Five Stages of Evolving β-Cell Dysfunction During Progression to Diabetes

Gordon C. Weir and Susan Bonner-Weir

This article proposes five stages in the progression of diabetes, each of which is characterized by different changes in β-cell mass, phenotype, and function. Stage 1 is compensation: insulin secretion increases to maintain normoglycemia in the face of insulin resistance and/or decreasing β-cell mass. This stage is characterized by maintenance of differentiated function with intact acute glucose-stimulated insulin secretion (GSIS). Stage 2 occurs when glucose levels start to rise, reaching ~5.0–6.5 mmol/l; this is a stable state of β-cell adaptation with loss of β-cell mass and disruption of function as evidenced by diminished GSIS and β-cell dedifferentiation. Stage 3 is a transient unstable period of early decompensation in which glucose levels rise relatively rapidly to the frank diabetes of stage 4, which is characterized as stable decompensation with more severe β-cell dedifferentiation. Finally, stage 5 is characterized by severe decompensation representing a profound reduction in β-cell mass with progression to ketosis. Movement across stages 1–4 can be in either direction. For example, individuals with treated type 2 diabetes can move from stage 4 to stage 1 or stage 2. For type 1 diabetes, as remission develops, progression from stage 4 to stage 2 is typically found. Delineation of these stages provides insight into the pathophysiology of both progression and remission of diabetes. *Diabetes* **53 (Suppl. 3):S16–S210, 2004**

Progression to diabetes can be viewed as having definable stages characterized by changes in various metabolic parameters and β-cell function. At the very beginning, fasting plasma glucose levels increase from perfectly normal values of ~4.5 mmol/l (80 mg/dl) to higher values that might be as low as 5.0 mmol/l (89 mg/dl). This change in glycemia would not be recognized as being clinically abnormal because it would fail to reach the official category of impaired fasting glucose (IFG; glucose level ≥5.6 mmol/l or 100 mg/dl) or impaired glucose tolerance (IGT; 2-h postglucose level of ≥7.8 mmol/l or 140 mg/dl) (1). Those destined to develop diabetes then progress to the IFG or IGT range, where they may remain for years before developing frank diabetes. Although this progression is mostly discussed in the context of type 2 diabetes, very similar changes occur as type 1 diabetes unfolds and as pancreas or islet transplants fail.

We propose that there are five stages in the progression of diabetes (Fig. 1), each of which is marked by important changes in β-cell mass, phenotype, and function. Although conventionally thought of as a continuum, we make the case that each has its own important characteristics. Stage 1 is best described as compensation: insulin secretion increases to maintain normal glucose levels in the face of insulin resistance resulting from obesity, physical inactivity, and genetic predisposition. Stage 2 occurs when glucose levels rise to levels of ~5.0–6.5 mmol/l (89–116 mg/dl)—a stable state of β-cell adaptation. Stage 3 is an unstable period of early decompensation in which glucose levels rise relatively rapidly to stage 4, which is characterized as stable decompensation. Finally, there is the severe decompensation of stage 5 that represents profound β-cell failure with progression to ketosis. Movement across stages 1–4 can be in either direction. For example, individuals with type 2 diabetes who undergo gastric reduction surgery can move from stage 4 to stage 1. Even conventional treatments with diet, exercise, and oral agents often return people to stage 2. For type 1 diabetes, as remission develops, progression from stage 4 to stage 2 is typically found.

