β-Cell Function and the Development of Diabetes-Related Complications in the Diabetes Control and Complications Trial

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In patients with type 1 diabetes, measurement of connecting peptide (C-peptide), cosecreted with insulin from the islets of Langerhans, permits estimation of remaining β-cell secretion of insulin. In this retrospective analysis to distinguish the incremental benefits of residual β-cell activity in type 1 diabetes, stimulated (90 min following ingestion of a mixed meal) C-peptide levels at entry in the Diabetes Control and Complications Trial (DCCT) were related to measures of diabetic retinopathy and nephropathy and to incidents of severe hypoglycemia. Based on the analytical sensitivity of the assay (0.03 nmol/l) and study entry criteria, the DCCT subjects were divided into four groups of stimulated C-peptide responses: ≤0.03, 0.04–0.20, 0.21–0.50 nmol/l at entry, and 0.21–0.50 nmol/l at entry and at least 1 year later (sustained C-peptide secretion). Uniformly in the intensive and partially in the conventional DCCT treatment groups, any C-peptide secretion, but especially at higher and sustained levels of stimulated C-peptide, was associated with reduced incidences of retinopathy (both a single three-step change and a repeated three-step change on the Early Treatment of Diabetic Retinopathy Study [ETDRS] scale at the next 6 month visit) and nephropathy (both albuminuria >40 mg/24 h once and repeated at the next annual visit). There were also differences in severe hypoglycemia across C-peptide levels in both treatment groups. In the intensively treated cohort there were essentially identical prevalences of severe hypoglycemia (~65% of participants) in the first three groups; however, those subjects with mixed-meal stimulated C-peptide level >0.20 nmol/l for at least baseline and the first annual visit in the DCCT experienced a reduced prevalence of ~30%. Therefore, even modest levels of β-cell activity at entry in the DCCT were associated with reduced incidences of retinopathy and nephropathy. Also, continuing C-peptide (insulin) secretion is important in avoiding hypoglycemia (the major complication of intensive diabetic therapy).

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The Diabetes Control and Complications Trial (DCCT) established the benefits of intensive treatment and a lower HbA_{1c} in the prevention or reversal of the microvascular complications of type 1 diabetes (1–3). However, the intensively treated DCCT subjects also faced a higher incidence of severe hypoglycemia (1). The DCCT Research Group has documented the distribution of baseline stimulated C-peptide levels at entry and for up to 6 years of follow-up (4,5), but these papers have emphasized differences between those with stimulated values above or below 0.20 nmol/l, the original upper limit of stimulated C-peptide allowed for participation in the DCCT. During the recruitment for the DCCT, the DCCT Research Group raised the permissible level of stimulated C-peptide to 0.50 nmol/l (from 0.20 nmol/l), thus allowing more patients to be eligible for the trial (4,6). They also conducted a 6-year ancillary study to follow annual stimulated C-peptide levels in those subjects who sustained stimulated C-peptide levels >0.20 nmol/l (4,5). Although there were clear advantages associated with higher levels of stimulated C-peptide (5), those subjects with at least measurable stimulated C-peptide at entry may also have experienced reduced incidents of the microvascular complications. In this paper, we examine the association of participants' stimulated C-peptide values and their rates of experiencing the microvascular complications and/or hypoglycemia over a 6-year period in an analysis that more finely classifies subjects' C-peptide levels than earlier analyses.

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Abbreviations: AER, albumin excretion rate; DCCT, Diabetes Control and Complications Trial; ETDRS, Early Treatment of Diabetic Retinopathy Study; HPLC, high-performance liquid chromatography.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

RESEARCH DESIGN AND METHODS

This study used data from the publicly released DCCT database (using the computer facilities of the General Clinical Research Center) and also additional data on levels of C-peptide in the annually repeated stimulation tests given to those subjects sustaining elevated poststimulation levels of C-peptide (kindly provided by Patricia Cleary of the Biostatistics Center, George Washington University).

Baseline stimulated C-peptide groups.

