

Natural History of β -Cell Function in Type 1 Diabetes

Nicole A. Sherry, Emily B. Tsai, and Kevan C. Herold

Despite extensive and ongoing investigations of the immune mechanisms of autoimmune diabetes in humans and animal models, there is much less information about the natural history of insulin secretion before and after the clinical presentation of type 1 diabetes and the factors that may affect its course. Studies of insulin production previously published and from the Diabetes Prevention Trial (DPT)-1 suggest that there is progressive impairment in insulin secretory responses but the reserve in response to physiological stimuli may be significant at the time of diagnosis, although maximal responses are more significantly impaired. Other factors, including insulin resistance, may play a role in the timing of clinical presentation along this continuum. The factors that predict the occurrence and rapidity of decline in β -cell function are still largely unknown, but most studies have identified islet cell autoantibodies as predictors of future decline and age as a determinant of residual insulin production at diagnosis. Historical as well as recent clinical experience has emphasized the importance of residual insulin production for glycemic control and prevention of end-organ complications. Understanding the modifiers and predictors of β -cell function would allow targeting immunological approaches to those individuals most likely to benefit from therapy. *Diabetes* 54 (Suppl. 2):S32–S39, 2005

Refinements in screening approaches have greatly improved the ability to identify individuals who will develop type 1 diabetes among first-degree relatives of affected individuals and possibly in the general population (1,2). In the recently completed Diabetes Prevention Trial (DPT)-1, a prospective study of first-degree (aged 3–35 years) and second-degree (aged 3–20 years) relatives of subjects with type 1 diabetes, ~3.7% of relatives of type 1 diabetic patients have islet cell autoantibodies (ICAs) (1). Among those ICA⁺ individuals, a high-risk cohort could be identified on the basis of insulin autoantibodies and diminished first-phase insulin response (FPIR) to intravenous glucose. These studies identified a group of individuals with a 60% risk of developing type 1 diabetes within a 5-year period, and the incidence may be higher with more observational time—a similar experience was seen in the European Nicotinamide Diabetes Intervention Trial (ENDIT) (3). Adding other

autoantibodies to screening may improve prediction of disease because the risk of diabetes appears to increase as the autoimmune response becomes diversified, characterized by multiple autoantibodies, which appear sequentially over time (4,5).

Although immunological markers have been extensively studied for their ability to predict diabetes, the metabolic course of pre-diabetes has not been as well characterized. Data from the NOD mouse have been conflicting. Sreenan et al. (6) reported an extended course of β -cell failure that begins with the onset of insulinitis. There were increased rates of β -cell proliferation before the onset of hyperglycemia, and there was evidence of β -cell dysfunction because insulin secretion was reduced to a greater extent than islet mass. In contrast, Shimada et al. (7) reported that, based on acquisition of the phenotype of destructive cytokines, β -cell destruction was a late event in the progression of the autoimmune process in the NOD mouse.

The evolution of β -cell failure is of more than theoretical interest and may affect the design of interventional trials to prevent or treat the disease. The timing of the interventional studies to prevent diabetes critically depends on the rate of progression of disease as well as the β -cell mass that is present at the time of the intervention. For example, it had been thought that because of the profound loss of insulin secretion at the time of onset of type 1 diabetes, intervention at diagnosis would have little clinical value and, therefore, prevention trials should primarily be pursued. However, the recent interest in persistent C-peptide production in patients with long duration of type 1 diabetes has led to the development of interventional studies to preserve or even increase β -cell mass long after the onset of disease. In this review, we will summarize our understanding of the natural history of insulin secretion in type 1 diabetes, from pre-diabetes to established disease, and discuss the implications of these findings for interventional trials.

METHODS TO MEASURE β -CELL FUNCTION IN TYPE 1 DIABETES

Plasma insulin levels. The concentration of plasma insulin after a glucose stimulus has been used to evaluate insulin secretion in high-risk individuals before the development of clinical diabetes. Two tests of this type are the intravenous glucose tolerance test (IVGTT) and oral glucose tolerance test. The IVGTT was standardized in the 1990s by the Islet Cell Antibody Registered Users (ICARUS) group (8,9). The FPIR, the sum of the plasma insulin at 1 and 3 min after the glucose load during an IVGTT, has been correlated with an increased risk of progressing from preclinical to clinical diabetes and was used in risk stratification in the DPT-1 (1,10).

Plasma C-peptide levels. There are limitations to the use of measurements of plasma immunoreactive insulin as a measure of insulin secretion. Insulin has a short half-life and variable first-pass hepatic extraction, and peripheral clearance makes the peripheral levels an inaccurate reflection

From the Naomi Berrie Diabetes Center and the Departments of Pediatrics and Medicine, College of Physicians and Surgeons, Columbia University, New York, New York.

Address correspondence and reprint requests to Kevan C. Herold, MD, Associate Professor of Medicine, Columbia University, 1150 St. Nicholas Ave., New York, NY 10032. E-mail: kh318@columbia.edu.

Received for publication 1 March 2005 and accepted in revised form 26 April 2005.

This article is based on a presentation at a symposium. The symposium and the publication of this article were made possible by an unrestricted educational grant from Servier.

DCCT, Diabetes Control and Complications Trial; DPT, Diabetes Prevention Trial; FPIR, first-phase insulin response; ICA, islet cell antibody; ISR, insulin secretory rate; IVGTT, intravenous glucose tolerance test; MMTT, mixed-meal tolerance test.

© 2005 by the American Diabetes Association.

