partitioned into TGs (107) and may therefore more effectively promote TG/FFA cycling. Furthermore, unlike saturated FAs, monounsaturated FAs do not cause accumulation of ceramides (98) or deplete mitochondrial cardiolipin levels (107), processes that have both been linked to cytotoxicity. β Cell lipid overload could potentially cause β cell apoptosis by suppressing the antiapoptotic factor Bcl-2 (108, 109).

Islet β cell exhaustion and ER stress. The compensating islet β cell places a high demand on the ER for the synthesis of proinsulin. The β cell exhaustion theory views an imbalance in the relationship between insulin secretion and production that results in depletion of releasable insulin to be a significant component of β cell failure in T2D (6, 54, S64, S65) (Figure 3). This is supported by the findings that resting of the β cell by somatostatin (110) or diazoxide (111) results in recovery of function. Additive to this is a defect in proinsulin synthesis, with ER stress as the potential mechanism (112, S64, S66). The unfolded protein response in the ER is protective in the early stages but can initiate cell death by apoptosis if severe (112, S64, S66). In support of a role of ER stress in T2D, it has been reported that the Akita mouse has a folding mutation in proinsulin that activates the ER stress response, resulting in diabetes with loss of β cell mass (113). In addition, chronic exposure of INS1 cells to palmitate causes lipid deposition in the ER and an ER stress response (114). It is certainly possible that excessive demands on ER — particularly if β cell mass is suboptimal or if there are inherited defects in the proinsulin gene, as in the Akita mouse — could cause β cell damage by this mechanism.

Mechanisms with progression roles in islet β cell damage in T2D

Glucotoxicity, O-glycosylation, glycation stress, islet inflammation, and amyloid deposition. Reversal of severe hyperglycemia in T2D subjects results in at least partial recovery of β cell function (115, S67). Similar observations have been made in animal studies (116). Studies have shown multiple pathways and mechanisms to be involved in glucotoxic damage (84). These include increased oxidative stress (84, S68), increased glucosamine pathway activity (84), increased ER stress (S64), accelerated glucolipotoxicity (4, 45), glycation stress (84, S68), activation of inflammatory pathways (55), and toxic accumulation of islet amyloid polypeptide (IAPP) (117). The end result is β cell de-differentiation and death by apoptosis (118) (Figure 3).

The glucosamine (or hexosamine) pathway is responsible for O-linked glycosylation of proteins important for normal function. Hyperglycemia may increase flux through this pathway, such that this pathway has also been implicated in β cell glucotoxicity (84). In support of such a role, islet adenoviral overexpression of glutamine:fructose amidotransferase, the rate-limiting enzyme of the glucosamine pathway, caused impaired GSIS in rats (119).

Chronic hyperglycemia can result in a browning reaction between glucose and the free amino acids on proteins and other large biological molecules resulting in the formation of advanced glycation end products (AGEs) (84, 120). AGEs are known to cause tissue damage through activation of inflammatory mediators such as IL-1β, IL-6, and TNF-α (121). AGEs have been implicated in diabetes complications (S69), but their role in islet β cell damage has not been established.

There is evidence that non–immune-mediated inflammatory pathways of β cell damage occur in vitro in human islets upon hyperglycemia. Thus, chronic elevated glucose has been documented as inducing IL-1β production and expression of Fas receptor in

human islets in association with cell death (53, 55). This, however, is not always confirmed in similar studies (S70). It remains to be evaluated in vivo whether nonimmune islet inflammation contributes to the progressive β cell demise.

Cytotoxicity from accumulation of IAPP may also be a factor in human T2D — some people consider T2D to be a form of islet Alzheimer disease. IAPP, the major component of islet amyloid, is co-secreted with insulin from β cells (117). In T2D this peptide aggregates to form intracellular microfibrils that can be toxic to islet tissue. The mechanism(s) responsible for islet amyloid formation in T2D are unclear. Enhanced secretion of IAPP, per se, is not sufficient. Other factors, such as impairment in the processing of proIAPP, have been proposed (122). Because amyloid deposits obtained from human specimens do not always correlate with the severity of diabetes (8, 123) and transgenic animal models with human IAPP have yielded inconsistent results (124), the role of IAPP in β cell dysfunction and demise remains to be established.

Concluding remarks

Islet β cell failure in T2D occurs when islets are unable to sustain β cell compensation for insulin resistance. The failure is progressive, particularly after hyperglycemia is established, which leads to poorly functioning, de-differentiated β cells and loss of β cell mass from apoptosis (Figure 3). Research effort needs to focus on the factors that make islets susceptible to dysfunction and failure, particularly those that are acquired in early life, as these may be preventable. Continued effort should also be directed toward the mechanisms that initiate islet damage, as it will be intervention at this stage of the disease that will prevent T2D from developing in subjects at risk. The roles of disordered mitochondrial function, TG/FFA cycling, AMPK/malonyl-CoA signaling, ER stress, and compensatory β cell growth all deserve attention. Pharmacological developments need to be directed at reversing β cell demise. GLP-1 receptor agonists and dipeptidyl peptidase–4 (DDP-4) inhibitors (16) and thiazolidinediones (125) show the most promise at present of being disease modifying rather than simply directed at normalization of hyperglycemia. Finally, the major cause of β cell failure in T2D continues to be overnutrition and lack of exercise.

Acknowledgments

We wish to thank Vern Vui (May) Pang for assistance in preparation of the figures and Jack Leahy for critical review of the manuscript. This work was supported by grants from Genome Quebec, Canada, the NIH (DK-63356), the Canadian Institute of Health Research, and the Private Practice Fund of The Canberra Hospital, Australian Capital Territory, Australia.

Note: References S1–S70 are available online with this article; doi:10.1172/JCI29103DS1.

Address correspondence to: Christopher J. Nolan, Department of Endocrinology and Diabetes, The Canberra Hospital, PO Box 11, Woden, ACT 2606, Australia. Phone: 61-2-6244-4224; Fax: 61-2-6282-5179; E-mail: christopher.nolan@anu.edu.au.

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