Joslin's

DIABETES MELLITUS

FOURTEENTH EDITION

C. RONALD KAHN
GORDON C. WEIR
GEORGE L. KING
ALAN C. MOSES
ROBERT J. SMITH
ALAN M. JACOBSON

LIPPINCOTT WILLIAMS & WILKINS

CHAPTER 25

β-Cell Dysfunction in Type 2 Diabetes Mellitus

Jack L. Leahy

Type 2 diabetes mellitus is a worldwide health crisis; the World Health Organization predicts an incidence of 300 million by 2025 (1). The past decade has seen great progress in our understanding of the pathogenesis of type 2 diabetes (Fig. 25.1). The initial event is a genetic predisposition for glucose intolerance. Although specific polymorphisms or mutated genes are not yet known, many that affect the liver, skeletal muscle, adipose, β-cells, or brain physiology will undoubtedly be uncovered. Lifestyle and environmental factors also determine whether glucose intolerance develops (2). An observation from many cross-sectional and longitudinal studies is the presence of insulin resistance (from obesity, a high-fat diet, inactivity, aging, or a genetic basis) early in the course, even predating the hyperglycemia (3–7). This observation is so well known that type 2 diabetes is understood by most students and practicing physicians to be a disease of insulin resistance. However, several lines of investigation, summarized below, have established a crucial role for β-cell dysfunction.

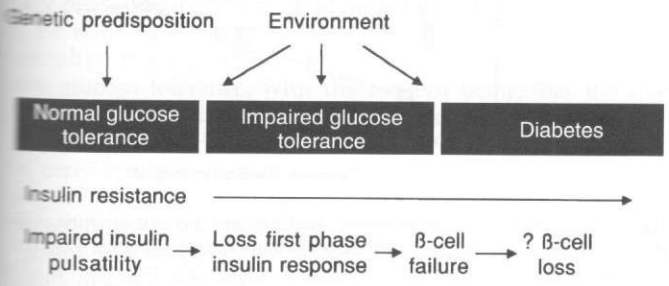


Figure 25.1. Proposed schema for the pathogenesis of type 2 diabetes. Predicted time points for the onset of various aspects of the β-cell dysfunction in this disease that are shown are based on the available literature, as reviewed in the chapter.

- β-Cell dysfunction is always found in type 2 diabetes. Furthermore, it occurs early and likely predates the hyperglycemia (8–10).
- Insulin resistance changes little during the progression from impaired glucose tolerance (IGT) to diabetes. In contrast, β-cell function undergoes substantial change. Cross-sectional and longitudinal studies have shown an increase in fasting insulin level and in insulin response to oral glucose in the early phases of the disease that keep glycemia normal despite the presence of insulin resistance, followed by a decrease when fasting glycemia surpasses 140 mg/dL (11,12). This inverted U-shaped curve of insulin levels (Fig. 25.2) was initially used to support the importance of insulin resistance in the early stages of the disease, as reflected in the increasing insulin output. However, more recent interest has focused on the decline—the so-called *β-cell failure*—as it coincides with, and is thought to cause, the progression from IGT to overt diabetes (9,10,13–15). Further, prospective study of persons with normal glucose tolerance (16) or IGT (17) showed that those with the lowest insulin response to a meal or to an oral glucose challenge (*low insulin responders*) carry the highest risk for developing type 2 diabetes later in life. These experimental observations highlight the importance of β-cell function in determining the glycemic status of at-risk persons.

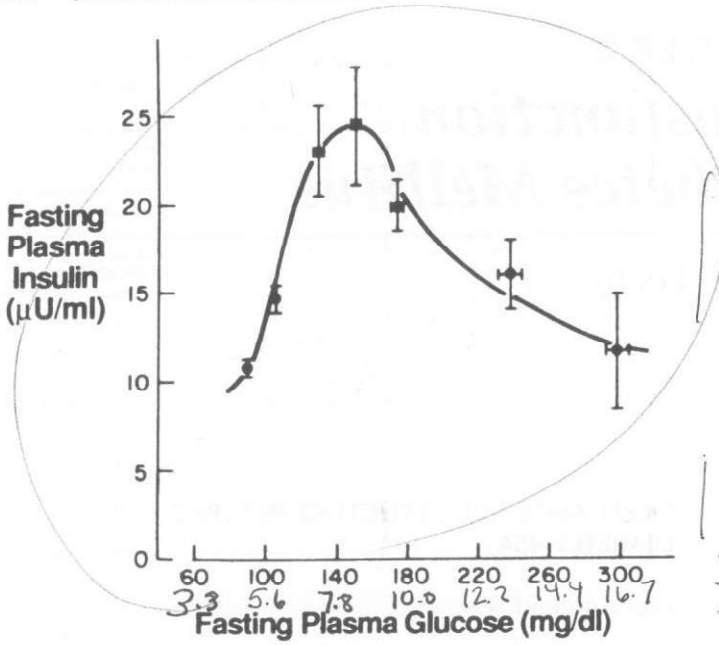


Figure 25.2. Fasting immunoreactive plasma insulin levels in nonobese subjects stratified by the level of glycemia. (From DeFronzo RA, Ferrannini E, Simonson DC. Fasting hyperglycemia in noninsulin-dependent diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake. *Metabolism* 1989;38:387–395, with permission.)

tance in the development of this disease; in the vast majority of affected persons, both must be present for the diabetes syndrome to occur (26). Whether defective insulin secretion and tissue resistance to insulin represent pleiotropic tissue effects of a single defect or multiple abnormalities is unknown. Another understanding is that type 2 diabetes is a progressive disease. Loss of β-cell function, and possibly of β-cell mass, is believed to underlie this progression, highlighting the pivotal role of the β-cell in determining the natural history of this disease. Many excellent reviews on β-cell dysfunction in type 2 diabetes are available (27–32).

NORMAL β-CELL FUNCTION

Pancreatic β-cells regulate the storage and metabolism of cellular fuels through their secretion of insulin. This crucial function is accomplished through a feedback loop whereby glycemia upregulates β-cell function—insulin secretion, proinsulin biosynthesis, processing of proinsulin to insulin, and β-cell replication rate—and the secreted insulin in turn lowers glycemia by inhibiting hepatic and renal glucose production and increasing glucose uptake into target organs, primarily skeletal muscle. Glucose regulation of insulin secretion occurs directly (*glucose-induced insulin secretion*) and also through modulation of the insulin response to insulinotropic hormones, nutrients, and neurotransmitters (*glucose potentiation of nonglucose secretagogues*; Fig. 25.3). These dual aspects of glucose-regulated insulin secretion are a potent modulatory system that ensures that the tissues' needs for insulin are exactly met in the fasting and

- Following the onset of diabetes, the β-cell dysfunction is reversible to a large degree by intensive glycemic control (18–20). In one of these studies (20), insulin pumps were used for 21 days in persons with type 2 diabetes. Improvement was observed not only in β-cell function but also in insulin-mediated suppression of hepatic glucose production and in some lessening of insulin resistance. These findings established the concept that part of the diabetes phenotype is acquired from metabolic derangements in the prediabetes/IGT phase of the disease through glucotoxicity (21,22), β-cell exhaustion (23), or less well defined mechanisms. Dysmetabolism-induced acquired tissue dysfunction explains why type 2 diabetes presents a relatively uniform clinical syndrome in different ethnic groups and populations despite presumed diverse genetic causes.
- Over time, type 2 diabetes becomes less responsive to oral hypoglycemic therapy in tandem with worsening β-cell function (24,25). The working assumption is that pathogenic elements that lead to a loss in β-cell mass become operative.
- There is little information from human studies regarding the biochemical and molecular basis for the β-cell dysfunction in type 2 diabetes because of the unavailability of pancreatic biopsy. Instead, animals—both rodents and larger animals—have been the major venue of investigation, as their β-cell (dys)function with diabetes closely resembles that of humans. Animal studies have identified a panoply of β-cell abnormalities with diabetes. We do not yet know which mechanisms operate in humans, but it seems certain that the β-cell dysfunction will be multifaceted and will entail multiple mechanisms.