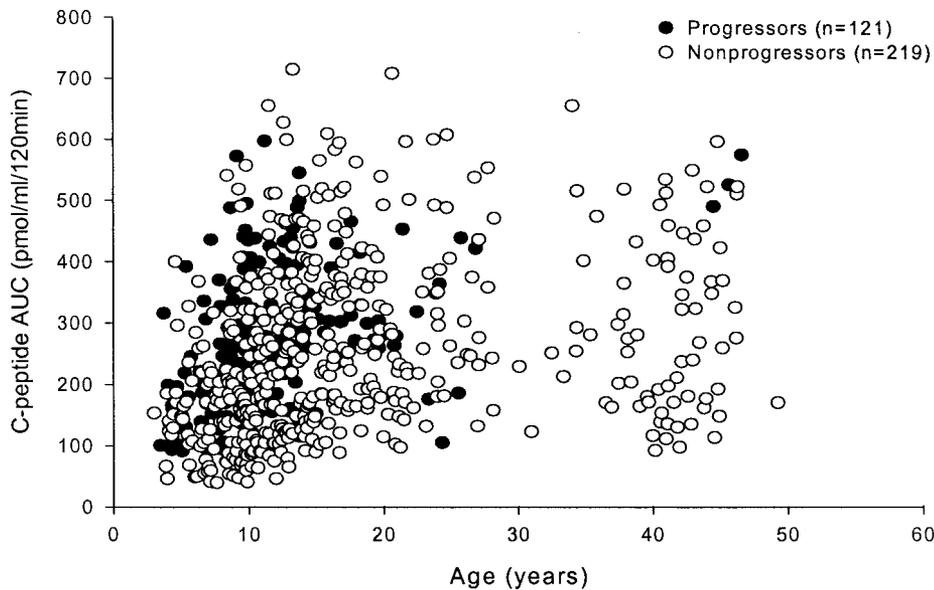


FIG. 1. Relationship between age and C-peptide response to an MMTT in subjects in the DPT-1 at risk of developing type 1 diabetes. Subjects who did (●, $n = 121$) and who did not (○, $n = 219$) progress to diabetes in the DPT-1 underwent one to five MMTTs during the follow-up period. The data shown represent all of the studies done at a time that subjects were not diagnosed with type 1 diabetes ($r = 0.27$, $P < 0.0001$). AUC, area under the curve.

tion of insulin secretion (11,12). It is difficult to distinguish insulin from proinsulin in most assays and values can be altered in the presence of insulin antibodies. C-peptide, which is cosecreted with insulin in equimolar concentrations, can be assessed more accurately and with higher reproducibility and greater sensitivity (10-fold) and has been recommended as the appropriate outcome measure for future clinical trials aimed at preserving β -cell function (13). The long half-life of C-peptide, however, makes evaluation of changes in insulin secretion over short time intervals under non-steady-state conditions difficult.

Calculated insulin secretory rates. Van Cauter, Polonsky, and colleagues (11,14–17) have developed standard kinetic parameters to be used in a two-compartment model to calculate the insulin secretory rate (ISR) under non-steady-state conditions. The parameters used for these calculations were derived from studies of the distribution of C-peptide in normal individuals and individuals with diabetes. These calculations have been used in adults and younger patients with type 2 diabetes as well as in younger patients with type 1 diabetes to provide both qualitative and quantitative information about insulin secretion (18,19). Whereas the parameters used in the two-compartment model derived from adults have been applied to studies with children, it is important to note that validation of these parameters in the pediatric population has not occurred, but they are not believed to be different.

Stimuli of insulin secretion other than glucose. β -Cell response to glucose, however, is only one measure of β -cell function. Just after onset of clinical type 1 diabetes, patients may have low or absent response to intravenous and oral glucose but retain near-normal response to arginine and glucagon (20). Glucagon or a mixed-meal tolerance test (MMTT) have generally been used after diagnosis, and studies have shown good correlation between the two stimuli (21,22). Some have advocated the use of intravenous arginine as a comparable and practically easier test (23). However, a detailed comparison of various stimuli showed that the manifestations of impairment in insulin secretion may be related to the stimulus used. Ganda et al. (24) showed that the rank order of response to stimuli was: intravenous arginine greater than intravenous glucagon greater than oral glucose greater

than intravenous tolbutamide greater than intravenous glucose. These studies highlighted that evidence of β -cell dysfunction is apparent well before complete unresponsiveness to intravenous glucose and that other secretagogues may be used to uncover abnormalities in insulin secretion. More recently, a number of studies have characterized β -cell function by indexes such as maximal insulin responsiveness to the potentiating effect of glucose and the incretin effect (the ability of gastrointestinal hormones and neural stimuli to augment the β -cell response beyond glycemic stimulation alone) (25–35).

INSULIN SECRETION IN PRECLINICAL TYPE 1 DIABETES

Early metabolic alteration: loss of expected rise in C-peptide with age. There have been few studies of insulin secretion in normal children and individuals at risk for diabetes. In a recently published interventional study of subjects at risk for type 1 diabetes, individuals in the control group who did not develop diabetes showed an increase in insulin responses with time, whereas those who progressed to diabetes did not (36). Gottsater et al. (37) studied β -cell function using intravenous glucose and glucagon stimulation in healthy subjects and found that C-peptide responses increased with age in subjects between the ages of 19 and 78 years. We have analyzed data from the DPT-1 and have found similar changes with age in response to an MMTT in nondiabetic at-risk subjects (Fig. 1). In nondiabetic subjects who are at risk for diabetes, with a median age of 12.8 years (range 3.0–49.2), we found a significant correlation between age and C-peptide response to an MMTT ($r = 0.271$, $P < 0.0001$) (Fig. 1). In view of these changes, the decrease in insulin secretion at diagnosis may be considered not just a loss in absolute terms, but also a failure to increase with age. It is possible that the increasing insulin secretory responses occur as a result of increasing insulin resistance, particularly during adolescence, but we did not find a significant relationship between age and the homeostasis model assessment for insulin resistance in a group of 25 individuals who progressed to type 1 diabetes (38).

Loss of FPIR in pre-diabetes. A reduced FPIR to intravenous glucose during an IVGTT occurs early in the preclinical phase of type 1 diabetes. A prospective study of 23 identical twins, discordant for type 1 diabetes, showed evidence of β -cell dysfunction including loss of FPIR (<1% of normal control subjects) and/or autoimmunity with development of diabetes after years of discordance (39). Likewise, in a prospective population-based study, following 52 genetically at-risk 1- to 5-year-old children for the development of type 1 diabetes, the type 1 diabetes prediction and prevention project (DIPP) found that the FPIR decreased shortly after the development of ICAs, in the first post-seroconversion IVGTT in 42% of subjects (40). Of the 13 children who went on to develop type 1 diabetes, the average FPIR was 20 mU/l (normal >38 mU/l) 1.9 years (range 1.2–4.5) before development of clinical disease. An additional 11 children with low FPIR had not developed type 1 diabetes during 1.5–4.4 years of follow-up after seroconversion to ICA, suggesting that individuals may be able to compensate for a low FPIR many months to years before the development of type 1 diabetes.

A reduced FPIR has been used to predict which genetically at-risk individuals will progress to clinical type 1 diabetes. The Islet Cell Antibody Registered Users group showed, in a retrospective study of 217 ICA⁺ first-degree relatives of subjects with type 1 diabetes, that for individuals with an FPIR of <50 mU/l, the risk of developing type 1 diabetes within 5 years was 85%, compared with 48% for those with an FPIR of 50–100 mU/l and 17% for those with an FPIR of >100 mU/l (41). Results from the DPT-1 showed that in the 1,622 subjects who had an IVGTT, low FPIR was correlated with the presence of ICAs and insulin autoantibodies and with increased levels of the titers (42). It is not clear whether this finding adds significantly to the predictive value of multiple autoantibodies. However, in the Childhood Diabetes in Finland Study (DiMe), a prospective study of 758 siblings of subjects with type 1 diabetes, the risk of developing type 1 diabetes could be estimated to a maximum of 66% when using a classification based exclusively on the number of antibodies but as high as 92% when grading according to both antibodies and FPIR (43).

