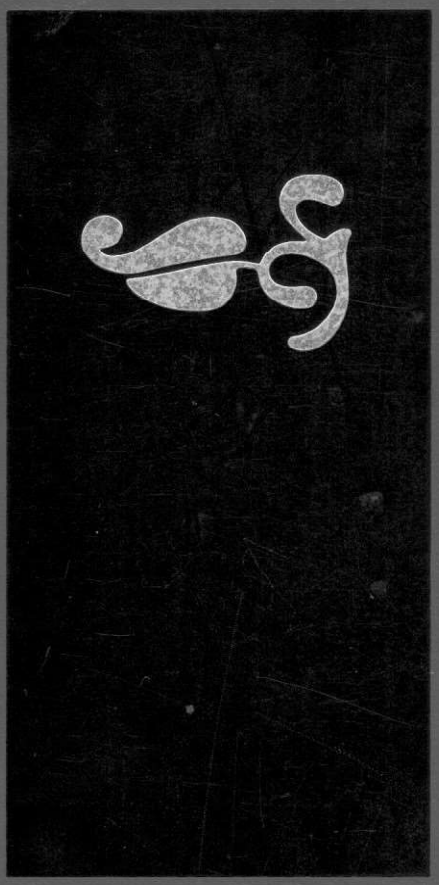

Diabetes Mellitus

A FUNDAMENTAL AND CLINICAL TEXT

3RD EDITION

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60

INSULIN SECRETION IN TYPE 2 DIABETES MELLITUS

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There is now general agreement about the essential contributions of both insulin resistance and β-cell failure to the pathogenesis of type 2 diabetes mellitus (DM). The definition of type 2 DM is phenotypic, which helps little in categorizing the different varieties of this heterogeneous syndrome, but it is hoped that genetic markers someday will lead to a more rigorous classification. A common pathogenic pathway appears to exist for most people with type 2 DM (Fig. 60.1). Genes play a major role, with those exerting control over insulin action, obesity, and the regulation of β-cell mass and function presumably being especially important. Environment also has a major impact, with the plentiful food and inactivity of affluent societies having a detrimental influence. Although definition of insulin resistance is somewhat arbitrary, the vast majority of individuals destined to develop type 2 DM are resistant. Nonetheless, most individuals with insulin resistance never progress to DM, although it could be argued that they would if they lived long enough. Only those whose β-cells fail to compensate for this insulin resistance develop type 2 DM. Therefore, even though hyperinsulinemia is almost always found in the prediabetic period, either absolute or relative insulin deficiency is always present when type 2 DM finally appears. Moreover, as shown by the United Kingdom Prospective Diabetes Study (UKPDS) and the Belfast diet intervention study (1,2), β-cell function progressively declines while the degree of insulin resistance changes little. Type 2 DM certainly has a heterogeneous etiology, yet the characteristics of insulin secretion found in this syndrome greatly resemble the secretory abnormalities found in early type 1 DM and with failing pancreas or islet transplants.

HETEROGENEITY WITHIN TYPE 2 DIABETES MELLITUS: AUTOIMMUNITY, MATURITY-ONSET DIABETES OF THE YOUNG, INSULIN MUTATIONS, AND MITOCHONDRIAL DNA MUTATIONS

Although most individuals with type 2 DM seem to have a similar phenotype, it has been fruitful to search for the variants within this heterogeneous syndrome. For example, in some populations, many patients diagnosed with type 2 DM have a syndrome called latent autoimmune diabetes in adults (LADA) (3). Autoimmune damage to β-cells can occur at any age and may be a slow-moving, incomplete process that can even become quiescent at some point. Thus, a self-limited asymptomatic autoimmune process occurring in childhood might somehow contribute to the development of type 2 DM later in life. It is often forgotten that overweight individuals are just as likely to develop autoimmune diseases as thin people. In Scandinavia, over 10% of subjects with type 2 DM have antibodies against β-cells. Because immune markers often disappear with time, more than 10%, perhaps even 20% or more, of patients with type 2 DM in populations in which autoimmune disease is common, such as in Northern Europe, may have some contribution from immune destruction. Insulin secretion in individual LADA subjects is virtually impossible to distinguish from that in typical type 2 DM.

Several mutations of the proinsulin gene have been described, including insulin Chicago (Phe-B25-Leu), insulin Los Angeles (Phe-B24-Ser), and insulin Wakayama (Val-A3-Leu) (3,4). These mutations obliterate biologic activity almost completely. Only heterozygotes have been found, and some do not even have diabetes, providing insight into the capacity of only one normal allele to provide sufficient insulin for decades. Other mutations lead to hyperproinsulinemia, which may or may not be associated with type 2 DM (4,5); these include Arg-C65-His and His-B10-Asp.

Maturity-onset diabetes of the young (MODY) is a heterogeneous condition that has become much better understood in the past few years, as the genetic etiology of about 60% of the cases has been identified (6). MODY, which appears to account for less than 5% of subjects with type 2 DM, is not the ideal name for these syndromes because most cases are now being found in adulthood. MODY-1 is caused by mutations of hepatocyte nuclear factor (HNF)-4α. MODY-2, the most common, is caused by mutations of the glucokinase genes. Glucokinase is known to play a key role in regulating the rate of insulin secretion, and a wide variety of mutations already have been defined (7–9). These subjects have mild diabetes that usually does not require insulin treatment. They have been found to have an altered set point for glucose-stimulated insulin secretion (GSIS), as is predicted by a reduction of glucokinase activity (9). Glu-

