ADA Workshop Report

C-Peptide Is the Appropriate Outcome Measure for Type 1 Diabetes Clinical Trials to Preserve β-Cell Function

Report of an ADA Workshop, 21–22 October 2001

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The underlying cause of type 1 diabetes, loss of β-cell function, has become the therapeutic target for a number of interventions in patients with type 1 diabetes. Even though insulin therapies continue to improve, it remains difficult to achieve normal glycemic control in type 1 diabetes, especially long term. The associated risks of hypoglycemia and end-organ diabetic complications remain. Retention of β-cell function in patients with type 1 diabetes is known to result in improved glycemic control and reduced hypoglycemia, retinopathy, and nephropathy. To facilitate the development of therapies aimed at altering the type 1 diabetes disease process, an American Diabetes Association workshop was convened to identify appropriate efficacy outcome measures in type 1 diabetes clinical trials. The following consensus emerged: While measurements of immune responses to islet cells are important in elucidating pathogenesis, none of these measures have directly correlated with the decline in endogenous insulin secretion. HbA_{1c} is a highly valuable clinical measure of glycemic control, but it is an insensitive measure of β-cell function, particularly with the currently accepted standard of near-normal glycemic control. Rates of severe hypoglycemia and diabetic complications ultimately will be improved by therapies that are effective at preserving β-cell function but as primary outcomes require inordinately large and protracted trials. Endogenous insulin secretion is assessed best by measurement of C-peptide, which is cosecreted with insulin in a one-to-one molar ratio but unlike insulin experiences little first pass clearance by the liver. Measurement of C-peptide under standardized conditions provides a sensitive, well accepted, and clinically validated assessment of β-cell function. C-peptide measurement is the most suitable primary outcome for clinical trials of therapies aimed at preserving or improving endogenous insulin secretion in type 1 diabetes patients. Available data demonstrate that even relatively modest treatment effects on C-peptide will result in clinically meaningful benefits. The development of therapies for addressing this important unmet clinical need will be facilitated by trials that are carefully designed with β-cell function as determined by C-peptide measurement as the primary efficacy outcome. *Diabetes* 53:250–264, 2004

Research over the last 25–30 years has clearly established that type 1 diabetes is a T-cell mediated autoimmune disease directed against the pancreatic islet β-cells. In the animal models of type 1 diabetes, the NOD mouse and the BB rat, immunomodulatory therapy can alter or block the autoimmune disease process and development of diabetes can be slowed or prevented (1). In humans, the two large-scale formal clinical trials testing whether parenteral insulin or nicotinamide could prevent development of overt type 1 diabetes failed to show protection by either drug (2,3). Studies in patients with recently diagnosed type 1 diabetes have reported beneficial effects of cyclosporin (4), azathioprine plus steroids (5), anti-CD3 monoclonal antibody (6), and the heat-shock protein peptide DiaPep277 (7) on β-cell function as measured by C-peptide. Studies in recently diagnosed patients are important not only because they identify treatments that likely alter the type 1 diabetes disease process and therefore may be successful in preventing type 1 diabetes, but also because preservation of β-cell function is recognized to result in better metabolic control and reduced end-organ complications (8).

For a therapy to receive regulatory approval, clinical trials must be conducted that show a meaningful and statistically significant effect on an appropriate primary

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ADA, American Diabetes Association; AER, albumin excretion rate; AIR, acute insulin response; AUC, area under the curve; DCCT, Diabetes Control and Complications Trial; ELISPOT, enzyme-linked immunosorbent spot-forming cell; FSIVGTT, frequently sampled intravenous glucose tolerance test; HOMA, homeostasis model assessment; ICA, islet cell antibody; LADA, latent autoimmune diabetes in adults; MMTT, mixed-meal tolerance test; RMSE, root mean square error.

outcome. Ideally, a treatment effect on the primary outcome will have an obvious and substantial clinical benefit. In some cases, the primary outcome may represent a direct clinical benefit, such as a reduction in mortality, hospitalization, or time to wound healing. In other cases, the primary outcome is an assumed or established risk factor for disease progression, such as hyperlipidemia or hypertension. Only recently have reductions of elevated lipid levels or blood pressure been shown to have clinical benefit. Early antilipidemic and antihypertensive agents were nonetheless approved for use based upon short-term improvements in these measures with the expectation that these effects would result in improved cardiovascular outcomes.

In yet other cases, the primary outcome is a measure of a necessary and sufficient intermediate pathophysiological state or process that determines future clinical status. One such outcome is level of glycemic control in diabetes. Results of the Diabetes Control and Complications Trial (DCCT) (9) and the U.K. Prospective Diabetes Study (10) have directly demonstrated that intervening to improve glycemic control as reflected by HbA_{1c} reduces diabetic complications and that the level of HbA_{1c} was directly related to the risk of such complications. Thus, it is now well established that exposure to hyperglycemia is the dominant factor in the etiology of microvascular complications in both type 1 and type 2 diabetes and that an agent that reduces the level of glycemia will have long-term clinical benefits. The approvals of drugs for glycemic control, including oral agents, insulins, and insulin analogs, have all been based on measures of the level of glycemia. For over 15 years, glycosylated hemoglobin (usually HbA_{1c}) has been the primary outcome measure for studies of antihyperglycemic agents in both type 1 and type 2 diabetes.

β-Cell function in newly diagnosed type 1 diabetes is a measurable outcome that likewise predicts long-term clinical status. Loss of β-cell function is the pathophysiological process that renders a normal person diabetic. In essence, diabetes is defined by loss of β-cell function below a level that is adequate to maintain euglycemia. Thus, an agent that leads to preservation of β-cell function can be expected to provide long-term clinical benefit. As a means of facilitating the development of therapies that target the underlying cause of type 1 diabetes, the American Diabetes Association (ADA) sponsored a workshop in Chicago, Illinois, on 21–22 October 2001 to assess the appropriate primary outcome for clinical trials of these therapies. The participants (see APPENDIX) reviewed published and unpublished data relevant to all candidate approaches. This report summarizes the data presented at and the recommendations of this workshop.

ASSESSMENT OF THERAPIES THAT TARGET PRESERVATION OF β-CELL FUNCTION

Failure of pancreatic β-cells to secrete insulin is the pathologic lesion common to both type 1 and type 2 diabetes, although the mechanisms responsible for this failure are different in the two types of diabetes. β-Cell failure is essentially the sole physiologic defect in type 1 diabetes. It is imperative that therapies directed at this fundamental defect be developed in order to reduce the risk of long-term diabetes complications, the adverse effects of intensive therapy (primarily hypoglycemia), and, in type 2 diabetes, the toxicities of glucose-lowering drugs. Ultimately, the cure for type 1 diabetes and a major advance in treatment for both type 1 and type 2 diabetes will come from therapies that restore and/or protect functioning β-cells.

Autoantibodies to islet antigens. Islet cell antibodies (ICAs) and other measures of β-cell autoimmunity could conceivably be used to assess the efficacy of interventions aimed at improving β-cell function. The most widely studied markers of autoimmune diabetes are autoantibodies. These immunoglobulins, predominantly of an IgG1 subclass, are not directly responsible for destruction of islet cells (11,12). However, more recent evidence, at least in the mouse models of type 1 diabetes, has underscored the potential importance of β-cells in the pathogenesis of the disease (13,14). Also GAD antibodies can alter presentation of GAD peptides to GAD-reactive T-cells (15).

Islet autoantibodies have been identified in individuals at risk years before the onset of disease and have been used to predict individuals at high risk for type 1 diabetes. Risk increases with the number of antibodies detected, and in the Diabetes Prevention Trial–Type 1, the risk of diabetes in individuals who had three or more autoantibodies was >50% after 5 years (16). This experience is consistent with several earlier reports from different laboratories. However, after onset of disease, the titers and frequencies of the different autoantibodies decline variably with time (17,18).

Some studies have suggested that autoantibodies may identify patients with type 1 diabetes with a rapid decrease in C-peptide level after clinical onset (19,20). In the Canadian-European cyclosporin trial, in those patients with type 1 diabetes who were not treated with cyclosporin, glucagon-stimulated C-peptide levels were >30% lower in GAD antibody–positive individuals than in GAD antibody–negative individuals (21). However, Jaeger et al. (17) reported that persistence of anti-GAD_{65} antibodies was not associated with residual β-cell function in disease of long duration. Recent data from islet transplantation trials have suggested that changes in autoantibodies may be useful predictors of islet allograft failure (22–24).