Later metabolic alteration: abnormal glucose tolerance. Abnormal glucose tolerance with preservation of fasting glucose is the next metabolic alteration in progression to clinical type 1 diabetes. An analysis of the data from the DPT-1 study has shown that of 585 first- and second-degree relatives of people with type 1 diabetes, who had positive ICAs and either positive insulin autoantibodies or decreased FPIR, 427 had normal glucose tolerance, 87 had impaired glucose tolerance, 3 had impaired fasting glucose, 7 had diabetes by fasting and 2-h oral glucose tolerance test criteria, and 61 had diabetes by 2-h oral glucose tolerance test criteria alone. There was a significant but not strong correlation of FPIR with categories of worsening glucose tolerance. Subjects with high FPIR had lower 2-h glucose values, those with low FPIR had a wide range of 2-h glucose values (2.8–30.5 mmol/l), and those with the highest 2-h glucose all had low FPIR, suggesting that low FPIR was followed by impaired tolerance to oral glucose (10). Also, there were indirect indications of defects in β -cell function with maximal stimulation at this intermediate stage. The 61 subjects with normal fasting glucose and diabetic oral glucose tolerance were found to have evidence of β -cell dysfunction in the

presence of hyperglycemia (decreased glucose potentiation of insulin response to arginine and decreased maximum insulin response to arginine) as well as decreased incretin effect and a defect in the suppression of glucagon levels (25).

In summary, the progression from preclinical to clinical type 1 diabetes may be characterized by a failure to increase insulin production with age, followed by the loss of FPIRs to intravenous glucose and finally oral glucose intolerance. The time of presentation with clinical disease may be affected by factors including the absolute level of insulin production and other metabolic needs as described below.

THE ACCELERATOR HYPOTHESIS: INSULIN RESISTANCE AS A PRECIPITATOR OF TYPE 1 DIABETES

When comparing the DPT-1 subjects with abnormal 2-h glucose values with and without abnormalities in fasting glucose, individuals with impaired fasting glucose were older, had increased fasting insulin, and had increased homeostasis model assessment for insulin resistance, suggesting that insulin resistance may play a role in the loss of the ability to maintain fasting euglycemia (10). The importance of insulin resistance in the development of type 1 diabetes has been suggested by others and advanced in the accelerator hypothesis by Wilkin (29). The hypothesis asserts that both type 1 and type 2 diabetes are primarily the same disorder with three accelerators at play to varying degrees in a given individual: 1) a constitutionally high rate of β -cell apoptosis, 2) insulin resistance, and 3) β -cell autoimmunity. In fact, the three may be linked together in that insulin resistance itself may increase β -cell apoptosis, which may release antigens and lead to β -cell autoimmunity in genetically susceptible individuals. In a retrospective study of 91 1- to 19-year-old children with type 1 diabetes, Kibirige et al. (30) found that BMI and weight change since birth were inversely related to age at diagnosis. A retrospective study of 99 7- to 14-year-old children with type 1 diabetes compared with age-matched control subjects demonstrated that higher energy intake based on diet history, covering the year before diagnosis, as well as higher weight-for-age were independently associated with increased risk of type 1 diabetes (31).

INSULIN SECRETION IN PATIENTS WITH NEW-ONSET TYPE 1 DIABETES

Insulin secretion at the time of diagnosis. It has been widely accepted that insulin secretion is profoundly impaired at the time of diagnosis of type 1 diabetes: previous estimates were that between 10 and 33% of normal β -cell mass remained based on pathological studies as well as measurement of C-peptide responses to feeding in young individuals with type 1 diabetes (44,45). However, the clinical approaches for metabolic control at the time that the data were collected may be different from those in current practice. The estimates of insulin secretion based on circulating C-peptide were done in individuals most likely with ambient hyperglycemia, which may exhaust the insulin secretory capacity of the β -cell or have a more direct inhibitory effect analogous to glucose toxicity described in type 2 diabetes (46).

Earlier studies of insulin secretion in type 1 diabetes recognized that some individuals with type 1 diabetes had persistent C-peptide after diagnosis that was positively

TABLE 1
Factors other than immunotherapies that may affect the natural course of β -cell failure in type 1 diabetes

	Effect	References
Age	Younger-aged subjects have lower residual insulin production at presentation.	19,53,57,65
Autoantibodies	More rapid deterioration in β -cell function occurs in ICA ⁺ individuals. Other autoantibodies may be less predictive.	56,57,65
Insulin resistance	Patients with type 1 diabetes often have evidence of insulin resistance, which may be an accelerator of the metabolic decompensation.	29,32,33
Sex	Some but not all studies have suggested more rapid rates of decline in males.	53,65
Management of diabetes	Aggressive management of type 1 diabetes has been shown to reduce the decline in insulin production.	60–62

correlated to age at onset and, importantly, metabolic control (see below) (45,47,48). Recent studies suggest that on the whole, insulin production may be more substantial at diagnosis than had been previously appreciated and that residual insulin production may persist in a subgroup of patients with autoimmune diabetes. For example, in our studies of insulin secretory responses to an MMTT in patients with new-onset type 1 diabetes, we found the average response to be 52% of the response of normal control subjects (19). All subjects had stimulated C-peptide levels that were >0.2 pmol/ml at diagnosis, a level that had been associated with improved glucose control in the Diabetes Control and Complications Trial (DCCT) (49). However, contemporary as well as older studies indicate that more provocative stimuli may uncover more profound losses of maximal insulin production, even in subjects with normal fasting glucose levels (24,25).

Thus, these studies suggest that the quantitative defect in β -cell function at diagnosis is less than originally indicated from pathological studies. The impairment in response to a physiological stimulus such as a mixed meal may be relatively modest ($\sim 50\%$ of normal response), but with maximal stimulation, the impairment can be shown to be more profound ($\sim 30\%$ of normal response).

