β-Cell Adaptation and Decompensation During the Progression of Diabetes

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Inadequate β-cell function is an essential component of all forms of diabetes. The most obvious problem is a failure to maintain sufficient β-cell mass and function to cope with whatever insulin resistance is present. The most striking functional defect is a loss of acute glucose-induced insulin secretion (GIIS). This review discusses the ways in which β-cells successfully adapt to increased demand and then decompensate as diabetes develops. Successful adaptation is achieved through increased β-cell mass and increased insulin secretion. The hypothesis is explored that β-cells exposed to the diabetic milieu lose their differentiation, which leads to loss of specialized functions such as GIIS. This concept has been strengthened by the finding of dedifferentiation of β-cells in a rat model of partial pancreatectomy that includes a reduction of insulin gene expression, which may further contribute to decreased insulin production. Another finding was increased expression of c-Myc, which probably contributes to an increase in the expression of lactate dehydrogenase and the development of β-cell hypertrophy. Arguments are developed that the β-cell changes found in diabetes are better correlated with increased glucose levels than with nonesterified fatty acid levels, thus supporting the importance of glucose toxicity. *Diabetes* 50 (Suppl. 1):S154–S159, 2001

Pancreatic β-cells have the remarkable ability to keep glucose levels within a very narrow range for the lifetime of most individuals, but failure of this capacity is a fundamental part of the pathogenesis of all forms of diabetes. In type 2 diabetes, the insulin resistance of obesity and reduced physical activity are major contributors, but diabetes develops only when β-cells fail to compensate for increased demand (1). New forms of diabetes with specific gene defects have been found that include maturity-onset diabetes of the young (MODY) 1–6 and mitochondrial diabetes; they too result from β-cell failure. Finally, type 1 diabetes and failure of pancreas and islet transplantation are also due to inadequate insulin secretion. This β-cell inadequacy results from a combination of deranged secretory function and inadequate β-cell mass.

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GIIS, glucose-induced insulin secretion; LDH, lactate dehydrogenase; NEFA, nonesterified fatty acid; PPAR, peroxisome proliferator–activated receptor; Px, pancreatectomy.

FOUR HYPOTHETICAL PHASES OF DETERIORATION

We view the changes that occur in β-cells during the progression from the normal state to severe diabetes as consisting of four phases. Although based on many studies in humans and rodents, these remain hypothetical but serve to emphasize the progressive and complex nature of the deterioration (Table 1).

Hypothetical phase 1: successful adaptation to increased demand. To cope with insulin resistance, β-cell mass increases to provide the required amount of insulin to keep glucose levels normal. β-Cell mass is determined by a balance between islet neogenesis, β-cell replication, β-cell hypertrophy, and β-cell apoptosis. During this adaptation to obesity and genetic insulin resistance, individual β-cells seem to function remarkably normally. With short-term glucose infusions in rats, we have found that β-cell mass increases leading to augmented insulin production, but the expression of a panel of key β-cell genes remains normal as long as glucose levels are kept normal. Although some β-cell hypertrophy might be present, the function of individual β-cells is almost normal, with glucose-induced insulin secretion (GIIS) remaining intact. A potentially important functional abnormality is that these β-cells probably have a modestly lowered set point, thus secreting more insulin at any given glucose level; this appears to be a very useful mechanism for keeping glucose normal (2). Thus, the increased insulin secretion found in obesity may be due to both increased β-cell mass and a changed set point for GIIS.