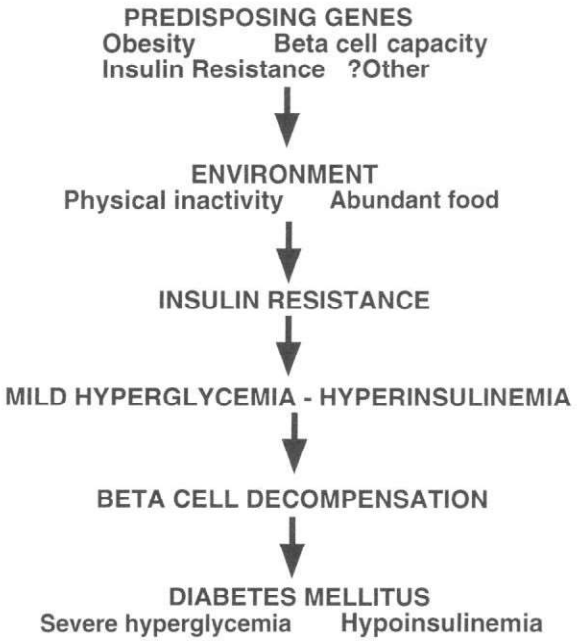

Figure 60.1. Sequence of events in the pathogenesis of type 2 diabetes mellitus.

cokinase is a critical enzyme for glucose handling by both β-cells and hepatocytes (10), but the relative contributions of these two tissues to the hyperglycemia of MODY have not yet been completely clarified (11). MODY-3 is caused by mutations of HNF-1α, MODY-5 by mutations of HNF-1β, and MODY-4 by mutations of PDX-1 (also known as IDX-1, STF-1, and IPF-1). The most recently discovered mutation is MODY-6, this being Neuro D, which, like PDX-1, is important for insulin gene expression and β-cell development (12).

Mutations of mitochondrial DNA can lead to the phenotype of both type 1 and 2 DM (13). Mitochondrial DNA contains 16,569 base pairs, which code for 37 genes, including those for 13 enzymes involved in oxidative phosphorylation. The mutation linked with DM is a substitution of guanidine for adenine at position 3243 of leucine transfer RNA (tRNA), which leads to problems with the synthesis of mitochondrial proteins. These mutations are passed on by maternal transmission and cause problems other than DM, including sensory hearing loss and the mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like symptoms (MELAS) syndrome. This form of DM, which is associated with insulin deficiency and not with insulin resistance, is rare and usually diagnosed in the second or third decade of life. Oxidative metabolism of glucose is required for insulin secretion, so it is not surprising that such a mutation would cause problems.

β-CELL SET POINT AND COMPENSATION FOR INSULIN RESISTANCE

β-cells are remarkably efficient at keeping glucose levels within a very narrow range, and yet the set point for GSIS—the dose-response relationship between glucose levels and the rates of insulin secretion—can vary considerably. As discussed, mutatio in glucokinase usually raise the set point, which typically lea to mildly elevated glucose levels. Recently, however, a glucok nase mutation was found that reduces β-cell set point, resulti in problematic hypoglycemia (14). An interesting puzzle co cerns subjects who are insulin resistant because of obesity a have far higher plasma insulin levels than normal weight co trol subjects, yet they have virtually identical glucose leve even after an oral glucose challenge (15). Concerns abo whether high insulin levels really reflect increased secreti rates have been put to rest with studies using sophisticated co volution techniques (15). So, if glucose levels are not elevat with insulin resistance, how can glucose be the signal to the cell? The answer seems to lie in a shift of the dose-respon curve that allows more insulin to be secreted at any given gl cose level. Thus, these compensating β-cells respond as if th were being exposed to higher glucose levels. This elegant mec anism for β-cell adaptation allows normal glucose levels to maintained in the face of the progressive insulin resistance th accompanies the modern Western lifestyle. The molecular ba for this change is not completely understood but could be d to activation of glucokinase (16).

To appreciate the role of insulin secretion in the pathogen sis of type 2 DM, it is necessary to understand that insulin resi tance, most often seen in the form of obesity, causes increas insulin secretion through increased demand. In fact, circulati insulin levels are actually an excellent indicator of the degree insulin resistance, with there being an excellent correlation b tween first-phase GSIS and insulin resistance (17,18). As i sulin resistance develops, glucose levels are kept normal by cell compensation, which is probably largely due to an increa in β-cell mass.

HYPOTHESIS: GLUCOSE-STIMULATED INSULIN SECRETION IN INDIVIDUALS WITH "NORMAL" GLUCOSE LEVELS HAS LITTLE PREDICTIVE VALUE FOR THE DEVELOPMENT OF DIABETES

Defining "normal" glucose levels is a major issue because must be recognized that important changes must occur as fa ing plasma glucose levels increase from 85 mg/dL (4.8 m*M*) 105 mg/dL (5.9 m*M*) in an individual who may or may not on their way to type 2 DM. To frame the argument, we take t position that 85 mg/dL is "normal" and 105 mg/dL is abno mal, but not yet at the level of impaired glucose toleran (IGT). Our contention is that study subjects are often call "normal" when they are not, which creates problems with inte pretation. In rodents an increase of only 20 mg/dL creat enough glucotoxicity to change β-cell function (19–2 Marked changes in β-cell function within such a narrow wi dow of glycemia also occur in humans. An important study adults with variable fasting glucose levels indicates that GSIS abolished once a level of 115 mg/dL is reached and that so impairment can occur at lower levels such as 100 mg/dL (2 This is in agreement with the findings of reduced insulin sponses to intravenous glucose tolerance tests (IVGTTs) in su

jects with pre–type 1 DM who have glucose levels that are "normal" at about 95 to 110 mg/dL (23) but probably higher than the 80 to 90 mg/dL they might have had before the autoimmune process began.