Despite their utility in identifying recurrent autoimmunity in recipients of islet or pancreatic grafts or in characterizing the aggressiveness of the autoimmune response in preclinical type 1 diabetes, islet autoantibodies have not been found to change with interventions that have shown clinical efficacy in attenuating the progressive loss of β-cell function in type 1 diabetes. In the French cyclosporin A trial, response to treatment, determined by reduced insulin requirements, was not correlated with anti-GAD or other anti-islet autoantibodies (25). In a trial of azathioprine and prednisone, age of onset, metabolic status at trial onset, and degree of lymphopenia were correlated with response to treatment, but immunological markers were not (5). In the Canadian-European cyclosporin trial, the prevalence of GAD_{65} antibodies and the median GAD_{65} antibody titer did not change in serum samples taken 3, 6, 9, or 12 months after study entry in either the cyclosporin-treated or control group, and the presence or absence of autoantibodies did not predict non–insulin-requiring remission in

either group (21,26). In a study of a non-FcR–binding anti-CD3 monoclonal antibody, changes in the titer or isotype of autoantibodies did not predict clinical response to anti-CD3 treatment (6). In fact, there was little change in these parameters over the 1st year of disease. In a small study of individuals at high risk for type 1 diabetes (the Schwabing Insulin Prophylaxis Pilot Trial) treatment of seven high-risk individuals with insulin delayed the onset of type 1 diabetes (27). However, the titers of ICAs and antibodies to GAD and tyrosine phosphatase–like protein IA2 remained unchanged. Interestingly, even high-dose glucocorticoid treatment of stiff-man syndrome, which is associated with high titers of antibodies against GAD_{65}, led to improved clinical status but failed to change the titer or epitope recognition of the anti-GAD_{65} antibodies (17). Thus, although autoantibodies are markers of the disease and may even predict its clinical course, there is little evidence that these immunoglobulins change with interventions that affect the natural history of the disease.

T-cells reactive to islet antigens. Most experimental evidence suggests that T-cells and/or their products are responsible for the islet damage and destruction of type 1 diabetes. However, conventional T-cell assays that use proliferative responses to antigens have been problematic. For example, in a recent T-cell workshop, cellular assays for proliferative responses to autoantigens were not found to be reliable or reproducible (28). Two newer approaches may be more informative. In a recent report from the 1st International NOD mouse T-Cell Workshop, the use of enzyme-linked immunosorbent spot-forming cell (ELISPOT) assay to detect individual antigen-reactive T-cells was felt to be a more sensitive assay to detect autoreactive cells (29), and this approach may also be useful in patients (30,31). Recently the DiaPep277 pilot study reported that DiaPep-induced preservation of C-peptide was associated with a TH_1 to TH_2 T-cell shift detected by ELISPOT (7). In addition, fluorochrome-labeled major histocompatibility complex tetramers may enable investigators to enumerate antigen-reactive T-cells (32). Shortcomings of this approach for identifying class II restricted cells (the restriction element for autoreactive $CD4^+$ T-cells) are that the studies can only be done in individuals with certain HLA types, with reactivity to specific antigen peptides, and that it is necessary to expand the cells in vitro because of the low precursor frequency in the peripheral blood. The use of these and other T-cell measurements to monitor disease activity and the effects of immunological therapy requires further development.

Glycemic control. HbA_{1c} as a measure of glycemic control should certainly be assessed in any trial aimed at showing a beneficial effect on β-cell function. However, given the importance of achieving good glycemic control to reduce complications and because glycemic control strongly influences the decline in β-cell function in type 1 diabetes (8), trials in recently diagnosed type 1 diabetes patients will involve treating all subjects to the same near-normal glycemic target. Consequently, differences in HbA_{1c} between treatment groups will be minimal even when an effective therapy is involved. Thus, HbA_{1c} or other measures of glycemia cannot serve as robust measures of efficacy in this setting.

Total daily insulin dose. In trials in which all patients are treated to the same glycemic target, a treatment that results in greater preservation of β-cell function could result in a commensurate reduction in total daily insulin doses required to reach the glycemic target. However, daily insulin dose as a reflection of improved β-cell function will be confounded by the large number of other factors that influence insulin dose. These include differences in insulin sensitivity, timing and frequency of insulin administration, type of insulin used, diet and exercise, intraindividual insulin pharmacokinetics and pharmacodynamics, and other variables. Thus, insulin dose is a very indirect reflection of β-cell function and is highly affected by subject compliance and other factors, and there is no established direct clinical benefit of a lower insulin dose.

Hypoglycemia. Near-normalization of glycemic control with current therapies is now possible, but carries an increased risk of serious hypoglycemia. As discussed under "The DCCT Experience: Clinical Benefits of Preserved β-Cell Function," the DCCT demonstrated that retention of β-cell function in individuals with type 1 diabetes is associated with a significantly reduced risk of serious hypoglycemia in the long term. Consequently, drug-induced improvement or stabilization of β-cell function would be expected to result in reduced rates of hypoglycemia. Reduction in the risk of this life-threatening complication of insulin therapy is therefore a major unmet need that would be provided by therapies that preserve or increase β-cell function.

However, demonstrating a positive treatment effect on severe hypoglycemia has proven to be very difficult—even for very large studies in which such differences were expected. Hypoglycemia encompasses a wide spectrum, from mild and usually poorly documented events to severe reactions resulting in coma and/or seizures. The assessment of hypoglycemia in clinical trials is consequently very difficult and complicated if other than the rigorous DCCT criteria for severe hypoglycemia are used. More importantly, the event rate of severe hypoglycemia by these criteria is very low during the first few years of diabetes, and events begin to occur more frequently only after ≥5 years of diabetes. Thus, very large trials of long duration would be required to demonstrate that a treatment that leads to preservation of β-cell function in newly diagnosed type 1 diabetes would lead to a long-term reduction in the risk of severe hypoglycemia.

Endogenous insulin secretion. Clearly the most direct measurement of improved β-cell function is endogenous insulin secretion itself. Accurate assessment of insulin secretion using peripheral blood insulin levels is limited because insulin undergoes variable and major (40–60%) first-pass hepatic extraction after secretion into the portal vein and variable peripheral clearance under various physiological circumstances. Furthermore, many insulin assays cannot differentiate insulin from proinsulin and proinsulin intermediates, are not accurate or precise in the low range typically found in patients with type 1 diabetes, and cannot differentiate endogenous from exogenous insulin. Also, therapeutic insulin can induce insulin antibodies, which can interfere with some insulin assays.

Measurement of C-peptide, however, provides a fully validated means of quantifying endogenous insulin secre-

tion. C-peptide is cosecreted with insulin by the pancreatic β-cells as a byproduct of the enzymatic cleavage of proinsulin to insulin. C-peptide and insulin are secreted into the portal circulation in equimolar concentrations. The pharmacokinetic parameters of C-peptide are well established. While the liver clears a significant portion of insulin in a first pass, C-peptide does not undergo hepatic extraction (33–35) and has constant peripheral clearance at various plasma concentrations and in the presence of alterations in plasma glucose concentrations (36,37). C-peptide is excreted exclusively by the kidney, and its plasma half-life of ~30 min contrasts sharply with the short plasma half-life of insulin (~4 min) (38). C-peptide assays are now widely available in which the relative molar cross-reactivity of proinsulin and proinsulin conversion products compared with C-peptide is ~10% and therefore contributes a negligible amount to total C-peptide immunoreactivity. The close relationship of C-peptide in the systemic circulation to endogenously secreted insulin in the portal system has been well established (39–42).

In addition, the relatively low variability and high reproducibility of C-peptide measurements make the assay suitable for precisely assessing the durability of a β-cell effect over long periods of time. For these and other reasons that will be detailed in later sections of this report, the workshop participants agreed that standardized measurements of C-peptide provided the most appropriate primary outcome for pivotal trials aimed at demonstrating the efficacy of therapies to preserve β-cell function in type 1 diabetes.