STAGE 1: COMPENSATION

The most common example of compensation is found with the insulin resistance due to obesity, which is accompanied by higher overall rates of insulin secretion (2) and increased acute glucose-stimulated insulin secretion (GSIS) following an intravenous glucose challenge (3). Much of the increase in insulin secretion undoubtedly results from an increase in β-cell mass, as has been found in autopsy studies in humans (4–7) and numerous rodent models (8,9). β-Cell mass is normally tightly maintained through a balance of β-cell birth (β-cell replication and islet neogenesis) and β-cell death through apoptosis (10). Most of the increase in β-cell mass with insulin resistance is probably due to increased β-cell number, but β-cell hypertrophy may also contribute. It is not yet clear if the higher plasma insulin levels can be entirely explained by the larger β-cell mass or whether there is also increased secretion per given unit of β-cell mass. Although compensation is usually thought of in the situation of insulin resistance, similar changes presumably occur in the early
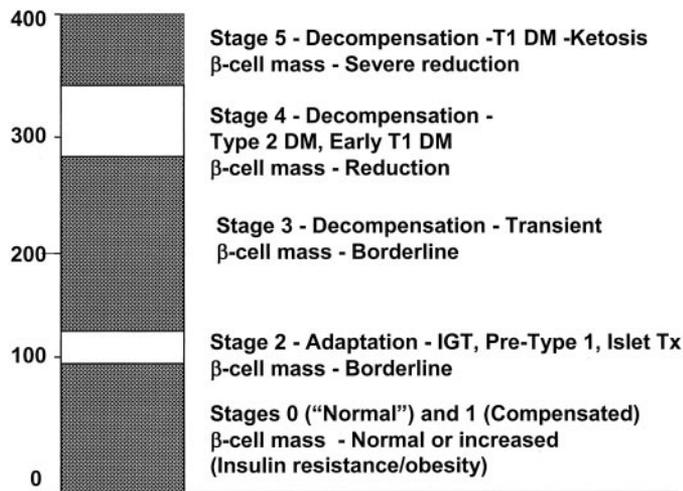
FIG. 1. Schema of five stages of progression of diabetes.

stages of autoimmune destruction. As β-cell mass falls, there must be a signal to increase mass and secretion, which presumably prolong the prediabetic period, which can last for years (11).

There is much interest in the signal leading to increased β-cell mass in this situation. The facile, but probably correct, explanation is that there is a feedback loop with insulin resistance producing increased glucose levels that stimulate β-cell secretion and growth. However, concerns are raised about how this can be compatible with seemingly normal glucose levels. One explanation is that the feedback loop is tightly regulated, like a thermostat that can maintain temperatures within a very narrow range. A molecular mechanism that could facilitate this control is activation of glucokinase in β-cells, the enzyme that controls the rate of glycolysis, thereby determining insulin secretory rates (12). Thus, very small elevations in glucose levels could lead to a change in the set point in GSIS that allows maintenance of "normal" plasma glucose levels (13).

The importance of β-cell differentiation. The unique differentiation of β-cells is responsible for the extraordinary efficiency of these cells in storing and secreting insulin to provide precise regulation of metabolism. With successful compensation for insulin resistance, little change in β-cell phenotype for the secretory machinery would be expected. The unique specialization of β-cells is presumably necessary for optimal GSIS; the impressively large acute insulin responses to glucose challenge seen in obesity strongly suggest the phenotype is maintained. The complexity of this differentiation is far from being understood, but some key features are apparent. The glucose transporter GLUT2 allows rapid equilibration between extra- and intracellular glucose levels. Glucose is phosphorylated by glucokinase with a K_m of ~8 mmol/l that allows it to function efficiently within the normal range of plasma glucose concentrations (14). Glucose metabolism is dominated by glycolysis with pyruvate directed to mitochondria for oxidation. There is little if any gluconeogenesis (15) or lactate production (16), which should be helpful for maximizing the efficiency of aerobic glycolysis. The β-cell also has specialized mitochondrial 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 β-cells the malate/pyruvate shuttle may facilitate the generation of NADPH, which could somehow enhance secretion. The need for these shuttles probably explains why β-cells have very high levels of mitochondrial glycerol phosphate dehydrogenase (mGPDH) and pyruvate carboxylase (17,18).

To maintain this degree of specialization, the genes that are highly expressed in β-cells include those of the secretory products (insulin and islet amyloid polypeptide [IAPP]), key genes for glucose metabolism (GLUT2 and glucokinase), key enzymes for the mitochondrial shuttles (glycerol phosphate dehydrogenase [mGPDH]) and pyruvate carboxylase, and critical transcription factors (PDX-1 and Nkx-6.1). Yet, enzymes that participate in unwanted pathways such as gluconeogenesis and lactate production are expected to be suppressed. Some of these include phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase and fructose-1,6-bisphosphatase, and lactate dehydrogenase. Hexokinase would also be unneeded, so its expression could also be suppressed. Clearly these changes in gene expression are just the tip of the iceberg, and a full picture of β-cell phenotype awaits gene array studies, which should emerge in the near future.