To take the argument further, consider the predictive value of GSIS for two individuals with truly normal glucose levels. A thin, physically trained individual with genetic insulin sensitivity and a fasting glucose level of 85 mg/dL will be expected to have a very low insulin response to a glucose challenge. Yet in spite of this low acute GSIS response, such an individual could have a secretory capacity that could cope with severe insulin resistance for a lifetime. In this situation the acute insulin response probably has no predictive value. In contrast, a young sedentary person with genetic insulin resistance who also has a fasting glucose level of 85 mg/dL, is expected to have a high secretory response to glucose, but this response should also provide little insight into whether β-cell function will deteriorate 20 years later.

The main problem in type 2 DM is that at some point, after many years of coping with insulin resistance, β-cells fail to produce enough insulin. We suspect that the production of insulin is largely dependent on β-cell mass, and that failure to maintain adequate β-cell mass is the crux of the problem in type 2 DM (Fig. 60.2). The as yet unidentified genes responsible for β-cell birth and death seem likely to play key roles. If the major problem is with growth capacity, then the failure to find useful information from secretory studies done in the stage of pre–type 2 DM is hardly surprising. In fact, it might even be expected that in subjects with pre–type 2 DM, perfectly normal plasma glucose levels would be associated with normal insulin responses to an array of secretory challenges. One exception to this rule is individuals with MODY-2, who have glucokinase mutations that give them an abnormal set point for GSIS. Another may be people with a genetic variant of the sulfonylurea receptor 1, who have decreased secretory responses to glucose and may be at increased risk for developing type 2 diabetes (24,25).

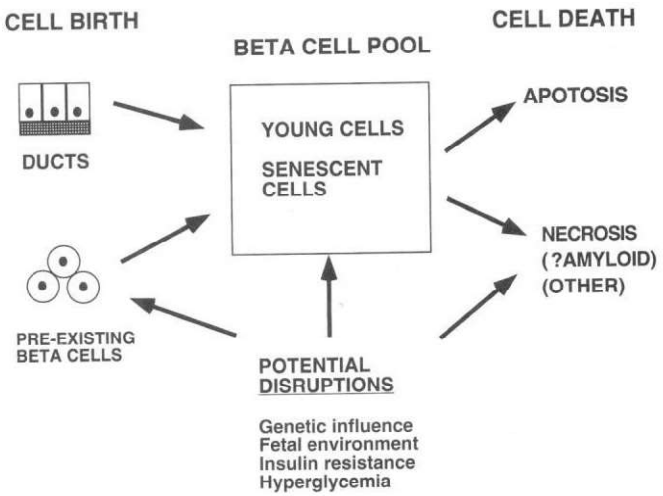

Figure 60.2. Regulation of β-cell mass and function. Sites of potential disruption associated with diabetes.

DIFFICULT TO INTERPRET STUDIES OF INSULIN SECRETION IN PREDIABETES

By the time that even the state of IGT is diagnosed, the critical events that led to the problem are blurred. If good-enough tools were available, we might be able to understand why a fasting plasma glucose value of 85 mg/dL deteriorates to 105 mg/dL, why there is further progression to the state of IGT, and why only some people go on to develop type 2 DM. Valuable studies have focused on people at risk for developing type 2 DM, such as the offspring of two parents with type 2 DM (26,27), Pima Indians (28), or first-degree relatives of patients with type 2 DM (29). In these and other studies, insulin resistance has been shown unequivocally to be a major risk factor. Conversely, those who were insulin sensitive had a very low risk for developing DM (26).

Although an impairment of insulin secretion to an IVGTT was not found in one offspring study (26), studies of Pima Indians (17,28) and several other prospective studies (30,31) have shown that impaired β-cell secretory responses to glucose were predictive of subsequent DM. Considering that some pathologic increase in glucose levels occur even before subjects can be officially diagnosed with IGT, one must be cautious about claims that abnormal secretion has ever been demonstrated in prediabetes when patients have unequivocally normal glucose levels in the fasting and post-nutrient state. There are, however, some studies that seem to challenge this contention. In an evaluation of the offspring of parents with early-onset diabetes, in one group of Pima Indians whose mothers developed diabetes after pregnancy, fasting glucose levels were only 85 mg/dL, but they had lower GSIS than matched controls (32). It is not clear that these eight subjects would also have developed type 2 DM early in life, but this study does raise questions as to whether abnormal acute GSIS in the "normal" state could in some situations be used to predict type 2 DM. Another challenging study was performed in 100 first-degree relatives of individuals with type 2 DM (33), but it is not clear if ideal controls were used.

For decades there has been a search for the first demonstrable change that predicts type 2 DM. The most convincing observed early change is insulin resistance, yet the genes that determine failure of β-cell compensation are present at birth, and key environmental influences probably occur at a very early stage. There is still much to learn about the earliest lesions that lead to type 2 DM.

β-CELL FUNCTION IN ESTABLISHED TYPE 2 DIABETES MELLITUS

Hyperinsulinemia and insulin resistance are usually found before the development of IGT and type 2 DM. In prospective studies, high fasting insulin levels have been found to be a risk factor for the development of type 2 DM (31), but these insulin levels fall as DM develops. For any level of insulin resistance,

subjects with type 2 DM will secrete less insulin than those without DM. Thus, obese individuals with type 2 DM can be expected to have higher plasma insulin levels than nondiabetic subjects of normal weight, but they will be found to be insulin deficient when matched with obese nondiabetic controls (34,35).