Participants were grouped according to their stimulated C-peptide value at baseline in the DCCT: "undetectable": C-peptide ≤0.03 nmol/l; "minimal": C-peptide 0.04–0.20 nmol/l; "baseline-only": C-peptide >0.20 nmol/l at baseline, but <0.20 nmol/l thereafter; and "sustained": C-peptide >0.20 nmol/l at baseline and again at least 1 year later. The minimal level of 0.03 nmol/l was selected because it represented the lower limit of detection of the radioimmunoassay of C-peptide (with the Novo M1230 antibody) used in the DCCT (4,5,7). The value of 0.20 nmol/l indicates the original upper limit allowed for entry into the DCCT, later raised to 0.50 nmol/l (4,5). We ex-

Table 1—*Demographic characteristics of the DCCT subjects*

	Stimulated C-peptide group			
	Undetectable	Minimal	Baseline-only	Sustained
n (%)	634 (44)	519 (36)	130 (9)	159 (11)
Intensive therapy* (%)	52	48	36	55
Retinopathy at baseline* (%)	73	41	8	19
Female (%)	50	45	41	49
Age (years) (%)	26 ± .3[a]	27 ± .3[a]	27 ± .3[ab]	29 ± .6[b]
Duration (years)	8.2 ± .1[a]	4.5 ± .1[b]	1.9 ± .3[c]	2.3 ± .3[c]
HbA_{1c} at eligibility	9.3 ± .1[a]	9.2 ± .1[a]	8.4 ± .1[b]	8.4 ± .1[b]
HbA_{1c} at baseline	9.0 ± .1[a]	9.0 ± .1[a]	8.4 ± .1[b]	8.2 ± .1[b]
AER (mg/24 h) (95% CI)	13.5[a] (12.6–14.4)	10.0[b] (9.4–10.7)	9.2[b] (8.3–10.7)	9.7[b] (8.7–10.7)

*Percentages differ significantly (χ^2 test, $P < 0.005$). Where stimulated C-peptide group means differed significantly ($P < 0.05$), the groups with the same letter were indistinguishable. Data are *n* (%), %, means ± SE, or means (95% CI). All statistical comparisons are between stimulated C-peptide groups within a single row.

amined the characteristics of these groups and compared their rates of developing retinopathy and nephropathy, the number of subjects experiencing hypoglycemia, and the rates of severe hypoglycemia during years 1–6 of the DCCT.

Definition of study end points.
Retinopathy events were indicated by a three-step change using a variation of the Early Treatment of Diabetic Retinopathy Study (ETDRS) protocol (1,2). Persistent retinopathy events were indicated by a three-step change that was confirmed at the subsequent 6 month visit. Nephropathy events (1,3) occurred when participants had an albumin excretion rate (AER) > 40 mg/24 h. Persistent nephropathy events occurred when participants had AER >40 mg/24 h at two or more consecutive annual visits. Severe hypoglycemia was defined by the DCCT Research Group as loss of consciousness, coma, or an unresponsive state relieved by the ingestion of sugar (1).

Estimation of event rates.
For each C-peptide group, the numerator for an event rate was the number of first events experienced within the first 6 years by a participant in that group, and the denominator was total patient-years at risk for the event summed across the group. The time to a participant's first event, or to year 6 if there was no event, comprised the interval that participant was at risk for that event (retinopathy, nephropathy, or hypoglycemia). To assess the effects of glycemic control on the rates, we used each participant's recorded monthly or quarterly HbA_{1c}, measured by high-performance liquid chromatography (HPLC) with ion-exchange resin (1). HbA_{1c} measurements over the first 6 months were omitted, because many participants, particularly those in the intensively treated group, experienced a change from baseline HbA_{1c} early in the DCCT. The recorded HbA_{1c} of a participant at the time an event occurred was used to assign events to three categories of HbA_{1c}: <7.5%, between 7.5 and 9.0%, and >9.0%. The denominator for each HbA_{1c} category was the total time participants spent in that category. For missing HbA_{1c} measurements, participants were presumed to have remained at their last recorded level until the next measurement. As their HbA_{1c} varied, a single participant may have contributed time to more than one HbA_{1c} category. Thus, time at risk for an event within a category of HbA_{1c} was the unit of analysis for the rates. Finally, event rates were calculated in this manner for the twelve combinations of C-peptide and HbA_{1c} categories.

Events with participant as unit.
For retinopathy and hypoglycemia, we also calculated the percentages of participants in each category who had a three-step change in retinal assessments or who experienced any symptoms of hypoglycemia at least one time. Event odds by logistic regression were adjusted for DCCT strata, age, duration of diabetes, and glycemic control. The adjustment for glycemic control used the average of the participant's HbA_{1c} levels from month 6 through year 6.