Maintenance of β-cell differentiation during stage 1 compensation. Some experimental data support the concept that much of the β-cell phenotype is kept intact during successful compensation for insulin resistance. When glucose was infused into rats for 4 days, compensation occurred and glucose levels remained normal during the latter stages of the infusion because of increases in insulin secretion that were accompanied by increased β-cell mass (19). While responding to the increased demand, the β-cell phenotype, as determined by analysis of a selected group of genes, remained remarkably similar to the control profile (19). There must have been induction of some genes required for β-cell replication, but the genes responsible for maintaining the machinery allowing GSIS did not seem to be perturbed. This preservation of phenotype, coupled with an increase in β-cell mass, is consistent with the exuberant GSIS seen in obesity.

STAGE 2: STABLE ADAPTATION

It is not possible to assign a precise glucose range for this stage of β-cell adaptation, but fasting levels between 5.0 and 7.3 mmol/l (89–130 mg/dl) are reasonable approximations. In stage 2, β-cells can no longer be considered to be compensating because truly normal glucose levels can no longer be maintained. However, this stage can be considered stable because, as shown in the Diabetes Prevention Program, individuals at the upper end of stage 2 with IGT progressed to diabetes at the rate of ~11% per year while those who adhered to a diet and exercise regimen progressed at a rate of only 5% per year (20). Thus, unless some process, such as autoimmunity, is producing rapid β-cell destruction, individuals in stage 2 usually evade progression to diabetes for years.

Nonetheless, as glucose levels rise to stage 2, important changes in β-cell function and differentiation occur. The

most striking and best studied is the loss of acute GSIS. The acute phase of secretion occurs ~3–10 min after the start of the glucose infusion and represents the first phase of GSIS (21). This experimental assessment has the pathophysiological correlate of impaired early insulin release during an oral glucose challenge, which contributes to glucose intolerance (22). An important study published in 1976 (23) showed that subjects maintained normal acute GSIS as long as fasting plasma glucose levels remained <5.6 mmol/l (100 mg/dl). However, acute GSIS dropped drastically when fasting plasma glucose values were above this level and disappeared completely once fasting glucose levels increased to only 6.4 mmol/l (114 mg/dl). This pattern has now been confirmed in countless studies in humans and animals. In contrast to this loss of acute GSIS, the second phase of insulin secretion is partially preserved (24), and acute insulin responses to nonglucose secretagogues, such as isoproterenol and arginine, remain largely intact (25).

Loss of GSIS in stage 2: the glucotoxicity hypothesis.
The concept of glucose toxicity is that β-cells normally function within a narrow range of plasma glucose levels and that even modestly higher glucose levels create an unnatural environment, which leads to alteration in function and most notably a loss of acute GSIS. On the molecular level, the exact mechanisms responsible for this loss of GSIS are not known; however, we postulate that a loss of specialized gene expression leads to complete disruption of the acute phase of GSIS. In contrast, the agents that stimulate insulin release through depolarization (arginine) or cyclic AMP (isoproterenol) work through more ubiquitous and less fragile mechanisms that are maintained (26). Although some have proposed that increased free fatty acid (FFA) levels are important for the β-cell dysfunction of diabetes (27), these levels correlate poorly with the loss of acute GSIS, whereas the correlation with glucose levels is very precise (26). This interpretation does not rule out important glucose-driven derangements of lipid pathways in β-cells that could justify use of the term "lipotoxicity."

Changes in β-cell differentiation in stage 2. The loss of acute GSIS seen in the diabetic state is accompanied by marked changes in β-cell phenotype demonstrated by changes in gene and protein expression. These changes have been found in islets from both diabetic ZDF rats (28) and rats following partial pancreatectomy in which residual β-cells are exposed to varying degrees of hyperglycemia (29–32). The alterations may be thought of as dedifferentiation, or loss of phenotype, in that the highly expressed genes in β-cells (mentioned above) are downregulated while those that are normally suppressed are upregulated in their expression. Some of the upregulated genes include glucose-6-phosphatase, fructose-1,6-bisphosphatase, lactate dehydrogenase, and hexokinase. In addition, a stress response is found with a variety of antioxidant, apoptotic, and proapoptotic genes being activated (31). These are accompanied by a marked increase in the expression of c-*myc* and activation of nuclear factor (NF)-κB. These changes are consistent with a recent report that interleukin (IL)-1 expression in β-cells is activated by high glucose levels (33). Another interesting facet is the presence of β-cell hypertrophy, which may be a

glucose-driven growth response stopping short of replication (29–32). Strengthening the hypothesis that these changes are secondary to chronic hyperglycemia are data showing that lowering of the elevated glucose levels with phlorizin, which inhibits renal glucose reabsorption, leads to reversal of virtually all of the changes in gene expression and hypertrophy (29).