THE DCCT EXPERIENCE: CLINICAL BENEFIT OF PRESERVED β-CELL FUNCTION

Numerous reports in relatively small numbers of patients have shown that preservation of β-cell function in patients with type 1 diabetes results in easier and better glycemic control and fewer end-organ complications, especially retinopathy (43–52). The strongest evidence that the preservation of β-cell function results in improved metabolic control, and consequently fewer end-organ complications, is provided by the DCCT. In an early report, the DCCT (53) examined the association between C-peptide levels at initial screening for potential participation in the study with metabolic control and insulin dose in patients cared for by community physicians. Patients were divided into groups according to mixed-meal–stimulated C-peptide: <0.05, >0.05–0.10, >0.10–0.20, and >0.20 nmol/l. Patients with stimulated C-peptide in the lower three groups had similar fasting glucose (206–222 mg/dl) (11.4–12.3 mmol/l) and similar HbA_{1c} (9.2–9.8%). In contrast, patients with stimulated C-peptide >0.2 nmol/l had significantly lower fasting glucose (177 mg/dl) (9.8 nmol/l) and HbA_{1c} (8.4%). Even though glycemic control was not significantly better in patients with stimulated C-peptide >0.1–0.2 nmol/l, they were treated with less insulin than patients with lower C-peptide levels. On the basis of this observation, the DCCT implemented a sub-study investigating the outcomes associated with preserved β-cell function defined as a C-peptide >0.2 nmol/l. This sub-study was confined to the subset of patients who entered the study with a range of 1–5 years' duration of diabetes (8).

These DCCT follow-up results are especially relevant to the evaluation of the clinical benefit of preservation of β-cell function in newly onset diabetes. If a study were initiated in newly onset diabetes, with up to 6 months' duration on entry, after 2 years of treatment with an experimental versus a control regimen, subjects would have an average of 2.25 years' duration of diabetes at the end of the study. This is approximately the same duration of diabetes as in the DCCT C-peptide sub-study, in which patients had a mean duration of diabetes of 2.6 years at DCCT baseline. Thus, the DCCT experience can be used to describe the expected impact of a therapy that yields a difference in stimulated C-peptide values after 2 years of treatment of newly onset type 1 diabetes.

TABLE 1
Baseline characteristics of C-peptide responders (≥0.2–0.5 nmol/l) vs. nonresponders (<0.2 nmol/l) among DCCT intensive treatment group subjects with 1–5 years' duration of diabetes on entry

Characteristic	Responders	Nonresponders	*P*
n	138	274	
Age (years)	28.2 ± 6.7	26.1 ± 7.4	<0.007
Female	50.7	48.2	<0.07
Duration (years)	2.1 ± 1.0	2.9 ± 1.2	<0.001
HbA_{1c} (%)	8.3 ± 1.6	9.2 ± 1.6	<0.001
Insulin ($U \cdot kg^{-1} \cdot day^{-1}$)	0.49 ± 0.20	0.69 ± 0.24	<0.001
Retinopathy present	12.3	19.3	>0.2
AER (mg/24 h)	10.4 ± 7.0	12.7 ± 11.4	>0.2

Data are means ± SD or % unless otherwise indicated.

While the DCCT enrolled a total of 1,441 subjects with 1–15 years' disease duration, the 855 with 1–5 years' duration on entry were required to have a C-peptide <0.5 nmol/l. Of these, 303 (138 intensively treated, 165 conventionally treated) were classified as C-peptide "responders," with values in the range of 0.2–0.5 nmol/l; 552 (274 intensively treated, 278 conventionally treated) had lost C-peptide response to a level <0.2 nmol/l, a level that had been shown to be clinically meaningful in prior analyses (53). These subjects were termed "nonresponders." The DCCT showed that compared with conventional therapy, intensive therapy, with a resulting reduction in HbA_{1c}, markedly reduced the risk of loss of C-peptide to a level <0.2 nmol/l (8).

Since intensive therapy is now the standard of care, the more important results from the DCCT are the comparisons of the clinical outcomes in the 138 C-peptide responders (0.2–0.5 nmol/l) versus the 274 nonresponders (<0.2 nmol/l) randomized to intensive treatment. Table 1 presents the characteristics of these intensively treated subjects at entry into the DCCT. Responders were significantly older and slightly more frequently female and had significantly shorter duration of diabetes and lower HbA_{1c} on entry, slightly but not significantly less retinopathy, and lower albumin excretion rates (AERs) (8). In an additional multivariate analysis (unpublished), the baseline HbA_{1c} obtained at initial eligibility assessment was significantly lower among those with C-peptide response above versus below 0.2 nmol/l (8.38 vs. 9.38%, respectively, $P < 0.0001$), adjusted for age, sex, and duration of diabetes. Lower HbA_{1c} was nominally significantly associated with increasing age ($P = 0.022$), but not with diabetes duration. C-peptide responders also had significantly

lower levels of AER ($P = 0.027$) and nearly significantly lower prevalence of retinopathy ($P = 0.057$) compared with nonresponders, adjusting for age and sex. The level of AER at baseline was also significantly associated with higher baseline HbA_{1c}, and the presence of retinopathy was associated with higher HbA_{1c} and longer duration. Neither baseline AER nor prevalence of retinopathy was associated with baseline C-peptide response after adjusting for these factors. This suggests that preservation of β-cell function has a direct effect on HbA_{1c} and an indirect effect on complications mediated in part by HbA_{1c}.

HbA_{1c}. The DCCT showed that the distribution of HbA_{1c} annually among intensively treated responders was nominally significantly ($P < 0.01$) different from that of nonresponders at baseline and over the first 4 years of follow-up (8). In an additional longitudinal analysis, the overall difference between responders and nonresponders within the intensively treated group during up to 9 years of follow-up was statistically significant (7.40 vs. 7.00%, $P < 0.0001$), with an average mean difference of 0.40%.

Thus, a therapy in newly onset type 1 diabetes patients that results in higher C-peptide levels after 2 years of treatment would be expected to show a difference in HbA_{1c} that would persist for ≥4 years. However, the magnitude of the effect on HbA_{1c} in intensively treated patients would also be expected to be quite small. The median stimulated C-peptide at DCCT baseline (2.6 years) was 0.32 nmol/l among the C-peptide responders, versus 0.06 nmol/l among the nonresponders, a 5.3-fold difference. This large difference was in turn related to median HbA_{1c} values of 7.8%, versus 9.1% at DCCT baseline. But after 1 year of intensive treatment, the difference in median HbA_{1c} was 7.1 − 6.6 = 0.5%. Thus, a 5.3-fold difference in C-peptide levels between responders and nonresponders at DCCT baseline yields an HbA_{1c} difference of only 0.5% at 1 year when patients are treated intensively.

An experimental treatment that yields a 50% higher mean C-peptide (0.3 vs. 0.2 nmol/l, for example), or a 1.5-fold difference, would likely be considered meaningful in a clinical trial testing efficacy of preserving β-cell function. However, this difference in C-peptide would yield a small difference in HbA_{1c} in intensively treated patients. In an additional longitudinal analysis, on average, over 9 years of follow-up, a 1.0-nmol/l increase in baseline C-peptide among patients treated intensively yields a 1.003% decrease in HbA_{1c}. Thus, a mean 0.1-nmol/l difference in C-peptide after 2 years of treatment in newly onset patients would be expected to yield a 0.1% difference in HbA_{1c} in intensively treated patients on average over follow-up.

Insulin dose. In the DCCT intensive treatment group at baseline, the lower HbA_{1c} among C-peptide responders versus nonresponders was achieved by regimens with a significantly lower exogenous insulin dose, 0.49 units · kg body wt^{-1} · day^{-1} among responders vs. 0.69 units · kg body wt^{-1} · day^{-1} among nonresponders (Table 1). Data from small-scale trials reporting preservation of β-cell function in recently diagnosed type 1 diabetes patients with DiaPep277 (7) and with a CD3 monoclonal antibody (6) have shown that the treated patients used less insulin but achieved comparable or better glycemic control compared with the control subjects.