Statistical methods.
Event rates for a subgroup of participants were computed using a Poisson model: if *D* is the total number of events among the subgroup of participants and *N* is their total time at risk, then the estimated event rate is *D*/*N* and the standard error of the estimated rate is $\sqrt{D}/N$. Rates were compared using a large-sample normal approximation (8,9). Percents of participants experiencing events were compared using the χ^2 test for independence. Adjusted odds ratios were estimated using logistic regression. The Statistical Analysis System (version 6.12) was used to perform all analyses.

RESULTS — Despite the revised eligibility criteria for stimulated C-peptide levels during enrollment in the DCCT, ~80% of participants at baseline had undetectable or minimal stimulated C-peptide, and these two groups contained proportionately more participants with mild retinopathy at baseline (i.e., were part of the secondary intervention cohort of the DCCT) (Table 1). Randomization failed to assign members of the C-peptide groups equally to the intensive and conventional treatment groups: a smaller percentage of baseline-only participants received intensive treatment than in the other three C-peptide groups. Other differences among the C-peptide groups were that baseline-only and sustained groups were slightly older with a duration of disease shorter than those with minimal or undetectable C-peptide; further, their baseline and eligibility HbA_{1c} levels were almost 1% lower (Table 1). Finally, during the trial the sustained C-peptide group experienced lower mean HbA_{1c} levels than most other C-peptide groups under both intensive and conventional treatment, and those treated intensively

Table 2—*Retinopathy (a single three-step change on the ETDRS scale) and albuminuria (a single event >40 mg/24 h) during the first 6 years of the DCCT, by stimulated C-peptide group*

	Stimulated C-peptide group				
	Undetectable	Minimal	Baseline-only	Sustained	All
Retinopathy					
All	7.8 ± 0.5[a]	5.7 ± 0.5[b]	5.2 ± 0.9[bc]	3.5 ± 0.7[c]	6.3 ± 0.3
Intensive	6.5 ± 0.7[a]	3.5 ± 0.5[b]	1.8 ± 0.9[bc]	1.4 ± 0.6[c]	4.5 ± 0.4
Conventional	9.2 ± 0.8[a]	7.9 ± 0.8[a]	7.3 ± 1.4[a]	6.3 ± 1.4[a]	8.2 ± 0.5
Albuminuria					
All	4.4 ± 0.4[a]	2.7 ± 0.3[b]	3.1 ± 0.7[ab]	1.4 ± 0.4[c]	3.3 ± 0.2
Intensive	4.0 ± 0.5[a]	2.3 ± 0.4[b]	1.7 ± 0.9[bc]	0.9 ± 0.5[c]	2.9 ± 0.3
Conventional	4.9 ± 0.6[a]	2.9 ± 0.5[b]	3.9 ± 1.0[ab]	2.1 ± 0.8[b]	3.8 ± 0.3

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 ($P < 0.05$), while rates sharing the same letter were indistinguishable.

evinced a smaller within-person variation in HbA_{1c} levels (data not shown).

Event rates for retinopathy and nephropathy.

Comparing the event rates (per 100 participant-years) for retinopathy (both a single three-step change and persistent three-step changes) and albuminuria (both a single increase >40 mg/24 h and persistent increases >40 mg/24 h) among C-peptide groups receiving intensive treatment, participants with minimal or greater stimulated C-peptide secretion uniformly experienced significantly lower rates of single (Table 2) and persistent (data not shown) events for both retinopathy and albuminuria than did the group with undetectable C-peptide. In subjects receiving conventional treatment, albuminuria rates (both single increases and persistent increases) showed a similar pattern of differences as those receiving intensive treatment (Table 2). Patterns were similar for retinopathy with conventional treatment; however, there were no significant comparisons (Table 2).

Percentages of subjects experiencing retinopathy.

The percentages of participants experiencing retinopathy were significantly lower in both the baseline-only and sustained C-peptide groups among intensively treated subjects but not among conventionally treated subjects (χ^2 analysis among the groups: intensive: $P = 0.001$; conventional: $P = 0.13$). These patterns were similar to those seen in expressing event rates for retinopathy (Table 2).

Hypoglycemic events and percentages of subjects experiencing hypoglycemia.

Among participants receiving intensive treatment, those with sustained C-peptide had significantly lower rates of hypoglycemia (Table 3) and lower percent of patients experiencing hypoglycemia than the other three C-peptide groups (Fig. 1), as well as significantly lower mean number of hypoglycemic incidents per patient than the undetectable and minimal C-peptide groups (data not shown). Compared with subjects receiving intensive treatment, subjects receiving conventional treatment had lower event rates and fewer incidents of hypoglycemia. However, the rate of hypoglycemia for intensively treated subjects with sustained C-peptide was much closer to the rates for all conventionally treated groups than to all other intensively treated groups (Fig. 1).