To summarize, there is now general acceptance that β-cell dysfunction plays a crucial and necessary role in type 2 diabetes. Indeed, most investigators in the field consider defective insulin secretion and tissue insulin resistance of equal impor-

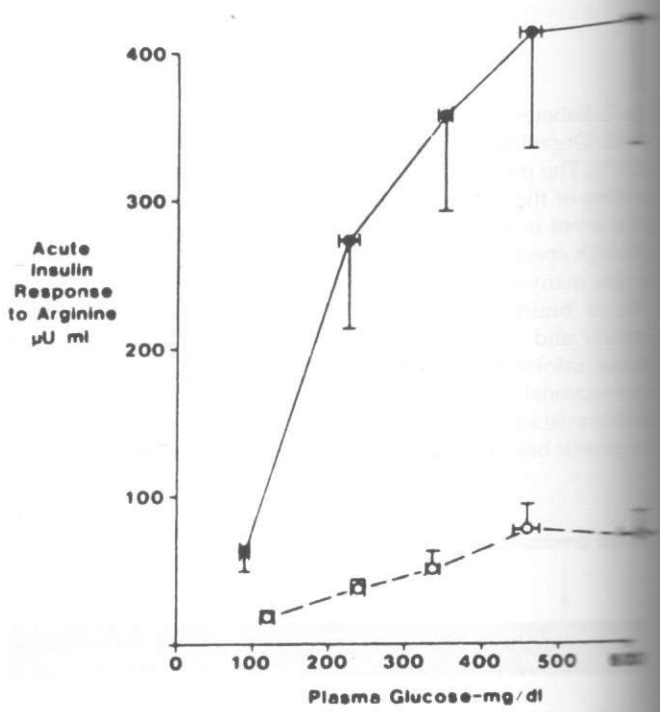


Figure 25.3. Acute insulin responses to 5 g of intravenous arginine at different glucose levels in eight subjects with type 2 diabetes (*open circles*) and eight control subjects (*closed circles*). Insulin responses at all the glucose levels are markedly attenuated in the subjects with type 2 diabetes. (From Ward WK, Bolgiano DC, McKnight B, et al. Diminished β-cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. *J Clin Invest* 1984;74:1318–1328, with permission from the American Society for Clinical Investigation.)

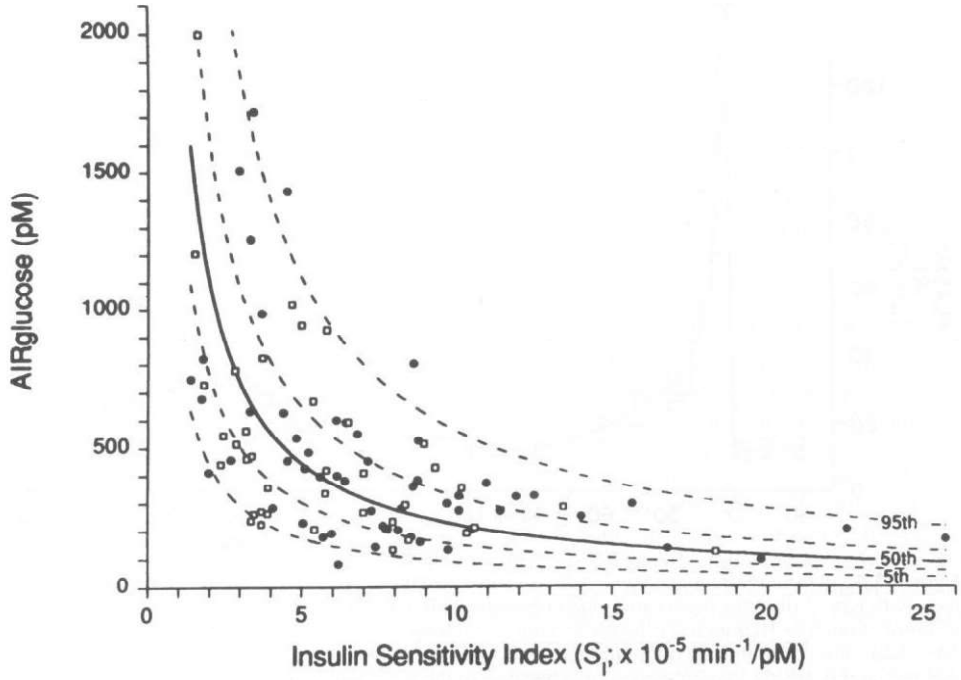


Figure 25.4. Curvilinear relationship between insulin sensitivity (S_I) and the first-phase insulin response (AIRglucose) in 93 normoglycemic subjects: 55 men (*closed circles*) and 38 women (*open squares*). Lines depicting the 5th, 25th, 50th, 75th, and 95th percentiles are shown. (Copyright © 1993 from the American Diabetes Association. From Kahn SE, Prigeon RL, McCulloch DK, et al. Quantification of the relationship between insulin sensitivity and β-cell function in human subjects: evidence for a hyperbolic function. *Diabetes* 1993;42:1663–1672, with permission from the American Diabetes Association.)

prandial states. The need for insulin is for the most part determined by the sensitivity of tissue to insulin—a curvilinear relationship exists with insulin secretion (Fig. 25.4) (33). Thus, even wide variations in insulin sensitivity such as the insulin resistance of puberty (34), pregnancy (35), and aging (36) do not normally affect glycemia. Also, dysfunction of the system can be determined by graphing insulin sensitivity and secretion from the experimental population to see if these values fall on the curvilinear curve (10,37,38). Another *in vivo* approach to testing the glucose-sensing character of β-cells uses a graded glucose infusion to produce a progressive increase in glycemia (39).

It is not only the amount of insulin released that is important. An acute increase in glycemia that can be approximated experimentally with intravenous glucose elicits a large burst of insulin secretion that lasts 5 to 7 minutes (*first phase*) followed by sustained insulin secretion that lasts for the duration of the hyperglycemia (*second phase*). Meals also induce a biphasic pattern of insulin secretion, although the phases are less distinct, with the early phase ascribed to the first 30 minutes and the later phase to the remaining postprandial hyperinsulinemia (1 to 2 hours normally). The biphasic pattern is necessary for normal mealtime glucose tolerance, with the concept being that the first phase primes the insulin-sensitive tissues for the coming food. Supporting this notion are studies that experimentally disrupted the early insulin response to a meal in otherwise healthy, normoglycemic subjects, causing impaired insulin-mediated tissue glucose disposal and excessive postprandial glycemia (40,41).

Insulin secretion occurs as oscillatory pulsations with a periodicity of 11 to 14 minutes, thought to be necessary to fully regulate hepatic glucose production (42,43). Also, large bursts of insulin release (*ultradian oscillations*) occur several times a day, particularly with meals (44), increasing the efficiency of nutrient clearance following meals (45).

Thus, the β-cell functions in a highly complex fashion that regulates the timing and overall insulin response to a meal to preserve normoglycemia. Quantitative insulin release and the pulsatile patterns can be tested *in vivo* by means of frequent insulin measurements to an appropriate stimulus. Also, the glucose-sensing and pulsatile-secretion characteristics can be jointly tested with an oscillatory glucose infusion that causes small increases and decreases in plasma glucose levels. Insulin secretion normally attains an oscillatory pattern termed *entrainment* (46,47). Failure of entrainment has been identified as an early defect in insulin secretion that precedes abnormal responses to more traditional tests. *In vivo* testing has been aided by several technical advances over the last few years. Insulin-specific assays are now widely available that have eliminated the cross-reactivity with proinsulin and its conversion intermediates (which are biologically inactive) that affected earlier assays. Also, insulin is secreted into the portal vein and undergoes a substantial first-pass clearance by the liver (approximately 50%). Thus, insulin levels in the peripheral circulation only approximate insulin secretion. Many investigators now analyze C-peptide values—the portion of proinsulin that is removed as it is converted to insulin. C-peptide is secreted with insulin in an equimolar ratio but undergoes minimal hepatic degradation and thus can be used for calculating true rates of insulin secretion (48,49).