Qualitative changes in insulin secretion that have been described in other forms of diabetes are also a feature of insulin secretion in type 1 diabetes at diagnosis. In our studies, there was relative preservation of basal insulin secretion (52% of normal control subjects), but the glucose-stimulated insulin secretion was severely impaired (26% of normal control subjects), consistent with the pattern of loss of stimulated insulin secretion in patients with type 2 diabetes (17). Moreover, a delayed ISR response to the MMTT was common in patients with type 1 diabetes (38%) (see below) (19). In a patient with recent-onset type 1 diabetes studied over time, Polonsky and colleagues (50–52) reported that the tight temporal coupling between ultradian oscillations in ISR and glucose observed in nondiabetic subjects was lost, reminiscent of the loss of ultradian oscillations in ISR that have been observed in patients with type 2 diabetes and those with polycystic ovary syndrome and a family history of type 2 diabetes. In response to mixed meals, the oscillatory pattern of secretion was preserved, but the magnitude of the secretory responses was reduced. Likewise, in a study of patients with new-onset type 1 diabetes, we found that 38% of subjects with type 1 diabetes had a delayed ISR to an MMTT, whereas only 6% (2/38) of normal control subjects showed this pattern (see below) (19).

Insulin secretion during progression of type 1 diabetes after diagnosis. With time, there is typically a continuous decline in β -cell function. Snorgaard et al. (53) reported that the fasting C-peptide at diagnosis of type 1

diabetes was 0.17 pmol/ml, increased annually by 0.16 pmol/ml per year to a peak of 0.28 pmol/ml, and then fasting and postprandial C-peptide declined at rates of 0.08 and 0.03 pmol/ml per year in a homogenous manner. In our analysis of insulin secretion over the first 2 years after diagnosis of type 1 diabetes, we found that the ISR area under the curve during a 4-h MMTT declined at a rate of 756 ± 132 pmol/ml per month to a level at 2 years that was $28 \pm 8.4\%$ of the response at diagnosis (19). Infrequently, C-peptide responses increased: of the 78 follow-up studies in 20 patients with type 1 diabetes, eight of the tests showed increased C-peptide responses to the MMTT after diagnosis compared with the study at diagnosis. However, the increases were not persistent over time—generally, they were not repeated in subsequent studies. By 24 months, 47% of patients had C-peptide levels that were below the lower limit of detection (0.03 pmol/ml). A similar experience was reported in the control group from the Canadian-European Cyclosporin trial, with only 27 and 10% of patients in a non-insulin-requiring remission at 3 and 12 months, respectively, after diagnosis (54).

FACTORS AFFECTING INSULIN SECRETION AT DIAGNOSIS AND ITS LOSS AFTERWARD

Age. Several factors have been studied as predictors of the natural history of β -cell response and decline. The most important of which is age. Karjalainen et al. (55) reported that type 1 diabetes that begins in adulthood (20–55.8 years) is characterized by a longer asymptomatic period before diagnosis and better preservation of residual β -cell function than type 1 diabetes beginning in childhood (1.3–18.2 years). Similarly, in our prospective study, we found that a greater insulin secretory reserve correlated with older age at presentation (the correlation between insulin secretory reserve and age at diagnosis was 0.54, $P = 0.012$), and in our current analysis of subjects in the DPT-1, the correlation between age at diagnosis and C-peptide area under the curve to an MMTT was similar ($r = 0.307$, $P < 0.01$, $n = 70$). In the DCCT, 48% of adult subjects had significant C-peptide levels (>0.2 pmol/ml) at the time of screening for entry into the study (duration 1–5 years), whereas 33% of adolescents had this level. Bonfanti et al. (56) found significantly reduced C-peptide levels in patients with type 1 diabetes diagnosed at less than 5 years of age compared with diagnosis at an older age (Table 1).

The inverse correlation of age with greater loss of insulin reserve at diagnosis may suggest that type 1 diabetes follows a more aggressive course in younger children. However, it could also be accounted for by other variables such as overall lower insulin responses in younger patients and/or greater insulin resistance in older patients, leading to a clinical presentation at an earlier

stage of disease. There are limited data on the natural history of insulin secretory function in patients with type 1 diabetes; however, the available data would suggest that the disease course, at least after diagnosis, follows a similar progression across ages. Our current analysis of the DPT-1 data indicates that the proportion of the C-peptide response at diagnosis relative to the response just before diagnosis is similar in younger and older subjects. Likewise, Wallensteen et al. (57) found that the slopes of the lines describing the changes in stimulated C-peptide over time were -0.017 pmol/ml per month in subjects 1–5 years of age, -0.019 pmol/ml per month in subjects 6–11 years of age, and -0.021 pmol/ml per month in subjects 12–17 years of age. Gottsater et al. (58) found that there was a similar decline in stimulated C-peptide in ICA⁺ patients with type 2 diabetes (age 50 ± 5 years) as in younger adult patients with type 1 diabetes (37 ± 5 years) but not in adult patients with type 2 diabetes who were ICA⁻ (age 52 ± 4 years), suggesting that in patients with evidence of autoimmunity, the disease progression is comparable from childhood through adulthood.

β -Cell dysfunction. β -Cell dysfunctional patterns have also been postulated to identify rates of disease progression. For example, we found that patients who had a delayed ISR to an MMTT (38% of patients with type 1 diabetes but only 5% of normal control subjects) had a slower rate of loss of ISR area under the curve to a 4-h MMTT response (336 ± 186 pmol/month) than those with a normal early response to the meal (936 ± 150 pmol/month) (19). A relatively high ratio of proinsulin/C-peptide has been identified in Scandinavian studies of patients with type 1 diabetes, but a prolonged partial remission was more common in patients with a low proinsulin/C-peptide ratio at onset (59).

Glucose control. The earliest studies of β -cell function in type 1 diabetes showed that strict glycemic control improved stimulated C-peptide responses but the effects were of short duration (60,61). An improvement secondary to glucose control was also seen in the DCCT, in which C-peptide responses were significantly greater in the intensively controlled group compared with individuals in the group receiving conventional therapy over a 5-year follow-up period (49). The mechanisms responsible for these observations remain unresolved. The expression of one autoantigen, insulin, might be expected to be reduced with more intensive metabolic control, but a more precise definition of mechanisms from animal studies will be needed to determine the precise effects of metabolic control on the disease process.

Other factors. There has not been consistency in the prediction of the natural history of disease using other parameters, although generally most studies have found enhanced rates of progression in autoantibody-positive individuals. Bonfanti et al. (62) reported that remission of diabetes was more common in older patients without detectable GAD antibodies but that sex, IA-2 autoantibodies, and HLA-DR were not independently associated with C-peptide secretion or insulin requirements. Likewise Wallensteen et al. (57) found that ICA⁻ patients had higher postprandial C-peptide levels at 1, 9, and 12 months after diagnosis compared with ICA⁺ patients. Similar findings were reported in studies by Torn et al. (63) and Decochez et al. (64), in which young age at diagnosis and high titer ICAs identified a group of patients with type 1 diabetes at high risk of rapidly losing residual β -cell function. However, Sabbah et al. (65) and Daneman et al. (66) reported

no significant difference in metabolic decompensation at diagnosis between autoantibody-positive and autoantibody-negative patients and suggested that the intensity of the humoral islet-directed immune response has little influence on the clinical characteristics at diagnosis. In addition to autoantibodies, male sex appears to identify higher rates of progression in some but not all studies (53,62–64).

