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β-Cell Failure in Diabetes and Preservation by Clinical Treatment

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There is a progressive deterioration in β-cell function and mass in type 2 diabetics. It was found that islet function was about 50% of normal at the time of diagnosis, and a reduction in β-cell mass of about 60% was shown at necropsy. The reduction of β-cell mass is attributable to accelerated apoptosis. The major factors for progressive loss of β-cell function and mass are glucotoxicity, lipotoxicity, proinflammatory cytokines, leptin, and islet cell amyloid. Impaired β-cell function and possibly β-cell mass appear to be reversible, particularly at early stages of the disease where the limiting threshold for reversibility of decreased β-cell mass has probably not been passed.

Among the interventions to preserve or "rejuvenate" β-cells, short-term intensive insulin therapy of newly diagnosed type 2 diabetes will improve β-cell function, usually leading to a temporary remission time. Another intervention is the induction of β-cell "rest" by selective activation of ATP-sensitive K+ (K_{ATP}) channels, using drugs such as diazoxide.

A third type of intervention is the use of antiapoptotic drugs, such as the thiazolidinediones (TZDs), and incretin mimetics and enhancers, which have demonstrated significant clinical evidence of effects on human β-cell function.

The TZDs improve insulin secretory capacity, decrease β-cell apoptosis, and reduce islet cell amyloid with maintenance of neogenesis. The TZDs have indirect effects on β-cells by being insulin sensitizers. The direct effects are via peroxisome proliferator-activated receptor γ activation in pancreatic islets, with TZDs consistently improving basal β-cell function. These beneficial effects are sustained in some individuals with time. There are several trials on prevention of diabetes with TZDs.

Incretin hormones, which are released from the gastrointestinal tract in response to nutrient ingestion to enhance glucose-dependent insulin secretion from the pancreas, aid the overall maintenance of glucose homeostasis through slowing of gastric emptying, inhibition of glucagon secretion, and control of body weight. From the two major incretins, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), only the first one or its mimetics or enhancers can be used for treatment because the diabetic β-cell is resistant to GIP action. Because of the rapid inactivation of GLP-1 by dipeptidyl peptidase (DPP)-IV, several incretin analogs were developed: GLP-1 receptor agonists (incretin mimetics) exenatide (synthetic exendin-4) and liraglutide, by conjugation of GLP-1 to circulating albumin. The acute effect of GLP-1 and GLP-1 receptor agonists on β-cells is stimulation of glucose-dependent insulin release, followed by enhancement of insulin biosynthesis and stimulation of insulin gene transcription. The chronic action is stimulating β-cell proliferation, induction of islet neogenesis, and inhibition of β-cell apoptosis, thus promoting expansion of β-cell mass, as observed in rodent diabetes and in cultured β-cells. Exenatide and liraglutide enhanced postprandial β-cell function.

The inhibition of the activity of the DPP-IV enzyme enhances endogenous GLP-1 action *in vivo*, mediated not only by GLP-1 but also by other mediators. In preclinical studies, oral active DPP-IV inhibitors (sitagliptin and vildagliptin) also promoted β-cell proliferation, neogenesis, and inhibition of apoptosis in rodents. Meal tolerance tests showed improvement in postprandial β-cell function.

Obviously, it is difficult to estimate the protective effects of incretin mimetics and enhancers on β-cells in humans, and there is no clinical evidence that these drugs really have protective effects on β-cells. (*Endocrine Reviews* 28: 187–218, 2007)

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Abbreviations: AGE, Advanced glycation end-product; AIR, acute insulin response; AIR_g, AIR to iv glucose; AUC, area under the curve; CoA, coenzyme A; CSII, continuous sc insulin infusion; DM2, type 2 diabetes mellitus; DPP-IV, dipeptidyl peptidase-IV; ER, endoplasmic reticulum; FA, fatty acid; FFA, free FA; FSIVGTT, frequently sampled iv glucose tolerance test; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; HbA1c, glycated hemoglobin; hIAPP, human IAPP; HOMA, homeostasis model assessment; HOMA-β, HOMA of β-cell function; HOMA-IR, HOMA of insulin resistance; IAPP, islet amyloid polypeptide; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; ISR, insulin secretion rate; NF-κB, nuclear factor κB; PACAP, pituitary adenylate cyclase-activating peptide; PDX-1, pancreas duodenum homeobox-1; PI/IRI ratio, proinsulin to total immunoreactive insulin ratio; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; S_i, sensitivity index; TZD, thiazolidinedione; ZDF, Zucker diabetic fatty (rat).

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I. Introduction

IT IS WELL KNOWN that there is a progressive deterioration in β-cell function over time in type 2 diabetes mellitus (DM2), as indicated by the United Kingdom Prospective Diabetes Study (UKPDS) (1, 2), regardless of therapy allocation, albeit conventional (mainly diet), insulin, chlorpropamide, glibenclamide, or metformin treatment. Moreover, the pancreatic islet function was found to be about 50% of normal at the time of diagnosis, independent of the degree of insulin resistance, with the reduction in function probably commencing 10–12 yr before diagnosis and aggravated by increasing fasting plasma glucose levels (3). In the cited work (1–3), along with other studies, particularly those of Butler *et al.* (4) evaluating human pancreatic tissue from 124 autopsies in obese impaired fasting glucose (IFG), obese DM2, obese nondiabetic, lean DM2, and lean nondiabetic subjects, and observing a reduction in β-cell mass in impaired glucose tolerance (IGT) (40%) being greater still in DM2 (60%) compared with their respective nondiabetic control group, the underlying mechanism was found to be increased β-cell apoptosis, whereas new islet formation and β-cell replication (normalized to relative β-cell volume) remained normal or increased. Also, islet amyloid deposits were present in the majority of DM2 cases, compared with nondiabetic controls, but were not increased in obese IGT subjects. The role of declining β-cell mass and function in the development of DM2 has drawn attention to the need for agents that can address this process. Emerging evidence suggests that several medical therapies could offer specific benefits by preventing or delaying the decline in β-cell mass/function, thereby representing a substrate for early intervention efforts to lower the burden of DM2. Moreover, in individuals with established DM2, the inhibition of the increased apoptosis may lead to restoration of β-cell mass because islet neogenesis appeared to be unaffected.

II. Decline of β-Cell Function

A. Epidemiology

As outlined above, β-cell function has been shown by the UKPDS (1–3) to be low at diagnosis and to decline, associated with a deterioration in glycemic control, with increasing duration of diabetes, notwithstanding the use of a number of therapies existing at the time the study was performed. Moreover, the importance of maintaining adequate β-cell function to offset the need for combination therapy is clearly demonstrated in the UKPDS findings; after 6 yr of sulfonylurea monotherapy, 62% of patients with baseline β-cell function [updated homeostasis model assessment (HOMA 2); Ref. 5] below 27% (in relation to a reference group of normoglycemic subjects aged 18–25 yr) required additional therapy to maintain glycemic targets. In contrast, only 28% of those patients with β-cell function above 55% required additional therapy (6, 7). The Skaraborg Hypertension and Diabetes Project, a Swedish community-based follow-up of 376 primary care patients with DM2, found glycated hemoglobin (HbA1c) levels to increase over time in association with a corresponding decline in β-cell function (8). An HbA1c level of 6.5% or higher was associated with impairment of β-cell function (HOMA) (9) and longer duration of diabetes, irrespective of age and gender. These associations are likely to be causal because β-cell function in DM2 is diminished, as indicated previously and discussed below.

B. Pathophysiology

Insulin resistance and impaired insulin secretion are usually present in patients with classical DM2 as well as in most individuals with IGT. The relative contributions of impaired β-cell function and insulin sensitivity in DM2 have been controversial, especially over what constitutes the primary genetic defect. Current evidence points to β-cell dysfunction as the first demonstrable defect with limited capacity to compensate for the presence of insulin resistance. However, the modulating effect of insulin sensitivity on β-cell function has to be considered for the assessment of insulin release in individuals at risk of developing DM2. The nature of this relationship is such that insulin sensitivity and β-cell function are inversely and proportionally related, whereby the product of these two parameters is constant, being referred to as the disposition index (10), and in turn can be interpreted as a measure of the ability of the β-cells to compensate for insulin resistance. Mathematically, this relationship is described by the hyperbolic relationship between the acute insulin response (AIR) and the metabolic action of insulin to stimulate glucose disposal (M) and is referred to as glucose homeostasis, with glucose concentration assumed to remain constant along the hyperbola. According to Stumvoll *et al.* (11), because glucose is one of the signals stimulating AIR in response to decreasing M, hypothetically, as with any normally functioning feed-forward system, AIR should not fully compensate for worsening M, since this would remove the stimulus for compensation. Evidence was provided from cross-sectional, longitudinal, and prospective data on Pima Indians (n = 413) and Caucasians (n = 60), demonstrating that fasting and postprandial glucose concentrations increase with decreasing M despite normal compensation of AIR. To denote this physiological adaptation to chronic stress (insulin resistance), Stumvoll *et al.* (11) have proposed the term "glucose allostasis." Allostasis (stability through change) would ensure continued homeostatic response (stability through remaining the same) to acute stress at some cumulative costs to the system. With increasing severity and over time, the allostatic load (increase in glycemia), while increasing the burden that persistent increases in insulin secretion place on the β-cell and perhaps other parts of the system, may have pathological consequences, such as the development of DM2. However, not all insulin-resistant subjects develop this disease where, in approximately 80% of cases, increased insulin secretion is sufficient to offset overt hyperglycemia (12). Nevertheless, in some individuals, failure of the β-cell to maintain adequate supplies of insulin triggers the development of DM2. Prospective studies (average follow-up of 7 yr) in Pima Indians, a very high-risk population for developing DM2, have shown that impaired β-cell function, as assessed by AIR to glucose (AIR_g), was a predictor of developing diabetes, independent of obesity and insulin resistance (prevalent in this population), evaluated by the hyperinsu-

linemic glucose clamp (13). In a longitudinal study of 48 Pima Indians with normal glucose tolerance followed for an average of 5 yr with multiple measurements performed during this period, 17 of these individuals progressed from normal glucose tolerance through IGT to diabetes and insulin secretion declining progressively by 78%, whereas insulin sensitivity declined by 14%, associated with an increase in mean body weight from 93.7 to 106.9 kg. In the remaining 31 individuals who did not develop diabetes, a similar 11% decrease in insulin sensitivity was associated with a 30% increase in insulin secretion. At baseline, however, when all subjects had normal glucose tolerance, those individuals who subsequently progressed to diabetes had β-cell function markedly decreased for their degree of insulin resistance compared with those subjects who did not progress over time (14). From this and other studies, some of them previously reviewed, substantial evidence has accumulated showing that deterioration in β-cell function typically precedes hyperglycemia and has an important influence on its course. Furthermore, most of the available evidence supports the view that DM2 is a heterogeneous disorder in which the major genetic factor is impaired β-cell function, whereas insulin resistance is the major acquired factor at least in Caucasian populations. However, this may differ in a high-risk non-Caucasian population with severe genetic predisposition to insulin resistance, *e.g.*, Indians (15). Compelling evidence that impaired insulin secretion is the major genetic trait has been provided via the assessment of the degree of β-cell compensation to reduced insulin sensitivity among normal glucose-tolerant offspring or siblings of Caucasian familial DM2 kindreds (*i.e.*, families with at least two siblings diagnosed with DM2 before age 65 yr) (16, 17). In effect, glucose-tolerant members of Caucasian familial DM2 kindreds (but not similar obese control subjects) exhibit impairment in β-cell compensation for obesity-related insulin resistance. Furthermore, the heritability of insulin secretion is 67% (16).

The demonstration of β-cell dysfunction before the onset of DM2 and the recognition of the confounding effect of obesity on insulin sensitivity cast doubt on the theory that insulin resistance is the primary cause of DM2 (15). Several studies of the insulin-secretory capacity of glucose-tolerant individuals with a predisposing ethnicity or a family history of DM2 have indicated that β-cell dysfunction, like insulin resistance, occurs in genetically predisposed individuals with normal glucose tolerance, well before the emergence of overt diabetes (see references cited in Ref. 15). Of note in these studies is the fact that there were no significant differences with respect to insulin sensitivity between the predisposed subjects and the control groups (glucose-tolerant subjects with no family history of DM2), suggesting that alterations in insulin secretion precede insulin resistance in patients at risk for developing DM2.

Considering that the vast majority (85–90%) of DM2 patients are obese and that intraabdominal obesity, being the major determinant of insulin resistance (not only in obese individuals but also in apparently nonobese individuals who have evidence of increased abdominal fat) represents a significant genetic component (18), then insulin resistance occurring as a result of this could be considered genetic. Nevertheless, most obese individuals who are insulin resistant are not diabetic and what distinguishes them from those who are diabetic is, as indicated previously, the ability of their pancreatic β-cells to compensate for insulin resistance. Although insulin resistance may be critical for the development of diabetes, irreversibly impaired insulin secretion plays, as expected, an essential role in diabetes persistence after weight loss and restoration to normal or near-normal insulin sensitivity (19).

Regarding the 10–15% of patients with DM2 who are not obese, whether they become diabetic depends on the balance between the severity of insulin resistance and the ability of the β-cell to compensate for the insulin resistance, as in the case of obese individuals. A spectrum could exist; at one extreme, the nonobese individual may be insulin resistant before or after the development of diabetes. High-fat diet, decreased physical fitness, increase in visceral fat, smoking, pregnancy, certain medications, and hyperglycemia itself (glucose toxicity) can all cause insulin resistance. At the other extreme of the spectrum, insulin resistance is absent, and the immediate cause of diabetes is then impaired insulin secretion. Only a minority of DM2 patients belong to this group, such as those with maturity-onset diabetes of the young and nonobese blacks and Swedes. There are no well-documented cases in which impaired insulin secretion is absent and insulin resistance is the immediate cause of DM2 (20).

The defect of insulin secretion in DM2 is related to two confounding components: insulin deficiency and disturbed kinetics of secretion combined with impaired glucose stimulus-secretion coupling (21).

1. Insulin deficiency. Insulin deficiency in DM2 is relative to the prevailing hyperglycemia and its measurement (immunoreactive or "true" insulin), at least in the early stages of the disease where these could show normal or increased values. However, because this level of insulin is insufficient to control the prevailing hyperglycemia, it would indicate that "insulin deficiency" is more evident when "true" insulin is measured. Besides, DM2 is characterized by an increased fasting ratio of proinsulin [long-chain precursor of insulin which is processed in the β-cell to produce equimolar quantities of mature ("true") insulin and C-peptide] to total immunoreactive insulin (reflecting all immunoreactive species including proinsulin and conversion intermediates) (PI/IRI) indicating a reduced processing of proinsulin, which correlates with β-cell dysfunction and is predictive of disease development (22). From the observed significant negative correlation between fasting PI/IRI ratio and the acute maximal insulin responses to arginine and glucose in DM2, it has been proposed that the PI/IRI ratio, a relatively easy parameter to obtain, indicates a reduced β-cell capacity in patients with DM2 (23).

The determination of insulin in fasting plasma samples has the caveat that in this situation the demand for insulin is at its lowest. Only after stimulation does the magnitude of the insulin deficiency become fully manifested.

2. β-Cell secretory defect. It is well recognized that the relatively selective loss of glucose stimulation manifested by the lack of the first phase of secretion and decreased second

phase (24) in DM2 patients as well in subjects with IGT, demonstrates multiple abnormalities in both qualitative and quantitative measures of insulin secretion. Prandial insulin release after an oral glucose load or mixed meal is also delayed (25), permitting the elevated postprandial glucose concentrations characteristic of these individuals, despite relatively normal fasting glucose levels in IGT (26). Sensitivity to nonmetabolizable stimuli such as arginine remains normal, although the magnitude of glucose-dependence of the insulin response may be attenuated. Baseline insulin secretion is normally pulsatile, with a periodicity of 5 to 10 min. The defective release of insulin in DM2 involves reduced diurnal oscillations (27), impaired ultradian oscillations (28), and their reduced entrainment (29) as well as impaired rapid pulsatile secretion (30). The hypothesis of impaired insulin oscillations as a primary β-cell defect in DM2 was supported by the observation of defective oscillatory insulin release in first-degree relatives of such patients (31). However, Ritzel *et al.* (32) observed that the parameters of pulsatile insulin secretion were similar in normal subjects and DM2 and IGT subjects by deconvolution, spectral, and autocorrelation analysis and approximate entropy. This could also partly account for some of the insulin resistance in a number of studies showing that equal amounts of insulin presented to target organs have improved action when delivered in a pulsatile manner (see references cited in Ref. 33). These defects of secretion, particularly the lack of response to glucose, acting in concert with the insulin deficiency along with disproportionate amounts of proinsulin, compromise the ability of residual β-cells to provide sufficient insulin to achieve euglycemia in the presence of insulin resistance or otherwise.