Hypoglycemia. Even modest retention of β-cell function in individuals with type 1 diabetes is associated with reduced risk of serious hypoglycemia. The DCCT (8) reported that in the intensive group, the rate of hypoglycemia resulting in coma and/or seizure was 6.6 per 100 patient-years of follow-up among the baseline C-peptide responders, versus 17.3 per 100 patient-years among nonresponders, a relative risk of 0.38, or a 62% risk reduction. The risk reduction was slightly greater after adjusting for the difference in HbA_{1c} levels (65%). Thus, even though the C-peptide responders maintained a lower level of HbA_{1c} during follow-up, they also experienced at least a 60% reduction in the risk of hypoglycemia. However, the bulk of these hypoglycemia events occurred after 2 or 3 years of follow-up. During the first 3 years of follow-up, only 9% of intensively-treated nonresponders experienced any severe hypoglycemia versus 6.5% of responders. The numbers experiencing hypoglycemia with coma and seizure were ~50% fewer.

Retinopathy and nephropathy. The DCCT (8) described the relative risk of progression of retinopathy and nephropathy, comparing baseline responders versus nonresponders separately within the intensive and conventional treatment groups. The analyses presented adjusted for the baseline level of HbA_{1c} and the level of retinopathy or AER at baseline. However, since the HbA_{1c} at baseline was significantly lower among C-peptide responders, this adjustment eliminates that part of the difference in risk between responders and nonresponders that is attributable to the difference in levels of HbA_{1c}. While such analyses show the *added* effect of C-peptide ≥0.2 nmol/l versus <0.2 nmol/l above and beyond the differences in HbA_{1c} over time, they do not show the *total* effect of C-peptide on risk of progression of complications. Thus, additional analyses within the intensive treatment group are presented herein.

Table 2 presents the relative risk reduction (1 − hazard ratio) of progression of retinopathy and nephropathy in the DCCT intensive treatment group, comparing baseline C-peptide responders (≥0.2–5 nmol/l) and nonresponders (<0.2 nmol/l) with no covariate adjustments, with adjustment for the baseline complication status and adjustment for the baseline HbA_{1c}. The unadjusted analysis presents the total effect of C-peptide status (responder versus nonresponder) at DCCT baseline on the risk of progression of complications.

Figure 1*A* shows that the cumulative incidence of any three or more–step progression of retinopathy during 9 years of follow-up reached 43.5% among C-peptide responders versus 27.6% among nonresponders who had been assigned to the intensive therapy group of the DCCT. The risk of any three or more–step retinopathy progression was reduced by 58% among C-peptide responders versus nonresponders ($P < 0.001$). Adjustment for the presence or absence of retinopathy at baseline status alone or with HbA_{1c} had no effect on this risk reduction. Figure 1*B* shows the cumulative incidence of sustained three or more–step progression, sustained at two consecutive 6-month visits, the primary retinopathy outcome used in the DCCT. For this outcome, the risk was reduced

TABLE 2
Risk reduction of progression of microvascular complications in the DCCT intensive treatment group comparing baseline C-peptide responders (≥0.2–0.5 nmol/l) vs. nonresponders (<0.2 nmol/l) with no adjustments, adjustment for baseline complication status, and adjustment for baseline status and HbA_{1c}

	Unadjusted	Adjusted for baseline status*	Adjusted for baseline status and HbA_{1c}
Retinopathy			
≥3-step progression	58 (27–76)	59 (29–77)	50 (12–72)
Sustained ≥3-step progression	79 (9–95)	79 (10–95)	71 (−26 to 93)
Nephropathy (AER >40 mg/24 h)	64 (−10 to 71)	38 (−22 to 69)	27 (−46 to 64)

Data are % risk reduction (95% CI). *Baseline status is presence or absence of retinopathy at baseline for analysis of retinopathy and the log(AER) at baseline for nephropathy.

by 79% ($P < 0.012$) among the responders. The risk reduction was similar after adjustment for baseline retinopathy status and remained significant ($P < 0.011$). The estimated risk reduction was also similar after adjustment for the baseline HbA_{1c}; however, the effect was not significant ($P < 0.053$), likely owing to the small number of outcome events (21 total).

While the unadjusted risk reduction for development of microalbuminuria (AER >40 mg/24 h) was comparable to that for retinopathy, the effect was not statistically significant ($P < 0.079$), again most likely due to the small number of such events (49 total). The risk reduction was lessened after adjustment, indicating that some of the difference in risk of nephropathy progression was attributed to differences between C-peptide responders versus nonresponders in the baseline levels of AER and HbA_{1c}.

Recently, Steffes et al. (54) published further analyses of the DCCT data. Patients were divided into four groups based upon the stimulated C-peptide levels at entry into the DCCT:

Undetectable: ≤0.03 nmol/l

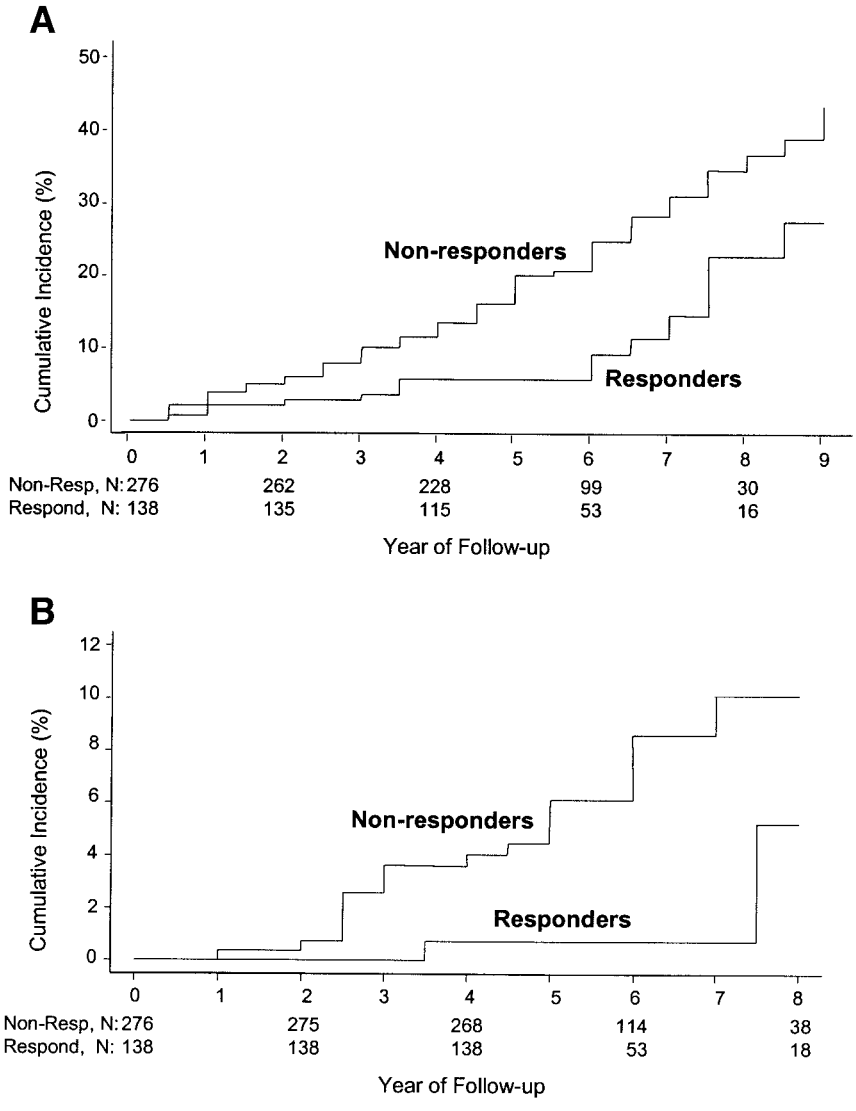

FIG. 1 ***A*** **Cumulative incidence of any three or more–step progression of retinopathy among baseline C-peptide responders versus nonresponders in the intensive treatment group of the DCCT.** ***B*****: Cumulative incidence of a sustained three or more–step progression.**

TABLE 3
Retinopathy (≥3-step change on Early Treatment Diabetic Retinopathy Study scale) and albuminuria (AER >40 mg/24 h) during the first 6 years of the DCCT, by stimulated C-peptide in intensively treated patients

	Undetectable	Minimal	Baseline-only	Sustained
Retinopathy	6.5 ± 0.7	3.5 ± 0.5	1.8 ± 0.9	1.4 ± 0.6
Unadjusted	a	b	bc	c
Adjusted	a	b	b	b
Albuminuria	4.0 ± 0.5	2.3 ± 0.4	1.7 ± 0.9	0.9 ± 0.5
Unadjusted	a	b	bc	c
Adjusted	a	ab	ab	b

Data are rates ± SE per 100 participant-years. Rates were compared (horizontally) between stimulated C-peptide groups. For each comparison, rates with different letters were significantly different ($P \leq 0.05$), without and with adjustment for multiple tests.