Insulin responses to an oral glucose tolerance test (OGTT) in mild type 2 DM are often relatively high, being thought of as representing increased insulin secretion, but the interpretation of this finding is complex. It is clear that insulin responses at 60 to 120 minutes may be higher than those of normal controls, a finding caused not only by insulin resistance, but also by the higher glucose levels seen at these time points. However, the early insulin responses at 30 minutes are typically lower in type 2 DM subjects, which leads to inefficient suppression of hepatic glucose output that partially accounts for the higher glucose levels found later in the OGTT (36). This impairment in the inhibition of hepatic glucose output is further contributed to by a less marked suppression of glucagon during this early time period. To compare insulin secretory capacity between individuals, both the degree of insulin resistance and the glucose level used for the challenge must be considered. This loss of early insulin secretion has been shown in various other studies to be an important contributor to the glucose intolerance found after the ingestion of oral carbohydrate (18,37), but to develop full-blown type 2 DM, more than a simple loss of early insulin release is required. Glucose levels can still be kept in a reasonable range because β-cells can respond to meals through signals from gut peptides, nutrients such as amino acids, and possibly through the parasympathetic nervous system. Even though "early" first-phase insulin secretion to glucose is gone, some later second-phase insulin secretion, which provides additional help, remains. In summary, the magnitude of GSIS in subjects with "normal" glucose levels vary greatly depending on the degree of insulin resistance, but GSIS responses will start to decline as glucose levels begin to climb toward the state of IGT.

Insulin responses to an intravenous glucose challenge are profoundly abnormal in diabetes. First-phase insulin secretion, that seen over a period of about 8 minutes, is absent, and in some subjects a paradoxic fall in plasma insulin concentration is found (22,38–41). A second phase of insulin release can be seen in all but the most severe cases of type 2 DM, but when glucose levels and the degree of insulin resistance are taken into account, these second-phase responses are deficient. The loss of first-phase GSIS is specific for glucose because β-cells can respond to acute challenges by other secretagogues such as arginine, isoproterenol, secretin, and tolbutamide. It is interesting that the insulin responses to these agents are usually of the same magnitude as those seen in subjects without diabetes. However, when glucose levels are experimentally raised in nondiabetic subjects, their insulin responses to arginine and isoproterenol greatly exceed those of comparable individuals with diabetes (42). Careful study of insulin secretory capacity suggests the presence of severe impairment in the diabetic state, even when β-cell mass is taken into account. For example, postmortem studies indicate that β-cell mass in typical type 2 DM is about 50% of normal (43,44), yet insulin response to maximal stimulation by a combination of arginine and glucose is only about 15% of that found in control subjects (42). Therefore, type 2 DM is characterized by both reduced β-cell mass and inefficient secretion from whatever β-cells remain.

PULSATILE INSULIN SECRETION

Insulin secretion is pulsatile with a periodicity of about 10 to 13 minutes, and these fluctuations are synchronized with oscillation in plasma glucose levels (45). In addition, less frequent large-amplitude pulses of insulin secretion have been described, occurring 10 to 15 times per day, with greater frequency after meals (46). The mechanisms responsible for the rapid oscillations are not known, but they are found with insulin secretion from the isolated perfused canine pancreas when glucose levels are held constant (47). It seems likely that there is a pancreatic neural network providing functional linkage between islets. The mechanisms responsible for secretory oscillations in individual β-cells is not fully understood, but interplay between calcium and glycolytic oscillations appears to be important (48,49). Both the short- and long-term pulsations are disrupted in type 2 DM, and even in the less severe state of IGT (50,51). It remains to be seen, however, if abnormalities can be found before IGT develops and if such abnormalities will turn out to have any predictive value for the development of type 2 DM. There are also interesting questions about the effect of these pulsations upon insulin action. When insulin is administered in a pulsatile fashion, hepatic glucose output is more efficiently suppressed than when delivery is continuous (52). Because the variations of insulin (and glucagon) concentration in the portal vein are substantial (53), these fluctuations may exert an important influence on hepatic metabolism. The oscillations of insulin levels in arterial plasma are more modest, and there must be further dampening when insulin leaves the vasculature to reach muscle or fat cells. Thus, the influence of pulsatile insulin secretion upon peripheral metabolism may be inconsequential.

CIRCULATING PROINSULIN-LIKE PEPTIDES

Although the cleavage of proinsulin to insulin and C peptide is very efficient, about 2% to 4% of the secreted insulin immunoreactivity consists of proinsulin and proinsulin intermediates. Because the clearance of these peptides is so much lower than insulin, they account for 10% to 40% of circulating immunoreactivity. About one-third of this is accounted for by intact proinsulin and two-thirds by des 32–33 split proinsulin; only small amounts of des 65–66 split proinsulin are detectable (54). For years it has been known that the ratio of circulating proinsulin-related peptides to insulin is increased in type 2 DM (32,55). Several studies have found this ratio to be positively correlated with the severity of hyperglycemia (56). In the state of IGT, modest elevations in the ratio have been found in some population groups (57). Failure to appreciate the contribution from the proinsulin-related peptides has led to overestimation of insulin levels in type 2 DM, but this is less of an issue in IGT, where hyperinsulinemia is most severe. Because these peptides have less than 5% of the biologic activity of insulin, their contribution to glucose homeostasis is probably minimal.

There has been interest in whether disproportionately elevated proinsulin levels could be useful markers for progression to diabetes in high-risk groups, but the changes are probably not consistent enough to be useful. The mechanisms responsible for the abnormal ratios have not been defined, but in hyperglycemic rodents the ratio of proinsulin-related peptides to insulin secreted directly from β-cells is increased (58). Although complex alterations in the cleavage events of β-cells may be taking place, it may be simply that increased secretory demand from hyperglycemia leads to depletion of mature granules and release of the contents of the available immature granules in which conversion is incomplete (55).

β-CELL MASS AND ISLET PATHOLOGY IN TYPE 2 DIABETES

β-Cell Mass

β-cell mass is tightly regulated, being maintained at about 1% of the weight of the pancreas in adults. Several large autopsy studies indicate that β-cell mass in type 2 DM is about 50% of normal (59–61). These earlier findings have now been confirmed with more comprehensive methodology (43,44). It is surprising that 50% would not be enough to maintain normoglycemia, but, as discussed earlier, these cells do not seem capable of secreting as much insulin as would be expected from that volume.