C. Decreased β-cell mass

Insights into changes that occur in the β-cells have been gained mainly from diabetic animal models that support reduced β-cell mass as a significant contributory factor to diminished insulin secretion in DM2. Many of the signals that regulate the balance of replication of the existing cells/neogenesis from stem cells and cell death through necrosis or apoptosis and thus determine net β-cell mass have been identified, but it is less clear which of these factors contribute to the failure of β-cell mass augmentation. Obviously, diminished proliferation or increased apoptosis or both will result in lower β-cell mass. There is evidence that increased β-cell apoptosis in the Zucker diabetic fatty rat (ZDF), an animal model of DM2, underlies the decreased β-cell mass seen in these animals (34). Autopsy data examining β-cell mass in obese and diabetic humans have been sparse and sometimes contradictory. It is difficult to distinguish between the two mechanisms, cell formation and cell death, in human tissue sections mainly because dead cells are removed rapidly from the islet by macrophages and neighboring cells, making it hard to quantify cell death. Although cell proliferation can be quantified in tissue sections using markers, this only provides a single snapshot in time that probably does not reflect accurately the complex dynamic of the process (21). Recently, the study by Butler *et al*. (4), as mentioned in *Section I*, showed relative β-cell volume to be increased by 50% in obese compared with lean nondiabetic pancreas and was attributed to increased neogenesis of islets from exocrine ductal tissue. Relative β-cell volume was decreased in obese IGT and more in obese DM2 compared with nondiabetic controls, being attributable to accelerated cell apoptosis. Other studies (35, 36) have also shown a reduction in β-cell mass in DM2, leading to a rapid emphasis of this etiological factor.

Potential mechanisms of β-cell adaptation can be summarized as follows (37): 1) functional up- and down-regulation of secretory machinery; 2) β-cell recruitment; 3) β-cell turnover and islet mass changes [evidence mainly from rodent work: increasing/decreasing cell size; β-cell turnover; *de novo* differentiation (neogenesis) of β-cells within islets and new islets budding from exocrine acinar cell pancreas; and β-cell death by necrosis *vs.* apoptosis (programmed β-cell death)].

Opinions diverge regarding the relative contribution of a decrease in cell mass *vs.* an intrinsic defect in the secretory machinery. A decrease in β-cell mass is likely to play a role in the pathogenesis of human DM2 as it does in rodent models of the disease, as indicated above. However, in contrast with type 1 diabetes, which has β-cell mass reduction of 70–80% at the time of diagnosis, DM2 initial pathological studies suggested β-cell loss of 25–50% (38, 39), although this has been disputed by others (40). Because β-cells cannot be measured *in vivo*, it remains unclear whether DM2 has a lower β-cell mass early in life, has failed to increase β-cell mass in the face of a given insulin resistance, or has a progressive β-cell loss as suggested by epidemiological evidence (1–3). Moreover, the secretion defect is probably more severe than could be accounted for solely by the reduction in β-cell mass in DM2 (41). Based on results obtained in rodent models of the disease and cultured rodent and human islets, it seems reasonable to assume that dyslipidemia and hyperglycemia negatively affect β-cell mass by increasing β-cell apoptosis in human DM2 (21, 42). *Section III* discusses recent hypotheses on the mechanisms of glucotoxicity and lipotoxicity besides other factors for progressive loss of β-cell function and mass.

Summary/conclusions. There is a progressive deterioration in β-cell function over time in DM2, independent of the type of treatment, where pancreatic islet function has been found to be about 50% of normal at the time of diagnosis, regardless of the degree of insulin resistance, as indicated by the UKPDS. The decline of β-cell function, the first demonstrable defect, is the limited capacity to compensate for the presence of insulin resistance, both usually present in classical DM2 as well as in most individuals with IGT. Most of the evidence supports the view that DM2 is a heterogeneous disorder in which the major genetic factor is impaired β-cell function where insulin resistance would be the major acquired factor at least in Caucasians. The defect of insulin secretion in DM2 is related to two confounding components: insulin deficiency and β-cell secretory defect. On the other hand, there is an impaired glucose sensing in the α-cells leading to hyperglucagonemia.

The reduction of β-cell mass is attributable to accelerated apoptosis. Regarding the relative contribution of a decrease in cell mass *vs.* an intrinsic defect in the secretory machinery, the latter is probably more severe than could be accounted for by the reduction in β-cell mass in DM2.

III. Factors for Progressive Loss of β-Cell Function and Mass

A. Glucotoxicity

Because glucose is the key physiological regulator of insulin secretion, it appears logical that it also regulates the long-term adaptation of insulin production by regulating β-cell turnover. However, it is important to stress that in human and *Psammomys obesus* β-cells *in vitro*, graded increase in glucose from a physiological concentration of 5.6 to 11.2 mmol/liter and above induces apoptosis (43, 44), whereas in rat islets the same graded glucose increment decreases apoptosis (45), indicating that glucose affects survival of islets differently in both species, which has lead to some confusion in the field. This difference in glucose sensitivity between *P. obesus* and rat islets highlights the importance of genetic backgrounds. In effect, although glucose was capable of inducing β-cell apoptosis in most batches of human islets studied by Donath *et al.* (21, 46), striking variations were observed in the magnitude of this response. All this points to a limitation in most studies performed in cell lines and rodent and human islets. Glucotoxicity of the islets can be defined as nonphysiological and potentially irreversible β-cell damage caused by chronic exposure to supraphysiological glucose concentrations along with the characteristic decreases in insulin synthesis and secretion caused by decreased insulin gene expression (47). In this context it is important to consider the possible detrimental effect on β-cell turnover of transient postprandial glycemic excursions early in the development of overt diabetes. This could perhaps underlie the decrease in β-cell mass documented in patients with IGT, the earliest manifest stage of DM2 (4). β-Cell exhaustion, according to Robertson *et al.* (47), refers to a physical depletion of β-cell insulin stores secondary to prolonged chronic stimulation with glucose or nonglucose secretagogues, so that insulin secretion is not possible, even if the β-cell were to become resensitized to glucose. An important distinction between β-cell exhaustion and glucose toxicity is that the exhausted islet has no defect in insulin synthesis, and therefore cell function fully recovers as it rests. Glucose toxicity, on the other hand, implies the gradual, time-dependent establishment of irreversible damage to cellular components of insulin production and consequently to insulin content and secretion (47).

The fact that glucose induces apoptosis in β-cells is probably linked to the relative specificity of this toxicity toward the β-cell but not to other islet or most nonislet cell types. The β-cell is extremely sensitive to small changes in ambient glucose. When these changes are of short duration and lie within the physiological range, such as after a meal, they lead to insulin secretion. When of longer duration and more pronounced in magnitude, perhaps they are translated by the β-cell glucose-sensing pathways into proapoptotic signals (21). One such signal might be endoplasmic reticulum (ER) stress triggered by an increase in insulin biosynthesis (48) leading to increased insulin secretion as well as increased proinsulin biosynthesis to replenish β-cell insulin stores. The increase in proinsulin biosynthesis in turn causes an increased flux of protein through the ER of the β-cell, which is quite high compared with other cell types even under physiological conditions, and any further increase is expected to be conducive to ER stress. Furthermore, chronic hyperglycemia could also lead to long-term increases in cytosolic Ca^{2+} [as opposed to the normal short-term increases arising from glucose-induced closure of ATP-dependent potassium channels (K_{ATP})] that could in turn be proapoptotic (49).

Long-term hyperglycemia also induces the generation of reactive oxygen species (ROS) leading to chronic oxidative stress because the islets express very low levels of antioxidant enzymes and activity. ROS, particularly hydroxyl radicals, interfere with normal processing PDX-1 (pancreas duodenum homeobox-1) mRNA, a necessary transcription factor for insulin gene expression and glucose-induced insulin secretion besides being a critical regulator of β-cell survival (47, 46). The generation of ROS (and reactive nitrogen species) will ultimately activate stress-induced pathways [nuclear factor κB (NF-κB), stress kinases, and hexosamines] to manipulate cell fate (50–52). Del Guerra *et al.* (53), studying islets isolated from the pancreata of 13 DM2 patients (age ranging from 49 to 75 yr, and diabetes duration from 2 to 8 yr, except one patient with 23-yr known duration of the disease) and 13 matched nondiabetic cadaveric organ donors, demonstrated several functional and molecular defects, confirming that DM2 islets release less insulin than control islets accompanied by altered expression of glucotransporters and of glucokinase, reduced activation of AMP-activated protein kinase and alterations in some transcription factors regulating β-cell differentiation and function. Markers of oxidative stress, such as nitrotyrosine and 8-hydroxy-2′-deoxyguanosine concentrations, were significantly higher in DM2 than control islets and correlated with the degree of glucose-stimulated insulin release impairment. The addition of glutathione in the incubation medium determined reduction of oxidative stress (as suggested by diminished levels of nitrotyrosine), improved glucose-stimulated insulin secretion, and increased insulin mRNA expression. Thus, Del Guerra *et al.* (53) concluded that the functional impairment of DM2 islets could be, at least in part, reversible by reducing islet cell oxidative stress. It is important to emphasize that in this study the percentage of β-cells was only slightly (~10%), although significantly, reduced in diabetic islets compared with control islets. As reasoned by Robertson *et al.* (47), if the steady decline in β-cell function in DM2 is attributable to any significant extent to ongoing apoptosis via chronic oxidative stress with no deterioration in β-cell replication, then interference with apoptosis by antioxidants or any other therapy, might provide a much needed new approach to conventional treatment that could stabilize β-cell mass.

B. Lipotoxicity

Diabetes is associated with dyslipidemia characterized by an increase in circulating free fatty acids (FFAs) and changes in lipoprotein profile. In healthy humans, besides the hyperinsulinemia induced by an acute elevation of FFAs, there is also an increase in glucose-stimulated insulin secretion after prolonged FFA infusion (48 and 96 h) (54, 55) but not in nondiabetic individuals genetically predisposed to developing DM2 (55). In healthy control subjects, the FFA-induced

insulin resistance was compensated by the enhanced insulin secretion, whereas persistently elevated FFAs may contribute to progressive β-cell failure (β-cell lipotoxicity) in individuals genetically predisposed to DM2. Santomauro *et al.* (56) have demonstrated that overnight administration of the nicotinic acid analog acipimox lowered plasma FFA as well as fasting insulin and glucose levels, reduced insulin resistance, and improved oral glucose tolerance with decreased insulin levels in lean and obese nondiabetic subjects and in subjects with IGT and DM2. The significant decrease in insulin levels suggested that plasma FFA support between 30 and 50% of basal insulin levels. A sustained (7-d) reduction in plasma FFA concentrations in DM2, with acipimox, was also associated with enhanced insulin-stimulated glucose disposal (reduced insulin resistance) associated with a decreased content of intramyocellular long-chain fatty acids (FAs) and improvement in oral glucose tolerance test with a slight decrease in mean plasma insulin levels (57). These data suggest that physiological increases in plasma FFA concentrations in humans potentiate glucose-stimulated insulin secretion and are unlikely to be "lipotoxic" to β-cells (58) but may contribute to progressive β-cell failure in at least some individuals who are genetically predisposed to developing DM2 (55). For all studies reported in relation to the FFA-β-cell interaction it is important to emphasize that the stimulatory effects on glucose-stimulated insulin secretion are physiological in nature, particularly during the fasted-to-fed transition. Circulating FFAs help maintain a basal rate of insulin secretion, keeping adipose tissue lipolysis in check.

In rodent islets, increased FFAs have been shown to be proapoptotic in β-cells (59). Exposure of cultured human islets to saturated FAs such as palmitate are highly toxic to the β-cell, inducing β-cell apoptosis, decreased β-cell proliferation, and impaired β-cell function. In contrast, the monounsaturated FAs such as oleate are protective against both palmitate and glucose-induced apoptosis and increase in proliferation. The deleterious effect of palmitic acid was mediated via ceramide-mitochondrial apoptotic pathways, whereas induction of the mitochondrial protein Bcl-2 by oleic acid may contribute to the protective effect of monounsaturated FAs, such as palmitoleic or oleic acids (60).

A similar balance between pro- and antiapoptotic effects is found for lipoprotein action on insulin-secreting cells from mouse pancreatic islets. The sole available study testing the hypothesis that lipoproteins modulate the function and survival of the cells has demonstrated that purified human very low-density lipoprotein and low-density lipoprotein particles reduced insulin mRNA levels and β-cell proliferation and were proapoptotic, whereas high-density lipoprotein protected β-cells against these proapoptotic effects. The protective effects of high-density lipoprotein were mediated, partially at least, by inhibition of caspase-3 cleavage and activation of Akt/protein kinase B, whereas proapoptotic lipoproteins seem to act via c-Jun N-terminal kinase (61). These results are highly suggestive that the changes in lipoprotein profile observed in DM2 could contribute to the pathogenesis and progression of β-cell failure.

Regarding the mechanism(s) by which lipotoxicity can impair β-cell function, the emerging evidence has suggested that long-chain fatty acyl-coenzyme A (CoA) might be involved in the β-cell dysfunction that occurs after prolonged exposure to FFAs, mediating its deleterious effects, at least in rodents, as initially proposed by Prentki and Corkey (62) and recently reviewed by Yaney and Corkey (63). According to these authors, the simultaneous presence of elevated glucose and FA results from glucose as an oxidative fuel, in accumulation of cytosolic citrate, the precursor of malonyl-CoA, which in turn inhibits carnitine-palmitoyl-transferase-1, the enzyme responsible for transport of FA into the mitochondria, blocking their oxidation and energy production, resulting in cytosolic accumulation of long-chain fatty acyl-CoAs. Thus, this model proposes that glucose concentration plays a critical role in the effect of FAs. Whether long-chain acyl-CoA accumulation directly affects β-cell function or whether it serves as a precursor for other active molecules such as diacylglycerols or phospholipids directly activating protein kinase C isoforms somehow synergizing with glucose to enhance insulin secretion was not characterized, whereas the nature of the effectors downstream of lipid metabolite accumulation remains unknown (62–64).

As reported by Poitout and Robertson (64), the "malonyl-CoA/long chain-acyl-CoA" proposed as a biochemical basis for lipotoxicity implies that the effects of FA are greatly influenced by concomitant glucose concentration. Therefore, in the presence of physiological glucose concentrations, elevated FA should be readily oxidized in the mitochondrion and should not harm the β-cell. Under circumstances in which both FA and glucose are elevated, accumulation of metabolites derived from FA esterification would inhibit glucose-induction insulin secretion and insulin gene expression. Thus, glucotoxicity and lipotoxicity are closely interrelated, in the sense that lipotoxicity does not exist without chronic hyperglycemia. Furthermore, the effects of glucose on lipid metabolism are so intense that according to Poitout and Robertson (64), lipotoxicity can be viewed as one mechanism of glucotoxicity. Hence, generation of ROS may be an alternative mechanism of both gluco- and lipotoxicity. In effect, exposure of islets to palmitate induces generation of ROS (65) and treatment of islets with metformin, which has antioxidant properties, protecting them from deleterious effects of FAs (66).

C. Proinflammatory cytokines and leptin

The chronic increase in inflammatory mediators observed in DM2 might not only affect insulin-sensitive tissues and blood vessel walls but could also affect pancreatic β-cells. In addition to genetic factors, development of DM2, with a central role for the functional β-cell mass, is strongly influenced by environmental factors, including decreased physical activity, nutrition, and obesity. This promotes the following factors, which are possible mediators of an inflammatory process (67).

1. Adipocyte-secreted factors. Locally produced hormones and cytokines possess important auto/paracrine properties. Some are also released into circulation and have endocrine effects. In particular, leptin, TNF-α, IL-6, and IL-1 receptor antagonist are produced and secreted by fat tissue, being increased in obesity, and have been causally linked to insulin resistance (18, 68).

Leptin is expressed primarily in adipose tissue, representing the most obvious exponent of the adipocyte. Recently, leptin has also been considered as a proinflammatory cytokine because of its structural similarity with other cytokines and its receptor-induced signaling pathways (69). In rodent islets, leptin induces β-cell proliferation and protects from FFA-induced β-cell apoptosis. In contrast, chronic exposure of human islets to leptin leads to β-cell apoptosis via increasing release of IL-1β and decreasing release of IL-1 receptor antagonist in the islets (70). Other cytokines released by adipocytes, including TNF-α and IL-6, may also modulate β-cell survival, although it is unclear whether the amount released into the circulation is sufficient to affect β-cells (70). Furthermore, it may well be that these cytokines are only effective in the presence of other cytokines.

2. Increased cell nutrients. Elevated glucose concentrations and FFAs, in addition to their role of cell nutrients, also have a dual effect on β-cell turnover. Depending on duration of exposure to glucose or FFA and on the genetic background of the islets, glucose and FFAs may induce β-cell proliferation and have pro- or antiapoptotic effects. According to Maedler *et al*. (50), elevated glucose concentrations induce β-cell production of IL-1β leading to β-cell apoptosis. Furthermore, chronic hyperglycemia increases production of ROS, which may cause oxidative damage in β-cells, as highlighted previously. Both IL-1β and ROS activate the transcription of the NF-κB, which plays a critical role in mediating inflammatory responses. On the other hand, increased concentrations of FFAs, particularly saturated, may affect the viability of the β-cells directly, as discussed earlier, or via obesity, *i.e*., adipocyte-secreted cytokines (TNF-α, IL-6, and leptin) may act directly on the β-cells or activate the innate immune system.

3. Innate immune system and autoimmunity. The innate immune system is considered to provide rapid host defenses until the slower adaptive immune response develops. In addition to the endocrine activity of the adipocytes, described above, macrophages and endothelium may contribute to increasing serum levels of IL-1β, IL-6, and TNF-α in DM2 (71) and may act on the pancreatic islets and impair β-cell secretory function or activate the innate immune system (67). Similarly, these cytokines induce the liver to produce acute-phase proteins such as C-reactive protein, haptoglobin, fibrinogen, plasminogen activator inhibitor, and serum amyloid A.