Minimal: 0.04–0.2 nmol/l
Baseline responder only: 0.21–0.50 nmol/l at baseline only, i.e., at entry into the DCCT but ≤0.2 nmol/l at year 1 of follow-up
Sustained responder: 0.21–0.50 nmol/l at entry and again at least 1 year later.

Table 3 compares the rates of retinopathy and nephropathy during the first 6 years of the DCCT among these groups. In intensively treated patients, the risk of complications increased successively as the level of C-peptide decreased, with the highest risk among those with undetectable C-peptide on entry. Patients with undetectable C-peptide had 4.6 times greater retinopathy progression and developed albuminuria 4.4 times more commonly than sustained C-peptide responders (group 1 vs. group 4). These differences were significant even after adjusting for multiple pairwise tests. While minimal C-peptide at entry afforded nominally significant protection against retinopathy and nephropathy compared with undetectable C-peptide, the differences were not significant after adjustment for multiple tests.

NATURAL HISTORY OF C-PEPTIDE IN HUMAN TYPE 1 DIABETES

Recently diagnosed type 1 diabetes. Measurement of baseline and stimulated C-peptide (after glucagon or a mixed meal) in patients with recent-onset type 1 diabetes is used in clinical settings as a measure of residual β-cell function. Comparing today's clinical onset of type 1 diabetes with that 10–15 years ago, it appears that diagnosis is probably made earlier in the natural history of the progressive failure of β-cells as assessed by C-peptide measurement, with less frequency of ketoacidosis and coma (55–57). It is not known whether this is due to greater population awareness of diabetes symptoms that leads patients to seek care earlier or because of other factors such as increased insulin resistance due to increased obesity. Patients diagnosed around puberty or as adults show consistent baseline and/or stimulated C-peptide levels ranging between 0.3 and 0.9 nmol/l and 0.6 and 1.3 nmol/l, respectively (57). In prepubertal children, however, several studies have demonstrated that average C-peptide levels at diagnosis are <0.2 nmol/l, implying more extensive destruction of β-cells (58–60).

Another important consideration in relation to C-peptide secretion is the implementation of intensive insulin therapy at disease diagnosis (61). Such a strategy has demonstrated that 1 year after diagnosis, it is possible in the majority of patients to preserve the residual β-cell function (assessed by C-peptide) found at the time of diagnosis (62). The behavior of C-peptide secretion in the 1st year of the disease has been taken as a primary outcome in numerous trials in patients with recent-onset type 1 diabetes (63,64). Previously, insulin-free clinical remission was considered the primary outcome in such trials; however, this can no longer be used, since it is now thought that maintenance of low doses of insulin at meals may help in protecting residual β-cell function by avoiding unnecessary simulation of endogenous insulin secretion.

1–15 years after diagnosis of type 1 diabetes. Although cross-sectional, the largest amount of data on C-peptide in the period 1–15 years postdiagnosis comes from the DCCT. Enrollment in the DCCT for patients with type 1 diabetes of 1–5 years' duration required that mixed-meal (Sustacal)–stimulated C-peptide (90-min) be <0.50 nmol/l, whereas for patients with type 1 diabetes of 5–15 years' duration, stimulated C-peptide had to be <0.2 nmol/l. To identify the 1,441 patients ultimately enrolled in the DCCT, Sustacal-stimulated C-peptide was evaluated in a total of 3,736 patients with type 1 diabetes. Much greater presentation of β-cell function, that is, higher C-peptide levels, was found than commonly expected. Figure 2 shows the stimulated C-peptide values upon initial evaluation for those 2,432 subjects who were at least 18 years of age at the time of diagnosis of type 1 diabetes. Among those with duration 1–5 years at the time of eligibility screening, stimulated C-peptide was >0.2 nmol/l in 48% and >0.5 nmol/l in 15%; for those with duration >5–15 years, stimulated C-peptide was ≥0.2 nmol/l in 8% and >0.5 nmol/l in 2%. As observed by many others, the stimulated C-peptide values at the time of DCCT screening were lower among those in whom the diagnosis of diabetes was made at <18 years of age (Fig. 3). Among these, 33% had stimulated C-peptide >0.2 nmol/l 1–5 years after diagnosis, but only 3% exceeded 0.2 nmol/l after 5–15 years of type 1 diabetes. These data were collected from 1983 to 1989. With the current emphasis on aggressive early glycemic control and since glycemic control reduces the decline in β-cell function in type 1 diabetes (8), β-cell function is now probably even more preserved in the years after diagnosis.

Latent autoimmune diabetes in adults, or type 1.5 diabetes. Latent autoimmune diabetes in adults (LADA), or type 1.5 diabetes, is usually defined as diabetes with onset after age 35 years with islet autoantibodies found in serum (usually GAD antibodies or ICAs) but without an immediate need for insulin. The natural course of β-cell function in LADA patients has been studied only in small cohorts. In general, recruitment of patients was not done in a way to avoid a selection bias. Prospective studies included only two or three time points, and the methods for assessing stimulated C-peptide responses differed widely (65–95).

Nonetheless, two important conclusions can be drawn from the available data. In LADA patients, levels of basal

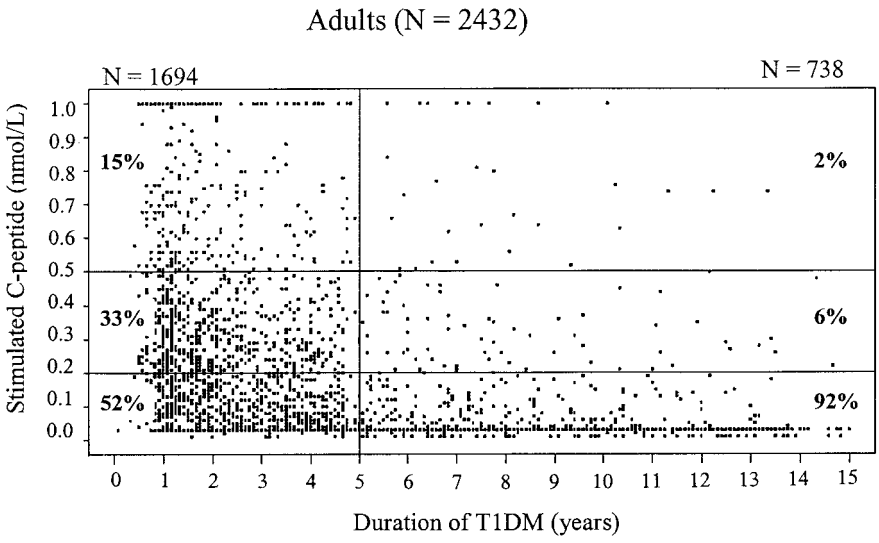

FIG. 2 Peak C-peptide during MMTT (2-h) in patients 18 years of age at onset of diabetes and with type 1 diabetes (TIDM) of 1-15 years' duration when screened for entry into the DCCT.

and stimulated C-peptide are higher at diagnosis than in classic type 1 diabetes.

There is a substantial loss of endogenous stimulated C-peptide secretion over a period of a few years after diagnosis of diabetes, almost approaching levels seen in type 1 diabetes.

For the aim of this discussion, it is important to note that the initial high endogenous C-peptide secretion corresponds with the initial non–insulin-dependent state of LADA patients. Secondly, the rapid progression to insulin dependency in LADA patients, the mean period often reported as ~5 years, is paralleled by a major loss of endogenous C-peptide secretion. Since serum C-peptide directly reflects insulin secretion, the association between the decrease in C-peptide levels and the progression of insulin dependency is not surprising.

However, (stimulated) C-peptide levels are more robust than insulin dependency as an outcome measure in clinical trials trying to halt the progression of LADA. C-peptide levels represent a continuous variable, while insulin dependency is categorical, yes/no. Furthermore, C-peptide levels are objective and quantitative, whereas insulin dependency is subjective. Hence, beneficial effects on residual β-cell function of a treatment modality would be easier to detect, and have much more statistical power, using C-peptide measurements. A major confounder is insulin resistance. Different levels of exercise, different types of diets, even different levels of stress or of systemic immune activation will render different patients or even the same patient insulin dependent or nondependent. Unfortunately, it is impossible to monitor and control for lifestyle closely enough to exclude this major confounder of outcome. Therefore, stimulated C-peptide levels as a measure of endogenous β-cell function would be a more appropriate outcome in LADA trials than the percentage of patients having progressed to insulin treatment in a given period.