β-CELL DYSFUNCTION IN TYPE 2 DIABETES

β-Cell Unresponsiveness to Glucose

The distinctive β-cell defect in type 2 diabetes is the loss of the first phase of glucose-induced insulin secretion (Fig. 25.5). This observation was first made in the late 1960s, when persons with

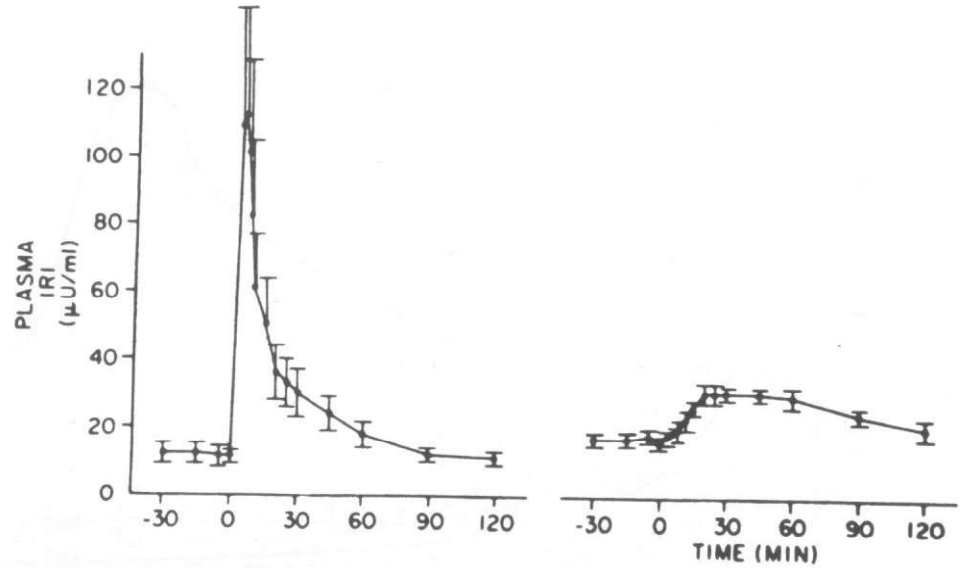


Figure 25.5. Plasma immunoreactive insulin response to 20-g bolus of intravenous glucose in nine subjects with type 2 diabetes (*right*) and nine normoglycemic control subjects (*left*). The first-phase insulin response (from 0 to 10 minutes) is totally lacking in the subjects with type 2 diabetes. In contrast, the second phase (from 10 minutes continuing for the duration of hyperglycemia) is intact in the subjects with diabetes and is greater than in the controls because of the persistent hyperglycemia in the subjects with diabetes following the glucose administration. (Reprinted from Pfeifer MA, Halter JB, Porte D Jr. Insulin secretion in diabetes mellitus. *Am J Med* 1981;70:579–588, with permission from Elsevier Science.)

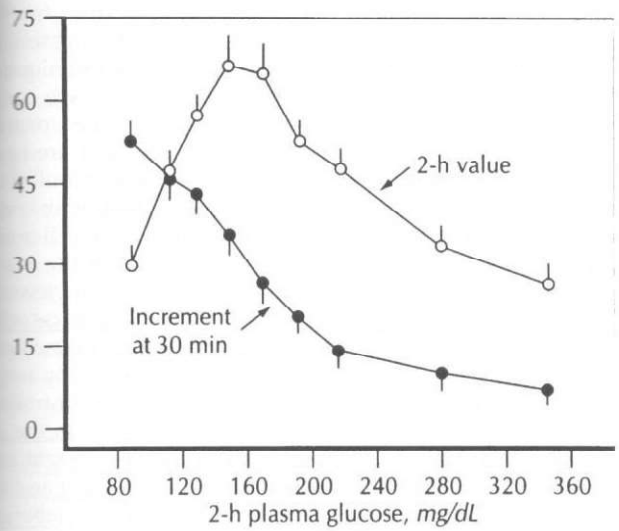
type 2 diabetes were noted to have a delayed insulin response to intravenous glucose, and later was recognized as loss of the first phase. The second phase is also impaired but to a lesser degree (50,51). Subsequent investigation showed that this defect was fully established by the time the fasting glucose level reached 115 mg/dL (52), clearly predating overt diabetes, but was not present in persons with truly normal glucose levels in whom type 2 diabetes developed later (3,6). Thus, it first appears in the prediabetes state, termed IGT, which is clinically manifest as excess postprandial excursions of glycemia. Given the importance of the first-phase insulin response for normal prandial glucose tolerance (40,41), it follows that this defect is an important cause of the IGT state. Attesting to this idea is a study that simulated a burst of insulin with a short-term insulin infusion at the beginning of a meal in persons with type 2 diabetes, which resulted in a marked improvement in postprandial glycemia (53).

A major advance in our understanding occurred with the demonstration that intensive glycemic control restored the first-phase insulin response in subjects with type 2 diabetes (19). At about the same time, glucose-induced insulin secretion was shown to be impaired in diabetic animals (discussed subsequently). An important observation, made in diabetic rats, is that phloridzin reverses the defect (54). Phloridzin promotes glycosuria; it is used experimentally in diabetic animals to restore normoglycemia without changing insulinemia, thus helping to identify pathogenic effects of hyperglycemia. The understanding that has evolved from these findings is that the defect in glucose-induced insulin secretion occurs when β-cells are exposed to a "toxic" environment of an abnormally high level of glycemia (so-called *glucose toxicity*).

Insulin responses to nonglucose secretagogues are less impaired than those to glucose. When the defective glucose-induced insulin secretion was first investigated, nonglucose agents were thought to be unaffected, a finding that led to the idea that the glucose response was uniquely deranged (*selective glucose unresponsiveness*). However, later studies that were more carefully controlled for glycemia in the subjects with and without diabetes made it clear that responses to nonglucose secretagogues also were impaired, although less so than those to glucose. This challenged the concept that glucose unresponsiveness is the prototypical β-cell abnormality in type 2 diabetes; however, subsequent investigation (55) showed that the basis for the defective nonglucose-mediated secretion was impaired glucose potentiation (Fig. 25.3). The time of onset for the glucose potentiation defect is not as well defined as that for glucose-induced insulin secretion, and it is not clear if these dual abnormal effects of glucose on insulin secretion represent a single defect or separate biochemical/molecular defects in glucose sensing by the β-cell. Viewed together, type 2 diabetes entails defective glucose regulation of insulin secretion through both pathways—glucose-induced insulin secretion (in particular the first phase) and glucose potentiation—emphasizing why fasting and postprandial hyperglycemia are defining characteristics of this disease.

For many years there was confusion about how β-cell dysfunction could be present in the prediabetes/IGT state, in particular the loss of the first-phase response, when countless studies had showed hyperinsulinemia (both fasting and after a glucose challenge or a meal) at that time (Fig. 25.2). Insight into this seeming paradox came with the understanding of the importance of the early insulin response for control of postprandial glycemia (40,41); loss of this early response results in an excessive meal-induced rise in glycemia, and this hyperglycemia causes the late insulin response to exceed that seen normally. Previous studies had generally looked at insulin levels 2 hours after a meal or oral glucose challenge and thus had missed the defect in early insulin secretion. This concept is shown in Figure 25.6, which shows the 30-minute and 2-hour insulin values after oral glucose challenge across a wide range of glycemia (29). Note the disparity as the early insulin response decreases with increasing glycemia at a time when later insulin release is increasing.

Thus, a characteristic feature of type 2 diabetes is loss of the first-phase insulin response to a meal. It occurs early in the



25.6. Comparison of 30-minute and 2-hour plasma insulin responses during oral glucose tolerance tests as a function of 2-hour plasma glucose values in 294 subjects. (From Gerich J, Van Haeften T. Insulin resistance versus impaired insulin secretion as the genetic basis for type 2 diabetes. *Curr Opin Endocrinol Diabetes* 1998;5:144–148, with permission.)

course of type 2 diabetes, predating fasting hyperglycemia, and is a major cause of the postprandial hyperglycemia that characterizes IGT and overt diabetes. This concept underlies the recent use of drugs for type 2 diabetes (meglitinides and phenylalanine derivatives) that are taken at meals to induce a rapid, short-lived insulin response.