As indicated by Donath *et al.* (67), when apoptotic cells are present in high enough numbers or when apoptosis is the consequence of exposure to cytokines such as IL-1β and TNF-α, they can provoke an immune response. Moreover, pronounced activation of the acute-phase response is associated with islet cell autoantibodies in patients with DM2 (72). After glucose and FFA-induced β-cell apoptosis, it is conceivable that depending on age and on genetic and/or environmental factors, some DM2 patients may show mobilization of T cells reactive to β-cell antigens, culminating in autoimmune destruction of β-cells similar to that observed during earlier stages in "classical" type 1 diabetes (21). This response may be so discrete and desynchronized in time and space that it has evaded detection in earlier autopsy studies. The precise role of the innate and acquired immune system in the ongoing process of β-cell demise in DM2 remains to be investigated.

D. Islet cell amyloid

The relevance of amyloid deposition in the deterioration of β-cell function has been the subject of debate for many years. As indicated in *Section I*, deposits composed mainly of islet amyloid polypeptide (IAPP), also known as amylin, have been reported in up to 90% of DM2 individuals, compared with 10–13% of nondiabetic counterparts (4). IAPP is a 37-amino acid, β-cell peptide that is costored and coreleased with insulin in response to β-cell secretagogues. The normally soluble peptide is found in the circulation at 5–15 pmol/liter concentration in man, and like C-peptide (but not insulin) it is excreted by the kidney; the relationship of circulating insulin-like molecules to IAPP would therefore be more accurate with C-peptide than insulin (73).

Several bodies of evidence support a potential role of IAPP in the pathophysiology of β-cell loss in DM2. In effect, spontaneous DM2 occurring in humans, monkeys, and cats shares close homology in IAPP sequence, which spontaneously forms amyloid fibrils in an aqueous environment. Nevertheless, proline substitutions in the region IAPP 24–29 that does not form fibrils in an aqueous solution, as found in rats and mice, do not spontaneously develop DM2 but instead require selective genetic manipulation to develop diabetes (see reference citations in Ref. 73). Transgenic mice expressing human IAPP (hIAPP) in β-cells when obese, spontaneously develop diabetes characterized by islet amyloid and decreased β-cell mass (74). Prospective studies in these mice support the hypothesis that the mechanism of the decreased β-cell mass is increased apoptosis (75). It has been suggested that fibril size of IAPP may determine the capacity of amyloid to cause cell death and that intermediate-sized oligomeric material is chiefly responsible for β-cell toxicity. Exposure of mouse islets to intermediate-sized aggregates of hIAPP caused disruption and vesiculation of cell membranes after 24 h, and both necrotic and apoptotic cells were evident after 48 h. These particles were more cytotoxic to mouse islet cells than matured particles containing large aggregates (76). The findings from the Mayo Clinic that only a minority (about 10%) of the cases with IFG had islet amyloid (evaluated in slides stained by Congo red and examined under polarized light for birefringence) present, while already having a deficit in presumptive β-cell mass of approximately 40%, is consistent with the suggestion that small IAPP oligomers (not detectable by light microscopy) are the cause of β-cell loss, whereas the large extracellular amyloid deposits visible by light microscopy are inert (4). Alternatively, it is possible that IAPP formation is secondary to the onset of hyperglycemia and not of primary importance in the pathophysiology of DM2 (4).

In the recently published review of islet amyloid (73), the authors concluded that in human DM2, islet amyloidosis largely results from diabetes-related pathologies (such as diabetes-associated abnormal proinsulin processing, which could contribute to destabilization of granular IAPP) and is

not an etiological factor for hyperglycemia. However, the associated progressive β-cell destruction leads to severe islet dysfunction and insulin requirement (Fig. 1).

E. Linkage of reduced β-cell mass and dysfunction

As reported previously (4), before IGT becomes apparent in humans (the earliest manifestation of incipient diabetes), there may have been loss of as much as approximately 50% of β-cell mass, and as overt diabetes develops, further loss might be limited to no more than an additional 10%, indicating a parallel impairment in β-cell function. In effect, marked impairment of first-phase insulin secretory responses to iv glucose at a very early stage in the development of hyperglycemia was shown, and by the time fasting plasma glucose exceeded 115 mg/dl, first-phase insulin responses were found mostly to be absent (77). When the first-phase insulin secretion was plotted as a function of fasting plasma glucose, in a study of Pima Indians, with progressive glucose intolerance from normal to DM2, it was apparent that very few persons with fasting plasma glucose above 110 mg/dl were able to mount even a minimal response to the iv glucose challenge (13). Hemi-pancreatectomy led to impaired β-cell function in healthy humans (78), whereas in rats, partial pancreatectomy results in impaired insulin secretion resembling that encountered in DM2 despite extensive regeneration of the remnant and only a modest increase in glycemia at the time of the study (79). It may be concluded from these studies that reduced β-cell mass can lead to impaired function, but the mechanism is not yet apparent, nor is it necessarily the sole factor (21).

Two possible explanations account for the impaired β-cell function consequent to decreased β-cell mass (21): 1) increased insulin demand on residual β-cells *per se* leading to changes in function (by ER stress or other mechanisms); and 2) hyperglycemia consequent to decreased β-cell mass driving the impairment in β-cell function. However, the decreasing β-cell mass experimentally more often than not leads to a more or less

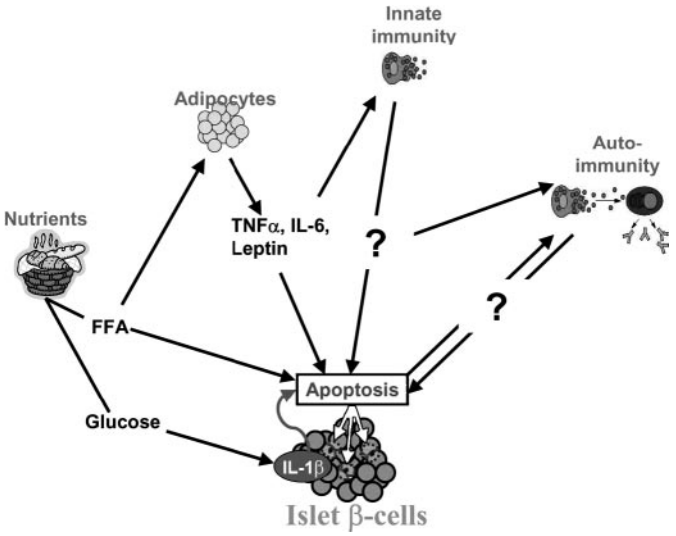

FIG. 1. Proposed model for the interplay between "aggressors" of the β-cell in the pathogenesis of DM2. [Reproduced with permission from M. Y. Donath *et al.*: *J Mol Med* 81:455–470, 2003 (67).]

prolonged period of hyperglycemia (79, 80). Moreover, in many instances there is compensatory regeneration of β-cells that might not be as well-differentiated as older, fully mature cells. Regardless, there are *in vitro* studies on islet and *in vivo* studies by glucose infusion showing that high glucose for prolonged periods of time leads to impaired β-cell mass and/or function. Therefore, accepting that glucose plays a central role among those factors contributing to β-cell demise whereas transient postprandial hyperglycemic excursions may predominantly induce β-cell proliferation in insulin-resistant individuals, this adaptive mechanism may fail in the long term and be overridden by β-cell apoptosis. However, it is unlikely that glucotoxicity acts alone, and the negative contribution of saturated FAs, lipoproteins, leptin, and circulating and locally produced cytokines will burn out the β-cells further. These factors will induce apoptosis and/or necrosis, which in the presence of proinflammatory cytokines may activate specific immunological phenomena, ultimately resulting in autoimmunity as indicated above (46).

Notwithstanding, DM2 in humans progresses over time, whereas impaired β-cell function appears to be reversible at least to a certain degree in the early stages of the disease. In effect, the overnight infusion of glucagon-like peptide 1 (GLP-1) in DM2 improved first- and second-phase insulin responses to a 2-h hyperglycemic clamp. All responses on GLP-1 were not significantly different from nondiabetic control subjects (81). Besides, a recovery of the first-phase insulin secretion was observed after the addition of rosiglitazone given for 6 months to failing sulfonylurea and metformin regimen in DM2 with a duration of diabetes of 7.6 ± 2.1 yr (82). Similarly, the iv administration of the incretin mimetic, exenatide, maintained for 30 min, to DM2 patients treated with diet/exercise, with a duration of the disease of 4 ± 2 yr, restored the first and second phases of insulin secretion, after glucose challenge, with a pattern similar to that found in healthy paired controls (83). These results are compatible with the view that the deficient pattern of insulin secretion is mainly functional in nature and that the reduction in pancreatic islet mass is moderate in patients with DM2 (4, 83). However, the recovery of insulin secretion should be tested in further studies focusing on patients with more advanced stages of DM2. Furthermore, if an individual with DM2, even with severe hyperglycemia, is rendered euglycemic by either pharmacological means or changes in lifestyle, β-cell function, in particular glucose responsiveness, can be restored, and this will be discussed later. This is particularly true in newly diagnosed DM2 (84, 85) where the limiting threshold for reversibility of decreased β-cell mass has probably not been passed. Rendering an individual with DM2 euglycemic interrupts the vicious circle linking decreased β-cell function with hyperglycemia. This might not only restore insulin secretory patterns but also allow for some restoration of β-cell mass. Although there are many reasons to believe that this may occur in humans as it does in animal models of reduced β-cell mass, this has never been documented by virtue of the fact that β-cell mass cannot be measured noninvasively at the time of writing.

Understanding the mechanisms of β-cell death and thus the decreased β-cell mass and impaired function has provided the basis for a new therapeutic target, β-cell preservation, particularly when one considers the reversibility of impaired β-cell function and possibly β-cell mass as dis-

cussed above. In this context, it should be noted that long-acting incretin mimetics, which besides enhancing glucose-sensing and insulin secretory capacity of the endocrine pancreas have also been shown in preclinical studies in rodents, have additional effects on β-cell mass by stimulating proliferation and inhibiting apoptosis (86).

Other examples include the thiazolidinediones (TZDs), which have been linked to not only improving insulin secretory capacity but also preventing the loss of β-cell mass by reducing apoptosis with maintenance of β-cell neogenesis in ZDF rats (87) as well as in other murine models of DM2 (88). In human beings, TZDs were able to preserve and recover β-cell function (82, 89) besides improving insulin sensitivity.

Summary/conclusions. Numbered among the factors for progressive loss of β-cell function and mass are glucotoxicity and lipotoxicity, which are closely interrelated in the sense that lipotoxicity does not exist without chronic hyperglycemia and, depending on duration of exposure to glucose or FFAs and on genetic background of the islets, may induce β-cell proliferation and have pro- or antiapoptotic effects.

Proinflammatory cytokines and leptin, produced by fat tissue, could also affect β-cells. In particular, leptin, TNF-α, IL-6, and IL-1 receptor antagonist are a linkage of obesity to DM2. Leptin in islets leads to β-cell apoptosis, whereas TNF-α and IL-6 may also modulate β-cell survival. Apoptotic cells can provoke mobilization of T cells reactive to β-cell antigens, culminating in autoimmune destruction of β-cells similar to that observed at earlier stages of type 1 diabetes.

Regarding the IAPP, several factors support its role in the pathophysiology of β-cell loss in DM2. Alternatively, it is possible that IAPP formation is secondary to the onset of hyperglycemia and not of primary importance in the pathophysiology of DM2.

The link of reduced β-cell mass to impaired function may be due to an increased demand on residual β-cells *per se* leading to changes in function (ER stress or other mechanisms) or related to the hyperglycemia consequent to decreased β-cell mass, driving the impairment in β-cell function. *In vitro* and *in vivo* studies suggested that persistently elevated glucose levels play a central role among those factors (FFAs, lipoproteins, leptin, and cytokines) contributing to β-cell demise.

Understanding the mechanisms of β-cell death and thus decreased β-cell mass and impaired function has provided the basis to β-cell preservation, particularly when one considers that the impaired β-cell function and possibly β-cell mass appear to be reversible, particularly at early stages of the disease where the limiting threshold for reversibility of decreased β-cell mass has probably not been passed.

IV. Clinical Intervention to Preserve or "Rejuvenate" β-Cells

A. Short-term intensive insulin therapy of newly diagnosed DM2

Optimal metabolic control, especially early intensive glycemic control, plays a role in the prevention of progressive β-cell dysfunction and possibly destruction of the β-cells with worsening of diabetes. Many reports have shown that induction of normoglycemia in DM2 results in both improved β-cell function and insulin resistance (90, 91). Rarely, however, has a prolonged benefit been demonstrated with virtually all patients becoming hyperglycemic again after a few weeks (92, 93). Until recently, it was unknown whether such outcomes pertained to new-onset DM2, although patients having failed diet therapy may show a good response to a short period of intensive insulin therapy by continuous sc insulin infusion (CSII) (94). Ryan *et al.* (84) recently reported that, in 16 severe (mean fasting plasma glucose of 239 mg/dl) newly diagnosed DM2 patients, a 2- to 3-wk course of intensive insulin therapy by multiple daily insulin injection (NPH plus regular) was able to maintain good glycemic control at 1 yr in seven of the subjects on diet therapy alone, whereas eight required oral hypoglycemic agent and one required insulin therapy. The distinguishing features of those who did not require oral agents or insulin treatment were that they required less insulin during the active insulin therapy phase (0.37 ± 0.05 *vs.* 0.73 ± 0.07 U/kg·d) and were able to attain a lower fasting serum glucose level at the end of the period of insulin therapy (106 ± 5 *vs.* 139 ± 7 mg/dl). It is interesting to highlight that the majority of the study patients (15 of 16) had an evident recovery of the area under the curve (AUC) insulin (oral glucose tolerance test) after the end of insulin therapy, being dramatically higher than study entry values associated with a significant reduction in AUC glucose by year end, probably related to the correction of gluco- and lipotoxicity (significant reduction in triglycerides and FFAs). Thus, the potential benefits of early, aggressive intervention with insulin therapy to counter both β-cell dysfunction (and insulin resistance) must be considered: effects of insulin therapy against chronic hyperglycemia and lipotoxicity-induced apoptosis of the β-cells.

In a similar study (85), 138 newly diagnosed DM2 patients with fasting glucose greater than 200 mg/dl were hospitalized and treated with CSII for 2 wk. After CSII, the patients were followed longitudinally on diet alone. Optimal glucose control was achieved within 6.3 ± 3.9 d in 126 patients. The remission rates (percentage of individuals maintaining near euglycemia) at the third, sixth, 12th, and 24th months were 72.6, 67.0, 47.1, and 42.3%, respectively. Patients who maintained glycemic control for more than 12 months (remission group) had greater recovery of β-cell function than those who did not (nonremission group) when assessed immediately after CSII. HOMA of β-cell function (HOMA-β) and AUC insulin during iv glucose tolerance test, as well as change in AIR, were significantly higher in the remission group. Furthermore, proinsulin decreased highly significantly; thus the proinsulin/insulin ratio was also reduced highly significantly (indication of an improvement in the quality of insulin secretion) as well as the HOMA of insulin resistance (HOMA-IR; surrogate for evaluation of the degree of insulin resistance) in the remission group. Li *et al*. (85) concluded that the improvement of β-cell function, particularly the restoration of the first-phase insulin secretion, could be responsible for the remission.

Summary/conclusions. Among the clinical interventions to preserve or "rejuvenate" β-cells, short-term intensive insulin therapy of newly diagnosed DM2 has been proposed: 2- to 3-wk intensive therapy with multiple daily insulin injections of NPH plus regular or CSII. The improvement of β-cell

function, especially the restoration of the first-phase insulin secretion, would lead to remission at least for a period of time. Furthermore, proinsulin decreased highly significantly as did PI/IRI ratio, indicating an improvement in the quality of insulin secretion.

B. Modulation of the β-cell ATP-sensitive K^+ (K_{ATP}) channel

It has been well demonstrated that insulin release by the β-cell is initiated by an increase in the intracellular Ca^{2+} concentration that is mediated by Ca^{2+} influx through voltage-gated Ca^{2+} channels in the plasma membrane. The opening and closing (gating) of these Ca^{2+} channels is determined by β-cell membrane potential, which is in turn regulated by the activity of the K_{ATP} channel. In the unstimulated β-cell, K_{ATP} channels are open and the outward movement of K^+ ions through these channels holds the membrane potential at a hyperpolarized level at which voltage-gated Ca^{2+} channels are closed. When plasma glucose concentration rises, glucose uptake and metabolism by the pancreatic β-cell are enhanced and, possibly through changes in intracellular adenine nucleotide concentrations, induce K_{ATP} channel closure. This leads to membrane depolarization, opening voltage-gated Ca^{2+} channels, and an increase in cytosolic Ca^{2+} that triggers the exocytosis of insulin. Drugs like the sulfonylureas act by inhibiting the K_{ATP} channel directly, and thereby through depolarizing the β-cell and stimulating Ca^{2+} influx they enhance insulin secretion. Another class of drugs is the K-channel openers. These comprise a structurally unrelated group of compounds that have the common property of opening K_{ATP} channels, which hyperpolarizes the β-cells and prevents insulin release, even in the presence of glucose. The most potent K_{ATP} channel opener in the β-cell used in clinical medicine is diazoxide (95).