Hypothetical phase 2: mild decompensation. The earliest signs of β-cell dysfunction in both type 1 and 2 diabetes appear as glucose levels start to increase, with fasting glucose levels around 100 mg/dl (5.6 mmol/l) and virtually complete loss of acute GIIS at ~115 mg/dl (6.4 mmol/l) (1). Thus, markedly abnormal insulin secretion can be found even before diabetes is diagnosed. This failure to keep glucose levels in a truly normal range presumably reflects a failure to sufficiently maintain or expand β-cell mass to meet demand. In this situation, there is a specific loss of GIIS, whereas secretion to other secretagogues such as arginine is better maintained. In this situation, although there is almost complete loss of acute first-phase insulin release, the later "second phase" is partially preserved. In a practical sense, as GIIS deteriorates, insulin changes with meals may become more dependent on other signals such as nutrients, gut hormones, and the autonomic nervous system. Insulin stores of β-cells are well preserved, suggesting that secretory mechanisms are more severely affected than those of insulin synthesis. A loss of

TABLE 1
Stages of β-cell decompensation in diabetes

Adaptation 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
Normal gene expression profile
Decompensation: mild hyperglycemia
Loss of acute glucose-induced insulin secretion
Preservation of responses to nonglucose secretagogues (arginine, etc.)
Near-normal insulin stores
Early β-cell dedifferentiation
Decreased gene expression of GLUT2, glucokinase, mGPDH, pyruvate carboxylase, voltage-dependent calcium channel, SERCA3, IP3R-II, and transcription factors (PDX-1, HNFs, Nkx6.1, and Pax6)
Increased gene expression of LDH, hexokinase, glucose-6-phosphatase, and the transcription factor c-Myc
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 expression of insulin, IAPP, glucokinase, Kir6.2, SERCA2B, PPAR-α, and transcription factor β2
Increased expression of glucose-6-phosphatase, 12-lipoxygenase, COX-2, PPAR-λ, uncoupling protein 2, fatty acid synthase, and the transcription factor C/EBPβ
Increased expression of stress genes inducible isoform of nitric oxide synthase, A20, and Heme oxygenase-1
Decompensation with structural damage
Apoptosis
Amyloid deposits
Lipid droplets
Glycogen deposits
Fibrosis

Based on the following studies: Weir and Bonner-Weir (1), Jonas et al. (8), and Laybutt et al. (13,14). C/EBPB, CAAT/enhancer binding protein-β; COX-2, cyclooxygenase-2; IAPP, islet amyloid polypeptide; IP3R-II, inositol phosphate 3 receptor; SERCA, sarcoendoplasmic reticulum Ca^{2+}-ATPase.

β-cell differentiation can be found at this stage, but insulin mRNA levels are protected, which probably allows insulin production to be reasonably well maintained for the degree of β-cell deficiency.

Hypothetical phase 3: severe decompensation. Severe decompensation occurs when glucose levels are clearly in the diabetic range. Except for β-cell hypertrophy, the islets look fairly normal. GIIS remains severely impaired, and now insulin responses to nonglucose secretagogues are inadequate when analyzed as a function of β-cell mass. β-Cells are degranulated, which coincides with a fall in insulin mRNA, pointing to decreased insulin synthesis. In this situation, β-cell differentiation is grossly deranged, with alteration of metabolic genes and key transcription factors, as well as increased expression of several important stress genes. Despite this result, some level of β-cell function and mass can be maintained for a long time, which allows enough insulin secretion to prevent complete metabolic decompensation with severe hyperglycemia and ketosis.

Hypothetical phase 4: decompensation with structural damage. Structural damage can be considered a separate group of phenomena, but it coexists with the functional compensation described above. Structural damage is still not well defined, and species differences make the situation complex. Studies in various rodent models show that islets can evolve from having relatively normal structure with abnormal function to a stage of obvious structural damage, with recently well-studied examples being sand rats (*Psammomys obesus*) (3), ZDF rats (4,5), and GK rats (6). Amyloid formation in human type 2 diabetes is a striking abnormality. Amyloid fibrils can have a destructive effect on β-cells, but we know little about the mechanisms responsible for their formation. Other pathology includes glycogen deposits, which used to be called hydropic degeneration. In some rodent models and in human pancreases, lipid droplets can be found in β-cells, which raises questions about lipotoxicity. Islet fibrosis can be found, but this too is poorly understood. There is much current interest in apoptosis of β-cells as a contributor to diabetes development. Unfortunately, it is difficult to quantitate apoptosis in chronic situations because the process is so short-lived. Although it is tempting to suggest that an increased rate of apoptosis must be important for the development of human type 2 diabetes, it is possible that limitations of islet neogenesis and β-cell replication are at least as important.