It should be emphasized that some changes of gene expression occur in stage 2, when glucose levels might be considered "normal." In some rats following a 90% partial pancreatectomy, fed plasma glucose levels of 6.9 mmol/l (124 mg/dl) were maintained over a 14-week period, whereas control rats maintained levels at 5.8 mmol/l (104 mg/dl) over the same time period (32). When considered in human terms, this increase of only 1.1 mmol/l (20 mg/dl) would probably not have reached the designation of IGT and therefore would be categorized as normal. However, the islets isolated from the pancreatic remnant of these rats 14 weeks after surgery had similar, albeit less marked, changes in gene expression as those of partially pancreatectomized rats with more severe hyperglycemia. Despite the minimal degree of hyperglycemia, these changes in gene expression correlate with the losses of GSIS seen in this and similar rodent models (34,35) and in humans (23), supporting the hypothesis that changes in β-cell phenotype are responsible for the disruption of GSIS. We recognize that blaming the loss of GSIS on altered phenotype may be an oversimplification. Certainly there could be important changes in signal transduction pathways that could be unrelated to changes in protein levels, but we expect that the changes in phenotype are of fundamental importance. In the future it should be possible to dissect out the many candidates that could contribute to β-cell dysfunction, such as mitochondrial shuttles, lactate dehydrogenase, glucose-6-phosphatase, uncoupling protein-2, peroxisome proliferator–activated receptor (PPAR)-α, and c-*myc*.

STAGE 3: UNSTABLE EARLY DECOMPENSATION
We postulate that during progression toward diabetes, stable stage 2 ends and glucose levels rise relatively rapidly from the range of 7.3 mmol/l (130 mg/dl), through an unstable transient stage 3 of decompensation, to a more stable stage 4 at ~16–20 mmol/l (285–350 mg/dl). Thus, individuals headed to type 2 diabetes can remain in stage 2 for many years, but when β-cell mass becomes inadequate at some critical point, glucose levels rise over a relatively short period of time to stage 4, which may or may not be associated with noticeable symptoms, such as polyuria and weight loss. A similar progression occurs in type 1 diabetes, but stage 2 does not last as long because autoimmune destruction of β-cells occurs much more rapidly than whatever process is responsible for the β-cell attrition in type 2 diabetes. Thus, a child nearing adolescence might be in stage 2, whereupon flu or some other stress might push glucose levels rapidly through stage 3 to stage 4. While in stage 4, the precipitating stress may recede, allowing the child to fall back through stage 3 to stage 2 for a remission, which would only last until further β-cell destruction results in a return to stage 3 and then stage 4. Stage 4 might not be long lasting because continued β-cell loss will usually lead to the severe decompen-
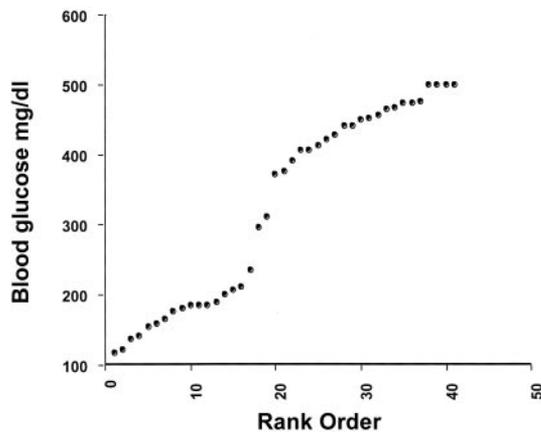
FIG. 2. Four weeks after transplantation of 750 syngeneic islets contained in an immunobarrier device into streptozocin diabetic B6AF1 mice, the recipients fall into two discrete groups: those with near-normal blood glucose levels and those with severe diabetes. Figure based on data of Suzuki et al. (38).

sation of stage 5, with propensity to ketoacidosis. The same pathophysiology probably occurs with pancreas and islet transplantation, in that loss of β-cells in the grafts would lead to progression through the above stages. While a successful pancreas transplant will typically bring a recipient to stage 1, an islet transplant performed according to the Edmonton approach (36) will usually place recipients in stage 2, where they will remain at risk for later decompensation (37).