The concept of deficient insulin stores as a contributing factor to β-cell dysfunction in DM2 was recognized 30 yr ago when diazoxide was used to inhibit insulin secretion (and induce β-cell "rest") in patients with DM2 receiving insulin, leading to restoration of the insulin response to a combined stimulation of β-cells with glucagon plus tolbutamide, not observed in control diabetics receiving placebo with insulin (96). Diazoxide has successfully been used to improve β-cell function in animal studies, such as the recovery of β-cell responsiveness in 90% pancreatectomized diabetic rats (97), and to prevent the progress of deranged β-cell function in rats with streptozotocin-induced diabetes (98). Besides, *in vitro* studies of human islets have demonstrated that chronic hyperglycemia desensitizes β-cells to glucose being accompanied by three major Ca^{2+} abnormalities: elevated basal Ca^{2+}, loss of glucose-induced rise in Ca^{2+}, and oscillatory activity disturbance with a decrease in glucose-induced slow oscillations. Relieving overstimulation with coculture with diazoxide (and 486 mg/dl glucose) significantly restored postculture insulin responses to glucose, lowered basal Ca^{2+}, and normalized glucose-induced oscillatory activity, but failed to restore Ca^{2+} during postculture glucose stimulation (99). Therefore, the induction of β-cell rest by selective activation of β-cell K_{ATP} channels preserving insulin stores and pulsatile insulin secretion may provide a strategy to protect β-cells from chronic overstimulation and to improve islet function. In effect, short-term diazoxide treatment of obese DM2 patients with poor metabolic control exerted moderate but beneficial effects on important parameters of insulin secretion: absent insulin response to an iv glucose challenge and glucose potentiation of arginine-induced insulin secretion after insulin treatment, whereas a response to both tests, albeit moderate, could be demonstrated after insulin plus diazoxide treatment. These effects could be dissociated from confounding effects of changes of glycemia because insulin infusion was used to achieve close to identical degrees of glycemia during the two treatment periods. Consequently, the results of this study indicate the beneficial effects of β-cell rest. Besides, a significant reduction of the molar ratio of proinsulin to insulin was observed only after diazoxide plus insulin (100). The response to glucose and glucose potentiation of arginine-induced insulin secretion has been shown to correlate to the functioning islet mass as demonstrated after islet autotransplantation in humans (101).

It was also observed that, in adults with autoimmune diabetes, diazoxide treatment at onset preserved some residual insulin secretion for up to 18 months, when compared with a placebo group, when both groups were also receiving multiple insulin injection therapy (102). A similar study was performed in childhood type 1 diabetes, demonstrating that partial inhibition of insulin secretion for 3 months with diazoxide at onset of the disease, in addition to multiple daily insulin injections, extended the period of remission and temporarily preserved residual insulin production compared with placebo (maximum at 6 months, followed by a progressive decline) (103).

Although the beneficial effect of diazoxide may be due to induction of β-cell "rest," it could also reflect in part the antiapoptotic effect of this type of drug because it was demonstrated that incubation of murine pancreatic islets and β-cells with glucose induced apoptosis of β-cells that is Ca^{2+} dependent, because introduction to the culture medium of diazoxide, which blocked glucose-induced Ca^{2+} increase, inhibited apoptosis (45). It is interesting to point out that the closure of K_{ATP} channels by the sulfonylureas tolbutamide and glibenclamide may also induce Ca^{2+}-dependent β-cell apoptosis in rodent and human islets (45, 104, 105), but this effect has only been observed *in vitro* and not consistently (106). The induction of β-cell rest by a new selective K_{ATP}-channel opener (NN414) preserved insulin stores and pulsatile insulin secretion without restoring the orderliness of insulin secretion in human islets, isolated from cadaveric organ donors, cultured at high glucose concentrations (198 mg/dl) (107). According to Ritzel *et al.* (107), the concept of β-cell rest may provide a strategy to protect β-cells from chronic overstimulation and improve islet function. The authors also provide evidence that impaired glucose-regulated insulin secretion in DM2 partially involves mechanisms distinct. Afrom insulin stores and insulin secretion rates (ISRs).

In line with findings indicated in *Section IV.A* related to insulin therapy of newly diagnosed DM2, a clinical study by Alvarsson *et al.* (108), comparing insulin and sulfonylurea (glibenclamide) treatment of recently (<2 yr) diagnosed DM2, showed that treatment with insulin given twice daily as premixed insulin (30% soluble and 70% NPH) preserved

β-cell function (glucagon-stimulated C-peptide response) more effectively than glibenclamide for the duration of the study (2 yr). On the other hand, the nonsulfonylurea secretagogues (meglitinides) repaglinide and natiglinide, which work similarly to sulfonylurea agents but have a more rapid onset and shorter duration of action, when applied for their respective circulating half-lives (less than 2 h) *in vitro*, appear not to have any apoptotic effect on cultured human islets. Thus, short-acting insulin secretagogues may be preferred to long-acting ones, such as glibenclamide, due to the fact that patients will be less exposed to proapoptotic stimuli during prolonged treatment with short-acting agents. This was according to *in vitro* studies of human islets, but *in vivo* confirmation is necessary because several additional factors including β-cell proliferation and regeneration may compensate for glibenclamide-induced apoptosis (105).

Summary/conclusions. The induction of β-cell "rest" by selective activation of β-cell K_{ATP} channels, using drugs such as diazoxide and the K_{ATP} channel opener NN414, that hyperpolarize β-cells, preserves insulin stores and pulsatile insulin secretion, thus protecting β-cells from chronic overstimulation and improving islet function besides reducing the PI/IRI ratio. Although the beneficial effect of those selective K_{ATP} channel openers may be due to inducing β-cell "rest," it could also reflect in part the antiapoptotic effect of these drugs by blocking the glucose-induced cytosolic Ca^{2+} increase, secondary to the β-cell K_{ATP} channels closure associated with the rise in plasma glucose, as shown in *in vitro* studies which have demonstrated that glucose-induced apoptosis of β-cells is Ca^{2+} dependent. The closure of K_{ATP} channels by sulfonylureas, tolbutamide and glibenclamide, may also induce Ca^{2+}-dependent β-cell apoptosis also in *in vitro* studies in rodent and human islets. If these findings translate to patients with DM2, at least some long-acting sulfonylureas may have adverse effects on β-cells, whereas short-acting ones will be preferred due to the fact that patients will be less exposed to proapoptotic stimuli during protracted treatment with these agents.

C. Antiapoptotic drugs

1. Leptin. As indicated earlier, leptin in ZDF rat islet protects the β-cell from FFA-induced apoptosis (109). However, it induces β-cell apoptosis in human islets by the mechanism previously discussed (70).

2. Aminoguanidine. Aminoguanidine acts as an antioxidant *in vivo* in diabetic animal models, preventing ROS formation and lipid peroxidation in cells and tissues, while also preventing oxidant-induced apoptosis (110). Moreover, at higher doses this inhibits inducible nitric oxide synthase with reduction in nitric oxide, which has been shown to mediate IL-1β-induced impairment of β-cell function and ultimately cause β-cell apoptosis as reported in cultured prediabetic ZDF islets (59, 111). Because ROS are known to increase intracellular advanced glycation end-products (AGEs) and antioxidants are known to inhibit AGE formation (110), presumably the time required for formation of immunodetectable intracellular AGEs is much longer than the time-course for oxidant-induced apoptosis, suggesting that intracellular AGE formation does not play a causal role in this process of oxidant-induced apoptosis. Furthermore, diabetic models using aminoguanidine have also shown a reduction in diabetes-related complications such as retinopathy, neuropathy, and nephropathy. Specifically, reduction in retinal microvessel formation, albuminuria, and the prevention or decrease in motor and sensory nerve conduction velocity have been reported (see references cited in Ref. 112). In line with previously indicated considerations (47), the prescription of antioxidants as adjunct therapy in DM2 is warranted to determine whether this approach would prevent continued deterioration in β-cell function, particularly when glycemic control is not satisfactory (47). Unfortunately, studies with antioxidant vitamins to date have not improved glycemic control. Additionally, aminoguanidine has not been used in humans because of its side effect profile in many organs.

Summary/conclusions. Aminoguanidine acts as an antioxidant *in vivo*, in diabetic animal models, preventing ROS formation and lipid peroxidation and oxidant-induced apoptosis and at higher doses inhibits inducible nitric oxide synthase with reduction in nitric oxide, which was shown to ultimately cause β-cell apoptosis in cultured prediabetic ZDF rat islets. Aminoguanidine has not been used in humans because of its side effect profile in many organs.

3. Effect of TZDs on β-cells. The TZDs are agonists of peroxisome proliferator-activated receptor γ (PPARγ), a nuclear receptor that regulates transcription of genes involved in lipid and glucose metabolism. Although predominantly expressed in adipose tissue, PPARγ is present in other insulin-sensitive tissues, including the pancreatic islet cells (113). Fully 10% of genes transcribed in adipose tissue are differentially expressed with the use of a PPARγ agonist, as well as 2% of all genes expressed in liver and 1% of those expressed in skeletal muscle (114). In adipose tissue, stimulation of PPARγ increases adipocyte differentiation, resulting in an increased number of small, insulin-sensitive adipocytes (115). The development of these insulin-sensitive cells enhances glucose uptake, improving glycemic control. Improved insulin sensitivity, reported for TZDs, both in monotherapy and combination in DM2, has the potential to protect β-cells because they may reduce the demand on the pancreas (see references cited in Ref. 116). Lowering plasma glucose may reduce the risk of glucotoxicity. In addition, by increasing adipose-tissue mass, TZDs promote FA uptake and storage in adipose tissue, thus reducing levels of circulating FFAs where this has the potential to alleviate lipotoxicity. Hence, TZDs retain fat where it belongs, according to the "FA steal" hypothesis (115). Reductions in FFAs may be mediated by suppression of TNF-α expression, which has been demonstrated by *in vitro* studies such as those of Arner (117), who has reported that rosiglitazone suppresses TNF-α to almost undetectable levels in human preadipocytes. Thus, according to Arner (117), TZDs reduce the release of FFAs from the adipocytes by two mechanisms: 1) increasing the insulin sensitivity of the cell, and thereby improving the antilipolytic effect of insulin; and 2) reducing levels of TNF-α, known to suppress the antilipolytic properties of insulin by inhibiting the insulin-signaling cascade.

Although rosiglitazone seems to be a pure PPARγ agonist,

the other TZD approved for the medical therapy is pioglitazone, which seems to also act like a partial PPARα agonist (118). In general, PPARα regulates genes involved in FA uptake and oxidation (liver, kidney, heart, and skeletal muscle), inflammation, and vascular function, whereas PPARγ, as shown, regulates genes involved in FA uptake and storage, inflammation, and glucose homeostasis (119).

a. Animal data. In obese ZDF rats, the overaccumulation of fat in the pancreatic islets induces β-cell dysfunction, reflecting a mutation of the leptin receptor that blocks the normal triglyceride-lowering action of leptin on islets, as discussed previously, leading to lipotoxicity through exaggerated production of nitric oxide (111). In isolated islets from obese ZDF rats, addition of troglitazone halved the triglyceride content, doubled insulin secretion stimulated by arginine, and produced a greater than 30-fold increase to that stimulated by glucose (120). This is consistent with the ability of TZDs to prevent TNF-α-induced inhibition of insulin signaling by islets. In the same animal model, rosiglitazone treatment was shown to maintain β-cell proliferation and to produce a 5-fold attenuation in the net rise in β-cell death, preventing the loss of β-cell mass indicated previously (87). Because excessive β-cell apoptosis is associated with excessive accumulation of intracellular triglycerides, as reported earlier (59), staining of islet cells for insulin in diabetic db/db mice has shown evidence of regranulation when the islet cell triglyceride content had been reduced with rosiglitazone (121). Furthermore, pioglitazone given to three murine models of DM2 improved insulin secretory capacity and prevented the loss of β-cell mass by reduction of oxidative stress with 1.5- to 15-fold higher levels of pancreatic insulin (122). The decrease of TNF-α expression by pioglitazone in adipose tissue in db/db mice (123) might be associated, as indicated above, with an improvement in β-cell dysfunction and survival. Treatment with rosiglitazone has also been shown to prevent islet amyloidosis in nondiabetic hIAPP transgenic mice (124).

In conclusion, the most relevant data in rodents indicated that the TZDs decrease β-cell apoptosis, maintaining its neogenesis and preventing islet amyloidosis.

b. In vitro data in human β-cells. PPARγ mRNA is expressed in isolated human pancreatic islets to a greater extent than PPARα mRNA, with predominance of $PPAR\gamma_2$. This could be interpreted as a condition favoring lipid accumulation and, hence, lipid-induced damage (125). The expression of $PPAR\gamma_2$ was markedly and time-dependently reduced by exposure to progressively higher concentrations of FFAs. This effect was not affected by the concomitant presence of high glucose. The presence of FFAs also produced deleterious cytostatic effect, inhibiting glucose-stimulated insulin release by 80%, as well as reducing islet insulin content by 75% and reducing insulin mRNA expression (125). However, incubation with rosiglitazone, a well-known PPARγ agonist, prevented FFA-induced down-regulation of PPARγ and insulin mRNA expression along with glucose-stimulation insulin release (125). In the same model, high concentrations of FFAs produced an almost 3-fold increase in rates of islet cell death, in association with significant increases in activity of the protease enzymes caspase 3 (apopain) and caspase 9, key mediators of apoptosis (104). Incubation with rosiglitazone at a concentration of 15 mg/ml attenuated islet cell death and normalized caspase activity levels (126).

Zeender *et al.* (127) were able to protect cultured human islets using pioglitazone (and sodium salicylate) against apoptosis and impaired function induced by IL-1β and high glucose by blocking NF-κB activation.

Lin *et al.* (128), noting the association of DM2 with increased β-cell apoptosis and the presence of islet amyloid derived from IAPP, showed that IAPP induces apoptosis in cultured human pancreatic islets and that the addition of rosiglitazone to the incubation prevented the IAPP-induced apoptosis.

c. Mechanism of action of TZDs on the β-cell

1) Indirect effects by amelioration of insulin sensitivity. TZDs consistently lower fasting and postprandial glucose concentrations in clinical studies as well as plasma FFAs in type 2 diabetics, with a reduction of gluco- and lipotoxicity. Furthermore, as mentioned above, TZDs may directly protect the β-cell from lipotoxic insult (125). Besides, insulin concentrations also decrease in the majority of trials, mirroring a decreased secretory demand on β-cells. Such changes indicate that TZDs act as insulin sensitizers, which has been confirmed by direct measurements in *in vivo* studies in humans. Although the insulin-sensitizing effect of TZDs is well established, less is known about their influence on insulin secretion. It now appears that TZDs normalize the asynchronous insulin secretion that characterizes β-cell failure. In a placebo-controlled study in patients with DM2, 13-wk treatment with rosiglitazone was shown to increase the ability of an oscillatory glucose infusion to program high-frequency pulsatile insulin secretion, despite the absence of any direct action on β-cell secretory capacity. It was postulated that the improvement in β-cell function could be related to a reduction in glucotoxicity due to the improved glycemic control and/or improved insulin sensitivity seen with TZDs (129). This could suggest an increased ability of the β-cell to sense and respond to glucose changes within the physiological range after TZD treatment.

2) Direct effects via PPARγ activation in pancreatic islets. As a class effect, TZDs consistently improve basal β-cell function, as measured by the HOMA model and as observed during TZD monotherapy and combination therapy (89, 130–134). Further evidence that TZDs exert beneficial effects on β-cells derive from a study in which 2-month treatment with pioglitazone restored the first-phase insulin response to an iv glucose tolerance test in patients with IGT and with frank DM2 (135). Similar results were reported in a randomized 6-month study comparing the addition of rosiglitazone *vs.* insulin in patients with DM2 who were inadequately controlled on glimepiride and metformin, using a frequently sampled iv glucose tolerance test (FSIVGTT) (82). At study end, the AIR_g was significantly increased with rosiglitazone, with no effect of exogenous insulin apparent. β-Cell function determined by the disposition index, calculated as the product of AIR_g and the insulin sensitivity index ($S_i = 1/HOMA\text{-}IR$) also increased greatly. It should be noted that the resto-

ration of the first-phase insulin response to glucose was independent of the correction of glucose toxicity.

Furthermore, extension studies with TZDs indicate that improvements in β-cell function are sustained in some individuals over time. Four 52-wk clinical trials involving more than 3,700 patients with DM2, reviewed by Campbell (136), showed that pioglitazone was an effective long-term treatment, both as monotherapy and in combination with metformin or sulfonylurea. As well as maintaining glycemic control over the long term, pioglitazone also yielded benefits in terms of improvements in fasting insulin (which was reduced as proinsulin and C-peptide), lipid parameters, and hypoglycemia compared with other monotherapies or combination treatments. The longest follow-up study was that by Bell and Ovalle (137, 138) of type 2 diabetics on sulfonylurea, metformin, and rosiglitazone for 60 months; 22 (68%) patients in the study who were put on the triple therapy remained relatively well controlled. The predictor of failure and the need for insulin after a mean duration of 30 months was the nonsignificant or lacking increase in the meal- or glucagon-stimulated C-peptide. At 60 months, a frequent finding was also a reduction in fasting C-peptide in the failure group.