HYPOTHESIS THAT THE DIABETIC MILIEU LEADS TO LOSS OF β-CELL DIFFERENTIATION RESULTING IN FUNCTIONAL AND STRUCTURAL ABNORMALITIES

The foundation for this hypothesis is the conviction that diabetes originates from the failure to sufficiently increase β-cell mass to meet demand, with the resultant prediabetic or diabetic plasma milieu leading to a critical loss of β-cell differentiation (7,8). The most obvious explanation for this change is a rise in blood glucose levels. Although increases in nonesterified fatty acids (NEFAs) may also contribute, we have argued that hyperglycemia per se is the main culprit, thus justifying the term "glucose toxicity" (1,8). β-Cells have evolved toward a unique differentiation that optimizes GIIS, insulin synthesis, and the capacity to expand β-cell mass. Those genes that are highly expressed make proteins that favor delivery of glucose metabolites to mitochondria with complementary increased activity of electron shuttles to further enhance the ATP/ADP ratio. In contrast, the capacity to make lactate is reduced by suppression of lactate dehydrogenase (LDH), and the gluconeogenic enzymes, which would interfere with optimal insulin secretion, are also suppressed. Our prediction was that exposure of β-cells to even mild hyperglycemia would cause a change (or loss) of differentiation, with downregulation of genes that are usually overexpressed and upregulation of suppressed genes, resulting in serious disruption of function and structure.

The rat partial pancreatectomy model. We have increasingly relied on the 85–95% partial pancreatectomy (Px) model to study β-cell adaptation and changes in gene expression (8,9). Although there is active regeneration in the first 10 days after surgery, by 4 weeks, well-formed islets have been exposed to hyperglycemia for enough time to have a stable reproducible model of diabetes. An important advantage of this

model is that it is a pure model of the effects of reduced β-cell mass, without the confounding genetic variables found in ZDF rats, GK rats, gerbils, etc., or potentially confusing effects of streptozocin, as in the neonatal streptozocin model (10).

Loss of GIIS and β-cell hypertrophy after partial Px. In earlier studies, we and others found marked loss of GIIS with some preservation of secretion to challenges such as arginine 4–6 weeks after partial Px (9). We also documented increase in β-cell mass during the days/weeks after partial Px, with early increases in neogenesis and β-cell replication. More recently, we found that an important component of the increase in β-cell mass is β-cell hypertrophy, with an 85% increase in β-cell size being found 4 weeks after partial Px (8). Hypertrophy of β-cells is seen in a variety of other rat models, including the last term of pregnancy (11), after 96 h of glucose infusion (12), and in male ZDF rats (5). At 4 weeks after partial Px, although β-cell hypertrophy is striking, β-cell turnover is surprisingly normal, with an unchanged replication rate. In addition, the frequency of apoptotic bodies is not obviously increased.

This situation seems to be a complex combination of deleterious decompensation and successful adaptation. On one hand, β-cell mass cannot be increased enough to prevent hyperglycemia, loss of GIIS is found, and there is partial loss of insulin gene expression that could limit overall insulin production. On the other hand, the hypertrophy that occurs must contribute to insulin production and there is no striking increase in apoptosis, which means that β-cell mass can be maintained at a level that prevents severe diabetic decompensation with ketosis but is inadequate to prevent hyperglycemia. Although we have intensively studied only the 4-week time point after partial Px, mild hyperglycemia persists for many months in this model. There are thus some parallels between the stability of the partial Px model and type 2 diabetes in humans in which β-cell function can persist for decades. It is not clear whether β-cell hypertrophy is often found in human type 2 diabetes, but somehow insulin production is at least partially preserved through mechanisms that allow maintenance of β-cell mass at a suboptimal level.