Table 3—*Hypoglycemia: first occurrence during the first 6 years of the DCCT, by stimulated C-peptide group and by treatment group*

	Stimulated C-peptide group				
	Undetectable	Minimal	Baseline-only	Sustained	All
All	13 ± 1[a]	10 ± 1[b]	7 ± 1[c]	6 ± 1[c]	12 ± 0.5
Intensive	21 ± 1[a]	16 ± 1[b]	17 ± 3[ab]	7 ± 1[c]	16 ± 0.8
Conventional	8 ± 1[a]	6 ± 1[b]	3 ± 1[c]	5 ± 1[bc]	6 ± 0.4

Data are rates ± SE per 100 participant-years. Rates were compared among stimulated C-peptide groups (horizontally) with each treatment group. For each comparison, rates with different letters were significantly different ($P < 0.05$), while rates sharing the same letter were indistinguishable.

Effect of glycemic control on hypoglycemia.

Stratifying by glycemic control (Table 4), intensive glycemic control (HbA_{1c} <7.5%) was associated with much lower hypoglycemia incidence rates in subjects who had sustained C-peptide levels compared with all other groups. Participants with undetectable or minimal C-peptide were most sensitive to lower levels of HbA_{1c}: they exhibited significant increases in their rates of hypoglycemia with each reduction in HbA_{1c} category (Table 4). Similarly, the effect of sustained C-peptide secretion substantially reduced the number of subjects experiencing any hypoglycemia in the intensive treatment group, while all other intensively treated groups experienced much higher percentages of participants with hypoglycemia (Fig. 1).

Adjustments for demographic factors and glycemic control.

Comparing retinopathy, albuminuria, and hypoglycemia incidences on a per-participant rather than per-participant-year basis, we estimated odds for each outcome for each C-peptide group: adjusted for DCCT treatment group, primary or secondary retinopathy stratum, age, duration of diabetes, HbA_{1c} at eligibility, and mean HbA_{1c} from month 6 through month 72. In those subjects receiving intensive treatment, the adjusted odds of retinopathy were 3.2-fold higher for those with undetectable C-peptide than for those in the sustained C-peptide group ($P < 0.02$); in those subjects receiving conventional treatment, the odds of retinopathy were no different among C-peptide groups. The adjusted odds of albuminuria were not different among the C-peptide groups in both DCCT treatment groups, although the trend was similar to that of retinopathy. The adjusted odds of a hypoglycemic incident among participants receiving intensive treatment were nearly threefold higher in all those without sustained C-peptide compared

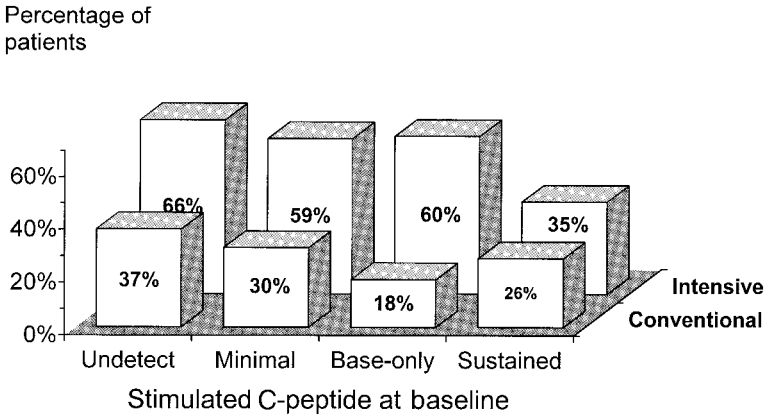

Figure 1—*Percentages of subjects who experienced at least one episode of severe hypoglycemia over the first 6 years of the DCCT. χ^2 analysis among the groups: intensive ($P = 0.001$); conventional ($P = 0.006$).*

with those with sustained C-peptide ($P < 0.02$ for each comparison); the odds were indistinguishable in the conventional treatment group. All these per-participant results (even those that did not reach statistical significance) are consistent with the unadjusted results per participant-year presented earlier.