TZDs may also improve insulin processing, as demonstrated by a reduction in the PI/IRI ratio, an important indicator of β-cell dysfunction outlined earlier (23). Rosiglitazone produced a decrease in the PI/IRI ratio in comparison with both placebo and sulfonylureas (139, 140), and similar results have also been reported for pioglitazone *vs.* placebo (141). Figure 2 summarizes the effects of TZDs on the β-cell (142).

d. Further clinical evidence of TZD effects on human β-cell function. Table 1 shows the clinical studies using rosiglitazone and pioglitazones, besides those presented above, previously published or in abstract form, that have demonstrated a beneficial effect of both agents in improving insulin sensitivity and recovery or improvement of β-cell function (141, 143–149). These results could suggest that the improvement in β-cell function could be, at least in part, secondary to the increase in insulin sensitivity (indirect effect on the β-cells). Further support for the beneficial effects of TZDs on β-cell function is provided by a study in which troglitazone

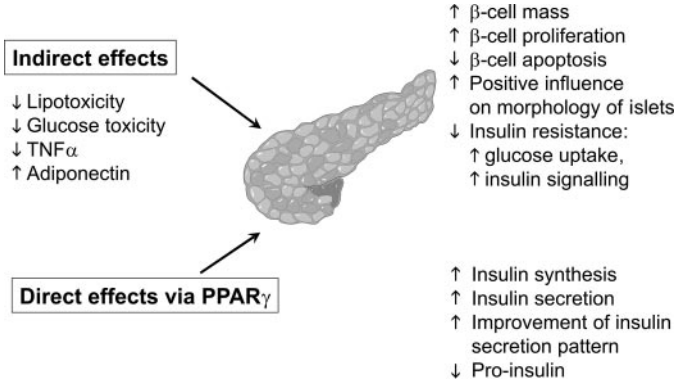

FIG. 2. Mechanism of action of TZDs on the pancreatic β-cell. [Reproduced with permission from H. Walter and G. Lübben: *Drugs* 65:1–13, 2005 (142).]

improved β-cell function (150), as evaluated by meal-stimulated C-peptide levels or expressed by C-peptide/glucose ratio, when given to DM2 patients failing on metformin plus sulfonylurea (n = 28). In contrast, there was no effect on β-cell function when metformin was given to patients failing on sulfonylurea monotherapy (n = 28). These observations were particularly striking because the troglitazone-treated patients had a longer duration of diabetes (mean of 16 yr) than the metformin plus sulfonylurea-treated subjects (8 yr) (150). These researchers have previously reported (82) that addition of rosiglitazone restored the first-phase insulin response to glucose in poorly controlled patients previously treated with maximum doses of sulfonylurea plus metformin. However, the addition of insulin had no effect on β-cell function. In both studies, the beneficial effect of TZDs on β-cell function was independent of glycemic control (because with a similar reduction in HbA1c, no improvement in β-cell function was found in the insulin-treated group), indicating that TZDs can promote recovery of β-cell function independently of the amelioration of insulin sensitivity.

e. TZDs in the prevention of diabetes

1) Troglitazone in Prevention of Diabetes (TRIPOD). In this study (151), 266 Hispanic women with previous gestational diabetes mellitus who were at high risk for DM2 were randomized into placebo (n = 133) or troglitazone (n = 133) groups. Troglitazone, approved in the United States in 1997, was the first TZD for treatment of DM2, and it was withdrawn from the market in 2000 because of hepatotoxicity. During a median follow-up of 30 months, average annual diabetes incidence rates were 12.1 and 5.4 in women assigned to placebo and troglitazone, respectively ($P < 0.01$). Eight months after troglitazone was withdrawn, 15% of the placebo subjects and 2.3% of the troglitazone group developed diabetes during a mean follow-up of 4.3 yr. The protective effect of the TZD was most prominent in women with a large reduction in insulin output during the FSIVGTT. Reducing the high secretory demands placed on β-cells through amelioration of chronic insulin resistance could preserve β-cell function and prevent DM2 for at least 4–5 yr.

2) Pioglitazone in Prevention of Diabetes (PIPOD). Eighty-nine Hispanic women with prior gestational diabetes mellitus who had completed participation in the TRIPOD study and were not diabetic (two thirds had IGT) participated in the PIPOD study for a planned 3 yr of drug treatment and 6 months of postdrug washout (152), with the annual diabetes incidence rate being 4.6%. The similarity of findings between the PIPOD and TRIPOD studies supports a class effect of TZDs reducing insulin secretory demands through a decrease in insulin resistance and preserving pancreatic β-cell function. The risk of diabetes proved lowest in the third of the women with the largest reduction in total FSIVGTT insulin AUC after 1 yr of treatment and highest in the third of women with the smallest reduction after l yr, thus indicating a "stabilization of β-cell function."

3) Diabetes Prevention Program (DPP). The DPP was a randomized clinical trial of prevention of DM2 in people at high risk of diabetes (153). From 1996 to 1998, 3,324 adults with

TABLE 1. Clinical trials evidencing TZD effects on insulin sensitivity and β-cell function

First author, year (Ref.)	Randomization	Study length (wk)	Insulin sensitivity	β-cell function	Proinsulin/ insulin
Publications					
Fonseca, 2000 (143)	Met + Rosi *vs.* Met + placebo (n = 348)	26	↑ HOMA-S	↑ HOMA-β	
Miyasaki, 2003 (144)	Dose response to Pio *vs.* placebo (n = 58)	26	↑ Insulin sensitivity	↑ Insulinogenic index	
Wallace, 2004 (141)	Pio *vs.* placebo (n = 19)	12	↑ HOMA-S ↑ Stimulated insulin sensitivity (euglycemic clamp)	↑ HOMA-β No change AIR_g	↓
Abstracts					
Pfützner, 2004 (145)	Pio *vs.* SU (glimepiride) (n = 87)	36	↓ HOMA score (insulin resistance marker)	↑ Insulin	↓
Rasouli, 2004 (146)	Pio *vs.* Met (IGT) (n = 10)	10	↑ S_i (FSIVGTT)	↑ AIR_g	
Vinik, 2004 (147)	Rosi + SU (glipizide) (n = 115) *vs.* SU + placebo (n = 110)	24	↑ HOMA-S or (↓ HOMA-IR)	↑ HOMA-β ↑ Insulinogenic index/HOMA-IR	
Hamdy, 2005 (148)	Pio *vs.* placebo/SU (n = 30), Pio *vs.* placebo (IGT) (n = 8)	16	↑ S_i (FSIVGTT)	↑ AIR_g	
De Winter, 2005 (149)	Pio *vs.* Met *vs.* SU (gliclazide) (n = 2406)	48	↑ HOMA-S	↑ HOMA-β	

SU, Sulfonylurea; Met, metformin; Rosi, rosiglitazone; Pio, pioglitazone; insulinogenic index, ΔAUC insulin/ΔAUC glucose [oral glucose tolerance test (OGTT)].

IGT and fasting plasma glucose from 100 to 140 mg/dl before June 1997 and from 95 to 125 mg/dl after this date were randomized to receive placebo (n = 582), metformin (n = 587), troglitazone (n = 585), or intensive lifestyle intervention (n = 589). The incidence of diabetes during an average follow-up of 2.8 yr (after which the study was interrupted) stood at 11.0, 7.8, and 4.8 cases per 100 person-years in the placebo, metformin, and lifestyle change groups, respectively. The incidence of diabetes was reduced by 58% in the lifestyle group and by 31% in the metformin group relative to standard lifestyle intervention (placebo). Over concern regarding its liver toxicity, the troglitazone arm was discontinued in June 1998 after 0.5–1.5 yr (mean, 0.9 yr) and then compared with other DPP interventions, considering both the short-term "in-trial" results and the longer term results after troglitazone was discontinued. During the mean 0.9 yr of troglitazone treatment, the diabetes incidence rate was 3.0 cases per 100 person-years, compared with 12.0, 6.7, and 5.1 cases per 100 person-years in the placebo, metformin, and intensive lifestyle participants ($P < 0.001$, troglitazone *vs.* placebo; $P < 0.02$, troglitazone *vs.* metformin; $P = 0.18$, troglitazone *vs.* intensive lifestyle). Indirect measures of insulin secretion and sensitivity, estimated from the oral glucose tolerance test, suggested that the effect of the TZD was, at least in part, due to improved insulin sensitivity with maintenance of insulin secretion. During the 3 yr after troglitazone withdrawal, the diabetes incidence rate was almost identical to that of the placebo group. Troglitazone, therefore, markedly reduced the incidence of diabetes during its limited period of use, but this action did not persist (154).

4) TZD therapy in the prevention/delay of DM2 in patients with IFG+IGT. A total of 101 patients with IFG+IGT received troglitazone for an average of 10 months (after which the drug was withdrawn from the U.S. market), then randomly switched to rosiglitazone (n = 39) or pioglitazone (n = 62) for a mean of 36 months (155). Patients with IFG+IGT who received no antidiabetic medication served as a control group (n = 71). Mean HbA1c and C-peptide levels measured at 2 yr and study end-point (3 yr) decreased from baseline (after troglitazone) for both TZDs at the 2-yr assessment, and reductions remained at that level at the end of the study. The cumulative incidence rate of diabetes after 3 yr was 2.97% after TZDs *vs.* 26.8% in the control group ($P < 0.001$). An estimated 4.2 patients would have to be treated with the TZDs, rosiglitazone or pioglitazone, for every one prevented case of diabetes in the 3-yr study period (134). According to the authors, the study provided support for the notion that early detection through routine screening and the proper preventative measures, including TZD treatment, can prevent or postpone the progression of high-risk patients with IFG+IGT of developing DM2.

5) The Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM) Trial. A total of 5269 adults aged 30 yr or more, with IFG and/or IGT and with no previous cardiovascular disease, received rosiglitazone (n = 2365) or placebo (n = 2634) and followed for a median of 3 yr (156). The primary outcome was a composite of incident diabetes or death. At the end of the study, taking into consideration the few individuals who dropped out from each group, 11.6% of the subjects given rosiglitazone and 26.0% receiving placebo developed the composite primary outcome (1.1% death and 10.6% DM2 in the rosiglitazone group *vs.* 1.3% death and 24.7% DM2 in the placebo group, $P < 0.0001$). A total of 50.5% of the individuals on rosiglitazone and 30.3% on placebo became normoglycemic ($P < 0.0001$).

Summary/conclusions. TZDs are agonists of PPARγ, a nuclear receptor that regulates transcription of genes involved in lipid and glucose metabolism. Regarding the effect of TZDs on β-cells, in murine models of DM2 and in isolated human pancreatic islets, there is an improvement of insulin

secretory capacity and decrease in β-cell apoptosis, maintaining its neogenesis and a reduction of islet amyloid.

In relation to the mechanism of action of TZDs on the β-cell, there are indirect and direct effects. The indirect effects are related to TZD action as insulin sensitizers, with a reduction in gluco- and lipotoxicity. Besides, it appears that TZDs normalize the asynchronous insulin secretion that characterizes β-cell failure. The direct effects are via PPARγ activation in pancreatic islet TZDs consistently improving basal β-cell function with significant improvement in AIR_g and increased disposition index ($AIR_g \times 1/HOMA\text{-}IR$), the restoration of the first-phase insulin response to glucose being independent of the correction of glucose toxicity. Clinical studies using rosiglitazone or pioglitazone have demonstrated a beneficial effect of both agents in improving insulin sensitivity and recovery or improvement of β-cell function, which are sustained in some individuals over time. The addition of rosiglitazone improved the first-phase insulin response to glucose in poorly controlled patients previously treated with maximum doses of sulfonylurea plus metformin. However, the addition of insulin had no effect on β-cell function, despite a similar reduction in HbA1c.

The several trials on prevention of diabetes with TZDs include the following. In the TRIPOD and PIPOD, troglitazone (withdrawn in 2000, because of hepatotoxicity) and subsequently pioglitazone delayed or prevented the onset of diabetes. The class effect of TZDs on reducing insulin secretory demands (decrease in insulin output during FSIVGTT) through a decrease in insulin resistance preserved β-cell function and prevented DM2. The DPP was a clinical trial of DM2 prevention, conducted from 1996 to 1998 in individuals at high risk of diabetes and involving adults with IGT who were randomized to placebo, metformin, troglitazone, or intensive lifestyle intervention. The diabetes incidence rate was 3.0 cases per 100 person-years in the troglitazone group (mean, 0.9 yr), compared with 12.0, 6.7, and 5.1 cases per 100 person-years in placebo, metformin, and intensive lifestyle participants. In the trial of TZD therapy in the prevention/delay of DM2 in patients with IFG+IGT, 101 subjects received troglitazone for an average of 10 months (at which point the drug was withdrawn), then randomly switched to rosiglitazone or pioglitazone for a mean of 36 months. The cumulative incidence rate of diabetes after the 3 yr was 2.97% after TZDs *vs.*26.8% in the control group: IFG+IGT ($P < 0.001$). The study provided support for the idea that early detection and proper preventive measures, including TZD treatment, can prevent or postpone the progression of high-risk patients with IGF+IGT from developing DM2. Finally, the DREAM trial, showed that rosiglitazone for 3 yr reduced significantly incident DM2 (10.6% *vs.* 24.7% on placebo) and increased the likelihood of regression to normoglycemia in adults with IFG and/or IGT.

4. Effect of incretin (GLP-1), incretin mimetics, and enhancers in β-cells. Incretin hormones are peptides released by the gastrointestinal tract in response to nutrient ingestion that enhance insulin secretion and aid in the overall maintenance of glucose homeostasis through slowing of gastric emptying, inhibition of glucagon secretion, and control of body weight (157). The two major incretins are GLP-1 and glucose-dependent insulinotropic polypeptide (GIP).

GLP-1 and GIP are small peptides, having 30 and 42 amino acids and released by the enteroendocrine L cells located in the distal ileum and colon and by the K cells in the duodenum, respectively. Both rapidly stimulate the release of insulin only when blood glucose levels are elevated, thereby enhancing the glucose-sensing and insulin secretory capacity of the endocrine pancreas during postprandial hyperglycemia (157). Although GLP-1 controls blood glucose via other actions besides stimulating glucose-dependent insulin release by inhibiting glucagon secretion and suppression of hepatic glucose output as well as decreasing the rate of gastric emptying, GIP decreases gastric emptying to a much lesser degree and does not inhibit glucagon secretion (157, 158). GLP-1 also activates regions in the central nervous system important for control of satiety (159). However, GLP-1 and GIP have also been shown in preclinical studies to exert significant cytoprotective and proliferative effects on the islets of Langerhans (157, 160, 161). The incretin hormones elicit their actions through direct activation of distinct G protein-coupled receptors expressed on islet β-cells (161, 162).

Native GLP-1 and GIP are rapidly inactivated by the ubiquitously expressed proteolytic enzyme dipeptidyl peptidase (DPP)-IV, which cleaves two N-terminal amino acids from both peptides to produce inactive metabolites (163). Regarding GLP-1, DPP-IV activation results in the inactivation of GLP-1 (7-36) amide and the generation of the metabolite GLP-1 (9-36) amide, which did not activate the GLP-1 receptor, thus not enhancing the insulin secretion and not blocking GLP-1 (7-36) amide enhancement of insulin secretion during an iv glucose tolerance test in healthy subjects (164). Recently, the suppression of hepatic glucose production by GLP-1 (9-36) amide not mediated by inhibition of glucagon secretion or through interaction with the known GLP-1 receptor has been shown in subjects with normal oral glucose tolerance tests (165). Thus, GLP-1 (9-36) amide may reduce postprandial glucose levels independent of insulin.

The short circulating half-life of bioactive intact GLP-1 and GIP initially limited enthusiasm for the potential use of incretin hormones in the treatment of diabetes. However, incretin analogs have been developed with significantly increased half-lives due to modification of the DPP-IV cleavage site and/or conjugation to large circulating proteins, such as albumin (*i.e.*, liraglutide). Furthermore, other GLP-1 receptor agonists are being tested in the treatment of DM2; presently available is synthetic exendin-4, structurally identical to native exendin-4 and originally isolated from the venom of a lizard, *Heloderma suspectum* (exenatide; Amylin Pharmaceuticals, Inc., San Diego, CA; and Eli Lilly and Co., Indianapolis, IN) (166–168).

Although both GLP-1 and GIP act as incretin hormones in normal subjects, only GLP-1 can be used to treat DM2 because diabetes is often associated with a blunted or absent response to GIP. It has been shown that whereas GLP-1 levels are significantly decreased in DM2, GIP values are normal, suggesting that DM2 patients are resistant to the biological effects of GIP, rendering it relatively ineffective (169). The mechanisms underlying the diminished GIP responsiveness

in experimental and clinical diabetes are not fully understood (170) but may involve down-regulation of GIP receptor expression (171) or receptor desensitization (172). Recent data have suggested that desensitization is not a major contributor to defective GIP action in diabetic patients (173).

A possible explanation for the decreased GLP-1 secretion in DM2 may be a decrease in gastric emptying rate, which hypothetically might increase the absorption in the proximal intestine, resulting in less food reaching the distal intestine where L cells are more numerous (169). Indeed, the opposite situation, increased exposure of carbohydrates to the distal intestinal mucosa by α-glucosidase inhibitors or accelerated gastric emptying, increases GLP-1 secretion (174, 175). However, the gastric emptying rate does not seem to exhibit consistent changes in DM2 and obesity but is more often reported as delayed (see references cited in Ref. 169). In the study reported by Toft-Nielsen *et al.* (169), the decreased GLP-1 secretion in DM2 was considered most likely a consequence of the disease.