Regulation of β-cell mass is a more dynamic process than generally realized (Fig. 60.2). It has not been possible to learn much about the replicative capacity of β-cells in humans, but much work has been carried out in rodents (62). The turnover of β-cells in adult humans is certainly slower than in rodents, but the general mechanisms for regulating β-cell mass should be similar. There is evidence for new islet formation from pancreatic ducts (neogenesis) during the autoimmune destruction period of type 1 DM, in subjects with obesity, in patients with liver disease, and even in individuals with established type 2 DM. Moreover, β-cell mass is increased in the insulin-resistant state of obesity. In adult rats there is a basal β-cell birth rate of about 2% to 3% per day, which can increase fivefold or more after partial pancreatectomy or during intravenous infusion of glucose. Not only can β-cell number increase (hyperplasia), but cell size can expand (hypertrophy) when confronted by chronic glucose stimulation (63). New β-cells are derived from two sources: from replication of existing β-cells and from differentiation of putative precursor cells found in pancreatic ducts. It has been estimated in rodents that the entire β-cell mass can turn over in about 6 weeks (64). Moreover, if β-cell mass were stable, the birth rate must be equaled by the death rate, which is thought to occur mainly through apoptosis. The birth rate of human β-cells from preexisting β-cells has been estimated to be only about 0.7% per day (65), but because these studies were in a mouse transplantation situation in which glucose levels were high compared with humans, the actual number is probably lower. The birth rate of β-cells from neogenesis is unknown, but if it were equal to the birth rate from β-cells, the combined birth rate from the two sources could be about 1%. If β-cell number is to be kept stable, the death rate must equal the birth rate, which means that if there are 2 billion β-cells in the pancreas of an obese individual, a loss of 20 million β-cells per day could be countered by a similar birth rate. Like other cell types, β-cells must have the capacity for only a limited number of divisions, which may be an important issue in the pathogenesis of type 2 DM. There must be genetic determinants of this capacity for replication and how it might be influenced by hyperglycemia or the aging process. Moreover, a variety of factors must influence the rate of β-cell death. Little is known about β-cell death in humans, but there are interesting rodent and gerbil models exhibiting accelerated β-cell apoptosis and β-cell dysfunction associated with hyperglycemia (66–68). It is highly likely that β-cell mass varies during adult life in humans, expanding to meet the demands of Western lifestyle and contracting with food restriction and regular exercise. This concept is supported by many studies in rodents, and a Korean autopsy study in humans showing a linear correlation between body mass index and β-cell mass (44). Many nongenetic factors could adversely influence the capacity of β-cells to maintain euglycemia for many decades, with some of the possibilities including a self-limited bout of autoimmune destruction, a period of obesity, exposure to β-cell toxins, a viral infection, or an episode of malnutrition.

Amyloid Deposition in Islets

Amyloid deposits in the islets of people with diabetes were first described in 1900, but only in 1986 was the peptide that forms this amorphous material identified (69,70). Islet-amyloid polypeptide (IAPP), also called amylin, consists of 37 amino acids with the sequence between positions 20 and 29 (position 25 being particularly important) determining the ability of this peptide to form amyloid deposits. Because of these structural requirements, amyloid deposits are found in primates and cats but not in many other species. Production of IAPP is restricted to β-cells, and its content is only about 1% of that of insulin, with this ratio being maintained during secretion (71).

Investigators have been searching for a physiologic role for IAPP, with suggestions being made that it exerts various effects upon the central nervous system, gastrointestinal track, and elsewhere, such that it should be injected therapeutically along with insulin (72). The mechanisms responsible for its deposition in some, but not all, of the islets of people with type 2 DM is unknown. It is not usually found in the islets of nondiabetic individuals with the insulin resistance of obesity. Perhaps there are clues in the findings of large amounts of amyloid in some insulinomas and in the islets of a patient with diabetes associated with extreme insulin resistance (73). The question of whether amyloid has a detrimental influence on islet function is important in light of recent studies showing that human IAPP fibrils have a toxic effect upon both rat and human islets (74,75). The amyloid deposits in islets presumably begin as small niduses that then enlarge. The IAPP that comprises these deposits could come from secreted IAPP or pro-IAPP, or from stored IAPP that is left as a sticky residue after apoptotic death of β-cells. Perhaps fibrils attached to patches of amyloid kill only the few adjacent β-cells with which they have direct con-

tact, allowing survival of most of the cells of an islet. Questions have been raised about whether intracellular IAPP accumulation can exert a toxic effect in normal β-cells (76). Such processes occurring slowly over years could contribute to a gradual decline in β-cell mass.

ADVERSE INFLUENCE OF THE DIABETIC MILIEU ON FUNCTION: GLUCOTOXICITY, LIPOTOXICITY, OR BOTH?

Major abnormalities of insulin secretion can be produced by chronic exposure of β-cells to the diabetic milieu (19,38, 77–81). Before 1960, a variety of investigators examined the question as to whether hyperglycemia had an adverse effect upon islet structure, but secretion and molecular changes have only been studied more recently. Hyperglycemia is virtually always associated with a reduction of GSIS. This abnormal secretion has been found in all forms of human diabetes, including type 2 DM, early type 1 DM, and individuals with failing pancreas or islet transplants. Similar abnormalities have been found in primate, dog, rodent, and gerbil models, with the best marker for functional impairment being the loss of acute GSIS. Now this phenomenon is called "glucose toxicity" or "glucotoxicity." More recently the concept of lipotoxicity has emerged, which suggests that elevated levels of circulating nonesterified fatty acids (NEFAs) have a deleterious influence on function (82). Probably the most clinically relevant and best demonstration of functional adversity of the diabetic state in humans comes from the findings of a Japanese study that insulin secretion improved during challenges with meals or oral glucose after hyperglycemia was reduced by diet, sulfonylurea treatment, or insulin administration (83), which is consistent with the findings of others (84). We believe that the major force leading to deranged β-cell function in diabetes is glucotoxicity, and that contributions made by elevations in plasma NEFAs have not yet been shown to be important.

