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Insulin secretion measured by stimulated C-peptide in long-established Type 1 diabetes in the Diabetes Control and Complications Trial (DCCT)/ Epidemiology of Diabetes Interventions and Complications (EDIC) cohort: a pilot study

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Abstract

Aims—To evaluate whether clinically relevant concentrations of stimulated C-peptide in response to a mixed-meal tolerance test can be detected after almost 30 years of diabetes in people included in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications cohort.

Methods—Mixed-meal tolerance tests were performed in a sample of 58 people. C-peptide levels were measured using a chemiluminescent immunoassay. This sample size assured a high probability of detecting C-peptide response if the true prevalence was at least 5%, a level that would justify the subsequent assessment of C-peptide in the entire cohort.

Results—Of the 58 participants, 17% showed a definite response, defined as one or more post-stimulus concentrations of C-peptide > 0.03 nmol/l, and measurable concentrations were found in all participants.

Conclusions—These results show that a stimulated C-peptide response can be measured in some people with long-term Type 1 diabetes. Further investigation of all participants in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study will help relate long-term residual C-peptide response to glycaemia over time and provide insight into the relevance of this response in terms of insulin dose, severe hypoglycaemia, retinopathy, nephropathy and macrovascular disease. Establishing the clinical

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Competing interests

None declared.

relevance of long-term C-peptide responses is important in understanding the impact that therapy to preserve or improve β -cell function may have in patients with long-term Type 1 diabetes.

Introduction

C-peptide concentration is widely accepted as an indicator of endogenous insulin secretion [1]. The Diabetes Control and Complications Trial (DCCT) showed that plasma C-peptide concentration was an important factor relating to glycaemia and complications in Type 1 diabetes [2,3]. The demonstrated benefits associated with higher concentrations of stimulated C-peptide include improved achievement of optimum glycaemic control with a lower incidence of hypoglycaemia and reductions in the incidence of retinopathy and nephropathy.

Although C-peptide falls to undetectable concentrations in some people with Type 1 diabetes within 5–10 years of diagnosis, it has been recognized since the 1970s that some patients with even longer-term Type 1 diabetes have persistent residual β -cell function [4,5]. During screening for the DCCT in 1983–1989, we found that 8% of adults with Type 1 diabetes 5–15 years after diagnosis had peak C-peptide concentrations during a mixed-meal tolerance test > 0.2 nmol/l [3]. In those included in the Joslin Medalists study [6], random C-peptide was at least detectable in $> 50\%$ of participants 50 years after diagnosis of Type 1 diabetes, and in many it was above the concentration associated with clinical benefit in the DCCT (0.2 nmol/l). The use of more sensitive C-peptide assays has identified patients with residual insulin secretion among those who would be classified as C-peptide-negative with standard assays [7–9]. In one of these studies, this very low C-peptide concentration may have been clinically significant, as those participants with very low C-peptide responses, detectable only with the highly sensitive assay, had less glycaemic variability and better counter-regulation and recovery from hypoglycaemia [7].

As a prelude to a more in-depth study, we report a pilot study of residual stimulated C-peptide among a selected cohort of 58 people included in the DCCT/ Epidemiology of Diabetes Interventions and Complications (EDIC) study with almost 30 years duration of diabetes.

Subjects and Methods

Subjects

The DCCT enrolled 1441 subjects during 1983–1993, half into a primary prevention cohort with no pre-existing retinopathy and 1–5 years' diabetes duration and half into a secondary intervention cohort with minimal pre-existing retinopathy and 1–15 years' diabetes duration. The DCCT ended in 1993 and the EDIC long-term follow-up study was initiated in 1994. In the EDIC study, subjects are evaluated annually when health status is documented and various assessments (e.g. HbA_{1c}) conducted. For the present pilot study, 92 people were selected who were considered likely to have some residual β -cell function, with the goal of testing 60 people. Owing to the need to analyse the data for submission of a grant application, the testing was terminated after 58 subjects had completed the mixed-meal tolerance test. Of these, 13 participants had a low mean HbA_{1c} concentration throughout the

DCCT/EDIC study (all < 43 mmol/mol, 6.1%). Four of these 13 also had a DCCT baseline stimulated C-peptide concentration > 0.2 nmol/l. The additional 45 subjects had stimulated C-peptide concentrations (> 0.2 nmol/L) at the DCCT baseline visit.