Abnormal Pulsatile Secretion of Insulin

The pattern of orderly oscillations of insulin secretion with an 8- to 14-minute periodicity is lost in type 2 diabetes (56). Relatives of persons with type 2 diabetes show this defect when their glucose tolerance is normal (57,58), showing that it occurs early in the course of the disease. This might imply a genetic etiology. Countering that idea is a recent study showing recovery of oscillations in persons with type 2 diabetes after an overnight infusion of somatostatin, which pharmacologically blocks insulin release—interpreted by the authors as indicating that depletion of a readily releasable pool of insulin granules underlies the oscillation defect (59).

It is now generally accepted that the abnormal insulin pulsatility impairs normal regulatory control of insulin over hepatic glucose production. However, this understanding is relatively recent. The importance of the pulsatility defect was uncertain when first observed, as the magnitude of the pulsations in the normoglycemic control subjects was quite small (56): It was difficult to appreciate that such a minor effect would have any physiologic importance. Those studies used blood from the peripheral circulation. Insulin is secreted into the portal vein and undergoes substantial hepatic extraction. The breakthrough came with the understanding that the pulses are in fact large and are damped by hepatic extraction so that only a small fraction escapes the liver (42,60). Thus, the abnormal peripheral pulsations in type 2 diabetes represent very distorted insulin delivery to the liver, fitting with the idea of major dysregulation to the insulin-hepatic system. Future treatments might target this abnormality. For example, glucagon-like peptide-1 (GLP-1), which is under investigation as a hypoglycemic therapy for type 2 diabetes, increases pulsatile insulin secretion (61).

The ultradian oscillations—the large bursts of insulin that occur every 1 to 2 hours and more frequently with meals—also are disrupted in type 2 diabetes (62,63). This fact is well established, but the impact on glucose homeostasis is not totally clear. Regardless, pulsatile delivery of insulin holds a physiologic advantage, as evidenced by studies that have shown a greater hypoglycemic effect of pulsed versus continuously infused exogenous insulin (64). As such, loss of the pulsatile pattern of insulin secretion in type 2 diabetes disrupts this aspect of the glucose homeostasis system.

Increased Proinsulin-to-Insulin Ratio

The blood levels of insulin precursors (proinsulin and its conversion intermediates, which have only weak biologic activity) are disproportionately increased relative to insulin in type 2 diabetes (65,66). The same observation has been made in patients with diabetes caused by cystic fibrosis (67), a finding that raises the possibility that this disproportionate increase is another manifestation of hyperglycemia-induced β-cell dysfunction. However, the relationship of this increased ratio to hyperglycemia has been confused by seemingly inconsistent data. Most cross-sectional data show that the increased proinsulin/insulin ratio in type 2 diabetes occurs after the onset of hyperglycemia (68,69), with the ratio increasing as glycemia worsens (69,70). In disagreement are reports of an increased proinsulin/insulin ratio in the absence of glucose intolerance in populations at high risk for type 2 diabetes (71,72). Treatment studies to determine how reversal of hyperglycemia affects the proinsulin/insulin ratio also have not clarified its dependence on abnormal glycemia, because both improvement (73) and no effect (74) have been reported. Insight into this conundrum has come from the previously mentioned study that administered an overnight infusion of somatostatin to persons with type 2 diabetes; the proinsulin/insulin ratio was normalized by this pharmacologic inhibition of insulin secretion (59). This observation suggests that excessive insulin secretion, as opposed to hyperglycemia per se, underlies the raised proinsulin/insulin ratio. Another study of type 2 diabetes (75) and a study in diabetic sand rats (76) made a similar conclusion.

Our laboratory has studied diabetic rats in an effort to gain insight into the mechanistic basis for the raised proinsulin/insulin ratio (77–79). We first studied rats that had 90% of their pancreas removed (90% pancreatectomy) and observed an increased abundance of proinsulin-like peptides relative to insulin in pancreatic extracts, suggesting that the underlying defect was the storage, and subsequent secretion, of material enriched in proinsulin (77). We made the same observation in normal rats that received 48-hour infusions of glucose (78). That study showed the increased ratio resulted from a decreased insulin content, not from an increased proinsulin content (Fig. 25.7). Also, co-infusion of diazoxide to inhibit insulin secretion blocked the increase in the proinsulin/insulin ratio, results analogous to those seen in humans infused with somatostatin (59). These findings are in accord with the previously stated concept that an ongoing hypersecretion of insulin leading to depletion of the releasable insulin stores is the cause of the enhancement in proinsulin secretion, likely through secretion of immature, proinsulin-enriched granules. Confirmatory evidence was obtained by infusing normal rats for 3 days with large amounts of the insulin secretagogue tolbutamide (plus glucose to keep them euglycemic) and observing a raised percentage of proinsulin in pancreatic extracts (78). Furthermore, this concept implies that there is no biochemical or molecular defect in the proinsulin-processing pathway in diabetes, which we demonstrated to be the case in glucose-infused rats (79).

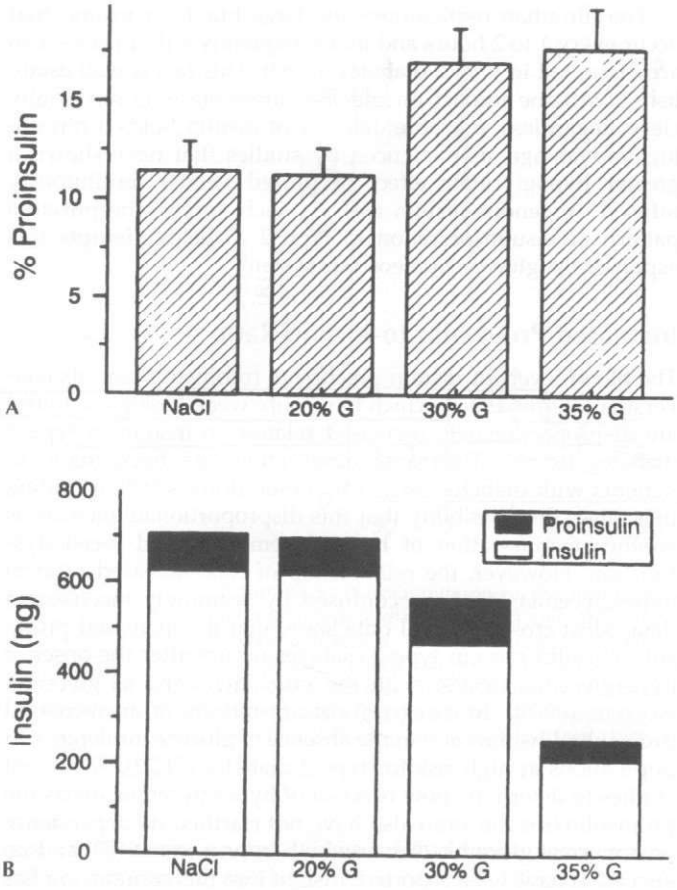


Figure 25.7. Relative proportion of proinsulin in pancreatic extracts from rats that received a 48-hour infusion with glucose. Pancreases underwent extraction, insulin/proinsulin precipitation, and separation of the insulins and proinsulins by high-performance liquid chromatography. Immunoreactive insulin and proinsulin were determined as the areas under the curve of the different peaks measured by insulin radioimmunoassay. **A:** % proinsulin = plasma immunoreactive insulin response (IRI) proinsulin/(IRI insulin + IRI proinsulin). **B:** IRI insulin and proinsulin in chromatography samples. Note that an increase in % proinsulin resulted from a decrease in insulin content not from an increase in proinsulin content. (Copyright © 1993, American Diabetes Association. From Leahy JL. Increased proinsulin/insulin ratio in pancreas extracts of hyperglycemic rats. *Diabetes* 1993;42:22–27. Reprinted with permission from the American Diabetes Association.)