Changes in gene expression of metabolism genes and transcription factors in the partial Px model. In an extensive recent study, our hypothesis about the importance of the loss of β-cell differentiation for the dysfunction of β-cells in diabetes has been greatly strengthened (8). Our predictions about which genes would be downregulated and which would be upregulated have been largely fulfilled. Thus, there was downregulation of the genes for insulin, GLUT2, glucokinase, mitochondrial glycerol phosphate dehydrogenase, and pyruvate carboxylase, as well as genes that were less predictable to us, such as the potassium channel Kir6.2, the voltage-dependent calcium channel α1D, and calcium ATPase (SERCA3). Genes involved in the transcription of insulin and various metabolic enzymes were also downregulated, including PDX-1, Nkx6.1, Pax6, Beta2, HNF1α, HNF4α1, HNF4α2/5, and HNF3β. In contrast, a group of suppressed genes was markedly upregulated including hexokinase 1, glucose-6-phosphatase, and LDH-A. We also made the potentially important observation that the expression of c-Myc was markedly increased.

Changes in expression of genes concerned with lipid metabolism. Using the 4-week time point after partial Px, it was found that expression of the peroxisome proliferator–activated receptors (PPARs) were altered. In particular, the expression of PPAR-γ, which is involved in lipogenesis, was threefold increased, whereas expression of PPAR-α, which is involved in lipid catabolism, was markedly suppressed (13). These changes were accompanied by modest increases in the gene expression of acetyl-CoA carboxylase, carnitine palmitoyltransferase-1, and fatty acid synthase and a reduction of acyl-CoA oxidase. An interesting and possibly related finding is that the expression of uncoupling protein 2 is elevated in the islets of the partial Px model. The influence of these changes on β-cell function is unknown, but surprisingly, islet triglycerides are normal.

Activation of stress genes in the diabetic state. Islets obtained 4 weeks after partial Px also had elevated gene expression of a variety of stress factors including the antioxidants Heme oxygenase-1, Mn-superoxide dismutase, and glutathione peroxidase, whereas catalase was unchanged (14). These changes were accompanied by a major increase in the expression of the inducible isoform of nitric oxide synthase (iNOS). Other interesting changes included an increase in the gene expression of Fas and the antiapoptotic gene A20, but there were no meaningful changes in Bcl-2 or heat shock protein 70. These findings indicate a profound change in the β-cell phenotype in this model of diabetes, which could be beneficial or detrimental. Some of these changes could make β-cells more vulnerable to immunological injury or to apoptosis and contribute to the pathogenesis of type 2 diabetes. On the other hand, some of the changes should be protective.

POTENTIAL MECHANISMS FOR β-CELL DERANGEMENTS IN THE PARTIAL Px MODEL

Chronic hyperglycemia and altered β-cell phenotype. The pathways from hyperglycemia to β-cell dedifferentiation, hypertrophy, and activation of the stress response are poorly understood, but some possible links are depicted in Fig. 1. Some of the leading candidates that could alter gene expression are calcium changes, redox activity, trichloroacetic acid cycle activity, or glycation that may or may not be linked to *O*-linked glycosylation through excessive production of glucosamine by the enzyme glutamine:fructose-6-phosphate amidotransferase. These candidates may activate the mitogen-activated protein kinases or protein kinase C and in other ways lead to the generation of reactive oxygen species.