CLINICAL IMPORTANCE OF RESIDUAL INSULIN SECRETION

The essential clinical question is the significance of even the small amount of residual insulin production on metabolic control of disease because metabolic control is ultimately the determinant of end-organ complications of type 1 diabetes. Clinical studies have consistently suggested that retention of some insulin secretion is associated with improved and more stable glucose control (48,67). In our study of patients with type 1 diabetes, within the first 2 years of disease, there was a significant inverse relationship between the insulin secretory response and the HbA_{1c} (A1C) level ($r = 0.401$, $P = 0.003$) (19). Earlier studies had suggested that only the subgroup with the highest levels (stimulated C-peptide levels >0.3 pmol/ml) had improved metabolic control compared with others (68), but data from the DCCT indicated that individuals with a low but clearly measurable stimulated C-peptide response (>0.2 pmol/ml) had improved metabolic control compared with individuals with stimulated responses below this level. (Note that all enrollees in the DCCT were required to have a stimulated C-peptide <0.5 pmol/ml.) At the time of study entry, the “responders” (i.e., stimulated C-peptide >0.2 pmol/ml) were older (28.2 vs. 26.1 year), had shorter duration of diabetes (2.1 vs. 2.9 years), used less daily insulin (0.49 vs. 0.69 units \cdot kg⁻¹ \cdot day⁻¹), and had lower A1C levels (8.3 vs. $9.2 \pm 1.6\%$) (13). Importantly, the DCCT data also indicated that patients with any C-peptide secretion, but especially those with the highest levels, had a reduced incidence of retinopathy and nephropathy (69). In addition, the incidence of hypoglycemia, the most common complication of insulin-treated diabetes, has been shown to be inversely related to residual insulin production. Improved glucagon secretory responses have been seen in patients with greater insulin secretory reserve (70). A relationship between α - and β -cell function in this setting has recently been suggested. During exogenous insulin administration, hypoglycemia is not followed by a decrease in the intra-islet insulin concentration, which could impair the stimulation of glucagon secretion (71).

The clear evidence of the clinical significance of residual β -cell function in stabilizing metabolic control and thereby preventing complications of diabetes has led an expert panel to conclude that C-peptide is an appropriate end point for clinical trials to preserve insulin production in type 1 diabetes (13).

INSULIN SECRETION IN PATIENTS WITH ESTABLISHED TYPE 1 DIABETES

Recent interest has focused on the prevalence of residual insulin secretion in patients with established type 1 diabetes, an observation that originated with the initial studies of C-peptide secretion in type 1 diabetes (48,72). The data from the DCCT suggested that this prevalence is infrequent in individuals diagnosed as children but is more frequent in individuals diagnosed over the age of 18 years,

possibly because of the higher insulin secretion in these patients at the time of diagnosis. In that cross-sectional data, 3% of adolescents and 8% of adults with disease duration of >5 years had stimulated C-peptide levels >0.2 pmol/ml. Pathological studies have drawn attention to the prevalence of residual insulin-positive cells in pancreata from patients with type 1 diabetes, suggesting that failure to progress to complete islet cell destruction may be more common than had previously been appreciated based on measurement of peripheral C-peptide levels alone. In a pathological study of pancreata from 26 patients with disease duration of 2–54 years, insulin-positive cells were found in 13 of 26 pancreata and in all pancreata from patients with diabetes duration less than 11 years, but near 40% from those with diabetes duration of more than 11 years (73). In this study, survival of the insulin-positive cells was not related to age at onset. A recent case report describes the recovery from type 1 diabetes in a 13-year-old male who initially presented with glycosuria and ketonuria (74). Finally, the improvement in C-peptide responses that have been identified within the first 6 months after diagnosis in some individuals suggests that repair of the loss of β -cell function and/or mass may occur early in the course of the disease. However, it should be emphasized that these examples are the exceptions rather than the general pattern of progression of the disease in the majority of patients.

The reason that any β -cells should persist, and hence insulin secretion, is not clear from our current understanding of the natural history of the autoimmune process and the described recurrence of disease in recipients of pancreas and possibly islet transplants. The relatively poor predictive value of a single autoantibody test in a prediabetic individual suggests that, at some point in the autoimmune process, progression may be halted. Perhaps, just as not all individuals with autoimmunity progress to disease, not all individuals with disease progress to complete loss of β -cell mass. Preservation of β -cells may occur because not all cells are equivalent targets of the response or because the response has been attenuated.

A second potential explanation for the persistence of insulin secretion is that there is renewal of β -cells. The extensive experience in patients with type 1 diabetes treated with immune-suppressive drugs to prevent kidney allograft rejection would suggest that recovery of significant β -cell function is not likely even in the setting of immune suppression. However, the drugs used in most transplant recipients, including cyclosporin A, FK-506, and glucocorticoids, have either direct inhibitory effects on insulin secretion or interfere with insulin action. Moreover, the immune suppression that may be needed to block autoimmune diabetes may be different from that needed to prevent allograft rejection.

The possibility that there is renewal of human islet cells in patients with type 1 diabetes remains an unresolved question. There have been several studies in animal models supporting this notion (75–77). In humans, data supporting β -cell proliferation is largely anecdotal, such as the finding of detectable C-peptide in patients with longstanding type 1 diabetes at the time of pregnancy and/or postpartum (78,79). Increased insulin production was thought to account for the development of hypoglycemia during the first trimester of pregnancy in 10 women with established type 1 diabetes when C-peptide levels were found to be 0.2 ± 0.02 pmol/ml and had been undetectable before pregnancy.

CONCLUSION

Clinical studies and those in animal models have changed previous concepts of the metabolic changes that occur during and after the development of type 1 diabetes. These studies have drawn attention to the quantitative and clinically important insulin secretory reserve that is found in individuals at risk for developing type 1 diabetes and at the time of presentation. Based on experience from the DCCT, intervention trials at the time of onset, if successful, would be expected to have significant effects on metabolic control of the disease and, if maintained, ultimately on development of secondary end-organ complications. In younger patients, a more aggressive approach to intervention may be needed because of the limited reserve that is present at the time of diagnosis, whereas older individuals may benefit from intervention even months after diagnosis. The potential for replication of β -cells is largely unknown but is consistently suggested by animal models and even by anecdotal human experience. Combinatorial therapy that could induce immunological tolerance and stimulate the replicative process would have important potential for treatment.