Experimental evidence for the instability of stage 3. When transplanting islets contained in immunoprotective devices into mice, we noted that a marginal number of islets produced either success with normal blood glucose levels or failure with glucose levels >15 mmol/l (280 mg/dl), with almost no glucose levels in between (38) (Fig. 2). A marginal number of syngeneic islets transplanted under the kidney capsule of streptozocin diabetic mice produced a similar pattern of either failure or success, with only a few glucose levels in the stage 3 range (data not shown). This concept was examined again in rats with a marginal reduction of β-cell mass following a 90% partial pancreatectomy (Fig. 3). Although a spectrum of glucose
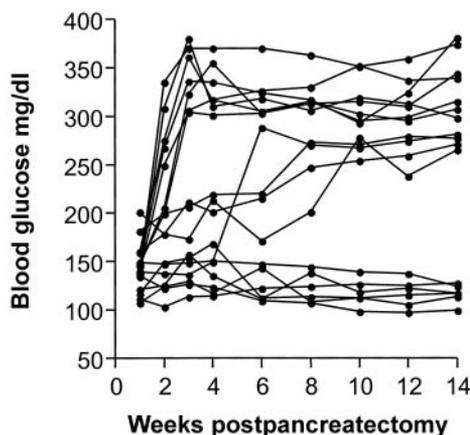

FIG. 3. Four- to six-week-old rats were subjected to near identical 90% partial pancreatectomies. Although glucose levels were scattered during the early stages, by 10 weeks two distinct groups emerged, one with near-normal blood glucose levels and one with unequivocal diabetes. Figure based on data of Laybutt et al. (32).

levels was seen at 4 weeks, the rats showed two distinct outcomes by 14 weeks: they were either near normal (glucose levels of 6.9 mmol/l [124 mg/dl] and 5.8 mmol/l [104 mg/dl] in fed and control rats, respectively) or unequivocally hyperglycemic (32). Somehow there were forces pushing these rodents to either stage 2 or 4, with their time in stage 3 being only transient.

Support is also provided by studies of islet transplantation in which canine islets were transplanted into the livers of young diabetic dogs (39). As the recipients matured, rapid decompensation to marked hyperglycemia was seen, presumably a result of the β-cell mass becoming inadequate to meet the insulin requirements of larger dogs. A recent study in humans also supports the concept that decompensation is not a continuum but can occur in a relatively short period of time. In studies on a population from the Mexico City Diabetes Study, it was concluded that within a 3-year timeframe the development of diabetes was often rapid rather than gradual (40). The investigators also thought the rapidity might have been underestimated because some of the subjects were receiving diabetes treatment.

Why is stage 3 unstable? At some point in stage 2, β-cells are no longer able to keep glucose levels in the prediabetic range. This failure presumably occurs because of a critical decline of β-cell mass and/or increase in insulin resistance. We postulate that there is a conspiracy of forces that push glucose levels upward, which include the insulin resistance associated with the diabetic state with its complex gluco- and lipotoxicity influencing the key insulin target tissues (i.e, liver, muscle, and fat) (41). The increases in glucose concentration probably also worsen glucotoxic effects on β-cells, leading to less efficient insulin secretion. For example, as insulin mRNA falls with increasing hyperglycemia, there is evidence that insulin biosynthesis becomes rate limiting for secretion (42). With these factors working synchronously, glucose levels rise to stage 4 but do not immediately progress to stage 5 because enough insulin production continues to prevent severe ketosis. By treating type 2 diabetes with diet, exercise, and drugs, individuals can quickly return to stage 2 and remain in that stable range for a considerable period of time as long as they continue the treatment and have no further decline in β-cell mass.

Why is the instability of stage 3 not more clinically apparent? Clinicians often see patients who are asymptomatic and have unremarkable glucose levels at one point and then present with glucose levels in the range of >16 mmol/l (285 mg/dl) with the absence or presence of symptoms. Certainly individuals progressing to type 1 diabetes often rapidly progress to very high glucose levels, but clearly many people with diabetes have glucose levels within the stage 3 range. The most obvious explanation is that external forces can overwhelm the processes that make stage 3 transient (in particular, treatment with oral medications and/or insulin). Even changes in caloric intake and exercise should allow individuals to move in and out of stage 3. Another point is that the transition from stage 2 to stage 4 is not instantaneous; even in our rodent studies (Fig. 3) progression took a few weeks. Nonetheless, we postulate that stage 3 is by nature transient.