Thus, the increased proportion of stored and circulating proinsulin in the diabetic rats appears to be secondary to a hypermobilization of granules, which leads to a rapid transit time and thus to incomplete processing to fully mature insulin. This hypersecretion scenario fits perfectly with the findings in patients with type 2 diabetes (59,75). However, enhanced insulin secretion per se is not enough to raise the proinsulin/insulin ratio, as is apparent from studies of nondiabetic obese subjects who are hyperinsulinemic but who have normal to lowered proinsulin/insulin ratios (68,80). Instead, some underlying element of β-cell dysfunction must be present as well.

β-CELL MASS AND STRUCTURE IN TYPE 2 DIABETES

The preceding discussion was of abnormalities that affect β-cell function. A second topic is whether the mass of β cells is lowered in type 2 diabetes and if so why? Relatively little is known about this subject, as investigation has been hampered by the inability to obtain pancreatic biopsy samples from free-living humans. Further, there is no noninvasive way to assess β-cell mass, although interest in the development of techniques that will allow that goal to be reached is growing. We are thus dependent on autopsy studies that have quantified pancreatic β-cell mass. However, these studies are few and are open to interpretation because of incomplete physiologic/clinical information about the subjects or of inexact matching with controls. Finally, the technical challenge of measuring β-cell mass in humans is substantial, which means that none of the available studies (81–83) are large. As such, they usually are viewed collectively, leading to the conclusion that the β-cell mass is modestly lowered in type 2 diabetes. Illustrating that conclusion is the study by Klöppel et al. (83), which is noteworthy not only because it is one of the largest but also because it controlled for obesity. They observed a doubling of β-cell mass in the obese versus nonobese control subjects (showing why weight matching of subjects is so important for this kind of study) and a 50% reduction in β-cell mass in the obese and nonobese diabetic subjects compared with their weight-matched controls (83).

That β-cell mass is decreased in type 2 diabetes is generally well accepted, but it is not known when it occurs or how it is temporally related to the β-cell failure that leads to the development of hyperglycemia. This early stage of diabetes is characterized by considerable recovery of β-cell function following intensive glycemic control (18–20), which has led some to conclude that abnormalities in β-cell function, not mass, are foremost. In contrast, the United Kingdom Prospective Diabetes Study (UKPDS), a study of intensive treatment in new-onset type 2 diabetes, reported increases in glycemia over time, in tandem with worsening of β-cell function, whether diet, sulfonylurea, metformin, or insulin therapy was used (24,25). This observation has led to speculation that the waning β-cell function in longstanding diabetes stems from a declining β-cell mass. The cause of that effect is not known. It is clearly not immune-mediated and is thus distinct from type 1 diabetes; morphologically, the islets appear relatively normal (except for amyloid infiltration, which is discussed below), and insulitis is never present (84). Also missing is evidence for the hyperactivity that might be expected in response to the hyperglycemia, with few mitotic figures found (84) and only modest degranulation seen (85). A recent finding in some diabetic animals is increased β-cell apoptosis (86–89), although its relevance for human type 2 diabetes is unknown.

Islet Amyloid

The best-studied mechanism that might lead to accelerated β-cell death in type 2 diabetes is the development of islet amyloid (90–93). Islet amyloid deposits were first described nearly 100 years ago by Opie (94). Although these deposits were found commonly in type 2 diabetes, they were thought to hold little significance until the seminal study of Howard that correlated islet morphology with the clinical and metabolic status of *Macaca nigra* monkeys as they went from nondiabetes to diabetes (95). Howard reported that the appearance of islet amyloid coincided with, or immediately preceded, hyperglycemia and concluded that amyloid-induced destruction of islet β-cells caused metabolic progression to diabetes. This relationship, which also was observed in cats (96), spurred substantial interest in islet amyloid.

A breakthrough in this field occurred with the identification and cloning, simultaneously by two groups, of the peptide that makes up the amyloid deposits. This peptide was termed *amylin*; the more common term today is *islet amyloid polypeptide* (IAPP) (97,98). IAPP is a 37-amino-acid peptide normally pro-

...ound in β-cells that is copackaged with insulin in secretory ...anules. The 25- to 28-amino acid sequence is conserved in ...mans, monkeys, and cats [Ala-Ile-Leu-Ser], all of which ...velop islet amyloid in tandem with diabetes. This amino acid ...quence is necessary for the formation of amyloid fibrils, as ...own by the lack of fibril formation *in vitro* or *in vivo* of IAPP ...m animals that lack this sequence (rats, guinea pigs, and ...se). This dichotomy has proven useful for studies in trans...nic mice that have overexpressed human IAPP in β-cells, as ...re is no risk of amyloid formation from the mouse's own ...PP (99,100). The normal function of IAPP remains controver...al. It slows gastric emptying—whether this effect is physio...gic or pharmacologic is debated—and clinical trials are in ...ogress of the ability of an analogue of IAPP to decrease post...andial glucose excursions in persons with diabetes (101).

The effects of IAPP on β-cells have been studied extensively ...*tro* and *in vivo*. When placed in solution, IAPP forms fibrils, and these aggregates have been shown to be cytotoxic to islets ...*tro* (102,103). However, the relevance of this observation *in ...o* is debated. Studies in transgenic mice that have overex...ssed human IAPP have resulted in amyloid deposition and ...abetes (99,100). However, a concern is that this requires very ...gh levels of IAPP expression (99) or an accompanying insult, ...ch as insulin resistance from growth hormone or dexametha...one treatment (104), or a high-fat diet (100). Whether this co...quirement shows that IAPP is nonpathologic under normal ...nditions or that physiologically nonrelevant circumstances ...e required to induce pathogenic outcomes is still not clear.

Crucial questions must be answered before confirmation of a ...thogenic role for IAPP and the amyloid deposits. There is no ...ubt that islets with amyloid deposits have a small β-cell mass ...th cellular distortion and destruction. The crucial question is ...ich came first? Is the deposition of amyloid an early event ...at precedes hyperglycemia or does it occur only after mild ...perglycemia (and thus the β-cell functional failure) has ...veloped? Do IAPP aggregates then induce functional and/or ...-cell structural damage? Alternatively, is type 2 diabetes asso...ated with β-cell death through an effect that is unrelated to ...PP such that the cellular stores of IAPP are released and form ...yloid extracellularly among the cellular debris? Currently, ...ere are no answers to these questions. However, studies of ...ncreas specimens that have been collected at autopsy from ...bjects who span the full range from nondiabetes to severe ...abetes are in progress. The key information obtained from ...ese studies should help determine the role of islet amyloid in ...is disease.

MECHANISMS OF β-CELL DYSFUNCTION

Glucose Toxicity

The hypothesis has been advanced that chronic hyperglycemia causes alterations in β-cell function, termed *glucose toxicity*. The idea started with the observation of substantial recovery of β-cell function in type 2 diabetes following treatments that restored normoglycemia (18–20). Also influential was the observation that diabetic animals have β-cell dysfunction similar to that in humans with type 2 diabetes (discussed below), supporting a causative effect of the metabolic environment. Subsequent biochemical and molecular studies of β-cell lines and islet tissue have uncovered plausible mechanisms for hyperglycemia-induced β-cell dysfunction. The term *glucose toxicity* was coined to represent tissue dysfunction from a hyperglycemic environment. Our own usage of the term is focused on the idea of a direct impairment effect of the raised glycemia on β-cell function as opposed to *exhaustion*, which is an indirect effect of hyperglycemia acting through a nonsustainable hypersecretion of insulin (23). The difference may seem subtle but becomes clear when reversal studies are performed with inhibitors of insulin secretion, as will be discussed subsequently. Generally, terminology that holds no exact mechanistic meaning is not advised. Regardless, the literature on type 2 diabetes and β-cell dysfunction is replete with these terms, and readers need to be familiar with their meanings.