ACKNOWLEDGMENTS

This study was supported by grants R01DK57846, R01DK068678, U19AI46132, P60DK20595, M01RR00645, and M01RR01271 and a Fellowship Award (to N.A.S.) from the Lawson Wilkins Society and the Juvenile Diabetes Research Foundation. Analyses using data from the DPT-1 were collected by the DPT-1 study group.

REFERENCES

1. Diabetes Prevention Trial—Type 1 Diabetes Study Group: Effects of insulin in relatives of patients with type 1 diabetes mellitus. *N Engl J Med* 346:1685–1691, 2002
2. Hagopian WA, Sanjeevi CB, Kockum I, Landin-Olsson M, Karlens AE, Sundkvist G, Dahlquist G, Palmer J, Lernmark A: Glutamate decarboxylase-, insulin-, and islet cell-antibodies and HLA typing to detect diabetes in a general population-based study of Swedish children. *J Clin Invest* 95:1505–1511, 1995
3. Gale EA: Intervening before the onset of type 1 diabetes: baseline data from the European Nicotinamide Diabetes Intervention Trial (ENDIT). *Diabetologia* 46:339–346, 2003
4. Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Jackson RA, Chase HP, Eisenbarth GS: Prediction of type 1 diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. *Diabetes* 45:926–933, 1996
5. Yu L, Rewers M, Gianani R, Kawasaki E, Zhang Y, Verge C, Chase P, Klingensmith G, Erlich H, Norris J, Eisenbarth GS: Antislet autoantibodies usually develop sequentially rather than simultaneously. *J Clin Endocrinol Metab* 81:4264–4267, 1996
6. Sreenan S, Pick AJ, Levisetti M, Baldwin AC, Pugh W, Polonsky KS: Increased beta-cell proliferation and reduced mass before diabetes onset in the nonobese diabetic mouse. *Diabetes* 48:989–996, 1999
7. Shimada A, Charlton B, Taylor-Edwards C, Fathman CG: Beta-cell destruction may be a late consequence of the autoimmune process in nonobese diabetic mice. *Diabetes* 45:1063–1067, 1996
8. Bingley PJ, Colman P, Eisenbarth GS, Jackson RA, McCulloch DK, Riley WJ, Gale EA: Standardization of IVGTT to predict IDDM. *Diabetes Care* 15:1313–1316, 1992
9. McCulloch DK, Bingley PJ, Colman PG, Jackson RA, Gale EA: Comparison of bolus and infusion protocols for determining acute insulin response to intravenous glucose in normal humans: The ICARUS Group: Islet Cell Antibody Register User's Study. *Diabetes Care* 16:911–915, 1993
10. Greenbaum CJ, Cuthbertson D, Krischer JP: Type 1 diabetes manifested solely by 2-h oral glucose tolerance test criteria. *Diabetes* 50:470–476, 2001
11. Polonsky K, Frank B, Pugh W, Addis A, Karrison T, Meier P, Tager H, Rubenstein A: The limitations to and valid use of C-peptide as a marker of the secretion of insulin. *Diabetes* 35:379–386, 1986
12. Polonsky KS, Given BD, Hirsch L, Shapiro ET, Tillil H, Beebe C, Galloway