Unequivocal hypoglycemic action of GLP-1 in type 1 diabetes was demonstrated in studies of iv infusion of the peptide in subjects with type 1 diabetes in the hyperglycemic postabsorptive state. Under these conditions, without the administration of insulin, parenteral infusion of GLP-1 was able to reduce plasma glucose significantly; this was associated with inhibition of glucagon secretion, and stimulation of residual insulin secretion, although statistically significant, was only marginal (176). However, three of the 11 patients examined were C-peptide-negative in the basal state and demonstrated no clear increment under the influence of GLP-1 (176). It was hypothesized that a major component of the glycemic effect is attributable to the known action of GLP-1 to inhibit gastric emptying and glucagon secretion (177). Studies of the effects of the GLP-1 receptor (GLP-1R) agonist, exendin-4, given together with established doses of insulin before a meal, supported the hypothesis. The more prolonged actions of exendin-4 were accompanied by greater and more prolonged reduction of meal ingestion glycemic effects in volunteers with C-peptide-negative type 1 diabetes receiving continuing intensive insulin therapy, demonstrating the capacity of the combination therapy to normalize blood glucose levels after ingestion of meals consistent with the dietary program of the volunteers, without apparent increased risk of hypoglycemia within a normal between-meals interval (178).

Similar findings were observed with an enhancer of GLP-1 (DDP-IV inhibitor, vildagliptin) in insulin-pump-treated type 1 diabetic patients, inducing a significant reduction in the postprandial glucagon in comparison with placebo. This finding provides further evidence that the glucagonostatic effect of GLP-1 and incretin mimetics is mediated via an endocrine effect on the α-cell rather than by a paracrine effect dependent on endogenous insulin release thought to tonically restrain glucagon secretion through a local endocrine/paracrine effect (179).

a. Animal data. Regarding the effect of GLP-1 on β-cells, it has been demonstrated that the acute effect is, as indicated earlier, potentiation of glucose-dependent insulin secretion (157), as also observed in healthy human subjects (170, 180), subjects with IGT (181), and patients with DM2 (81, 170, 180). Its subacute effect is to enhance insulin biosynthesis and stimulation of insulin gene transcription and to increase expression of mRNA for glucose transporter-2 and glucokinase, as shown in *in vitro* studies in rodent models. The chronic GLP-1 action is stimulation of β-cell proliferation and induction of islet neogenesis from precursor ductal cells, which are associated with an expansion of β-cell mass. Finally, an inhibitory effect of GLP-1 was found in β-cell apoptosis. These effects were observed in rodent models of diabetes as well as in cultured β-cells (37, 182–184). Similar findings were obtained using the GLP-1R agonist exendin-4 in rats rendered diabetic by partial pancreatectomy (185). In Wistar rats, the age-dependent decline in β-cell function and the subsequent impairment of glucose tolerance were reversed by GLP-1 administration (186). It should be mentioned that the antiapoptotic effect of GLP-1 is independent from its glucose-lowering activity, as demonstrated by *in vitro* experiments in which GLP-1 was capable of inhibiting H2O2-dependent apoptosis in cultured mouse insulinoma cell lines. GLP-1 reduced DNA fragmentation and improved cell survival (184).

In vivo studies have demonstrated that GLP-1 and exendin-4 may improve glucose tolerance in animal diabetic models as well as in diabetic subjects (see references cited in Ref. 187). These beneficial effects were maintained long after the termination of GLP-1 or exendin-4 infusion. This finding indicates that GLP-1 induces long-term changes that cannot be due simply to the modulation of preformed insulin secretion but must involve more substantial modifications in the functional activity of β-cells. Recent findings from *in vivo* studies have shown that the beneficial long-lasting effects of GLP-1 can be partly attributed to changes in β-cell mass due to β-cell growth and differentiation (182). However, the ability to stimulate β-cell neogenesis was not associated with the promotion of proliferative lesions in a 2-yr rodent safety study (188) (Fig. 3).

The GLP-1-dependent regulation of glucose homeostasis appears to be based on biological mechanisms far more complex than the simple modulation of insulin secretion. Indeed, GLP-1 also affects the expression of insulin and other β-cell-specific genes whose products are involved in the regulation of glucose utilization. The mechanism by which GLP-1 modulates β-cell-specific gene expression is unknown, but recent studies strongly suggest involvement of the homeodomain transcription factor *PDX-1*. The capability of GLP-1 to regulate the expression of *PDX-1*, a major determinant in pancreatic organogenesis, has led investigators to propose that GLP-1 may be involved in the regulation of the mechanism(s) that governs endocrine phenotype specification during β-cell differentiation and growth (187).

Therapy with GLP-1R agonists has also been associated with expansion of β-cell mass via stimulation of β-cell proliferation, promotion of islet cell neogenesis, and inhibition of β-cell apoptosis (185, 190). Because GLP-1R is widely expressed and the observation that activation of GLP-1R signaling is coupled to stimulation of cell proliferation and enhanced cell survival, it is viewed as a positive aspect of GLP-1 action in the context of promoting expansion and survival of functioning β-cells (86) and has generated great

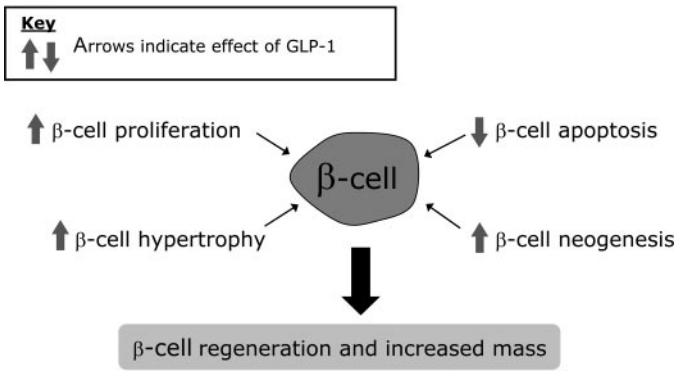

FIG. 3. GLP-1 stimulated β-cell regeneration and mass in animal models. [Reproduced with permission from A. Bahnsali: International Investigator's Meeting of the Clinical Study NN 2211-1697 (Liraglutide) Zurich, Switzerland, April, 2006 (189).]

interest in the development of GLP-1R agonists for treatment of DM2. However, the proliferative and antiapoptotic action of these agents may have theoretical consequences on tumor formation in either the endocrine or exocrine pancreas, considering that GLP-1R may also be expressed in the pancreatic ductal epithelium, a potential precursor site for the development of pancreatic cancer. Recently it was shown that although human pancreatic cancer cell lines may express a functional GLP-1R, activation of GLP-1R signaling by synthetic exendin-4 was not coupled to stimulation of cell proliferation or resistance to cell death in short-term studies (191). The levels and duration of exendin-4 exposure in the study in question were considerably different from levels of exposure to exendin-4, recently introduced in the treatment of DM2, that might be expected in human subjects.

Furthermore, it was shown that human and nonhuman primate islet culture with excendin-4 and daily exendin-4 administration to transplant recipients (nondiabetic or diabetic mice) increase both the survival and function of islets transplanted into the liver (192).

In Table 2 are presented the animal data relative to the effects of GLP-1 and incretin mimetics (exendin-4 and liraglutide) on β-cells.

b. In vitro data in human β-cells. Farilla *et al.* (195) found that GLP-1 delayed the morphological changes that occurred in human islets in culture, indicated by a longer-lasting preservation of their three-dimensional structure, along with maintenance of the noncellular membrane that surrounds healthy human islets. GLP-1 promoted a time-dependent increase in the expression of the antiapoptotic protein bcl-2 and a down-regulation of the intracellular levels of the active form of caspase-3. A similar effect was observed at the mRNA level for bcl-2 and caspase-3. It was also shown that by improving cell viability, Farilla *et al.* (195) were able to show a significant amelioration of islet cell function. Indeed, GLP-1-treated human islets contained more insulin and were capable of greater glucose-dependent insulin secretion. However, because of the short-term nature of the experiment (the islets were cultured up to 5 d), an increase in the size or number of islets was not observed in response to GLP-1.

Furthermore, it was demonstrated by Buteau *et al.* (196) that GLP-1 prevented glucose- and palmitate-induced apoptosis, singly or combined, in overnight cultured human islet cells isolated from healthy organ donors, between 50 and 65 yr old. The antiapoptotic action was mediated via activation of the phosphatidylinositol-3 kinase/protein kinase B signaling pathway, enhanced downstream target NF-κB DNA binding activity, and possibly stimulating the expression of IAP-2 and Bcl-2, two antiapoptotic genes under the control of NF-κB. The same antiapoptotic mechanism was observed earlier by Hui *et al.* (184) in cultured mouse insulinoma cell line and, as stated above, in human islets (195). Inhibition of NF-κB abolished the prevention of glucolipotoxicity by GLP-1. The antiapoptotic action of GLP-1 and incretin mimetics, in addition to their incretin effect, are of great interest as therapeutic agents in the treatment of DM2, particularly in the presence of glucolipotoxicity, which is an important factor for β-cell decompensation in the development of obesity-associated DM2 (196). It would be interesting to mention that GLP-1-induced β-cell proliferation has not yet been reported in human islets, only in rodent.

Microarray analysis performed on cultured human islet preparations by Hui *et al.* (197) showed that the long-acting GLP-1 analog liraglutide modulated the expression of multiple members of the TGF-β pathway.

c. Clinical evidence of incretin mimetic effects on human β-cell function

1) GLP-1 receptor agonists. In DM2 individuals, like native GLP-1, GLP-1 receptor (GLP1-R) agonists enhance insulin release and inhibit glucagon secretion, in addition to delaying gastric emptying, improving insulin sensitivity and activating regions in the central nervous system that control satiety and reduces body weight (198). More recently, studies with animal models suggest that GLP-1R agonists may also enhance insulin-independent glucose disposal in peripheral tissues, potentially via stimulation of glucose sensors located in the portal vein and subsequent activation of neuronal pathways that modify glucose clearance (199). In addition to glucoregulatory actions, GLP-1R agonists exhibit protective

TABLE 2. Effects of incretin (GLP-1) and incretin mimetics (exendin-4 and liraglutide) on β-cells: animal studies

Experimental model	Ref.	GLP-1/incretin mimetic	Replication	Proliferation	Neogenesis	Differentiation	Apoptosis	Mass
Rodent diabetes or cultured β-cells	37, 86, 184, 187, 190	GLP-1/exendin-4	↑	↑	↑	↑	↓	↑
Partially pancreatectomized rats	185, 193, 194	Exendin-4, liraglutide	↑		↑		↓	↑
Aging Wistar rats	186, 190	GLP-1			↑	↑	↓	↑

effects in the cardiovascular and nervous systems after experimental injury (see references cited in Ref. 198).

Exenatide. In clinical studies, exenatide (synthetic exendin-4) exhibited actions that are similar to those of GLP-1, as outlined above: stimulation of insulin secretion only when glucose concentrations are elevated, suppression of postprandial glucagon secretion, slowing of gastric emptying, and promoting of satiety (157). The significant attenuation of postmeal plasma glucose elevation after exenatide infusion was related to a reduction in the rate of oral glucose appearance in the systemic circulation and enhancement of the suppression of endogenous glucose production; half of the decrease in endogenous glucose production results from the inhibition of glucagon secretion and half from increased insulin secretion, as observed in a study of six DM2 patients taking metformin plus sulfonylurea, with HbA1c approximately 8.5% (200).

Three similarly designed 6-month placebo-controlled trials form the basis of the approval of exenatide for use in patients with DM2 who exhibit unacceptable glycemic control while on metformin and/or a sulfonylurea, testing the addition of exenatide to metformin alone, sulfonylurea alone, or metformin and a sulfonylurea together (166–168). The placebo-adjusted decline of HbA1c from baseline levels of 8.2–8.6% was approximately 1.0% in each trial. A mean placebo-adjusted weight loss of 2.5 kg occurred when exenatide was added to metformin, 1.0 kg when the drug was added to a sulfonylurea, and 0.9 kg when it was added to metformin plus a sulfonylurea. All patients began the treatment with 5 μg injected twice daily sc, before the morning and evening meals, for the first month, followed by 10 μg twice daily thereafter (166–168.) After 30 wk, a majority of the patients continued in an open-labeled extension for 82 wk.

A subset of 73 subjects from the exenatide plus metformin and exenatide plus metformin plus sulfonylurea groups had postprandial β-cell function evaluated at baseline and after 6 months (201). Glucose excursions after meal tolerance tests were significantly reduced after exenatide but not after placebo. To assess β-cell function independent of confounding effects from postprandial plasma glucose differences, mathematical modeling was performed incorporating three main components of β-cell function: rate sensitivity (early ISR), dose-response relating ISR to plasma glucose concentration (glucose sensitivity), and a potentiation factor, which is a function of time and may reflect the actions of nonglucose secretagogues along with other factors (202). The mathematical model indicated a greater insulin response for any glucose concentration. Also, exenatide increased potentiation at 2 h after a meal compared with placebo, presumably due to its incretin effect because it is only partially affected, whereas the β-cell function is inherently impaired in DM2 (203). In summary, exenatide enhanced postprandial β-cell function in patients with DM2 treated with metformin or metformin and sulfonylurea.

It was demonstrated that iv exenatide enhanced first- and second-phase insulin secretion in response to an iv glucose bolus in subjects with DM2 treated with diet/exercise, metformin, or acarbose and compared with healthy subjects with normal oral glucose tolerance test not receiving exenatide. Exenatide-treated patients with DM2 had a similar secretory pattern (first- and second-phase insulin secretion) but higher insulin levels than healthy subjects, in contrast to patients with DM2 treated with placebo who had blunted first-phase insulin secretion compared with healthy controls. This effect is consistent with the ability of exenatide to improve β-cell function acutely (83).

Liraglutide. Liraglutide has 97% homology with GLP-1 and resists DPP-IV degradation by FA acylation and albumin binding (204). Single-dose kinetic studies in DM2 subjects revealed a half-life of 10.0 ± 3.5 h, allowing for single daily-dose administration (205), whereas native GLP-1 with a very short half-life of 1.5 ± 0.35 min has limited clinical value (206). It was demonstrated that bedtime administration of a single dose of liraglutide in well-controlled DM2 (compared with placebo) resulted in a significant reduction in fasting glucose while restoring β-cell responsiveness to physiological hyperglycemia, although β-cell function was grossly impaired in the absence of liraglutide (207). Once-daily dosing of 6 μg/kg sc for 1 wk significantly reduced overall glycemia while improving first-phase insulin response to glucose and almost doubling the disposition index, with a fall in PI/IRI ratio along with diminishing glucagon levels and glycogenolysis, whereas gluconeogenesis was unaltered (208). At this dose, no effect on gastric emptying and no nausea were noted (184).

A phase II 14-wk study was performed to assess the efficacy and safety of liraglutide once-daily in 165 DM2 patients (HbA1c at randomization, 8.1–8.5%), drug-naive or on single oral antidiabetic agent (washout for 4 wk) randomized to one of three doses of liraglutide (0.65, 1.25, or 1.9 mg) as monotherapy or placebo. Significant improvement in HbA1c was achieved in all actively treated groups *vs.* placebo, with an estimated difference *vs.* placebo of 1.74% at the highest dose. Dose-dependent weight reduction was achieved in the highest dose with the estimated change in body weight being −2.99 kg from baseline (209).

To characterize β-cell function improvement by liraglutide, 24-h triple-meal tests were performed in 13 DM2 subjects, with liraglutide being administered for 7 d before tests. ISR was calculated from C-peptide deconvolution, and β-cell function was assessed using the mathematical model with three main components of β-cell function: 1) early (first-phase) ISR (rate sensitivity); 2) glucose sensitivity = dose-response curve; and 3) a potentiation factor (function of time, expressing relative ISR increase), as outlined previously (202). Modeling analysis indicated that rate sensitivity did not change significantly, but there was a marked effect on β-cell dose-response, which was shifted upward and became steeper (increase in glucose sensitivity). In addition, liraglutide stimulated potentiation during the first meal (210). In conclusion, liraglutide enhanced several β-cell function parameters, and the enhancement was correlated with the improvement in glycemic control. The mechanisms of liraglutide action, as expected, appear to be analogous to those exerted by endogenous incretins and incretin mimetics (201).

Rodent studies have indicated that liraglutide can increase β-cell mass in rat models of β-cell deficiency (male ZDF rats and in 60% pancreatectomized rats), but it may in part depend on the metabolic state of the animals, those with higher blood

glucose levels presenting greater β-cell mass (193). Furthermore, using primary neonatal rat islets, it was shown that native GLP-1 and liraglutide inhibited both cytokine- and FFA-induced apoptosis in a dose-dependent manner, thus playing a role in the maintenance of β-cell mass in diabetes (194).