Multiple rat models of experimental hyperglycemia have been studied for β-cell function, including the administration of streptozotocin during the neonatal period (105–107), partial pancreatectomy (108,109), and *in vivo* glucose infusions (110,111). Studies have also been carried out in a variety of rodents with spontaneous hyperglycemia, including GK rats (112), Zucker diabetic fatty (ZDF) rats (113), Otsuka Long-Evans Tokushima fatty (OLETF) rats (114), and many others. A universal finding is that secretion in response to glucose is impaired as opposed to secretion in response to nonglucose secretagogues such as arginine, glucagon, or tolbutamide, which are relatively unaffected (Fig. 25.8). This pattern of selective unresponsiveness to glucose closely resembles the pattern in human type 2 diabetes, as described. Just as important in terms of the concept of glucose toxicity is the complete absence of β-cell unresponsiveness to glucose with normoglycemia, as determined in studies of animals without a genetic predisposition to diabetes or before the onset of hyperglycemia in animals that later go on to develop diabetes (113,114). This concept is further supported by the finding that glucose-induced insulin secretion *in vitro* or *in vivo* is recovered with therapies that reverse the hyperglycemia (115–117). The most influential of these studies in terms of identifying hyperglycemia as the causative factor was a study of phloridzin. As previously discussed, phloridzin promotes glycosuria and restores normoglycemia without changing plasma insulin or other metabolic factors and thus identifies tissue dysfunction related to hyperglycemia. Rossetti et al. (54) reported correction of the β-cell dysfunction in 90% pancreatectomized diabetic rats after phloridzin treatment.

Many biochemical and molecular mechanisms for the induction of β-cell dysfunction by hyperglycemia have been proposed based on studies of islet tissue from diabetic animals or the use of superphysiologic glucose concentrations *in vitro*. The proposed mechanisms include excess glycogen storage (118), impaired glucose transport into the β-cell (119), impaired activity of key signaling pathways such as the glycerol phosphate shuttle (120,121) or pyruvate carboxylase (121,122), defective ATP-sensitive channel activity (123–125), reduced expression of voltage-dependent calcium channels (126,127), defective hydrolysis of membrane inositol phospholipids (128), cycling of glucose-6-phosphate back to glucose through increased glucose-6-phosphatase activity (129–131), altered Na^+-K^+-ATPase activity coupled with reduced myoinositol uptake (132), and loss of β-cell differentiation (133). That there are so many possible mechanisms clearly demonstrates how "toxic" a hyperglycemic environment is for β-cells. Stated another way, it is almost certain that the β-cell dysfunction in human type 2 diabetes will stem from multiple biochemical/molecular defects.

β-Cell Exhaustion

A related concept is *β-cell exhaustion* or *overwork*, which we (23,134) and others (59) view as β-cell dysfunction from a nonsustainable hyperstimulation of insulin secretion. In that case, hyperglycemia is the stimulus for the β-cell dysfunction but is not the operative mechanism. This subtlety is revealed when reversal strategies are undertaken that use inhibitors of insulin

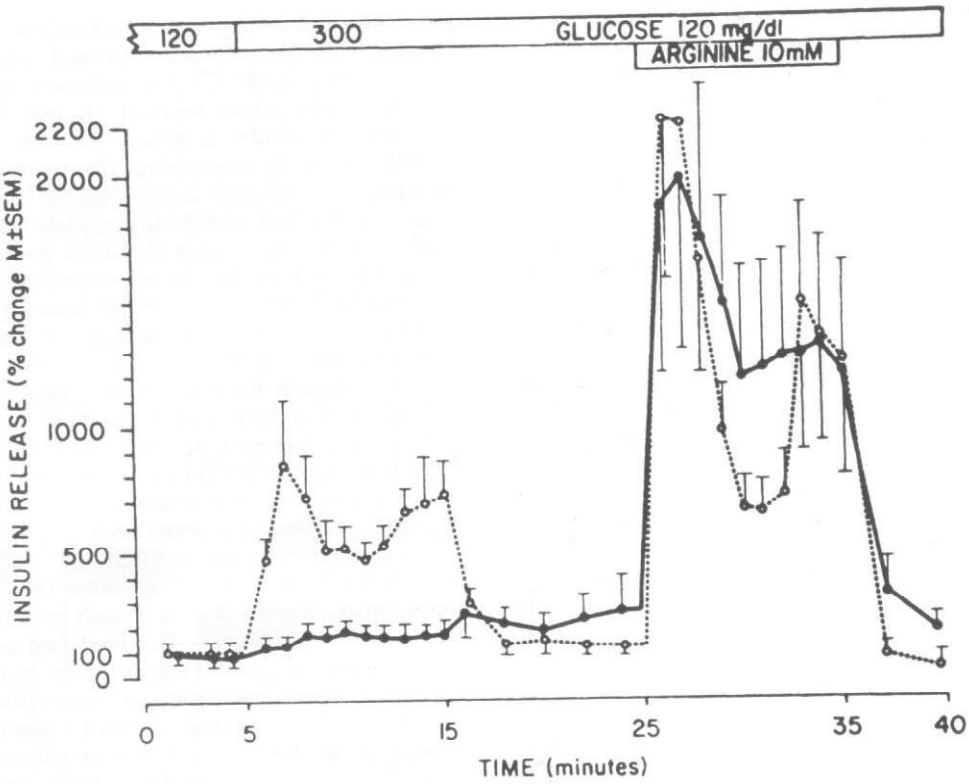
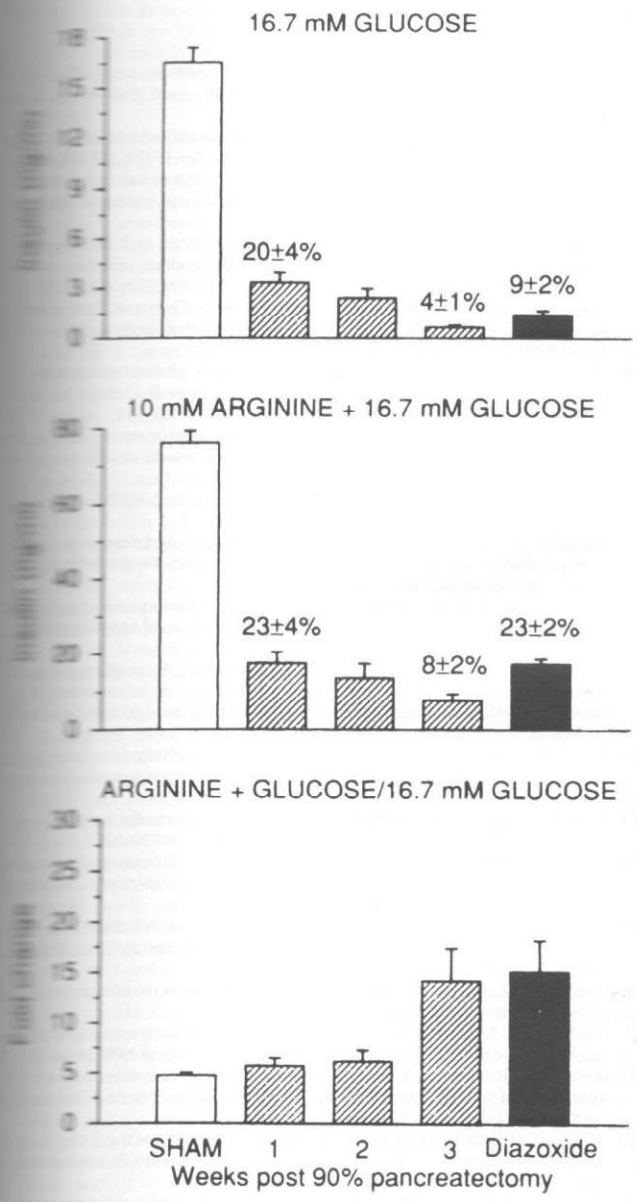


Figure 25.8. Insulin secretory responses to glucose and arginine in the perfused pancreatic remnant of 90% pancreatectomized diabetic rats (*solid line*) and normoglycemic control rats (*dotted line*). Studies were performed 8 to 11 weeks after pancreatectomy or sham surgery. Note the pattern of selective glucose unresponsiveness in the diabetic rats, as reflected in the brisk insulin response to arginine but the absence of response to a high level of glucose. (From Bonner-Weir S, Trent DF, Weir GC. Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. *J Clin Invest* 1983;71:1544–1553, with permission from the American Society for Clinical Investigation.)

secretion. The most studied inhibitor is diazoxide, although somatostatin has also been used. Glycemia is unchanged in these studies, or sometimes worsens, so that detrimental tissue effects of hyperglycemia versus hyperstimulated insulin secretion can be identified separately.