STAGE 4: STABLE DECOMPENSATION

Once individuals progress through stage 3 to the unambiguous diabetes of stage 4, they typically have enough insulin secretion to remain in this stage rather than progressing to ketoacidosis. In most cases this stage lasts a lifetime for people with type 2 diabetes, while the rapid progressive autoimmune destruction of β-cells in type 1 diabetes can lead to stage 5 relatively quickly. Individuals with failing islet transplants can probably stay in stage 4 for relatively long periods of time if the immunosuppression regimens allow sufficient β-cell survival.

Changes in β-cell mass, function, and differentiation in stage 4. Morphometric studies on postmortem pancreases of people with type 2 diabetes provide convincing evidence that β-cell mass is reduced to ~50% of that of control subjects (4–6). Data generated in rodents suggest β-cell dedifferentiation is more severe at higher glucose levels (29), which should result in less efficient insulin secretion. This lack of efficiency fits with insulin secretion studies in type 2 diabetes that have indicated that the capacity for secretion is considerably less than the 50% reduction in β-cell mass (43).

Important questions are raised by the finding that a stress response is induced by chronic hyperglycemia (31). Because the stress response is characterized by the expression of a mixture of proapoptotic, antiapoptotic, and antioxidant genes, it is difficult to know if β-cells in this situation would be more or less susceptible to the apoptosis. For destruction by autoimmunity and/or transplant rejection, the stress response could make β-cells either more vulnerable or provide protection. For type 2 diabetes, this phenotypic change may accelerate β-cell loss. Even if accelerated, the rate of β-cell apoptosis in type 2 diabetes is certainly very slow, given the assumption that the β-cell death rate must be similar to β-cell birth rate, which is probably considerably less than 1% per day (4,44). Even less is known about the rate of neogenesis, which is probably very slow as well (4). Somehow, regardless of β-cell loss in typical type 2 diabetes, apoptosis rarely progresses to near-complete loss, which leads to ketosis, even after decades of the disease. Although it is often assumed that accelerated apoptosis of β-cells is important for the pathogenesis of type 2 diabetes, limitations in β-cell replication and/or neogenesis could be just as important. Although some rodent models of diabetes exhibit increased rates of apoptosis (8,45), in rats following 90% partial pancreatectomy with glucose levels in the stage 4 range of diabetes, rates of β-cell replication and the incidence of apoptotic bodies were no different than in control rats (46).

STAGE 5: SEVERE DECOMPENSATION

In this last stage of diabetes, the marked loss of β-cells is so severe that people become ketotic and truly dependent on insulin for survival. Glucose levels are typically >22 mmol/l (350 mg/dl) but will vary with eating and hydration. This situation is typically found with type 1 diabetes or patients with pancreas or islet transplants when β-cells have been mostly destroyed by the immune system. It can also occur in unusual situations, such as exposure to certain toxins or very severe pancreatitis, but it rarely occurs in typical type 2 diabetes.

SUMMARY

The development of the concept of five stages of β-cell function that characterize progression of diabetes has emerged from clinical observation, awareness of clinical investigation by many workers, and work with animal models of diabetes. This perspective provides only a broad picture of these stages and will hopefully stimulate studies that will more precisely define the mechanisms leading to diabetes.