MEASUREMENT OF β-CELL FUNCTION

There are several ways to measure the function of the β-cell. In normal subjects, the β-cells respond to *oral* glucose, fats, and proteins with an increase in insulin values. When measured in 30-min postmeal intervals, the peak response is usually before 90 min, with a return to baseline insulin values by 120 min. In subjects with impaired β-cell function, the response is reduced as measured by the area under the curve (AUC) and/or the peak value. In addition, there may be a temporal shift in the

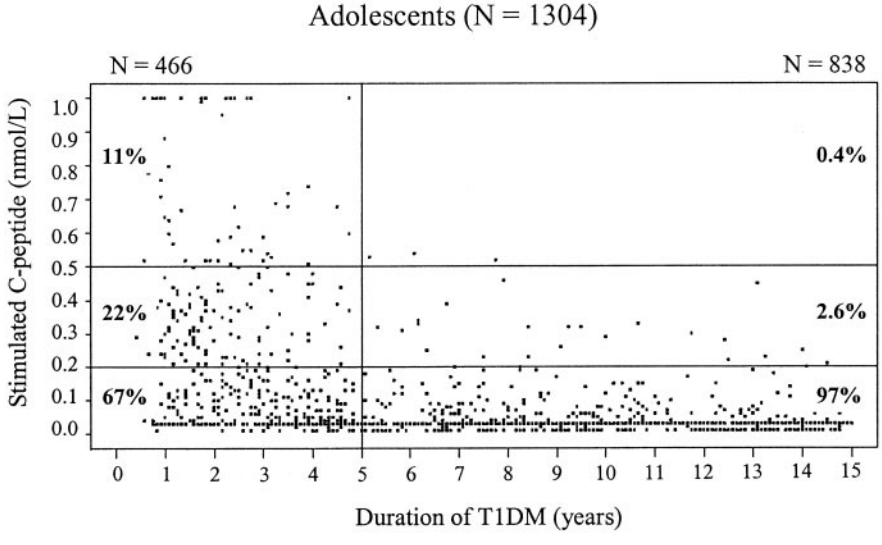

FIG. 3 Peak C-peptide during MMTT (2=h) in patients <18 years of age at onset of diabetes and with type 1 diabetes (T1DM) of 1–15 years' duration when screened for entry into the DCCT.

response such that the peak insulin value occurs later and may not return to baseline values until 4 h. Such a shift in the curve reflects a deficit in early insulin secretion, which is thought to be important in maintaining carbohydrate tolerance.

The β-cell also responds to *intravenous* stimulation. The insulin response within the first 10 min after glucose stimulation is referred to as the first-phase insulin response or the acute insulin response (AIR) to glucose. In normal subjects, insulin levels peak several-fold above baseline. In subjects at risk for subsequent clinical type 1 diabetes, the AIR to glucose is frequently diminished. At the time of clinical diagnosis, this is often very low or absent. In patients with type 2 diabetes, an absent AIR to glucose occurs when fasting glucose is >115 mg/dl (6.4 mmol/l) (96). At the time that AIR to glucose is absent, however, patients with diabetes may still increase C-peptide in response to intravenous arginine and glucagon (97–101). This is particularly the case soon after diagnosis and during the honeymoon phase of the disease.

Another characteristic of the β-cell is the ability of glucose to augment the response to nonglucose stimuli. For example, administration of arginine at baseline glucose levels results in an acute release of insulin, and administration of the same dose of arginine after the glucose level is raised results in an augmented response. This ability of elevated glucose to alter the response to a second stimulus, however, underlies the concern about the potential complicating effects of the prevailing glucose level on β-cell responses (102,103). Hypoglycemia inhibits β-cell responses. Although less of a factor in measurements of responses to mixed meals, hypoglycemia will inhibit the insulin response to intravenous glucagon. Thus, the Immunology of Diabetes Society recommends that tests of β-cell function be conducted in the absence of hypo- or hyperglycemia, between 70 and 200 mg/dl (3.9–11.1 mmol/l) (104).

The selection of a measure of β-cell function to be used as a primary outcome in clinical trials depends on balancing the need for scientific validity with the practical realities of performing the tests in a clinical trial setting. While fasting C-peptide alone is easy to obtain and correlates with stimulated C-peptide, it may be insufficient to detect subtle effects of therapy. After clinical diagnosis, the appropriate test may include the stimulated C-peptide response to a nonglucose secretagogue. Europeans have most often used intravenous-glucagon–stimulated testing. This has the advantage of being a short test (6 min), but the disadvantage of occasionally causing transient nausea. Others have used C-peptide responses to a liquid mixed meal (Sustacal/Boost). Though the mixed meal does not induce nausea, the test does require more time (most studies have used a 2-h testing period and some have advocated a 4-h period).

Recent data suggest that the rate of fall of glucagon-stimulated C-peptide among subjects receiving intensive insulin treatment is very slow (62). Whether this is due to insensitivity of the glucagon stimulation test because of supraphysiological stimulation or to a changing natural history of the disease with intensive treatment is not known. C-peptide responses to mixed-meal tests also seem to fall less rapidly in studies conducted after the advent of intensive therapy than in older investigations. Two recent studies reported C-peptide responses to both intravenous glucagon and mixed meal stimulation. Schnell et al. (105) compared these outcome measures in subjects receiving intensive insulin treatment with measures in an experimental group also receiving high-dose intravenous insulin for 2 weeks at time of diagnosis. In that study, there were no significant changes at 1 year as compared with baseline by either measure of C-peptide function (105). Similar results using both glucagon and mixed-meal responses were reported by Chaillous et al. (106) in a trial testing oral insulin and placebo in newly diagnosed subjects. Unfortunately, there are few other reports of direct comparisons between the two tests. It is therefore the current recommendation of the Immunology of Diabetes Society to choose one test as the primary outcome measure, but to perform both tests at baseline and annually to have comparison data in future trials (104).

Measurement of immunoreactive insulin in serum or plasma is still the standard method for evaluating pancreatic β-cell function in people not receiving exogenous insulin therapy. Unfortunately, a number of factors limit the utility of peripheral insulin concentrations as a measure of β-cell function. These include the substantial hepatic extraction of insulin (~50% on the first pass), the inability of many insulin assays to differentiate insulin from proinsulin and proinsulin intermediates, the variable peripheral clearance of insulin under physiological circumstances, the inability to obtain accurate measurements in the presence of anti-insulin antibodies, and the inability of insulin assays to differentiate endogenous from exogenous insulin. Since participants in clinical trials of therapies for type 1 diabetes are frequently being treated with exogenous insulin and/or have anti-insulin antibodies, in this setting, an alternative method of evaluating β-cell function must be used. Measurement of peripheral concentrations of C-peptide is the most common approach.

C-peptide is considered to be a good marker of insulin secretion because of its equimolar secretion with insulin, negligible hepatic extraction (33–35), and constant peripheral clearance at different plasma concentrations and in the presence of alterations in plasma glucose concentrations (36,37). The use of plasma C-peptide levels as an index of β-cell function is dependent on the critical assumption that the mean clearance rates of C-peptide are constant over the range of C-peptide levels observed under normal physiological conditions. This assumption has been shown to be valid in both dogs and humans (33,39).

The characteristics of the C-peptide assay used are also important and must be defined and monitored to ensure accurate and reproducible C-peptide measurements. The assay should have a low level of cross-reactivity with proinsulin and proinsulin breakdown products, or the measured C-peptide level could be elevated by cross-reacting proinsulin that is detected in the assay. C-peptide concentrations are generally substantially higher than proinsulin levels in the periphery, and as long as the degree of cross-reactivity of proinsulin in the C-peptide assay is <10%, proinsulin would not be expected to contribute to immunoreactive C-peptide under physiological circumstances. The presence of anti-insulin antibodies with a large capacity to bind proinsulin could falsely

elevate the measured C-peptide, but this does not commonly occur.