CONCLUSIONS — These observations emphasize the benefits of higher and sustained levels of C-peptide (and thereby insulin) secretion to reduce the incidences of the microvascular complications of type 1 diabetes. Altogether the findings in participants with sustained C-peptide secretion uniquely underline the possibility of reaching glycemic goals of intensive treatment in the DCCT accompanied by fewer incidences of the major complication of intensive insulin therapy, severe hypoglycemia. Even modest β-cell activity was associated with decreased incidences of the microvascular complications in the intensively treated group. Finally, the results and conclusions remained consistent overall after adjusting for duration of diabetes, glycemic control, and other factors. Nevertheless, the findings of this retrospective analysis need replication prospectively in other populations of new-onset patients with type 1 diabetes.

Table 4—***Hypoglycemia: first occurrence during the first 6 years of the DCCT, by stimulated C-peptide group and by HbA_{1c} category***

Stimulated C-peptide group	HbA_{1c} category		
	<7.5%	7.5–9.0%	>9.0%
Undetectable	23 ± 2[a]	13 ± 1[b]	4 ± 1[c]
Minimal	20 ± 2[a]	8 ± 1[b]	3 ± 1[c]
Baseline-only	21 ± 4[a]	4 ± 1[b]	2 ± 1[b]
Sustained	8 ± 1[a]	6 ± 0.2[a]	1 ± 0.7[b]
All	19 ± 1	9 ± 0.7	3 ± 0.4

Data are rates ± SE per 100 participant-years. Rates were compared among HbA_{1c} categories (horizontally) within each stimulated C-peptide group. For each comparison, rates with different letters were significantly different ($P < 0.05$), while rates sharing the same letter were indistinguishable.

For the microvascular complications and the reciprocal risk of severe hypoglycemia, the greatest benefit lies at glycemic levels approaching those of the nondiabetic subject; i.e., the incidences of severe hypoglycemia in those intensively treated patients with sustained β-cell function were remarkably low. Few DCCT participants reached normal levels of HbA_{1c}; however, for those who could approach that goal (i.e., those with values <7.5%), sustained islet function permitted a near-optimal outcome (universal benefits in reduced microvascular complications and fewer incidences of severe hypoglycemia). These analyses build upon earlier studies (10,11) by following a larger group of subjects for a longer period of time, as compared with other studies (11–13), and by more finely categorizing subjects with respect to islet cell function (4,5).

Alternatively sustained C-peptide secretion may have directly affected the microvascular complications, as has been demonstrated by others (14,15), most recently in a small clinical trial (16). In this scenario, C-peptide may have directly impacted the development and/or progression of the microvascular complications. However, the weaker benefit of sustained C-peptide secretion in the conventional compared with the intensive treatment group suggests glycemic control is potentially a more important factor in imparting the benefit of continuing β-cell function than the direct effect of C-peptide itself; i.e., both groups secreted comparable levels of C-peptide, but the intensively treated patients had lower levels of HbA_{1c}.

Although clearly not confirmed in this or any other study, the benefits of fewer incidents of hypoglycemia with sustained β-cell activity may have arisen from sustained α-cell activity. Under normal physiologic conditions, the increased glucagon secretion in response to falling glucose levels can stimulate glucose production and prevent severe hypoglycemia (17,18). During the progression of type 1 diabetes, α-cells can secrete glucagon following administration of a secretagogue (i.e., an amino acid) (19). However, with increasing duration of diabetes the α-cells can no longer respond to decreased circulating glucose levels with secretion of glucagon (19), perhaps because functioning β-cells help sustain α-cell function. Thus, the diabetic patient experiences two deficiencies: no tonic and responsive secretion of insulin to allow better glycemic control; and no response of glucagon to avoid severe hypoglycemia. Also, the increased likelihood of severe hypoglycemia with falling levels of glucose reduces the capacity to increase administration of insulin to reach glycemic targets (20,21).

It is unlikely that intensive control in the DCCT substantially altered the course of destruction of the β-cells in the islets; however, this remains speculative. Thus, the most likely interpretation of these observations involves enhancing insulin (and C-peptide) secretion from the remaining β-cells, possibly by allowing the β-cells to function within more normal physiologic parameters with intensive diabetic management. Since most type 1 diabetic patients eventually lose β-cell function, the group with essentially no stimulated C-peptide subsumes most type 1 patients with duration >10 years. Therefore, all type 1 (and possibly many type 2) diabetic patients may benefit from any success in maintaining β-cell (and thereby α-cell) activity after onset of disease (21). From the data presented here, the best current and practical method

subsumes the best possible control for each diabetic patient.

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