REFERENCES

1. American Diabetes Association: Diagnosis and classification of diabetes mellitus. *Diabetes Care* 27 (Suppl. 1):S5–S10, 2004
2. Polonsky KS, Given BD, Hirsch L, Shapiro ET, Tillit H, Beebe C, Galloway JA, Frank BH, Karrison T, Van Cauter E: Quantitative study of insulin secretion and clearance in normal and obese subjects. *J Clin Invest* 81:435–441, 1988
3. Karam JH, Grodsky GM, Forsham PH: Excessive insulin response to glucose in obese subjects as measured by immunochemical assay. *Diabetes* 12:196–204, 1963
4. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC: Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52:102–110, 2003
5. Yoon KH, Ko SH, Cho JH, Lee JM, Ahn YB, Song KH, Yoo SJ, Kang MI, Cha BY, Lee KW, Son HY, Kang SK, Kim HS, Lee IK, Bonner-Weir S: Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea. *J Clin Endocrinol Metab* 88:2300–2308, 2003
6. Maclean N, Ogilvie RF: Quantitative estimation of the pancreatic islet tissue in diabetic subjects. *Diabetes* 4:367–376, 1955
7. Kloppel G, Lohr M, Habich K, Oberholzer M, Heitz PU: Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. *Surv Synth Pathol Res* 4:110–125, 1985
8. Pick J, Clark J, Kubstrup C, Pugh W, Bonner-Weir S, Polonsky K: Role of apoptosis in failure of beta cell mass compensation for insulin resistance and β cell defects in the male Zucker diabetic fatty (ZDF) rat. *Diabetes* 47:358–364, 1998
9. Bruning JC, Winnay J, Bonner-Weir S, Taylor SI, Accili D, Kahn CR: Development of a novel polygenic model of NIDDM in mice heterozygous for IR and IRS-1 null alleles. *Cell* 88:561–572, 1999
10. Bonner-Weir S: Life and death of the pancreatic beta cells. *Trends Endocrinol Metab* 11:375–378, 2000
11. Bleich D, Jackson RA, Soeldner JS, Eisenbarth GS: Analysis of metabolic progression to type I diabetes in ICA+ relatives of patients with type I diabetes. *Diabetes Care* 13:111–118, 1990
12. Matschinsky FM: A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. *Diabetes* 45:223–241, 1996
13. Chen C, Hosokawa H, Bumbalo LM, Leahy JL: Regulatory effects of glucose on the catalytic activity and cellular content of glucokinase in the pancreatic B cell. *J Clin Invest* 94:1616–1620, 1994
14. Thorens B, Charron MJ, Lodish HF: Molecular physiology of glucose transporters. *Diabetes Care* 13:209–218, 1990
15. MacDonald MJ, McKenzie DI, Walker TM, Kaysen JH: Lack of glyconeogenesis in pancreatic islets: expression of gluconeogenic enzyme genes in islets. *Horm Metab Res* 24:158–160, 1992
16. Sekine N, Cirulli V, Regazzi R, Brown LJ, Gine E, Tamarit-Rodriguez J, Girotti M, Marie S, MacDonald MJ, Wollheim CB, Rutter GA: Low lactate dehydrogenase and high mitochondrial glycerol phosphate dehydrogenase in pancreatic B-cells. *J Biol Chem* 269:4895–4902, 1994
17. MacDonald MJ: Elusive proximal signals of beta cells for insulin secretion. *Diabetes* 39:1461–1466, 1990
18. MacDonald MJ: Feasibility of a mitochondrial pyruvate malate shuttle in pancreatic islets. *J Biol Chem* 270:20051–20058, 1995
19. Steil GM, Trivedi N, Jonas JC, Hasenkamp WM, Sharma A, Bonner-Weir S, Weir GC: Adaptation of beta-cell mass to substrate oversupply: enhanced

function with normal gene expression. *Am J Physiol Endocrinol Metab* 280:E788–E796, 2001

20. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM: Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 346:393–403, 2002

21. Curry DL, Bennett LL, Grodsky GM: Dynamics of insulin secretion by the perfused rat pancreas. *Endocrinology* 83:572–584, 1968

22. Mitrakoku A, Kelley D, Mokan M, Veneman T, Pangburn T, Reilly J, Gerich J: Role of suppression of glucose production and diminished early insulin release in impaired glucose tolerance. *N Engl J Med* 326:22–29, 1992

23. Brunzell JD, Robertson RP, Lerner RL, Hazzard WR, Ensinck JW, Bierman EL, Porte D Jr: Relationships between fasting plasma glucose levels and insulin secretion during intravenous glucose tolerance tests. *J Clin Endocrinol Metab* 42:222–229, 1976

24. Cerasi E, Luft R: Plasma insulin response to sustained hyperglycemia induced by glucose infusion in human subjects. *Lancet* 2:1359, 1963

25. Porte D Jr: B-cells in type II diabetes mellitus. *Diabetes* 40:166–180, 1991

26. Weir GC, Laybutt DR, Kaneto H, Bonner-Weir S, Sharma A: Beta-cell adaptation and decompensation during the progression of diabetes. *Diabetes* 50 (Suppl. 1):S154–S159, 2001

27. Prentki M, Corkey BE: Are the B-cell signaling molecules malonyl-CoA and cytosolic long-chain acyl-CoA implicated in multiple tissue defects of obesity and NIDDM? *Diabetes* 45:273–283, 1996

28. Tokuyama Y, Sturis J, Depaoli AM, Takeda J, Stoffel M, Tang J, Sun X, Polonsky KS, Bell GI: Evolution of beta cell dysfunction in the male Zucker diabetic fatty rat. *Diabetes* 44:1447–1457, 1995