Another potential pitfall that must be considered in the design and execution of clinical trials that use peripheral levels of C-peptide as an efficacy outcome is the fact that C-peptide is a small linear peptide that is susceptible to cleavage by proteolytic enzymes. As a result, investigators must exercise care to ensure that plasma for C-peptide measurement is separated from the blood sample within a short time (no more than a few hours) and that the assay is performed within the 1st month. C-peptide immunoreactivity does fall with prolonged storage and with repeated freezing and thawing of the plasma sample. The speed and extent of the reduction in C-peptide is variable and may differ in different assays. Accuracy of the C-peptide levels should be validated under the conditions of each study with the assay that is to be used.

Maintenance of normoglycemia is actually the result of the interplay of islet β-cell secretion and insulin sensitivity of the periphery and liver (with additional contributions from the effect of glucose to facilitate its own uptake). Individuals with normal β-cell function can alter their insulin secretion to accommodate different degrees of insulin sensitivity and so maintain normal blood glucose concentrations. Cross-sectional studies in healthy young adults have demonstrated a curvilinear relationship between insulin secretion and insulin sensitivity (107). This relationship highlights an important caveat in interpretation of β-cell function in intervention trials. Most trials measure β-cell function with the assumption that increased secretion is due to a direct therapeutic effect of the intervention on the immune-mediated β-cell destructive process. However, an increase in insulin secretion could also be seen if the therapy caused insulin resistance, resulting in a physiological compensatory increase in insulin secretion by the remaining β-cells (assuming sufficient residual secretory capacity) (108).

It would therefore be advantageous that some measurement of insulin sensitivity also be performed. The gold-standard measurement for insulin sensitivity involves use of a glucose clamp, which is impractical in the setting of a large multicenter clinical trial. Similarly, though somewhat easier to obtain, insulin sensitivity measured by the frequently sampled intravenous glucose tolerance test (FSIVGTT) (109,110) is also not practical in this role. In contrast, insulin sensitivity as estimated by the homeostasis model assessment (HOMA) is practical because it relies on fasting glucose and insulin or C-peptide and has been shown to correlate reasonably well with clamp results (111) in normal subjects and patients with type 2 diabetes. Recent studies have suggested that both insulin sensitivity and β-cell function can be determined from a modified OGTT (112–114), although this method has also not yet been validated in type 1 diabetes. Despite the difficulties in performing the tests, it may be useful to perform either the clamp or FSIVGTT in a subset of patients in clinical trials for comparison with analyses obtained using the HOMA determination of insulin sensitivity.

TABLE 4
Baseline characteristics of subjects with MMTT and post-stimulus value, those with MMTT and calculation of 2-h or 4-h AUC, and those with glucagon infusion

	MMTT (peak or post-stimulus)	MMTT and 2-h AUC	MMTT and 4-h AUC	Glucagon
n	143	91	34	119
AUC (nmol/l × time)	—	39.60	121.12	—
Mean*nmol/l	0.4650	0.3300	0.5047	0.4337
Age (years)	12.50	12.94	16.78	22.52
Male	56.2%	61.4%	59.7%	73.1%

*For AUC, mean = AUC/minutes, minutes = 120 for 2-h test and 240 for 4-h test.

SAMPLE SIZE FOR STUDIES OF C-PEPTIDE IN NEWLY ONSET TYPE 1 DIABETES

In 2001, the National Institutes of Health established the Type 1 Diabetes TrialNet to conduct studies of therapies aimed at preservation of β-cell function in patients with recently diagnosed type 1 diabetes and at prevention of diabetes in subjects at increased risk of future type 1 diabetes. TrialNet has adopted C-peptide as the principal outcome measure for clinical trials in newly onset diabetes.

To evaluate the sample size for such studies, TrialNet assembled a database consisting of longitudinal measures of C-peptide in 262 recently diagnosed patients who received conventional or intensive insulin therapy but no investigational therapy (J.M. Lachin, P. Friedenberg, unpublished observations). For those subjects in whom mixed-meal tolerance tests (MMTTs) were performed, a single post-stimulus measure was available in 143, multiple measures over 2 h in 91, and a 4-h test in 34. An additional 106 subjects received only a glucagon-infusion test, and 13 subjects received both an MMTT and a glucagon-infusion test. Table 4 presents a summary of the patient characteristics from the eight studies and the baseline C-peptide measures.

Figure 4 displays the distributions of the 2-h peak values from the MMTT, the most frequently measured value in these studies, stratified by age. The corresponding percentiles are presented in Table 5. The distribution is shifted toward lower values for those <12 years of age and toward higher values for those ≥18 years of age. Figure 5 displays the distributions of the glucagon-stimulated values obtained from 211 subjects ≥12 years of age. There were only six subjects <12 years of age. These data show little difference between those 12–17 versus ≥18 years of age.

The majority of the patients (225 of 262) were followed for 12 months, some with interim assessments at 3, 6, and/or 9 months, but only 156 at 18 months and 95 at 24 months. Longitudinal analysis showed that the baseline C-peptide assessment (at initiation of study) had the strongest association with C-peptide levels over time. While age at diagnosis of type 1 diabetes had a strong association with the baseline value obtained from the MMTT (Fig. 4), it did not have an effect on the rate of change in C-peptide over time. Sex, BMI, and duration of diabetes had little effect, although these covariates were available in only 51 subjects.

The various summary measures from the MMTT (1-h value, peak over 2 h, peak over 4 h, AUC over 2 h, and AUC over 4 h) were highly correlated, with the smallest R^2 (variation explained) being 0.928 between the 1-h value and the 2-h peak. The R^2 for MMTT measures versus the glucagon-stimulated values was much lower, ranging from

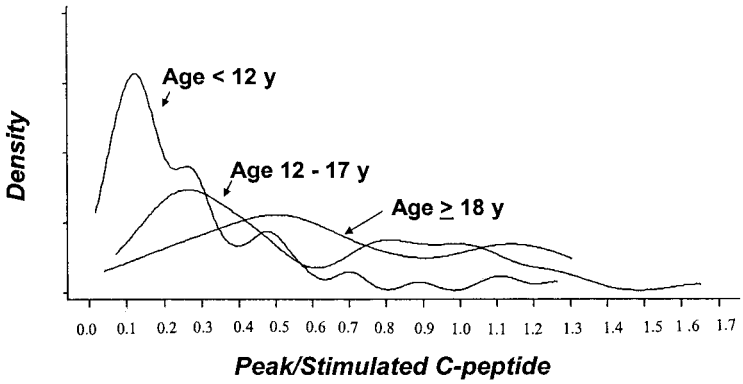

FIG. 4. Distribution of peak of stimulated C-peptide values from an MMTT within each of three age strata.

0.531 for the 4-h peak to 0.692 for the 4-h AUC for the 13 control group subjects from the Miami study who had both procedures.

From this database, components of variation were estimated that would serve as the basis for a sample size or power computation for an analysis of mean differences after a period of treatment, of slopes over time, or of the incidence of threshold events, such as falling below 0.2 nmol/l. After consideration of these various analytic approaches, TrialNet decided that the primary analysis for studies in newly onset diabetes would be the difference in the distributions after 2 years of treatment. The principal outcome chosen is the AUC of the C-peptide values at 0, 30, 60, 90, and 120 min from a 2-h MMTT. Since the unit of measurement for the AUC is nanomoles per liter per minute, the weighted mean C-peptide, computed as AUC mean = AUC/120, is used to convert the measurement back to nanomoles per liter. Further, since some values may be zero or unmeasurable, the analysis is based on the 24-month log(AUC mean + 1), with an adjustment for the baseline MMTT log(AUC mean + 1).

Table 6 presents the geometric mean and the root mean square error (RMSE) of the $\log(x + 1)$ values from the MMTT or glucagon test at 12 months, adjusting for baseline measure, age, and sex. The results from the smaller number of subjects evaluated at 24 months were similar. These values allow determination of sample size or power for an analysis of mean differences using the $\log(x + 1)$ transformation based on different exclusion criteria with respect to the baseline level and age. In general, the geometric mean, but not RMSE, increases with increasing baseline C-peptide eligibility limits and increases with a lower age limit of 12 years.