Why is GIIS lost in the diabetic state? It is not surprising that dedifferentiation of β-cells would lead to a more serious loss of GIIS than non–glucose-mediated secretion. The mechanisms that evolved for GIIS appear to be unique and probably sensitive to perturbation, whereas the pathways required for non–glucose-mediated secretion are probably ubiquitous and robust. We still do not know whether one defect plays a primary role in destroying GIIS; moderate changes at multiple points could paralyze the system. If LDH were active, there could be a leak of carbon molecules from glucose that could otherwise be oxidized in mitochondria to make ATP, thus making glucose less effective. Another possibility is that LDH activity could oxidize NAD(P)H when producing lactate from pyruvate, thus limiting the shuttling of electrons to mitochondria with resultant reduction of ATP formation. It could be predicted that overexpression of LDH would lead to defective GIIS. Indeed, impairment of GIIS was found when LDH was overexpressed in MIN-6 cells (15). However, when

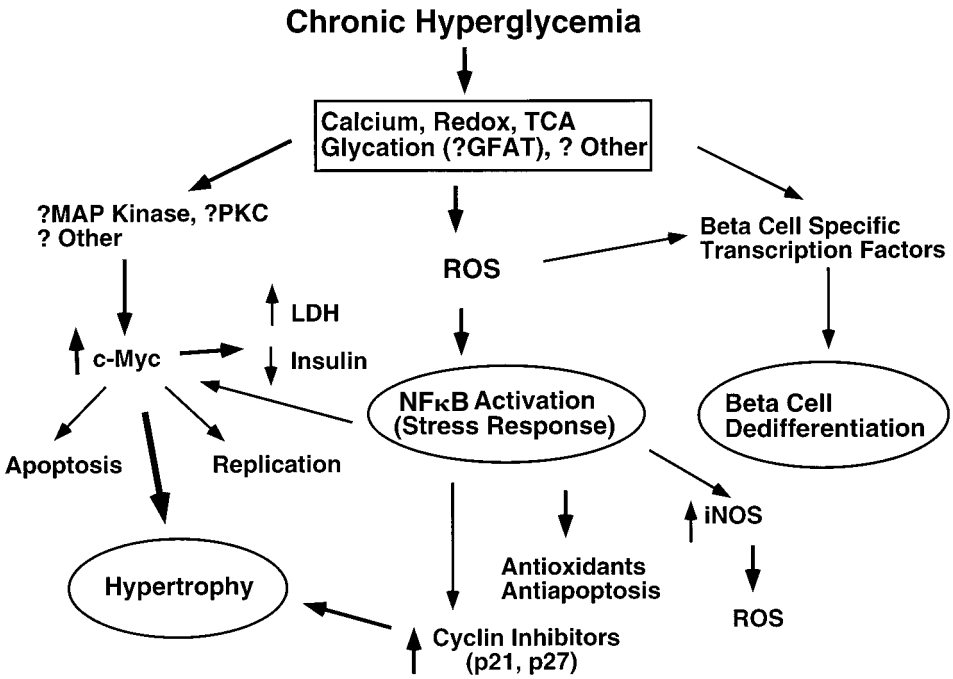

FIG. 1. Hypothetical scheme about how chronic hyperglycemia might lead to alterations of the β-cell phenotype in diabetes, which include β-cell dedifferentiation, hypertrophy, and activation of stress response genes. GFAT, fructose-6-phosphate amidotransferase; iNOS, inducible isoform of nitric oxide synthase; MAP, mitogen-activated protein; PKC, protein kinase C; ROS, reactive oxygen species; TCA, trichloroacetic acid.

rabbit LDH-A was overexpressed in INS-1 cells with adenovirus infection, GIIS was unaffected, in spite of a marked increase in enzyme activity (16). It may be questioned whether INS-1 cells behave as normal β-cells and whether LDH is active within the transfected cells, even though cell homogenates have abundant active enzyme.

Potential roles for c-Myc in β-cells. c-Myc has an important influence on cell cycle progression, cell differentiation, and the process of apoptosis (17–20). Myc proteins are regulatory proteins with high binding affinity to E-box related sequences (18). The *c-myc* gene is typically activated when quiescent cells are pushed toward proliferation. A well-demonstrated role for c-Myc in glycolysis is for activation of LDH-A (21,22); therefore, it seems likely that increased expression of c-Myc in β-cells could influence multiple enzymes of importance for insulin secretion.