- JA, Frank BH, Karrison T, Van Cauter E: Quantitative study of insulin secretion and clearance in normal and obese subjects. *J Clin Invest* 81:435–441, 1988
13. Palmer JP, Fleming GA, Greenbaum CJ, Herold KC, Jansa LD, Kolb H, Lachin JM, Polonsky KS, Pozzilli P, Skyler JS, Steffes MW: C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve beta-cell function: report of an ADA workshop, 21–22 October 2001. *Diabetes* 53:250–264, 2004
 14. Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels: comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41:368–377, 1992
 15. Polonsky KS, Licinio-Paixao J, Given BD, Pugh W, Rue P, Galloway J, Karrison T, Frank B: Use of biosynthetic human C-peptide in the measurement of insulin secretion rates in normal volunteers and type I diabetic patients. *J Clin Invest* 77:98–105, 1986
 16. Polonsky KS, Rubenstein AH: C-peptide as a measure of the secretion and hepatic extraction of insulin: pitfalls and limitations. *Diabetes* 33:486–494, 1984
 17. Polonsky KS, Given BD, Hirsch LJ, Tillil H, Shapiro ET, Beebe C, Frank BH, Galloway JA, Van Cauter E: Abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. *N Engl J Med* 318:1231–1239, 1988
 18. Heptulla RA, Tamborlane WV, Cavaghan M, Bronson M, Limb C, Ma YZ, Sherwin RS, Caprio S: Augmentation of alimentary insulin secretion despite similar gastric inhibitory peptide (GIP) responses in juvenile obesity. *Pediatr Res* 47:628–633, 2000
 19. Steele C, Hagopian WA, Gitelman S, Masharani U, Cavaghan M, Rother KI, Donaldson D, Harlan DM, Bluestone J, Herold KC: Insulin secretion in type 1 diabetes. *Diabetes* 53:426–433, 2004
 20. Chaillous L, Rohmer V, Maugendre D, Lecomte P, Marechaud R, Marre M, Guilhem I, Charbonnel B, Sai P: Differential beta-cell response to glucose, glucagon, and arginine during progression to type I (insulin-dependent) diabetes mellitus. *Metabolism* 45:306–314, 1996
 21. Chaillous L, Lefevre H, Thivolet C, Boitard C, Lahlou N, Atlan-Gepner C, Bouhanick B, Mogenet A, Nicolino M, Carel JC, Lecomte P, Marechaud R, Bougneres P, Charbonnel B, Sai P: Oral insulin administration and residual beta-cell function in recent-onset type 1 diabetes: a multicentre randomised controlled trial: Diabete Insuline Orale group. *Lancet* 356:545–549, 2000
 22. Schnell O, Eisfelder B, Standl E, Ziegler AG: High-dose intravenous insulin infusion versus intensive insulin treatment in newly diagnosed IDDM. *Diabetes* 46:1607–1611, 1997
 23. Greenbaum C, Seidel K, Pihoker C: The case for intravenous arginine stimulation in lieu of mixed-meal tolerance tests as outcome measure for intervention studies in recent-onset type 1 diabetes. *Diabetes Care* 27:1202–1204, 2004
 24. Ganda OP, Srikanta S, Brink SJ, Morris MA, Gleason RE, Soeldner JS, Eisenbarth GS: Differential sensitivity to beta-cell secretagogues in “early,” type I diabetes mellitus. *Diabetes* 33:516–521, 1984
 25. Greenbaum CJ, Prigeon RL, D’Alessio DA: Impaired beta-cell function, incretin effect, and glucagon suppression in patients with type 1 diabetes who have normal fasting glucose. *Diabetes* 51:951–957, 2002
 26. Yki-Jarvinen H, Koivisto VA: Natural course of insulin resistance in type I diabetes. *N Engl J Med* 315:224–230, 1986
 27. Martin FI, Hopper JL: The relationship of acute insulin sensitivity to the progression of vascular disease in long-term type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 30:149–153, 1987
 28. Greenfield JR, Samaras K, Chisholm DJ: Insulin resistance, intra-abdominal fat, cardiovascular risk factors, and androgens in healthy young women with type 1 diabetes mellitus. *J Clin Endocrinol Metab* 87:1036–1040, 2002
 29. Wilkin TJ: The accelerator hypothesis: weight gain as the missing link between type I and type II diabetes. *Diabetologia* 44:914–922, 2001
 30. Kibirige M, Metcalf B, Renuka R, Wilkin TJ: Testing the accelerator hypothesis: the relationship between body mass and age at diagnosis of type 1 diabetes. *Diabetes Care* 26:2865–2870, 2003
 31. Pundziute-Lycka A, Persson LA, Cedermarck G, Jansson-Roth A, Nilsson U, Westin V, Dahlquist G: Diet, growth, and the risk for type 1 diabetes in childhood: a matched case-referent study. *Diabetes Care* 27:2784–2789, 2004
 32. Libman IM, Pietropaolo M, Arslanian SA, LaPorte RE, Becker DJ: Changing prevalence of overweight children and adolescents at onset of insulin-treated diabetes. *Diabetes Care* 26:2871–2875, 2003
 33. Libman IM, Pietropaolo M, Arslanian SA, LaPorte RE, Becker DJ: Evidence for heterogeneous pathogenesis of insulin-treated diabetes in black and white children. *Diabetes Care* 26:2876–2882, 2003
 34. Gungor N, Saad R, Janosky J, Arslanian S: Validation of surrogate estimates of insulin sensitivity and insulin secretion in children and adolescents. *J Pediatr* 144:47–55, 2004
 35. Veening MA, van Weissenbruch MM, Heine RJ, Delemarre-van de Waal HA: Beta-cell capacity and insulin sensitivity in prepubertal children born small for gestational age: influence of body size during childhood. *Diabetes* 52:1756–1760, 2003
 36. Schatz D, Cuthbertson D, Atkinson M, Salzlter MC, Winter W, Muir A, Silverstein J, Cook R, Maclaren N, She JX, Greenbaum C, Krischer J: Preservation of C-peptide secretion in subjects at high risk of developing type 1 diabetes mellitus: a new surrogate measure of non-progression? *Pediatr Diabetes* 5:72–79, 2004
 37. Gottsater A, Landin-Olsson M, Fernlund P, Gullberg B, Lernmark A, Sundkvist G: Pancreatic beta-cell function evaluated by intravenous glucose and glucagon stimulation: a comparison between insulin and C-peptide to measure insulin secretion. *Scand J Clin Lab Invest* 52:631–639, 1992
 38. Cutfield WS, Jefferies CA, Jackson WE, Robinson EM, Hofman PL: Evaluation of HOMA and QUICKI as measures of insulin sensitivity in prepubertal children. *Pediatr Diabetes* 4:119–125, 2003
 39. Verge CF, Gianani R, Yu L, Pietropaolo M, Smith T, Jackson RA, Soeldner JS, Eisenbarth GS: Late progression to diabetes and evidence for chronic beta-cell autoimmunity in identical twins of patients with type I diabetes. *Diabetes* 44:1176–1179, 1995
 40. Keskinen P, Korhonen S, Kupila A, Veijola R, Erkkila S, Savolainen H, Arvilommi P, Simell T, Ilonen J, Knip M, Simell O: First-phase insulin response in young healthy children at genetic and immunological risk for type I diabetes. *Diabetologia* 45:1639–1648, 2002
 41. Bingley PJ: Interactions of age, islet cell antibodies, insulin autoantibodies, and first-phase insulin response in predicting risk of progression to IDDM in ICA+ relatives: the ICARUS data set: Islet Cell Antibody Register Users Study. *Diabetes* 45:1720–1728, 1996
 42. Chase HP, Cuthbertson DD, Dolan LM, Kaufman F, Krischer JP, Schatz DA, White NH, Wilson DM, Wolfsdorf J: First-phase insulin release during the intravenous glucose tolerance test as a risk factor for type 1 diabetes. *J Pediatr* 138:244–249, 2001
 43. Mrena S, Savola K, Kulmala P, Akerblom HK, Knip M: Staging of preclinical type 1 diabetes in siblings of affected children: Childhood Diabetes in Finland Study Group. *Pediatrics* 104:925–930, 1999
 44. Gepts W: Pathologic anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes* 14:619–633, 1965
 45. Madsbad S, Krarup T, Regeur L, Faber OK, Binder C: Insulin secretory reserve in insulin dependent patients at time of diagnosis and the first 180 days of insulin treatment. *Acta Endocrinol (Copenh)* 95:359–363, 1980
 46. Leahy JL: Natural history of beta-cell dysfunction in NIDDM. *Diabetes Care* 13:992–1010, 1990
 47. Faber OK, Binder C: B-cell function and blood glucose control in insulin dependent diabetics within the first month of insulin treatment. *Diabetologia* 13:263–268, 1977
 48. Ludvigsson J, Heding LG, Larsson Y, Leander E: C-peptide in juvenile diabetics beyond the postinitial remission period: relation to clinical manifestations at onset of diabetes, remission and diabetic control. *Acta Paediatr Scand* 66:177–184, 1977
 49. Diabetes Control and Complications Trial Research Group: Effect of intensive therapy on residual beta-cell function in patients with type 1 diabetes in the Diabetes Control and Complications Trial: a randomized, controlled trial. *Ann Intern Med* 128:517–523, 1998
 50. Ehrmann DA, Sturis J, Byrne MM, Karrison T, Rosenfield RL, Polonsky KS: Insulin secretory defects in polycystic ovary syndrome: relationship to insulin sensitivity and family history of non-insulin-dependent diabetes mellitus. *J Clin Invest* 96:520–527, 1995
 51. O’Meara NM, Sturis J, Van Cauter E, Polonsky KS: Lack of control by glucose of ultradian insulin secretory oscillations in impaired glucose tolerance and in non-insulin-dependent diabetes mellitus. *J Clin Invest* 92:262–271, 1993
 52. O’Meara NM SJ, Herold KC, Ostrega DM, Polonsky KS: Alterations in the patterns of insulin secretion before and after diagnosis of IDDM. *Diabetes Care* 18:568–571, 1995
 53. Snorgaard O, Lassen LH, Binder C: Homogeneity in pattern of decline of beta-cell function in IDDM: prospective study of 204 consecutive cases followed for 7.4 yr. *Diabetes Care* 15:1009–1013, 1992
 54. Martin S, Pawlowski B, Greulich B, Ziegler AG, Mandrup-Poulsen T, Mahon J: Natural course of remission in IDDM during 1st yr after diagnosis. *Diabetes Care* 15:66–74, 1992
 55. Karjalainen J, Salmela P, Ilonen J, Surcel HM, Knip M: A comparison of childhood and adult type I diabetes mellitus. *N Engl J Med* 320:881–886, 1989
 56. Bonfanti R, Bazzigaluppi E, Calori G, Riva MC, Viscardi M, Boggetti E,