To identify genes involved in the antiapoptotic activity of liraglutide, human islets freshly isolated from three cadaveric donors were treated with liraglutide for 22 h while the same number of untreated islets from the same donors served as a control. Microarray analysis, indicated that the expression of 96 genes was up-regulated, whereas the expression of 257 genes was down-regulated in the islets exposed to liraglutide. Of the genes with significant change in response to liraglutide, five were involved with cell proliferation; cell survival mechanisms and resistance to apoptosis were found to be increased 2-fold or more. The expression of four proapoptotic genes was also suppressed, and two genes related to inhibition of caspase were also down-regulated. It was concluded that liraglutide inhibits cell death in human isolated islets by regulating the expression profile of apoptosis-related genes (211).

In summary, liraglutide, can control diabetes by multiple actions through increasing insulin and lowering glucagon, yet it has a rapid and sustained glycemic effect and is associated with weight reduction. All actions are predictably strictly glucose-dependent, with low hypoglycemia risk and counterregulatory response to hypoglycemia not being impaired. Liraglutide may potentially delay disease progression, considering the β-cell function improvement in DM2 and β-cell mass shown to increase in animal models. Finally, it is well tolerated, with mild and transient gastrointestinal symptoms.

Table 3 indicates the overlapping and distinct properties of the two GLP-1R agonists exenatide and liraglutide, the former presently available and the latter in phase III evaluation, for the treatment of DM2 (212).

Table 4 shows the clinical evidence of incretin (GLP-1) and incretin mimetics (exendin-4 and liraglutide) effects on β-cell function in humans.

Summary/conclusions. Incretin hormones are peptides released from the gastrointestinal tract in response to nutrient ingestion to enhance glucose-dependent insulin secretion from the pancreas and aid in the overall maintenance of glucose homeostasis through slowing of gastric emptying, inhibition of glucagon secretion, and control of body weight. The two major incretins are GLP-1 and GIP. GIP decreases gastric emptying to a much lesser degree and does not inhibit glucagon secretion, although it does exert a potent stimulatory effect in normal rodents and human β-cells, the diabetic β-cell being resistant to GIP action. Thus, the majority of efforts to develop incretin-based therapies are focused on GLP-1. The major component of the glycemic effect of GLP-1 or GLP-1R agonists, exendin-4 and the GLP-1 conjugated to albumin (liraglutide), is attributable to the inhibition of gastric emptying and glucagon secretion, with the glucagonostatic effect of GLP-1 and incretin mimetics being mediated via an endocrine effect on the α-cell.

The acute effect of GLP-1 and GLP-1R agonists on β-cells in rodent models of diabetes and in cultured β-cells is stimulation of glucose-dependent insulin release, whereas the subacute effect is enhancing insulin biosynthesis and stimulation of insulin gene transcription. Their chronic action is stimulation of β-cell proliferation, induction of islet neogenesis from precursor ductal cells, and inhibition of β-cell apoptosis, thus promoting an expansion of β-cell mass.

The GLP-1R agonist, exenatide (synthetic exendin-4), exhibited similar actions to those of native GLP-1. The addition of exenatide to DM2 patients with unacceptable glycemic control while on metformin alone, sulfonylurea alone, or metformin plus sulfonylurea in 6-month placebo controlled trials demonstrated a placebo-adjusted decline of HbA1c and a placebo-adjusted weight loss.

Postprandial β-cell function evaluated in a subset of 73 subjects from exenatide plus metformin and exenatide plus metformin plus sulfonylurea groups, at baseline and after 6 months, using a mathematical model incorporating the three main components of β-cell function (rate sensitivity, glucose sensitivity, and potentiation factor), indicated that exenatide enhanced postprandial β-cell function, presumably due to its incretin effect in DM2. Furthermore, iv exenatide enhanced first- and second-phase insulin secretion in response to an iv glucose bolus in DM2 treated with diet/exercise, metformin, or acarbose.

Liraglutide has 97% homology with GLP-1 and resists DPP-IV degradation by FA acylation and albumin binding, with a half-life of 10 ± 3.5 h, allowing for a single daily-dose administration, whereas native GLP-1 with a very short half-life of 1.5 ± 0.35 min has limited clinical value. To characterize β-cell function improvement by liraglutide, 24-h triple meal tests were performed in DM2 after 1-wk liraglutide treatment, where insulin secretion was assessed by the mathematical model with three components of β-cell function showing an enhancement of several β-cell function parameters that correlated with improvement in glycemic control, analogous to the mechanisms exerted by endogenous incretins and incretin mimetics.

Rodent studies have indicated that liraglutide can increase β-cell mass in rat models of β-cell deficiency. This was demonstrated in human islets freshly isolated from three cadaveric donors treated with liraglutide, where microarray analysis indicated that liraglutide inhibited cell death by regulating the expression profile of apoptosis-related genes.

TABLE 3. Incretin mimetics: exenatide *vs.* liraglutide

	Exenatide	Liraglutide
Administration	Injection	Injection
Half-life (h)	≈2–4	≈12–14
Frequency of injection	Twice daily	Once daily
Dose per injection	5–10 μg	Up to 2 mg
DPP-IV substrate?	No	No
Insulin secretion[a]	↑	↑
Glucagon secretion[a]	↓	↓
Fasting glucose	↓	↓ ↓ ↓
Weight reduction	Yes	Yes
Gastric empting	↓	(↓)
Antibody production	Yes (≈ 45%)	No

Reproduced with permission from M. A. Nauck: Proc of the 66th Scientific Sessions, American Diabetes Association, June 11, 2006 (212).

[a] Glucose-dependent.

TABLE 4. Clinical evidence of incretin (GLP-1) and incretin mimetics (exendin-4 and liraglutide) effects on β-cell function in humans

Peptide	Administration	Ref.	Subject	Findings
GLP-1	Overnight + 2 h iv	81	DM2	↑ First and second phase insulin secretion
	Extended (12 h) iv	181	IGT, DM2	↑ Ability of the β-cell to sense and respond to subtle changes in plasma glucose (IGT > DM2)
	Extended (5 h) iv	180	Normal, DM2	↑ First and second phase insulin secretion
Exendin-4	Extended (5 h) iv	83	Normal, DM2	↑ First and second phase insulin secretion (greater than normal)
	6 months sc exanetide + oral metformin or oral metformin + sulfonylurea	201	DM2	Postprandial β-cell function ↑ Insulin response/any glucose level ↑ Potentiation at 2 h after meal (incretin effect)
Liraglutide	1 dose sc	207	DM2	↑ β-cell sensitivity (graded glucose infusion) ↓ Proinsulin/insulin ratio ↑ Insulin secretion area (ISR) and slope of ISR *vs.* plasma glucose (≈ nondiabetic controls)
	1 wk sc (1 dose/d)	208	DM2	Unchanged 24-h ISRs at lower plasma glucose ↑ First phase insulin response ↑ Disposition index[a] ↓ Proinsulin/insulin ratio
	1 wk sc (1 dose/d)	210	DM2	Postprandial β-cell function (24 h triple meal tests) ↑ β-cell dose response curve (↑ glucose sensitivity) ↑ Potentiation (relative ISR with time)–incretin effect

[a] $S_i \times AIR_g$.

2) DPP-IV inhibitors ("incretin enhancers"). The major therapeutic drawback of using native GLP-1 is its very short half-life of less than 2 min after exogenous administration, as previously indicated, due in part to the protease DDP-IV (157). A result, preventing the degradation of native GLP-1 by inhibiting the activity of the DDP-IV enzyme, has emerged as a therapeutic strategy for enhancing endogenous GLP-1 action *in vivo*.

DPP-IV is a ubiquitously expressed serine protease that exhibits postproline or alanine peptidase activity (213). The requisite for cleavage is that an alanine or a proline must be present as the second amino acid from the N-terminal end, in which case the two N-terminal amino acids are cleaved from the rest of the peptide. When this cleavage results in a loss of activity, such as with GLP-1 and GIP, DPP-IV acts as an inactivation enzyme. However, because exogenous GIP is comparatively less effective than GLP-1 at stimulating insulin secretion in DM2, whereas the insulinotropic action of GP-1 is well preserved, as shown earlier, much of the current research has focused on enhancing GLP-1 action for the treatment of DM2. However, it is possible that the effects of DPP-IV inhibition in diabetes are mediated by inhibition of bioactive peptides other than GLP-1. One puzzling finding might support this, as indicated by Ahrén (214): in patients with DM2, concentrations of active GLP-1 after meal ingestion are doubled by DPP-IV inhibition (compared with placebo), whereas glucose control improves (215). In contrast, when similar increases in GLP-1 levels are produced by exogenous infusion, there is little or no effect on insulin secretion or glucose levels (216). This suggests that mediators other than GLP-1 may contribute to the therapeutic effect of DPP-IV inhibition. Alternatively, recent findings indicate that GLP-1 may work indirectly through activation of the autonomic nervous system (217), an effect possibly mediated by local activation of afferent nerve terminals. If this proves to be an important mechanism, comparison of circulating levels of GLP-1 under different conditions becomes less relevant because the key factor would be the local concentration of active GLP-1 in the vicinity of nerve terminals rather than the concentration in the circulation (218).

Concerning the potential mediators of the therapeutic effects of DPP-IV inhibition, Nauck and El-Ouaghlidi (218) presented five arguments suggesting that GLP-1 is not the only, or at least not the major, mediator of the glucose-lowering actions of DPP-IV inhibition observed in clinical studies (215, 219, 220); *i.e.*, DPP-IV inhibition: 1) causes little increase in circulating endogenous GLP; 2) has little effect on gastric emptying; 3) does not cause nausea/vomiting as do GLP-1 and incretin mimetics; 4) meal-stimulated GLP-1 fall; and 5) has delayed effects on glucose homeostasis (218). In the same issue of *Diabetologia*, Holst and Deacon (221) acknowledge these arguments but maintain that current evidence nonetheless supports the dominant role of GLP-1.

Table 5 summarizes the overlapping and distinct properties of GLP-1R agonists *vs.* DPP-IV inhibitors for the treatment of DM2 (222).

Ahrén (214) has suggested that neuropeptides, biologically active peptides localized to islet nerve terminals and functioning as neurotransmitters, may be substrates for DPP-IV (213), thus contributing to the actions of DPP-IV inhibitors in diabetes. Neuropeptides that are stored in islet nerve terminals and affect islet function would be of particular relevance in this regard, and the islet autonomic nervous system could be an important regulator of islet function (223). One neuropeptide of potential importance is pituitary adenylate cyclase-activating peptide (PACAP), which is localized to islet nerves and has several actions relevant to glucose homeostasis (224). Because PACAP is also a substrate for DPP-IV, it could contribute to the therapeutic benefits of DPP-IV inhibition recently shown in mice whereby PACAP-induced insulin secretion increased after inhibition of DPP-IV by valine pyrrolidide (225).

The potential risks associated with DPP-IV inhibitors include the prolongation of the action of other peptide hor-

TABLE 5. GLP-1R agonists *vs.* DPP-IV inhibitors

	GLP-1R	DPP-IV inhibitors
Administration	Injection	Orally available
GLP-1 concentrations	Pharmacological	Physiological
Mechanism of actions	GLP-1	GLP-1 + GIP
Activation of portal glucose sensor	No	Yes
↑ Insulin secretion	+++	+
↓ Glucagon secretion	++	++
Gastric emptying	Inhibited	+/−
Weight loss	Yes	No
Expansion of β-cell mass in preclinical studies	Yes	Yes
Nausea and vomiting	Yes	No
Potential immunogenicity	Yes	No

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mones, neuropeptides, growth factors, cytokines, and chemokines cleaved by the protease (213) along with their interaction with DPP-IV-related proteases. Moreover, DPP-IV has effects beyond its proteolytic action, including T cell activation and proliferation. However, to date, side effects resulting from the prolongation of the action of messengers such as those related to inflammation, blood pressure, and allergic reactions from DPP-IV inhibition have not been observed in preclinical animal and clinical human studies (226).

In preclinical studies with normal and diabetic animals, several orally active DPP-IV inhibitors have been shown to increase plasma levels of intact, biologically active GLP-1 and GIP, resulting in stimulation of insulin and inhibition of glucagon secretion in a glucose-dependent manner as well as promotion of β-cell proliferation, neogenesis, and inhibition of apoptosis (198).

Several studies examining the efficacy of the two DPP-IV inhibitors sitagliptin (Januvia, Merck & Co., Whitehouse Station, NJ) and vildagliptin (Galvus, Novartis, East Hanover, NJ) in the treatment of DM2 are now on phase III trials and will be reviewed below.

Sitagliptin (MK-0431). A preclinical study has demonstrated that 8–12 wk of treatment of diabetic mice with an analog of sitagliptin (des-fluoro-sitagliptin) produced restoration of β-cell mass and morphology, increased islet insulin content, and improved glucose-stimulated insulin secretion in isolated islets, similar to that observed in preclinical studies after administration of GLP-1 or GLP-1R analogs (227).

Single doses of 100 mg or higher, with an apparent half-life of 8–14 h, resulted in inhibition of plasma DPP-IV activity over 24 h by at least 80%. Sitagliptin increased the meal-associated rise in active plasma GLP-1 concentration by approximately 2-fold without causing hypoglycemia (228). Therefore, sitagliptin possesses pharmacokinetic and pharmacodynamic characteristics that support a once-daily dosing regimen, stimulating insulin secretion, inhibiting glucagon secretion, and reducing glycemic excursions after an oral glucose load in mild DM2 (229).

There are recent reports from the ongoing phase III program with sitagliptin involving three large studies. In one study, sitagliptin monotherapy reduced significantly HbA1c and improved glycemic control in the fasting and postprandial state after 24 wk in 741 patients with DM2, randomized to placebo or sitagliptin. Postmeal insulin and C-peptide AUC, ratio of insulin AUC/glucose AUC, and HOMA-β, which are all measures of β-cell function, were significantly increased with sitagliptin relative to placebo. Moreover, PI/IRI ratio was significantly reduced with sitagliptin compared with placebo (230). In another study, when sitagliptin was added to ongoing metformin therapy administered to 701 patients with DM2 and inadequate glycemic control on metformin who were randomized to receive either placebo or sitagliptin, a significant reduction in HbA1c, fasting plasma glucose, and 2-h postmeal plasma glucose was observed after 24 wk .The same indices of β-cell function, evaluated as in the above indicated report, were also significantly increased, and the PI/IRI ratio was significantly decreased with sitagliptin relative to placebo (231).

The effect of sitagliptin on β-cell function in DM2 was assessed using frequently sampled (9 points) meal tolerance tests in substudies from three phase III trials (two monotherapy studies and an add-on to metformin study) using the C-peptide minimal model, which allowed for the estimation of the ISR and the characterization of the insulin secretion response into basal (β-cell responsiveness to basal glucose concentrations), static (β-cell responsiveness to above-basal glucose concentrations after a meal), and dynamic (β-cell responsiveness to the rate of increase in above-basal glucose concentrations after a meal) components. Insulin sensitivity was assessed with a validated composite index. The disposition indices were also assessed. When used as monotherapy or added to ongoing metformin therapy, sitagliptin produced improvements in basal and static components and overall responsiveness to the β-cell to glucose. The dynamic component showed numerical, but not statistically significant, improvements with sitagliptin. Disposition indices were also broadly improved with the DPP-IV inhibitor relative to placebo. Despite enhanced β-cell sensitivity to glucose, the low rate of hypoglycemia observed in clinical trials with sitagliptin indicated, according to the authors, that the increase in β-cell function remained glucose-dependent (232).

Finally, the addition of sitagliptin to 353 DM2 patients who were not well-controlled with pioglitazone monotherapy and were randomized to placebo or sitagliptin (100 mg/d) to ongoing pioglitazone produced a significant placebo-subtracted reduction in HbA1c over 24 wk, maintaining the glycemic control over the study period. Proinsulin levels and PI/IRI ratio were significantly reduced with sitagliptin compared with placebo, suggesting improvement in β-cell function without additional weight gain (233).

Vildagliptin (LAF-237). In preclinical studies, the ability of vildagliptin to augment β-cell mass by enhancing endogenous incretin action was assessed in neonatal rats, a model for rapid β-cell turnover and growth. Neonatal rats were treated once daily with vildagliptin or vehicle (control) for 21 d. Vildagliptin increased the number of replicating islet cells significantly and reduced the number of apoptotic islet cells. Additionally, there was a significant increase in pancreatic insulin content and β-cell mass even 1 wk after discontinuation of treatment (234). The head-to-head comparison of vildagliptin with injectable exenatide on *in vivo* β-cell regulation in streptozotocin-induced β-cell injury in mice demonstrated that both drugs are equally effective in reducing the streptozotocin-induced proliferative response, a protective effect against β-cell injury as well as in promoting early differentiation of pancreatic progenitor cells, in increasing formation of ductal β-cell and improving glucose tolerance to a similar extent in streptozotocin-diabetic mice. Furthermore, the effect was maintained after the treatment washout (235).