β-Cell proliferation, hypertrophy, and apoptosis: potential contributions of c-Myc. The factors that push β-cells toward division or hypertrophy are poorly understood. Newly formed β-cells must have a finite number of potential divisions and then reach terminal senescence compatible with normal function for some finite period of time. At some point, apoptosis occurs but at a rate that equals the rate of β-cell formation if β-cell mass is to be kept stable.

β-Cell hypertrophy is a compensatory mechanism activated by increased demand, which probably occurs in β-cells that are in the stage of terminal senescence, thus not able to divide. Mechanisms of cellular hypertrophy are complex and seem to differ among various tissues, but all involve cdk inhibitors, which interfere with the normal progression to the G_1 and S phases of the cell cycle (23–27). The finding that c-Myc increases are associated with β-cell hypertrophy suggests that c-Myc plays an important role but must work in concert with other signaling pathways. Factors other than glucose are known to influence β-cell replication, with the best factors studied being growth hormone, prolactin, and glucagon-like peptide 1 (28,29). Other factors that orchestrate this process will be discovered, but our bias is that glucose will have a dominant influence, with the other factors playing a more supportive role. The links between glucose metabolism and the signal transduction pathways responsible for cell replication and hypertrophy are ill-defined. An influence from c-Myc could be exerted at several points (21,22), including activation of enzymes important for DNA synthesis such as ornithine decarboxylase. Another control point is at the level of translation (23).

Paradoxically, c-Myc can also induce apoptosis by mechanisms partially independent of those of proliferation (23,24). Fibroblasts with activated *c-myc* have been rendered sensitive to tumor necrosis factor–induced apoptosis (22). When c-Myc is conditionally overexpressed in the β-cells of adult transgenic mice, apoptosis and diabetes appear (30). This raises important questions about why the elevated c-Myc in the partial Px model is associated with hypertrophy but not increased apoptosis.

ADVERSE INFLUENCE OF THE DIABETIC MILIEU ON β-CELL FUNCTION: GLUCOSE TOXICITY, LIPOTOXICITY, OR BOTH?

Current debate about β-cell dysfunction in diabetes focuses on the phenomena of glucose toxicity (1,31) and lipotoxicity (32,33). With glucose toxicity, it is thought that chronically elevated levels of glucose can exert toxic effects on β-cells, whereas with lipotoxicity, it is postulated that elevated levels of circulating NEFAs and possibly other lipid moieties have a deleterious influence on function (32,33). Probably the best demonstration of functional adversity of the diabetic state in humans is improved insulin secretion in response to meals or

oral glucose after hyperglycemia was reduced by diet, sulfonylurea treatment, or insulin administration (34). Hyperglycemia is virtually always associated with a reduction of GIIS (1,31). This abnormal secretion has been found in all forms of human diabetes, including type 2 diabetes, early type 1 diabetes, and diabetes in individuals with failing pancreas transplantation. Similar abnormalities have been found in primates, dogs, and many rodent models.

Intracellular lipids. Intracellular lipids seem very important for β-cell function. There has been much interest in the hypothesis that extracellular NEFAs can enter β-cells and be converted to acyl-CoAs, which can be further modified to serve as mediators of secretion (32,33). Other intracellular lipid mediators must also be important, such as phospholipids, diacylglycerol, prostaglandins, or leukotrienes, which can be activated or suppressed by signals such as nutrients, hormones, or neurotransmitters. 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 to serve as intracellular lipid mediators of insulin secretion (35). However, the importance of this pathway has recently been questioned (36). 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, which could influence insulin secretion. Another pathway of interest is the production of fatty acids from β-cell triglyceride stores through activation of hormone-sensitive lipase.

Effects of extracellular NEFAs in the fasting state. Probably the clearest demonstration of an important influence of NEFAs on β-cell function comes from studies of the fasting state. In both rats and humans, NEFAs play a crucial role in maintaining insulin secretion during fasting when glucose levels are low, which may provide an important brake to excessive ketogenesis (33,37,38). Some level of NEFA must be needed to act in a permissive manner on β-cell metabolism, but it is not clear how much influence can be exerted by fluctuation of NEFA levels within the physiological range.