Nonesterified Fatty Acids and β-Cells

Correlating elevated NEFA levels to the loss of acute GSIS in diabetes is far more difficult. Nonetheless, NEFAs are no doubt very important for normal β-cell function acting through as yet uncertain mechanisms (82,85). One hypothesis suggests that increased glucose metabolism in β-cells leads to increases in malonyl CoA levels, which can inhibit fatty acid entry into mitochondria, thus making fatty acids in the cytosol more available for synthesis of lipid mediators (86). The importance of this pathway, however, has been called into question (87). The contribution of fatty acid oxidation to insulin secretion is unclear, but in the presence of low glucose levels, some fatty acid oxidation does take place and may be important. NEFAs have been found to play a crucial role in maintaining insulin secretion during fasting when glucose levels are low, perhaps providing a brake to excessive ketogenesis (85,88,89). Even during the fed state, NEFAs probably work with glucose and other mediators, such as amino acids, neurotransmitters, and gut hormones, to provide optimal β-cell function.

Animal Studies

In rodents, both *in vivo* and *in vitro* studies indicate that NEFA can have a short-term stimulatory effect on insulin secretion (90), but with *in vivo* NEFA infusions for 48 hours or the addition of NEFA to cultured islets, inhibitory effects can be found (85). Inhibition of proinsulin biosynthesis has also been reported (91–93) *in vitro*, but confirmatory *in vivo* changes have not been demonstrated with infusions of glucose into rats (94). Unfortunately, the relative contributions of hyperglycemia and elevated NEFA to the β-cell dysfunction of diabetes have not been worked out. One complexity is that the two may have synergistic or complementary inhibitory influences (90,95). In studies with a rat partial pancreatectomy model, a surgical reduction of β-cell mass, the characteristic selective loss of GSIS can be found even with relatively modest elevations of plasma glucose levels (96,97). These secretory changes are tightly associated with rising glucose concentrations, but elevated NEFA levels were not found in this model (63).

Human Studies

A contribution of NEFAs to the abnormal insulin secretion of diabetes in humans continues to be poorly defined, since it has proved difficult to convincingly correlate the loss of GSIS in IGT and early diabetes to increased NEFA levels. For example, normoglycemic obese subjects have elevated NEFAs and increased GSIS, with there being evidence that the NEFAs in this situation contribute both to hyperinsulinemia and the increased GSIS (98). However, a complete loss of GSIS is found with only modest increases in plasma glucose concentration, levels that do not even meet the criteria for IGT (22). No studies have yet shown that the NEFA levels in this state of mild hyperglycemia with abolished GSIS are higher than those seen in normoglycemic obesity with its increased GSIS.

Thus, until more detailed studies are performed, it must be concluded that the early loss of GSIS in humans is well correlated with rising plasma glucose levels but poorly correlated with NEFA levels. These findings implicate glucotoxicity rather than lipotoxicity in the early loss of GSIS in humans but do not rule out an important role for NEFA as a contributing variable or permissive factor, nor exclude a role for glucose-induced abnormalities in the lipid pathways of β-cells.

HYPOTHESIS THAT ALTERED β-CELL PHENOTYPE LEADS TO ABNORMAL β-CELL FUNCTION IN DIABETES

For β-cells to make and store insulin and then secrete large amounts of insulin in response to glucose and a variety of other signals, evolution has provided unique differentiation, with the expression of various genes being either enhanced or depressed (63,99,100) (Table 60.1) . Some critical characteristics of normal β-cells include the following:

1. Specialized mechanisms for glucose uptake and phosphorylation, which include increased expression of GLUT-2 and glucokinase and suppression of hexokinase.

Unimpeded glycolytic flux so that pyruvate can enter mitochondria without being diverted to lactate via lactate dehydrogenase (LDH). Consistent with this, LDH is minimally expressed in normal β-cells (101).

ncreased expression of special shuttles. The glycerol phosphate shuttle allows reduced nicotinamide dinucleotide NADH) to be oxidized by mitochondria, thereby contributing to adenosine triphosphate (ATP) formation. The oxidation of NADH should also enhance glycolytic flux. In 3-cells the malate/pyruvate shuttle may facilitate the generation of NADPH, which could somehow enhance secretion. The need for such shuttles probably explains why β-cells have very high levels of mitochondrial glycerol phosphate dehydrogenase (mGPDH) and pyruvate carboxylase (102, 03).

Suppression of the gluconeogenic pathway. Gluconeogenesis r glucose recycling could interfere with the maximum efficiency of glycolysis required for insulin secretion (104). This would be accomplished by decreased expression of phosphonolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase, and fructose-1,6-bisphosphatase.

ome of this specialized machinery is altered in the diabetic . A striking abnormality is the profound reduction in the ll glucose transporter (GLUT)-2 found in all rodent models f hyperglycemia (19). Unfortunately, it is not known why JT-2 expression is reduced or whether it leads to a change SIS. Glucokinase plays a critical role in the regulation of ate of insulin secretion (10), and increases in the intrinsic ity of this enzyme are found in hyperglycemic states (16). , there is interest in possible impairment of glucose oxidathat may be linked to reduced activity of the mitochondrlycerol phosphate shuttle (105). Other potential trouble s include increased glucose cycling with conversion of glu-6-phosphate to glucose, which could lead to less efficient olysis (106). Unfortunately, none of these alone or any r single defect (see Table 60.2 for a list of potential defects) likely to fully explain the loss of GSIS.