C-peptide

The mixed-meal tolerance test (BoostTM Nutritional Drink, Nestle, Vevey, Switzerland) was conducted after a 10-h fast, preceded by a 3-day consumption of a high carbohydrate diet of 150 g, and a fasting blood glucose of 70–200 mg/dl the morning of the test. The mixed meal was given at a dose of 6 ml per kg body weight, with a maximum dose of 360 ml. Timed collections were obtained at –10, 0, 15, 30, 60, 90, 120, 180 and 240 min relative to the ingestion of the stimulus at time zero. All samples were frozen on the day of collection at –70° C and thawed only once at the time of assay. C-peptide was assayed from plasma at all of these times with the Roche Elecys 2010 Analyzer (Roche Diagnostics Corp., Indianapolis, IN, USA) using a chemiluminescent immunoassay method (Roche Diagnostics Corp.) in the DCCT/EDIC Central Biochemistry Laboratory at the University of Minnesota. The laboratory interassay coefficient of variation is 2.7% at low concentrations. We report all C-peptide concentrations, with the lowest at 0.004 nmol/l, in effect the lower limit of detection.

Statistical analysis

If all 60 subjects were non-responders to the mixed-meal tolerance test during the EDIC study, the upper (one-sided) 95% confidence limit on the true probability of a C-peptide response is 0.049. In this case, with 95% confidence we could conclude that the true probability is < 5%, a level below which it was considered unlikely that residual C-peptide could have a strong effect on DCCT/EDIC outcomes. The empirical distributions of measured basal and stimulated C-peptide concentrations were computed along with exact 95% confidence limits on the probability of having measurable concentration(s). Participants with at least one post-stimulus C-peptide measurement in plasma from the mixed-meal tolerance test >0.03 nmol/l, as measured by the high-sensitivity immunoassay, were classified as responders, as concentrations below 0.03 nmol/l were associated with a markedly higher risk of retinopathy progression in the DCCT [3].

Results

Characteristics of the participants in the present study at the time of the mixed-meal tolerance test are shown in Table 1. The mean (range) participant age was 55 (37–68) years with a mean (range) Type 1 diabetes duration of 29 (24–42). Of the 58 participants, 57 were members of the DCCT intensive treatment group.

A measurable level of C-peptide was obtained in all participants. Ten participants (17.2%, 95% confidence limit 8.6–29.4%) showed a clinically meaningful C-peptide response consisting of one or more post-stimulus concentrations > 0.03 nmol/l (Fig 1a), all showing a definite rise over the time of the test from the level at baseline. All responders had a 90-min stimulated C-peptide concentration > 0.2 nmol/l at DCCT entry. Among the 48 participants classified as non-responders by the above criteria, four were probable responders with a

small rise in C-peptide concentrations post-stimulus, although the peak was in the range of 0.005 to 0.03 nmol/l (Fig 1b). It is of interest that all participants that met the criteria for response had a mean fasting C-peptide concentration > 0.02 nmol/l, whereas those with no mixed-meal response had a mean fasting C-peptide concentration < 0.02 nmol/l.

Among the 58 participants, only one had an estimated GFR < 60 ml/min/1.73m² (31.2 ml/min/1.73m²), and that participant was not a responder.

Discussion

These results confirm that a stimulated C-peptide response can be detected in some people with long-term Type 1 diabetes. The 58 people assessed in the present study, with a mean diabetes duration of 29 years, were selected from among people considered likely to have some residual β -cell function. These people either had a mean HbA_{1c} concentration < 43 mmol/mol (<6.1%) and/or had above average stimulated C-peptide concentrations (>0.2 nmol/l) at DCCT baseline, at a mean of 25 years before the current assessment.

A stimulated C-peptide value of > 0.03 nmol/l was used to define C-peptide response because a previous study of the DCCT cohort showed that the risk of microvascular disease progression was markedly higher among patients who entered with values below this level [3]. Of the 58 participants in the present pilot study, 17% (95% CI 9, 29%) had a definite C-peptide response to the stimulus. In most cases this response consisted of a rise to a peak, followed by some decay rather than a single isolated stimulated value. This demonstrates that this response could not be explained by assay variation and that it represents a true functional response of β cells to the stimulus.

The prevalence of such residual function would be expected to be lower in the full cohort, and a lower value would be expected to apply in a general population of subjects with Type 1 diabetes of ~30 years' duration. Nevertheless, even if the true prevalence was lower than the lower confidence limit of 9%, further study in the complete DCCT/EDIC cohort would be justified.