- Meschi F, Bosi E, Chiumello G, Bonifacio E: Parameters associated with residual insulin secretion during the first year of disease in children and adolescents with type 1 diabetes mellitus. *Diabet Med* 15:844–850, 1998
57. Wallensteen M, Dahlquist G, Persson B, Landin-Olsson M, Lernmark A, Sundkvist G, Thalme B: Factors influencing the magnitude, duration, and rate of fall of B-cell function in type 1 (insulin-dependent) diabetic children followed for two years from their clinical diagnosis. *Diabetologia* 31:664–669, 1988
58. Gottsater A, Landin-Olsson M, Lernmark A, Fernlund P, Sundkvist G: Islet cell antibodies are associated with beta-cell failure also in obese adult onset diabetic patients. *Acta Diabetol* 31:226–231, 1994
59. Ludvigsson J, Heding L: Abnormal proinsulin/C-peptide ratio in juvenile diabetes. *Acta Diabetol Lat* 19:351–358, 1982
60. Madsbad S, Krarup T, Regeur L, Faber OK, Binder C: Effect of strict blood glucose control on residual B-cell function in insulin-dependent diabetics. *Diabetologia* 20:530–534, 1981
61. Madsbad S, Krarup T, Faber OK, Binder C, Regeur L: The transient effect of strict glycaemic control on B cell function in newly diagnosed type 1 (insulin-dependent) diabetic patients. *Diabetologia* 22:16–20, 1982
62. Bonfanti R, Bognetti E, Meschi F, Brunelli A, Riva MC, Pastore MR, Calori G, Chiumello G: Residual beta-cell function and spontaneous clinical remission in type 1 diabetes mellitus: the role of puberty. *Acta Diabetol* 35:91–95, 1998
63. Tom C, Landin-Olsson M, Lernmark A, Palmer JP, Arnqvist HJ, Blohme G, Lithner F, Littorin B, Nystrom L, Schersten B, Sundkvist G, Wibell L, Ostman J: Prognostic factors for the course of beta cell function in autoimmune diabetes. *J Clin Endocrinol Metab* 85:4619–4623, 2000
64. Decochez K, Keymeulen B, Somers G, Dorchy H, De Leeuw IH, Mathieu C, Rottiers R, Winnock F, ver Elst K, Weets I, Kaufman L, Pipeleers DG: Use of an islet cell antibody assay to identify type 1 diabetic patients with rapid decrease in C-peptide levels after clinical onset: Belgian Diabetes Registry. *Diabetes Care* 23:1072–1078, 2000
65. Sabbah E, Savola K, Kulmala P, Veijola R, Vahasalo P, Karjalainen J, Akerblom HK, Knip M: Diabetes-associated autoantibodies in relation to clinical characteristics and natural course in children with newly diagnosed type 1 diabetes: the Childhood Diabetes in Finland Study Group. *J Clin Endocrinol Metab* 84:1534–1539, 1999
66. Daneman D, Sochett E, Pak CY, Yoon JW: Relationship of islet cell and islet cell surface antibodies to the presentation and early course of IDDM in childhood. *Diabetes Res Clin Pract* 4:127–132, 1988
67. Daneman D, Clarson C: Residual beta-cell function in children with type 1 diabetes: measurement and impact on glycemic control. *Clin Invest Med* 10:484–487, 1987
68. Madsbad S, McNair P, Faber OK, Binder C, Christiansen C, Transbol I: Beta-cell function and metabolic control in insulin treated diabetics. *Acta Endocrinol (Copenh)* 93:196–200, 1980
69. Steffes MW, Sibley S, Jackson M, Thomas W: Beta-cell function and the development of diabetes-related complications in the Diabetes Control and Complications Trial. *Diabetes Care* 26:832–836, 2003
70. Fukuda M, Tanaka A, Tahara Y, Ikegami H, Yamamoto Y, Kumahara Y, Shima K: Correlation between minimal secretory capacity of pancreatic beta-cells and stability of diabetic control. *Diabetes* 37:81–88, 1988
71. Raju B, Cryer PE: Loss of the decrement in intraslet insulin plausibly explains loss of the glucagon response to hypoglycemia in insulin-deficient diabetes: documentation of the intraslet insulin hypothesis in humans. *Diabetes* 54:757–764, 2005
72. Rother KI, Harlan DM: Challenges facing islet transplantation for the treatment of type 1 diabetes mellitus. *J Clin Invest* 114:877–883, 2004
73. Lohr M, Kloppel G: Residual insulin positivity and pancreatic atrophy in relation to duration of chronic type 1 (insulin-dependent) diabetes mellitus and microangiopathy. *Diabetologia* 30:757–762, 1987
74. Karges B, Durinovic-Bello I, Heinze E, Boehm BO, Debatin KM, Karges W: Complete long-term recovery of beta-cell function in autoimmune type 1 diabetes after insulin treatment. *Diabetes Care* 27:1207–1208, 2004
75. Zorina TD, Subbotin VM, Bertera S, Alexander AM, Haluszczak C, Gambrell B, Bottino R, Styche AJ, Trucco M: Recovery of the endogenous beta cell function in the NOD model of autoimmune diabetes. *Stem Cells* 21:377–388, 2003
76. Ryu S, Kodama S, Ryu K, Schoenfeld DA, Faustman DL: Reversal of established autoimmune diabetes by restoration of endogenous beta cell function. *J Clin Invest* 108:63–72, 2001
77. Dor Y, Brown J, Martinez OI, Melton DA: Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 429:41–46, 2004
78. Ilic S, Jovanovic L, Wollitzer AO: Is the paradoxical first trimester drop in insulin requirement due to an increase in C-peptide concentration in pregnant type I diabetic women? *Diabetologia* 43:1329–1330, 2000
79. Singer F, Horlick M, Poretsky L: Recovery of beta-cell function postpartum in a patient with insulin-dependent diabetes mellitus. *N Y State J Med* 88:496–498, 1988