LE 60.1 UNIQUE β-CELL DIFFERENTIATION

ased expression	Decreased expression
-2	Glucose-6-phosphatase
kinase	Hexokinase
DH	Lactate dehydrogenase
ate carboxylase	PEPCK
n	Fructose-1,6-bisphosphatase
?	C/EBPβ
1?	*c-myc*
5.1	

is a partial list based on references 63, 109, and 116.
; CCAT/enhancer binding protein; GLUT, glucose transporter; IAPP, amyloid polypeptide; mGPDH, mitochondrial glycerol phosphate degenase; PDX-1, transcription factor pancreatic and duodenal homeene 1; PEPCK, phosphoenolpyruvate carboxykinase.

TABLE 60.2 POTENTIALLY IMPORTANT β-CELL DEFECTS IN TYPE 2 DIABETES MELLITUS

GLUT-2 reduction
GLUT-2/glucokinase dysfunction
Glucokinase alteration
Glucose cycling
Glucose-6-phosphatase increase
Phosphofructokinase decrease
Lactate dehydrogenase increase
Mitochondrial glycerol phosphate dehydrogenase decrease
Triglyceride accumulation
Long-chain fatty acids (mediators)
Malonyl CoA increase
Glycogen accumulation
Insulin gene expression decrease
Ion channel dysfunction

CoA, coenzyme A; GLUT, glucose transporter.

Rather than trying to explain β-cell dysfunction on the basis of a single defect, it seems more productive to think of there being multiple defects caused by a general loss of the unique differentiation of β-cells that is required for optimal GSIS (107). Therefore, when β-cells successfully compensate for insulin resistance with increased insulin secretion, they maintain near normal differentiation, but as this effort fails and β-cells are chronically exposed to the diabetic milieu, they undergo dedifferentiation that damages the normal delicately balanced metabolic machinery that permits GSIS while preserving the more hardy, less specialized secretory pathways required for nonglucose secretagogues, such as arginine.

The hypothesis predicts that β-cells exposed to the diabetic milieu would have downregulation of those transporters and enzymes that are highly expressed and upregulation of those that are normally suppressed. Changes in expression of the transcription factors that control this expression should also be found. Evidence supporting this hypothesis can be found in studies of islet gene expression carried out in diabetic Zucker fatty rats (108) and in partially pancreatectomized rats (63, 100,109), the latter not having the potentially confounding influence of mutated leptin receptors. Indeed, decreased expression of GLUT-2, glucokinase, mGPDH, pyruvate carboxylase, and insulin are found, while increases are seen with hexokinase, LDH, and glucose-6-phosphatase (63,108,110). Although it is not yet possible to understand the transcriptional control of these changes, it is noteworthy that transcription factors with decreased expression include PDX-1, HNFs (1a, 4a, and 3b), Nkx6.1, Pax6, and Beta2, and that transcription factors with increased expression include *c-myc* and CCAAT/enhancer binding protein β (C/EBPβ) (63,111). PDX-1, Beta2, and the HNFs are important for expression of insulin and glycolytic enzymes. C/EBPβ may inhibit insulin gene expression, and *c-myc*

may be responsible for the increase of LDH and suppression of insulin.

Such changes should have a crippling effect on insulin secretion. Expression of LDH could allow some pyruvate to be diverted to lactate and therefore not reach mitochondria. Cytoplasmic NADH might be oxidized by LDH, thereby lessening the shuttling of reducing equivalents to mitochondria. The expression of gluconeogenic enzymes might allow wasteful glucose recycling, and loss of specialized shuttles could further impair the linkage between metabolism and insulin secretion in β-cells.

The molecular mechanisms responsible for these changes in gene expression are starting to be unraveled (Fig. 60.3). Increased calcium levels, increased reactive oxygen species (ROS) produced via the GFAT pathway, and changes in protein kinase C β2 activity have all been implicated as upstream mediators (112–114). More distal mediators may be the JNK pathway, c-Myc, and nuclear factor β (21,100,115,116).

STAGES OF β-CELL DECOMPENSATION

The changes that occur in β-cells when confronted initially by increased demand and then later by the diabetic milieu can be thought of as having three stages (Table 60.3):

1. Compensation for insulin resistance. The hyperinsulinemia of insulin resistance is caused by an increased rate of insulin secretion (15) and is an effective mechanism for maintaining normoglycemia. It is not known how much of this increased secretion can be accounted for by increased secretion from individual β-cells and how much is dependent on increased β-cell mass. In studies of situations with increased β-cell demand in humans and experimental animals, there is evidence of increased β-cell mass as well as increased β-cell size, so both hyperplasia and hypertrophy seem to be important (63,117). Another key component of compensation appears to be a shift to the left of the dose-response curve for GSIS, so that β-cells respond as if they were seeing higher levels of glucose. As discussed earlier, this may be due to increased activity of glucokinase. Interestingly, with successful compensation in a glucose infusion model, the level of expression of the genes important for β-cell differentiation is virtually unchanged (94), which fits with GSIS being intact in obesity.
2. Adaptation with mild hyperglycemia. Some β-cell decompensation, as evidenced by a loss of GSIS, starts to occur with very mild elevations of glucose levels, values that might not even meet the criteria for IGT. Indeed, in humans when fasting glucose levels climb to only about 115 mg/dL, acute GSIS is virtually abolished, while acute responses to nonglucose secretagogues such as arginine are preserved. Work in rodents indicates that the insulin stores of β-cells (insulin content per given mass of β-cells) are conserved (96), suggesting that the transcription and translation mechanisms for insulin biosynthesis are mostly intact. For example, in 90% partially pancreatectomized rats with mild hyperglycemia, insulin gene expression is not reduced (63). Nonetheless, altered β-cell gene expression is found, which probably disrupts the machinery that is critical for optimal GSIS. In particular, there is decreased gene expression of GLUT-2, glucokinase, mGPDH, pyruvate carboxylase, VDCC, SERCA3, IP3R-II, and various transcription factors (PDX-1, HNFs, Nkx6.1, Pax6). In contrast, some genes that are normally expressed at relatively low levels in β-cells are found to have increased expression, including LDH, hexokinase, glucose-6-phosphatase, and the transcription factor c-*myc*.
3. Decompensation with severe hyperglycemia. When severe hyperglycemia develops, even more severe decompensation occurs, as evidenced by markedly reduced insulin stores referred to as degranulation, suggesting that the mechanisms of proinsulin biosynthesis cannot keep up with secretory demand (118,119). With reduced insulin stores, it is not surprising that secretion even by nonglucose secretagogues is reduced. In this situation, an obviously increased ratio of proinsulin to insulin is found in plasma, which reflects proportionally increased secretion of proinsulin (58). This state of severe hyperglycemia is associated with even more marked dedifferentiation of β-cells than was found with an environment of mild hyperglycemia. β-cells also may be more susceptible to death, as is suggested by the increased rate of apoptosis seen in hyperglycemic Zucker diabetic fatty rats treated with dexamethasone (120), yet other studies suggest other hyperglycemic rats are not as vulnerable to dexamethasone (100).