29. Jonas J-C, Sharma A, Hasenkamp W, Ilkova H, Patane G, Laybutt R, Bonner-Weir S, Weir GC: Chronic hyperglycemia triggers loss of pancreatic beta cell differentiation in an animal model of diabetes. *J Biol Chem* 274:14112–14121, 1999

30. Laybutt DR, Sharma A, Sgroi DC, Gaudet J, Bonner-Weir S, Weir GC: Genetic regulation of metabolic pathways in beta-cells disrupted by hyperglycemia. *J Biol Chem* 277:10912–10921, 2002

31. Laybutt DR, Kaneto H, Hasenkamp W, Grey S, Jonas JC, Sgroi DC, Groff A, Ferran C, Bonner-Weir S, Sharma A, Weir GC: Increased expression of antioxidant and antiapoptotic genes in islets that may contribute to beta-cell survival during chronic hyperglycemia. *Diabetes* 51:413–423, 2002

32. Laybutt DR, Glandt M, Xu G, Ahn YB, Trivedi N, Bonner-Weir S, Weir GC: Critical reduction in beta-cell mass results in two distinct outcomes over time: adaptation with impaired glucose tolerance or decompensated diabetes. *J Biol Chem* 278:2997–3005, 2003

33. Maedler K, Sergeev P, Ris F, Oberholzer J, Joller-Jemelka HI, Spinas GA, Kaiser N, Halban PA, Donath MY: Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. *J Clin Invest* 110:851–860, 2002

34. Trent DF, Fletcher DJ, May JM, Bonner-Weir S, Weir GC: Abnormal islet and adipocyte function in young B-cell deficient rats with near-normoglycemia. *Diabetes* 33:170–175, 1984

35. Leahy JL, Bonner-Weir S, Weir GC: Minimal chronic hyperglycemia is a critical determinant of impaired insulin secretion after an incomplete pancreatectomy. *J Clin Invest* 81:1407–1414, 1988

36. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 27:230–238, 2000

37. Ryan EA, Lakey JR, Paty BW, Imes S, Korbutt GS, Kneteman NM, Bigam D, Rajotte RV, Shapiro AM: Successful islet transplantation: continued insulin reserve provides long-term glycemic control. *Diabetes* 51:2148–2157, 2002

38. Suzuki K, Bonner-Weir S, Trivedi N, Yoon K-H, Hollister-Lock J, Colton CK, Weir GC: Function and survival of macroencapsulated syngeneic islets transplanted into streptozocin-diabetic mice. *Transplantation* 66:21–28, 1998

39. Alejandro R, Cutfield RG, Shienvold FL, Polonsky KS, Noel J, Olson L, Dillberger J, Miller J, Mintz DH: Natural history of intrahepatic canine islet cell autografts. *J Clin Invest* 78:1339–1348, 1986

40. Ferrannini E, Nannipieri M, Williams K, Gonzales C, Haffner SM, Stern MP: Mode of onset of type 2 diabetes from normal or impaired glucose tolerance. *Diabetes* 53:160–165, 2004

41. Rossetti L: Glucose "toxicity": Effect of chronic hyperglycemia on insulin action. In *Diabetes Mellitus: A Fundamental and Clinical Text*. LeRoith D, Taylor SI, Olefsky JM, Eds. Philadelphia, Lippencott-Raven, 1996, p. 544–560

42. Leibowitz G, Uckaya G, Oprescu AI, Cerasi E, Gross DJ, Kaiser N: Glucose-regulated proinsulin gene expression is required for adequate insulin production during chronic glucose exposure. *Endocrinology* 143:3214–3220, 2002

43. Ward WK, Bolgiano DC, McKnight B, Halter JB, Porte D Jr: Diminished B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. *J Clin Invest* 74:1318–1328, 1984

44. Tyrberg B, Ustinov J, Otonkoski T, Andersson A: Stimulated endocrine cell proliferation and differentiation in transplanted human pancreatic islets: effects of the ob gene and compensatory growth of the implantation organ. *Diabetes* 50:301–307, 2001

45. Donath MY, Gross DJ, Cerasi E, Kaiser N: Hyperglycemia-induced beta-cell apoptosis in pancreatic islets of *Psammomys obesus* during development of diabetes. *Diabetes* 48:738–744, 1999

46. Xu G, Stoffers DA, Habener JF, Bonner-Weir S: Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased β-cell mass and improved glucose tolerance in diabetic rats. *Diabetes* 48:2270–2276, 1999