It should be noted that other recent studies have reported that a high proportion of subjects with long-established diabetes have measurable levels of C-peptide: 43% in one study in people with an average of 15 years' diabetes duration [8] and 73% in another in people with an average of 30 years' diabetes duration [9]. In the latter study, a measurable level was defined as a stimulated value of 0.0033 nmol/l in a mixed-meal tolerance test. By this criterion, all 58 (100%) of the participants in the present study had measurable C-peptide concentrations, as the lowest value we report using our assay was 0.004 nmol/l, and prevalence would be 11% in the study by Oram *et al.* [9] if a C-peptide concentration >0.03 nmol/L and >30 years' diabetes duration were used; however, the clinical impact of a measurable value below the level used to define C-peptide response in the present study, i.e. a value between 0.0033 and 0.03 nmol/l is unknown.

A weakness of the present study is that neither insulin nor islet cell antibodies, nor human leukocyte antigen type, were measured at baseline, factors that would more definitively establish this as a true Type 1 diabetes cohort; however, all participants with up to 5 years'

diabetes duration had a baseline stimulated C-peptide concentration < 0.5 nmol/l, all those with 5–15 years' diabetes duration had a baseline stimulated C-peptide concentration < 0.2 nmol/l, and all participants were insulin-dependent.

Further investigation of all surviving participants in the DCCT/EDIC study, using the highly sensitive assay used in the present pilot study, will provide insights into the clinical relevance of retained C-peptide concentrations after three decades of Type 1 diabetes, in terms of treatment (e.g. insulin dose and long-term HbA_{1c}) and the development of microvascular and macrovascular complications. Furthermore, establishing the clinical relevance of low concentrations of C-peptide is important in understanding what impact therapies targeting β -cell regeneration or preservation may have in people with long-term Type 1 diabetes.

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What's new?

- Residual β -cell function assessed according to stimulated C-peptide concentrations during the first 5 years of Type 1 diabetes is associated with better glycaemic control and a lower incidence of microvascular disease and hypoglycaemia, but it is not known whether a residual β -cell response to a mixed-meal tolerance test remains in those with long-term Type 1 diabetes.
- We show that low concentrations of C-peptide can be detected after nearly 30 years of Type 1 diabetes.
- This justifies future research on factors associated with preservation of β -cell function and the role of β -cell regenerative therapy in people with long-term Type 1 diabetes.

FIGURE 1.

Plasma C-peptide concentrations from the timed collections of the 4-h mixed-meal tolerance test for the 10 people who met the criterion for response vs the 48 who were non-responders. (a) C-peptide in plasma among responders and non-responders. Dashed line represents 0.03 nmol/L. (b) C-peptide in plasma among non-responders with a rise post-stimulus.

Table 1

Characteristics of the 10 people in the pilot cohort who met the criterion for C-peptide response vs those of the 48 people who were non-responders

	Non-responders N=48	Responders N=10
Age at diagnosis, years		
Mean $\pm$ SD	24.4 $\pm$ 7.5	28.6 $\pm$ 5.7
Median (range)	24.4 (8–38)	28.6 (20–37.5)
Mean $\pm$ SD age at time of EDIC C-peptide draw	54.2 $\pm$ 7.0	57.0 $\pm$ 4.5
Mean $\pm$ SD diabetes duration at study entry, years	3.1 $\pm$ 3.3	2.7 $\pm$ 1.0
Mean $\pm$ SD duration at mixed-meal tolerance test, years	29.0 $\pm$ 4.2	27.5 $\pm$ 2.0
Female, <i>n</i> (%)	25 (52)	3 (30)
Intensive therapy group, <i>n</i> (%)	47 (98)	10 (100)
Primary cohort, <i>n</i> (%)	38 (79)	7 (70)
Mean $\pm$ SD 90-min C-peptide concentration at DCCT entry, nmol/l	0.26 $\pm$ 0.13	0.35 $\pm$ 0.10
90-min C-peptide concentration at DCCT entry > 0.2 nmol/l, <i>n</i> (%)	39 (67)	10 (100)
Mean $\pm$ SD HbA _{1c} at DCCT entry, mmol/mol	667 $\pm$ 18	61 $\pm$ 13
Mean $\pm$ SD HbA _{1c} at DCCT entry, %	8.2 $\pm$ 1.6	7.7 $\pm$ 1.2
Average HbA _{1c} concentration < 43 mmol/mol (6.1%) during DCCT/EDIC, <i>n</i> (%)	11 (23)	2 (20)
Mean $\pm$ SD insulin use during EDIC (units/kg/24 h)	0.71 $\pm$ 0.03	0.60 $\pm$ 0.06

DCCT, Diabetes Control and Complications Trial; EDIC, Epidemiology of Diabetes Interventions and Complications.

All comparisons between responders and non-responders were non-significant ($P > 0.05$).