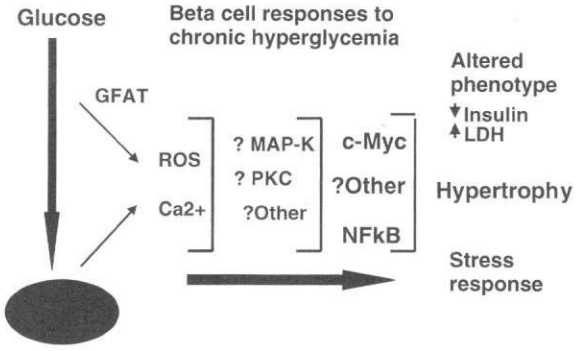

Figure 60.3. Potential molecular basis of β-cell adaptation and decompensation. *GFAT,* glutamine:fructose-6-phosphate amidotransferase; *ROS,* reactive oxygen species, *MAP-K,* mitogen activated protein kinase; *PKC,* protein kinase C; *NFkB,* nuclear factor κB; LDH, lactate dehydrogenase.

CONCLUSION

Although insulin resistance plays a critical role in the pathogenesis of type 2 DM, β-cell failure, either in a relative or absolute sense, is the sine qua non for the development of the diabetic state. Much is known about the altered characteristics of insulin secretion in type 2 DM, but the actual causes of the β-cell failure are still poorly understood. It might be helpful to consider two mechanistic categories. First, the genes responsible for the maintenance of β-cell mass, insulin synthesis, and the complex machinery of insulin secretion could be important. Second, the

TABLE 60.3 STAGES OF β-CELL DECOMPENSATION IN DIABETES

I. Compensation for insulin resistance
β-cell hypertrophy
β-cell hyperplasia
Shift to the left of glucose dose-response curve
"Normal" or increased glucose-induced insulin secretion
II. Adaptation: mild hyperglycemia
Loss of glucose-induced insulin secretion
Preservation of responses to nonglucose secretagogues (arginine, etc.)
Near-normal insulin stores
Early β-cell dedifferentiation
Decreased gene expression of GLUT-2, glucokinase, mGAPDH, pyruvate carboxylase, VDCC, SERCA3, IP3R-II and transcription factors (PDX-1, HNFs, Nkx6.1, Pax6)
Increased gene expression of LDH, hexokinase, glucose-6-phosphatase, and the transcription factor c-*myc*
III. Decompensation: severe hyperglycemia
Loss of glucose-induced insulin secretion
Impairment of responses to nonglucose secretagogues (arginine, etc.)
Increased ratio of secreted proinsulin to insulin
Reduced insulin stores (degranulation)
More severe β-cell dedifferentiation
Decreased gene expression of insulin, IAPP, glucokinase, Kir6.2, SERCA2B, and transcription factor Beta2
Increased gene expression of glucose-6-phosphatase, 12-lipoxygenase, fatty acid synthase, and the transcription factor C/EBPb

Based on refs. 63, 100, 109, and 116.
HNF, hepatocyte nuclear factor; IP3R, inositol-1,4,5 triphosphate receptor; LDH, lactate dehydrogenase; SERCA, sarcoendoplasmic reticulum Ca^{2+}ATPase; VDCC, voltage-dependent Ca^{2+} channels.

mechanisms of compensation, adaptation, and decompensation of β-cells in both the prediabetic and diabetic states, which may be very complex, may be responsible.

Therapeutic approaches to type 2 DM could be targeted to enhancing the action of insulin or somehow increasing the production of insulin. Agents that enhance insulin action, insulin sensitizers, or agents that inhibit gluconeogenesis could have a major impact because a substantial amount of β-cell capacity can still be present after many years of DM. It is not uncommon to see patients with long-standing type 2 DM who lose large amounts of weight either voluntarily or because of illness and no longer need treatment. Drugs that enhance insulin secretion through pathways different from those affected by sulfonylureas might be useful. Studies with glucagon-like peptide-1 and exendin-4 provide an example of one such approach (25,121). Not only can they improve glycemic control by enhancing insulin secretion, but they can stimulate β-cell replication and neogenesis (122). Gene therapy targeting specific genes to β-cells might be exploited in some manner, such as to counter apoptosis or oxidant injury. A search for drugs that inhibit the purported damaging effects of amyloid formation could be undertaken. Finally, because β-cell failure is a root cause of type 2 DM, both β-cell replacement with transplantation and the development of an automatic mechanical insulin delivery system should be considered potential options for the future.

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