Effects of NEFAs in obesity and diabetes: the complexity of the lipotoxicity hypothesis. The hypothesis that high NEFA levels can have a deleterious effect on β-cell function is supported by the finding of high NEFA levels and increased islet content of triglycerides in ZDF diabetic rats (39). It spite of the correlation often found between high NEFA levels and impaired β-cell function, such as a loss of GIIS, it has been difficult to prove a cause-and-effect relationship and to understand how NEFA levels are related to the phenomenon of glucotoxicity. For example, there are several situations in which there is no clear relationship between NEFAs and the altered β-cell function associated with hyperglycemia. In our studies with a rat partial Px model, the characteristic selective loss of GIIS can be found even with relatively modest elevations of plasma glucose levels (9). These secretory changes, which are associated with loss of β-cell differentiation, are tightly associated with rising glucose concentrations, but NEFA levels were not found to be increased in this model (8) nor were there increases in plasma triglyceride levels or islet triglyceride content. In another approach, glucose was infused into rats for 48 h, which led to suppression of NEFA levels, but abnormal GIIS could still be demonstrated (40). These studies indicate that the typical abnormalities of insulin secretion found in diabetes can occur in the absence of any increase in plasma NEFAs.

In rodent studies using exogenous lipids, it was found both in vivo and in vitro that NEFAs can have a short-term stimulatory effect on insulin secretion (41). However, with in vivo NEFA infusions for 48 h or the addition of NEFAs to cultured islets, inhibitory effects have been found (33). In addition to a chronic inhibitory effect of NEFAs on secretion, inhibition of proinsulin biosynthesis has been reported (42–44). It is postulated that the insulin deficiency of diabetes (either absolute or relative) is responsible for circulating NEFA levels that are even higher than those found in obesity, thus producing an inhibitory rather than stimulatory effect on β-cells (33,41). Another complexity is that the combination of high glucose and NEFA levels may have synergistic or complementary inhibitory influences on insulin secretion (41).

To understand the effects of lipids, it is important to distinguish between the effect of endogenous versus exogenous fatty acids. Studying the effects of exogenous fatty acids is problematic because it is difficult to mimic physiological or pathophysiological changes; circulating NEFAs contain a variety of fatty acids, each of which could exert a different effect on insulin secretion, as found for fatty acid length (33). When NEFA levels are raised with the infusion of a lipid emulsion, the effects on insulin secretion might be much different from a comparable rise in endogenous NEFA. The situation is even more problematic with in vitro studies in which it is next to impossible to mimic normal concentrations of fatty acids that are not protein bound.

Effects of elevated NEFA levels in human obesity and diabetes. It has yet to be shown that the decrease of GIIS in impaired glucose tolerance and early diabetes is related to increased NEFA levels. Normoglycemic obese subjects have elevated NEFA levels and increased GIIS, with evidence that the NEFAs in this situation contribute to both hyperinsulinemia and increased GIIS (1). In contrast, complete loss of GIIS is found, with only modest increases in plasma glucose concentrations that do not even meet impaired glucose tolerance criteria (45). NEFA levels in this state of mild hyperglycemia with abolished GIIS are not higher than those in normoglycemic obesity. Thus, until more detailed studies are performed, it must be concluded that the early loss of GIIS in humans is well correlated with rising plasma glucose levels but poorly correlated with NEFA levels. These findings implicate glucose toxicity rather than lipotoxicity in the early loss of GIIS in humans but do not rule out an important role for NEFAs as a contributing variable or permissive factor or exclude an important role for NEFAs when the diabetic state is more severe. Although some pieces of the puzzle are starting to fall into place, there is much to be learned about how glucose and lipids contribute to the deterioration of β-cell function in diabetes.

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