These values can be used to determine the sample size for a study as follows. Let M refer to the mean of the $\log(x + 1)$ values. Then the corresponding geometric mean (in nanomoles per liter) is $G = \exp(M) - 1$. Consider the analysis of the 2-h AUC mean, with no exclusions for age or C-peptide, with control group geometric mean $G_c = 0.196$ nmol/l. For a 50% improvement in the experimental treatment group, $G_e = 1.5 \times 0.196 = 0.294$ nmol/l. The corresponding means on the $\log(x + 1)$ scale are $M_c = \log(1.196) = 0.179$ and $M_e = 0.258$. Then the total N for a two-group study is provided by the standard equation (115) for the difference between means (0.179 vs. 0.258) with SD equal to RMSE (0.172). For a two-sided test at the 0.05 level and 85% power, with corresponding Z values of 1.96 and 1.04, the total sample size in a two-group study is provided by $N = [\{(1.96 + 1.04) \times 2 \times 0.172\}/(0.258 - 0.179)]^2 = 171$ rounded up to 172, or 86 per group. To allow for 10% losses, the total sample size would be $n = 172/(0.90) = 191$ (rounded to 192). Larger sample sizes would be required to provide similar power to detect smaller differences.

TABLE 5
Percentiles of distribution of peak or post-stimulus C-peptide values from the MMTT or glucagon infusion within age-groups

	n	Percentile				
		5%	25%	50%	75%	95%
MMTT						
Age <12 years	62	0.0500	0.1200	0.2104	0.4356	0.7012
12 ≤ age <18 years	41	0.0745	0.2257	0.4800	0.9600	1.2000
Age ≥18 years	21	0.0400	0.4600	0.5470	1.0800	1.3020
Glucagon infusion						
12≤ age <18 years	58	0.2900	0.3800	0.4400	0.7200	1.1600
Age ≥18 years	153	0.1500	0.2550	0.3300	0.4365	0.8800

Data from the DCCT predict that a treatment effect on C-peptide levels would have a beneficial effect on other outcomes; however, it may not be feasible to design a study that would demonstrate such effects.

HbA_{1c}. As discussed previously, the DCCT showed an average reduction in HbA_{1c} of 0.1% per 0.1 nmol/l greater level of C-peptide in intensively treated patients. Thus, a treatment that produces a 50% greater C-peptide after 2 years of treatment (0.3 vs. 0.45 nmol/l) would be expected to provide a mean difference in HbA_{1c} of 0.15%. The SD of the HbA_{1c} values in the DCCT intensive treatment group was 1.6. Using the above expression, a sample size of $n = 4{,}096$ would be required to provide 85% power to detect this difference in HbA_{1c}.

Hypoglycemia and retinopathy. As discussed previously, there is a significant relationship between the level of C-peptide and the risks of hypoglycemia and retinopathy. However, the rate of both outcomes is low, and differences among C-peptide responders versus nonresponders evolve slowly. Thus, large trials of long duration would also be required to detect significant differences in these outcomes with a therapy that leads to preservation of β-cell function after 2 years of treatment.

RECOMMENDATIONS AND CONCLUSIONS

Development of therapies directed at retaining or improving β-cell function in patients with type 1 diabetes will be facilitated by establishing appropriate efficacy outcome measures. The retention of endogenous insulin secretion

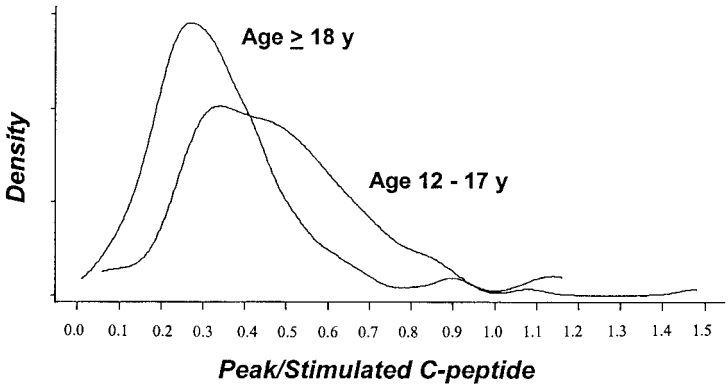

FIG. 5. Distribution of stimulated C-peptide values from a glucagon infusion within each of two age strata.

in patients with type 1 diabetes has been shown to be associated with improved glycemic control, reduced hypoglycemia, and reduced development of retinopathy and nephropathy. The amount of residual β-cell function that confers these clinical benefits is relatively small and is present in many patients during the first few years of clinical disease.

Measurements of various immune responses, while useful in characterizing the pathogenesis of the disease process and for identifying individuals at risk for developing type 1 diabetes, do not predict the clinical benefit of therapies aimed at preserving or restoring β-cell function. Therapies that preserve residual β-cell function will improve the ability to achieve good glycemic control. However, the standard measure of glycemic control, HbA_{1c}, is not a suitable primary outcome in clinical trials of such therapies. It is ethically required that all subjects receive intensive insulin therapy with the target of near-normal glycemia. Such treatment will tend to minimize the treatment effect on HbA_{1c} irrespective of the therapy's effect on C-peptide. Even though a treatment that preserves β-cell function would be expected to reduce hypoglycemia and to reduce retinopathy and other complications in the long term, the event rates will remain low for many years after diabetes onset. Therefore, the use of severe hypoglycemia or retinopathy as a primary outcome would require large numbers of subjects and long duration to adequately power the trial.

The most appropriate measurement of endogenous insulin secretion and β-cell function is measurement of C-peptide under standardized conditions. The amount of preserved C-peptide has been positively correlated with improved clinical outcomes. C-peptide levels post-stimulation are a validated means of assessing endogenous insulin secretion. Sensitive, reproducible assays for measuring C-peptide are readily available.

Based on a review of current research evaluating new therapies for type 1 diabetes, the group of international experts convened by the ADA concluded that assessment of β-cell function, as measured by C-peptide levels, is the most suitable primary outcome for pivotal intervention studies of therapies aimed at preservation of β-cell function in patients with type 1 diabetes.

Several other international activities complement the conclusions of this ADA workshop and will facilitate implementation of its recommendation. The Immunology of Diabetes Society has suggested specific recommendations for performing C-peptide–stimulation tests (104). These recommendations have been summarized in this workshop report. To determine the relative properties of glucagon compared with MMTT stimulation of C-peptide secretion, a direct comparison study is being conducted.

The ADA workshop also recommended a direct laboratory comparison to determine the optimal assay format for measuring C-peptide and to standardize assays worldwide. The National Institutes of Health and the Centers for Disease Control and Prevention are supporting a comparison that is being coordinated by the University of Missouri. This comparison will have four phases. The first phase, which has been completed, is to survey the assays performed by laboratories measuring C-peptide worldwide. The second stage is to evaluate conditions for optimal specimen collection and storage, such as serum or plasma, need for the addition of aprotonin, and freezing at −20°C vs. −70°C. The third stage will be a plasma or

TABLE 6
Geometric mean and RMSE of the $\log(x + 1)$ value for peak or stimulated C-peptide, 2-h AUC mean, and glucagon post-stimulus value at 12 months, adjusted for baseline value, age, and sex

	MMTT (peak or post-stimulus)		MMTT and 2-h AUC mean		Glucagon (post-stimulus)	
Eligibility	*n*	GM (RMSE)	*n*	GM (RMSE)	*n*	GM (RMSE)
Any age						
Any C-peptide	127	0.253 (0.223)	129	0.196 (0.172)	90	0.289 (0.150)
>0.2 pmol	88	0.289 (0.200)	89	0.228 (0.162)	80	0.299 (0.153)
>0.3 pmol	65	0.300 (0.215)	66	0.237 (0.176)	62	0.324 (0.156)
Age 12 years						
Any C-peptide	61	0.303 (0.247)	62	0.240 (0.200)	88	0.289 (0.149)
>0.2 pmol	54	0.314 (0.217)	55	0.248 (0.179)	78	0.300 (0.151)
>0.3 pmol	43	0.305 (0.258)	44	0.238 (0.191)	60	0.326 (0.143)

GM, geometric mean.

serum exchange between participating laboratories, and the final stage will be analysis and reporting of results.

In this workshop report, we have summarized the data underlying the unanimous conclusion of the workshop's participants that in type 1 diabetes clinical trials to assess endogenous β-cell function, the preferred outcome is measurement of C-peptide.

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APPENDIX: WORKSHOP PARTICIPANTS

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