In clinical studies with vildagliptin, postmeal glucose levels were significantly decreased, but postmeal insulin levels were unchanged (220). Although such findings might be interpreted to suggest that DPP-IV inhibitors do not improve insulin secretion in patients with DM2, it should be recognized that circulating insulin levels are not a direct measure of insulin secretion and that insulin secretion must be considered in the context of ambient glucose levels. Accordingly, β-cell function can be improved without appreciable changes in circulating insulin levels, particularly if glucose values are reduced (236). The effect of 4-wk treatment with vildagliptin (100 mg, twice daily) on meal-related β-cell function was examined in drug-naive patients with DM2, using the mathematical model previously presented, which derives multiple parameters of β-cell function relating ISRs and glucose levels (β-cell dose response), change in glucose with time (derivative component), and a potentiating factor (201). Vildagliptin was found to improve β-cell function by increasing insulin secretion at any given glucose level (secretory tone), whereas the slope of the β-cell dose response, the derivative component, and the potentiation factor were not affected. This would suggest that insulin secretion at a given glucose concentration is augmented by vildagliptin (236). The same type of study was performed over 52 wk in metformin-treated DM2 subjects (inadequately compensated). Patients were randomized to either metformin and placebo or metformin and vildagliptin, 50 mg once daily (237). Meal tests were performed at study commencement and again after 12, 24, and 52 wk to evaluate meal-related β-cell function, calculating several parameters: the insulin secretion; insulin sensitivity during meal ingestion (238); the adaptation index, which gives a figure for the ability of the β-cell to adapt insulin secretion to the ambient insulin sensitivity (insulin secretion × insulin sensitivity) (239). Administration of metformin and vildagliptin for 52 wk was associated with reduced fasting and postprandial plasma glucose (with a sustained highly significant reduction in HbA1c), enhanced postprandial insulin secretion, increased dynamic insulin sensitivity in relation to meal intake compared to fasting (static) insulin sensitivity and adaptation index, and a reduced PI/IRI ratio compared with baseline values. In contrast, for the metformin-placebo-treated groups, fasting plasma glucose increased (as well as HbA1c values), postmeal insulin secretion and adaptation index decreased, but insulin sensitivity during meal ingestion was not altered, and no net change was observed for the PI/IRI ratio (237).

Although vildagliptin improves β-cell function and reduces fasting and postprandial glucose in patients with DM2, it did not increase absolute postmeal insulin levels either during 4–12 wk of treatment in patients with DM2, as described above, or after a single dose given before a 75-g oral glucose tolerance test in healthy subjects (240). In diet-treated DM2 patients, an acute insulinotropic effect of vildagliptin (100 mg) was observed only when an oral glucose load (75 g) provided an intense glucose stimulus for insulin secretion when compared with a standard breakfast meal test (incremental glucose AUC ~5-fold greater) (241).

Regarding the mechanisms by which a single-dose administration of vildagliptin (100 mg) before a mixed evening meal improves glucose tolerance and reduces fasting plasma glucose in DM2, it was shown that increasing ISR, despite a significant reduction in plasma glucose (increase in insulinogenic index) and suppressing plasma glucagon, leads to enhanced suppression of endogenous glucose production. During the overnight period, endogenous glucose production was significantly reduced, and its decline was positively correlated with the decrease in fasting plasma glucose (242). It was demonstrated that 12-wk treatment of drug-naive DM2 with vildagliptin (50 mg twice daily), compared with placebo, restored the AIR_g and the S_i determined during FSIVGTT. The disposition index ($AIR_g \times S_i$) increased more than 4-fold where part of this effect remained after a 2- to 4-wk washout, suggesting that vildagliptin may exert some disease-modifying effect (243).

Recently, some results from ongoing phase III programs with vildagliptin have been presented in the same line described in the sitagliptin section. One study comprising 780 patients compared vildagliptin monotherapy (50 mg twice daily) with metformin. After 52 wk, HbA1c levels were reduced significantly with both drugs. Vildagliptin was better tolerated than metformin. Both treatments were weight neutral (244). In a second study comprising 416 patients, vildagliptin (50 mg once or twice daily) or placebo was added to metformin, improving glycemic control when the incretin enhancer was given in comparison to placebo in a 24-wk study. Vildagliptin was well-tolerated and mitigated metformin-induced gastrointestinal side effects whenever present (245).

A third study comprised 256 patients and compared vildagliptin with placebo in inadequately controlled DM2 requiring insulin. After 24 wk, vildagliptin 100 mg/d plus insulin reduced insulin requirements and HbA1c slightly more in comparison with the patients treated with placebo plus insulin. The hypoglycemic events were less common and less severe in the vildagliptin-treated patients (246).

Although sitagliptin is not quite as potent as vildagliptin, the available data from single-dose studies indicate that both compounds have similar clinical efficiency in reducing glucose excursion after oral glucose administration (247). Data are not yet available to enable comparison of the efficacies of

both DPP-IV inhibitors following chronic dosing. Preclinical data suggest that sitagliptin may have a longer duration of action than vildagliptin, since its half-life in monkeys is >2-fold longer than that of vildagliptin. Thus, in clinical trials with vildagliptin as monotherapy a twice daily dosing has been used while therapy with sitagliptin an once daily dosing regimen should be indicated. Enzyme inhibitory data released has shown that sitagliptin has more selectivity for DPP-IV than vildagliptin. If sitagliptin is superior to vildagliptin in this respect, the biological consequences of inhibiting DPP-IV related enzymes remains poorly understood. However, inhibition of DPP VIII/IX may be associated with considerable toxicity in preclinical studies (247, 248). Given the absence of serious side effects in clinical studies either with sitagliptin or vildagliptin, any differences in selectivity between inhibitors is likely to be of more academic, rather than clinical importance (247).

In Table 6 are shown the effects of incretin enhancers on β-cells in animal studies. Table 7 shows the clinical evidence of the effects on β-cell function in humans with DM2.

Summary/conclusions. Preventing the degradation of native GLP-1 by inhibiting the activity of the DDP-IV enzyme has emerged as a therapeutic strategy for enhancing endogenous GLP-1 action *in vivo*. The effects of DPP-IV inhibition could be mediated not only by GLP-1 but also by other mediators of the glucose-lowering actions of DPP-IV inhibition in clinical studies because it causes little increase in circulating endogenous GLP-1, has little effect on gastric emptying, does not cause nausea/vomiting like GLP-1 and GLP-1R agonists, and is not associated with weight loss. In preclinical studies, oral active DDP-IV inhibitors have been demonstrated to promote β-cell proliferation, neogenesis, and inhibition of apoptosis in rodents similar to that observed after the administration of GLP-1 or GLP-1R agonists.

In contrast with GLP-1R agonists, which are administered via injection, the DPP-IV inhibitors, sitagliptin and vildagliptin, accepted for review by the U.S. Food and Drug Administration, are administered orally and are well tolerated. Major adverse effects have not been reported in clinical trials examining the efficacy of DPP-IV inhibitors for the treatment of DM2.

In healthy subjects, a single dose of 100 mg or higher of sitagliptin resulted in inhibition of plasma DDP-IV activity over 24 h by at least 80%, increasing a meal-associated rise in active plasma GLP-1 levels 2-fold without hypoglycemia. Phase III studies with sitagliptin plus ongoing metformin and sitagliptin plus ongoing pioglitazone have indicated an improvement in HbA1c and glycemic control as well as postmeal indices of β-cell function. The effect of sitagliptin on β-cell function, assessed by frequently sampled meal tolerance tests in three substudies from phase III using the C-peptide minimal model, showed improvement in β-cell responsiveness to basal and above-basal glucose concentration components after the meals. The dynamic component (β-cell responsiveness to the rate of increase in above-basal glucose levels after a meal) showed numerical but not significant improvements with sitagliptin. Disposition indices were also improved.

Preclinical studies have demonstrated that vildagliptin increased β-cell mass by increasing the number of replicating islet cells and reducing the number of apoptotic islet cells as assessed in neonatal rats, a model for rapid β-cell turnover and growth. The head-to-head comparison of vildagliptin with exenatide in a mouse model of streptozotocin-induced β-cell injury showed that both had a protective effect against β-cell injury, promoted early differentiation of pancreatic progenitor cells, and increased formation of ductal β-cells, improving glucose tolerance with the effect being maintained after the treatment washout.

Although vildagliptin reduced postprandial glucose in DM2, it did not increase postmeal insulin levels during 4–12 wk of treatment. In diet-treated DM2, oral glucose load (75 g) provided an intense glucose stimulus for insulin secretion when compared with a standard breakfast meal test. In drug-naive patients, 4-wk treatment with vildagliptin (100 mg twice daily) improved β-cell function by increasing insulin secretion at any glucose level, whereas the slope of β-cell dose response and the potentiation factor were not affected using a mathematical model. In a similar study over 52 wk of vildagliptin added to metformin-treated DM2, an improvement was demonstrated in β-cell function, with enhanced postprandial insulin secretion and increased insulin sensitivity and disposition index. Twelve-week treatment of drug-naive DM2 with vildagliptin (50 mg twice daily) restored the AIR_g and S_i determined by FSIVGTT. The disposition index ($AIR_g \times S_i$) increased >4-fold, and part of its effect remained after a 2- to 4-wk washout, suggesting that vildagliptin may exert some disease-modifying effect. Three recent studies have been carried out in the same line as for sitagliptin. One study compared vildagliptin (50 mg twice daily) monotherapy with metformin over 52 wk, with better sustained control with the DPP-IV inhibitor. In the second study, vildagliptin (50 mg daily or twice daily) was added to metformin, with improvement of glycemic control slightly better with the larger dose. The third study compared vildagliptin (100 mg daily) with placebo in insulin-requiring DM2. After 24 wk, there was a reduction in HbA1c and in the mean insulin doses.

TABLE 6. Effects of incretin enhancers (sitagliptin and vildagliptin) on β-cells: animal studies

Experimental model	Ref.	Incretin enhancer	β-cell
Diabetic mice	227	Des-fluoro-sitagliptin	↑ β-cell mass ↑ Ratio of insulin positive β-cell/total islet area Restoration of β/α-cell distribution
Neonatal rats	234	Vildagliptin	↑ Replication ↓ Apoptosis ↑ β-cell mass
Streptozotocin-diabetic mice	235	Vildagliptin Exenatide	↑ Glucose tolerance ↑ Differentiation of progenitor ductal cells → ↑ ductal β-cell

TABLE 7. Clinical evidence of incretin enhancer (sitagliptin and vildagliptin) effects on β-cell function in humans with DM2

Peptide	Added medication	Ref.	Findings
Sitagliptin	Monotherapy (24 wk)	230	Postmeal ↑ Insulin and C-peptide AUC ↑ Ratio insulin AUC/glucose AUC ↑ HOMA-β ↓ PI/IRI ratio
	Monotherapy or + metformin (24 wk)	231	Postmeal ↑ Insulin and C-peptide AUC ↑ Ratio insulin AUC/glucose AUC ↑ HOMA-β ↓ PI/IRI ratio
		232	Postprandial β-cell function ↑ β-cell responsiveness to basal glucose ↑ β-cell responsiveness to above-basal glucose after meal ↑ Disposition index[a]
Vildagliptin	Monotherapy (4 wk)	236	Postmeal β-cell function ↑ Insulin secretion at any given glucose level No change in slope of β-cell dose response and other model parameters
	+ Metformin or metformin alone (52 wk)	237	Postmeal β-cell function Vildagliptin + metformin: ↑ postprandial insulin secretion, ↑ insulin sensitivity to meal intake, ↑ adaptation index (insulin secretion × insulin sensitivity) Placebo + metformin: ↓ postprandial insulin secretion and ↓ adaptation index. No change in insulin sensitivity during meal intake
	Monotherapy (12 wk)	243	FSIVGTT ↑ AIR_g, ↑ S_i ↑ Disposition index[a]

[a] $S_i \times AIR_g$.

Single-dose studies indicate that sitagliptin and vildagliptin have similar clinical efficiency in reducing the glucose excursion after oral glucose. Data are not yet available to enable comparison of the efficacies of both products in chronic use.

d. Potential effects on preservation and augmentation of β-cell mass. Regarding the most important question concerning both GLP-1 analogs, GLP-1R activators and DPP-IV inhibitors (incretin enhancers), incretin mimetics, is whether they will be able to slow or prevent the apparently inevitable progress of the DM2, considering their trophic effects on β-cells (249), not only stimulating their proliferation (185, 250) but also enhancing the differentiation of new β-cells from progenitor cells in the pancreatic duct epithelium (251) and, perhaps most importantly, being capable of inhibiting apoptosis of the β-cells including human β-cells (195). Because the normal number of β-cells is maintained in a balance between apoptosis and proliferation, the protective effects of incretin mimetics could be relevant under conditions in which β-cell apoptosis is increased, such as in DM2 (4). Because, for obvious reasons, it is difficult to estimate the protective effects of incretin mimetics on β-cells in humans, there is no clinical evidence that these drugs really have protective effects on β-cells (252). Furthermore, the rate at which β-cell proliferation occurs in humans is not known. Thus, will incretin mimetics protect β-cells or promote their regeneration in clinical use, as appears to be the case in animal studies (157)? A question put forward by Holst in his Claude Bernard Lecture 2005 to the European Association for the Study of Diabetes (252) is how long should one wait for such effects to unfold? Nevertheless, there are two observations addressing this question that were put forward by Holst (252). In the study in which vildagliptin was administered for 52 wk to patients inadequately treated with metformin, subjects showed a sustained improvement in HbA1c, whereas an increase was observed in the control group (215). Similarly, over 82 wk of treatment with exenatide, HbA1c levels remained constant at approximately 7% (253) although, according to the UKPDS, an increase would have been expected over this time span. These sustained effects could be the first indication that incretin mimetics have β-cell protective effects that persist after the plateau, reached 3 to 4 months after initiation of therapy, suggesting that continued exposure to incretin mimetics is required for further improvements in metabolic control. At any rate, these observations, according to Holst (252), tell us that incretin mimetics should be started as early in the clinical course as possible, before β-cell function has deteriorated to unacceptable levels.

Incretin mimetics may exert an indirect effect on β-cell mass by reducing hyperglycemia, which, as indicated before, can cause β-cell dysfunction and apoptosis. Another effect on β-cell mass could be the result of a nonspecific growth factor effect of augmented insulin supply itself (254). A direct effect on β-cell mass has been demonstrated, at least in rodents, by stimulating both β-cell replication and neogenesis and inhibiting cell apoptosis, thus increasing β-cell mass. Because GLP-1R is found in pancreatic ducts, a presumed site of origin of β-cell precursors (162), neogenesis is probably derived from these precursors (Fig. 4).

Regarding glucagon secretion (254), there is abundant evidence that hyperglucagonemia plays a role in the development of hyperglycemia in DM2, contributing to an excessive glucose production by the liver through its action in association with a reduced insulin secretion. However, the efforts to understand and correct the abnormal glucagon secretion

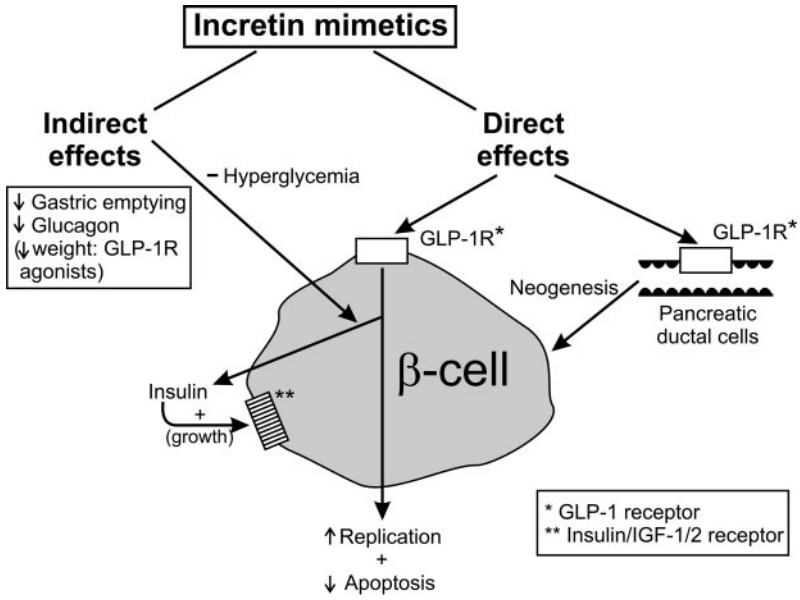

FIG. 4. Pathways supposedly involved in insulin secretion and β-cell mass increase by the action of incretin mimetics.

have been overshadowed by the emphasis on insulin secretion and action. The recognition that GLP-1 and mimetics exert opposing effects on glucagon and insulin secretion has revived interest in glucagon, the neglected partner in the "bihormonal hypothesis" proposed by Unger and Orci 30 yr ago to explain the origin of hyperglycemia in diabetes (255). The defect in the α-cell function that occurs in DM2 reflects impaired glucose sensing, which contributes to the increased rate of hepatic glucose output in DM2. Another manifestation of impaired glucose sensing by the α-cell is the loss of hyperglycemia-induced suppression of glucagon release. Besides, a very important aspect of α-cell dysfunction in diabetes is a failure to respond appropriately to hypoglycemia (256). GLP-1 and incretin mimetics inhibit glucagon secretion, and this action may be as important as its insulinotropic effect in terms of its therapeutic properties. Most of the limited available evidence is consistent with the notion that GLP-1 improves α-cell glucose sensing in DM2, but the α-cell response to incipient hypoglycemia is not suppressed by GLP-1. Furthermore, the ability of GLP-1 and mimetics to suppress glucagon secretion in patients with DM2 appears to be maintained with chronic treatment (256, 257).

The recent description of hyperinsulinemic hypoglycemia and nesidioblastosis together with increased circulating levels of GLP-1 in a few patients after gastric bypass surgery emphasizes the importance of understanding the long-term consequences of prolonged activation of GLP-1 receptors in human subjects (182, 258).

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