Support for the concept of β-cell exhaustion began with Sako and Grill (135), who reported that diazoxide prevented β-cell dysfunction in normal rats that were made hyperglycemic by a 48-hour glucose infusion *in vivo*. These investigators also showed a protective effect of diazoxide during long-term *in vitro* incubation of islets with high glucose and made the additional observation that the diazoxide effect resulted from preventing the insulin content of β-cells from declining below a critical level (136). Glucose-infused rats are markedly hyperinsulinemic, and the hyperglycemia is by necessity short term, so the applicability of the Sako and Grill study to the more usual situation of normal to subnormal plasma insulin levels with long-term diabetes was unclear. We treated rats that were diabetic from a 90% pancreatectomy with diazoxide for 5 days: Glucose-potentiated insulin secretion in response to arginine (a commonly used nonglucose secretagogue) normalized (Fig. 25.9). In contrast, the direct effect of glucose on insulin secretion improved minimally. The recovery of glucose potentiation occurred in tandem with normalization of the insulin content for the β-cell mass (137). A second mode for lowering insulin secretion, a 40-hour fast, produced similar results—a marked improvement in glucose potentiation but no change in glucose-induced insulin secretion—this time using GLP-1^{7-37} as the nonglucose secretagogue (138). GLP-1^{7-37} is a member of the incretin family of gut-released peptides that potentiate meal-induced insulin secretion (139,140). Both of these studies noted a linear correlation between the insulin content of the pancreas and glucose-potentiated secretion of insulin before and after treatments, findings that agree with those of Grill's laboratory concerning the crucial role of insulin content in the exhaustion concept (136). To further test the exhaustion hypothesis, we investigated the prediction that upregulation of insulin secretion in nondiabetic rats sufficient to lower the insulin content should impair glucose-potentiated secretion of insulin. Normal rats received a 48-hour infusion of high-dose tolbutamide (insulin secretagogue) plus enough glucose to maintain normoglycemia: The insulin content of the pancreas declined 50%, and as predicted, glucose-potentiated insulin secretion in response to arginine declined exactly in parallel (141).

These findings support a causative role for excessive insulin release in the defective glucose-potentiated secretion of insulin with chronic hyperglycemia. The concept is that a substrate, cofactor, cellular fuel, or other required substance is depleted, resulting in a lowered insulin response to meals. Our studies have focused on the potential role of the releasable pool of insulin stores as that factor. As already discussed, the same pathogenic mechanism has been linked to the increased proinsulin to insulin ratio and the abnormal pulsatile insulin secretion in type 2 diabetes (59). It is important to note that results supporting the exhaustion concept have been obtained in studies of human type 2 diabetes. Diazoxide therapy (142), a 4-day fast (143), and overnight infusion of somatostatin (59) all improved insulin secretion as opposed to their normal



Increased insulin secretion with diazoxide therapy in rats made by 90% pancreatectomy. Insulin secretion in response to 16.7 mM glucose and 16.7 mM glucose/10 mM arginine was assessed with the *in vitro* perfused pancreas at weekly intervals after a 90% pancreatectomy. The percentage values above the bars are fractional output calculated from the results for the sham-operated control rats (*open bars*). Note the decrease in insulin responses at 3 weeks after surgery, showing the onset of defective glucose responsiveness and glucose potentiation. A 5-day treatment period with diazoxide partially blocked the decrease in glucose-induced insulin secretion and normalized that to glucose/arginine (*solid bars*). (Data from Leahy JL, Bumbalo LM, Chen C. Diazoxide causes recovery of β-cell glucose responsiveness in 90% pancreatectomized diabetic rats. *Diabetes* 1992;41:173–179; and Leahy JL, Bumbalo LM, Chen C. Beta-cell hypersensitivity for glucose precedes loss of glucose-induced insulin secretion in 90% pancreatectomized rats. *Diabetologia* 1993;36:1238–1244.)

inhibitory effect, replicating the results in studies of diabetic rats (137,138).

Impaired Proinsulin Biosynthesis

It has been suggested that transcriptional control of proinsulin biosynthesis is impaired by chronic hyperglycemia and that this may cause of the lowered insulin secretion in type 2 diabetes (144,145). This idea stems from extensive *in vitro* studies of β-cell lines and isolated islets that established that several factors related to a high glucose environment cause defective activation of proinsulin transcription; these factors include lowered expression/binding of the activators PDX-1 (pancreatic duodenum homeobox factor-1; also termed somatostatin transcription factor-1 and islet duodenum homeobox-1) (146) and rat insulin promoter element 3b1-activator (147) and increased expression of the inhibitor C/EBPβ (148). Additional support has come from studies in diabetic rats. Zangen et al. (149) reported impaired proinsulin transcription that paralleled lowered expression of PDX-1 in 90% pancreatectomized diabetic rats. Seufert et al. (150) reported similar results in ZDF rats in association with upregulation of C/EBPβ expression (150). Harmon et al. (151) prevented hyperglycemia in ZDF rats with troglitazone, thereby eliminating the lowered gene expression of proinsulin, in association with recovery of PDX-1 expression/binding. A recent cross-sectional study of diabetic rats with various levels of hyperglycemia suggested that inhibition of gene expression of proinsulin requires severe hyperglycemia (133). We speculate that this effect plays a role in the β-cell dysfunction of markedly hyperglycemic type 2 diabetes but not in the β-cell failure of new-onset diabetes, in which hyperglycemia is typically mild for most patients.

Lipotoxicity

Other metabolic disruptions beside hyperglycemia make up the diabetic environment, including hypertriglyceridemia and elevated circulating and tissue levels of free fatty acids. A hypothesis about the pathogenesis of β-cell dysfunction secondary to these factors, called *lipotoxicity*, has evolved based on several experimental findings (152–155). Islets cultured with elevated levels of fatty acids develop β-cell dysfunction reminiscent of that in type 2 diabetes, namely lowered glucose-induced insulin release, impaired proinsulin synthesis, and accelerated β-cell apoptosis (156,157). Plausible biochemical and molecular mechanisms for the β-cell dysfunction were identified soon afterward. Fatty acids were shown to lower expression of IDX-1 (also called PDX-1), which is a key transcription factor for β-cell development, glucose metabolism, and proinsulin synthesis (158). Also, the well-known effect of fatty acids to impair glucose oxidation through lowered activation of pyruvate dehydrogenase, the so-called *Randle cycle* (159,160), was shown to be operative in fatty acid-cultured islets and was implicated in the lowered glucose-induced secretion of insulin (161). The most influential studies have been the extensive studies of ZDF rats by the Unger laboratory (155,162,163). These rats are obese and hyperlipidemic, and the males have large stores of triglyceride in islets in tandem with the spontaneous development of diabetes. These investigators have identified a well-characterized biochemical sequence of altered islet triglyceride metabolism that was shown to correlate with β-cell dysfunction and apoptosis. Unclear, however, is whether the findings from these studies of ZDF rats can be applied to other hyperlipidemic states, since the genetic abnormality in ZDF rats is a mutated leptin receptor that blocks leptin action and a leptin deficiency plays a central role in their identified pathogenic sequence (164,165).

Despite these findings, there remains uncertainty about the lipotoxicity concept. Of concern are the observations from several studies that used long-term lipid infusions in nondiabetic humans; most found minimal to no detrimental effect on insulin secretion (166–169). This is not surprising, as nondiabetic insulin-resistant states entail supernormal β-cell function/insulin secretion (33) despite the common occurrence of

hypertriglyceridemia (170). Also, reexamination of the biochemical defects that were purported to cause the fatty acid–induced β-cell dysfunction has led to questioning of some of the original findings. We (171) and others (172) observed no Randle cycle–induced impairment of glucose oxidation in fatty acid–cultured islets or β-cells. Also, the reported inhibitory effect of fatty acids on proinsulin biosynthesis (156) was not confirmed by an in-depth analysis (173). Finally, hyperlipidemia or raised islet triglyceride stores are clearly not mandatory for β-cell dysfunction in diabetic rats (174). None of these negative data eliminate the possibility of lipid-induced β-cell dysfunction. However, the concept needs to be investigated further to determine the role of lipid-induced dysfunction (if any) in type 2 diabetes.

SUMMARY

Debate about the importance of β-cell dysfunction in the pathogenesis of type 2 diabetes is over. Prospective studies of the progression to type 2 diabetes have highlighted the crucial role played by β-cell dysfunction. A notable example is the study from Weyer et al. that monitored insulin action, insulin secretion, and endogenous glucose output in 17 Pima Indians as they progressed from normal glucose tolerance to diabetes, compared with 31 Pima Indians who retained normal glucose tolerance over the same time (10). In those in whom diabetes developed, defects in both insulin secretion and insulin action were present when they were normoglycemic, but it was the lowering of the insulin response to intravenous glucose that best correlated with the progression from normoglycemia to diabetes. In addition, there is now a clear understanding that β-cell dysfunction continues to exert a major influence once diabetes develops, with a particular focus on the progression from oral monotherapy, to therapy with a combination of oral agents, to insulin therapy likely reflecting deteriorating β-cell function (24,25).

The past decade has seen considerable progress in our understanding of potential pathogenic mechanisms, and we are optimistic that we are on the threshold of identifying prevention and/or therapeutic strategies that will preserve β-cell function in this disease. However, major challenges remain, the foremost being the determination of the molecular, biochemical, and genetic bases for the β-cell dysfunction. Animal models have been the major investigative tools until now, but their relevance to human disease is still uncertain. Ways must be found to identify the defects that occur in humans and to design experimental systems that reproduce the human pathogenic condition. We must clarify the role of β-cell dysfunction versus loss of β-cell mass in the disease. The identification of ways of imaging β-cells noninvasively for both function and mass is a key requirement. Biochemical and molecular investigation into normal β-cell function and development must continue. Some of the most important recent advances are based on defining how β-cells work normally, for example, how β-cells grow and develop (175,176) and the role of islet neogenesis (177) are important topics of active investigation. Also, a functional role for "the insulin signaling cascade" within β-cells was identified just a few years ago (178–180). It is plausible that future breakthroughs will take advantage of signaling pathways or other aspects of β-cell physiology that are not yet known to exist.

It is almost certain that in the next decade our treatment of type 2 diabetes will change to take advantage of the incredible, and ongoing, scientific advances. Much attention will be focused on prevention. A reasonable prediction is that β-cell–directed therapies will play a crucial role in both endeavors.

REFERENCES

1. King H, Rewers M, WHO Ad Hoc Diabetes Reporting Group. Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. *Diabetes Care* 1993;16:157–177.
2. Hamman R. Genetic and environmental determinants of non-insulin-dependent diabetes mellitus (NIDDM). *Diabetes Metab Rev* 1992;8:287–338.
3. Lillioja S, Mott DM, Howard B, et al. Impaired glucose tolerance as a disorder of insulin action: longitudinal and cross-sectional studies in Pima Indians. *N Engl J Med* 1988;318:1217–1225.
4. Lillioja S, Mott DM, Spraul M, et al. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: Prospective studies of Pima Indians. *N Engl J Med* 1993;329:1988–1992.
5. Eriksson J, Franssila-Kallunki A, Ekstrand A, et al. Early metabolic defects in persons at increased risk for non-insulin-dependent diabetes mellitus. *N Engl J Med* 1989;321:337–343.
6. Warram JH, Martin BC, Krolewski AS, et al. Slow glucose removal rate and hyperinsulinemia precede the development of type II diabetes in the offspring of diabetic parents. *Ann Intern Med* 1990;113:909–915.
7. Martin BC, Warram JH, Krolewski AS, et al. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *Lancet* 1992;340:925–929.
8. Leahy JL. Natural history of beta-cell dysfunction in NIDDM. *Diabetes Care* 1990;13:992–1010.
9. Mitrakou A, Kelley D, Mokan M, et al. Role of reduced suppression of glucose production and diminished early insulin release in impaired glucose tolerance. *N Engl J Med* 1992;326:22–29.
10. Weyer C, Bogardus C, Mott DM, et al. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest* 1999;104:787–794.
11. DeFronzo RA, Ferrannini E, Simonson DC. Fasting hyperglycemia in non-insulin-dependent diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake. *Metabolism* 1989;38:387–395.
12. Saad MF, Knowler WC, Pettitt DJ, et al. Sequential changes in serum insulin concentration during development of noninsulin-dependent diabetes. *Lancet* 1989;1:1356–1359.
13. Saad MF, Knowler WC, Pettitt DJ, et al. A two-step model for development of non-insulin-dependent diabetes. *Am J Med* 1991;90:229–235.
14. Beck-Nielsen H, Groop LC. Metabolic and genetic characterization of prediabetic states. Sequence of events leading to non-insulin-dependent diabetes mellitus. *J Clin Invest* 1994;94:1714–1721.
15. Swinburn BA, Gianchandani R, Saad MF, et al. In vivo beta-cell function at the transition to early non-insulin-dependent diabetes mellitus. *Metabolism* 1995;44:757–764.
16. Efendic S, Grill V, Luft R, et al. Low insulin response: a marker of prediabetes. *Adv Exp Med Biol* 1988;246:167–174.
17. Kadowaki T, Miyake Y, Hagura R, et al. Risk factors for worsening to diabetes in subjects with impaired glucose tolerance. *Diabetologia* 1984;26:44–49.
18. Kosaka K, Kuzuya T, Akanuma Y, et al. Increase in insulin response after treatment of overt maturity-onset diabetes is independent of the mode of treatment. *Diabetologia* 1980;18:23–28.
19. Vague P, Moulin J-P. The defective glucose sensitivity of the β-cell in non-insulin dependent diabetes: improvement after twenty hours of normoglycemia. *Metabolism* 1982;31:139–142.
20. Garvey WT, Olefsky JM, Griffin J, et al. The effect of insulin treatment on insulin secretion and insulin action in type II diabetes mellitus. *Diabetes* 1985;34:222–234.
21. Rossetti L, Giaccari A, DeFronzo RA. Glucose toxicity. *Diabetes Care* 1990;13:610–630.
22. Yki-Järvinen H. Toxicity of hyperglycaemia in type 2 diabetes. *Diabetes Metab Rev* 1998;14 [Suppl 1]:S45–S50.
23. Leahy JL. β-cell dysfunction with chronic hyperglycemia: the "overworked β-cell" hypothesis. *Diabetes Rev* 1996;4:298–319.
24. U.K. Prospective Diabetes Study Group. U.K. prospective diabetes study. Overview of 6 years' therapy of type II diabetes: a progressive disease. *Diabetes* 1995;44:1249–1258.
25. Turner RC, Cull CA, Frighi V, et al. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). *JAMA* 1999;281:2005–2012.
26. Weir GC. Which comes first in non-insulin-dependent diabetes mellitus: insulin resistance or beta-cell failure? Both come first. *JAMA* 1995;273:1878–1879.
27. Polonsky KS, Sturis J, Bell GI. Seminars in Medicine of the Beth Israel Hospital, Boston. Non-insulin-dependent diabetes mellitus—a genetically programmed failure of the beta cell to compensate for insulin resistance. *N Engl J Med* 1996;334:777–783.
28. Ferrannini E. Insulin resistance versus insulin deficiency in non-insulin-dependent diabetes mellitus: problems and prospects. *Endocr Rev* 1998;19:477–490.
29. Gerich J, Van Haeften T. Insulin resistance versus impaired insulin secretion as the genetic basis for type 2 diabetes. *Curr Opin Endocrinol Diabetes* 1999;6:144–148.
30. Kjos SL, Buchanan TA. Gestational diabetes mellitus. *N Engl J Med* 1999;341:1749–1756.