Trends of Earlier and Later Responses of C-peptide to Oral Glucose Challenges with Progression to Type 1 Diabetes in Diabetes Prevention Trial-1 Type 1 Participants Running Title: C-peptide with Progression to Type 1 Diabetes

Jay M. Sosenko, MD (1); Jerry P. Palmer, MD (2); Lisa E. Rafkin-Mervis, MS CDE (1); Jeffrey P. Krischer, PhD (3); David Cuthbertson, MS (4); Carla J. Greenbaum, MD (5); George Eisenbarth, MD, PhD (6); Jay S. Skyler, MD (1) and the Diabetes Prevention Trial-1 Study Group

1. Division of Endocrinology, University of Miami, PO Box 016960 (D110), Miami, FL 33101
2. Division of Endocrinology/Metabolism, University of Washington, Seattle, WA
3. Division of Informatics and Biostatistics, University of South Florida, Tampa, FL
4. Pediatrics Epidemiology Center, University of South Florida, Tampa, FL
5. Benaroya Research Institute at Virginia Mason, Seattle, WA
6. HLA/DNA Laboratory, University of Colorado, Aurora, CO

Corresponding Author
Jay M. Sosenko, MD
Email: jsosenko@med.miami.edu


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Objective. We studied the C-peptide response to oral glucose with progression to type 1 diabetes (T1D) in Diabetes Prevention Trial-Type 1 (DPT-1) participants.

Methods. Among 504 DPT-1 participants <15 years of age, longitudinal analyses were performed in 36 progressors and 80 non-progressors. Progressors had oral glucose tolerance tests (OGTTs) at baseline and every 6 months from 2.0 years to 0.5 years before diagnosis; non-progressors had OGTTs over similar intervals prior to their last visit. Sixty-six progressors and 192 non-progressors were also studied proximal to and at diagnosis.

Results. The 30-0 minute C-peptide difference from OGTTs performed 2.0 years before diagnosis in progressors was lower than the 30-0 minute C-peptide difference from OGTTs performed 2.0 years before the last visit in non-progressors (p<0.01), and remained lower over time. The 90-60 minute C-peptide difference increased at every OGTT before diagnosis in progressors, whereas it declined at every OGTT before the last visit in non-progressors (p<0.01 at 2.0 years). The percentage whose peak C-peptide occurred at 120 minutes was higher in progressors at 2.0 years (p<0.05); this persisted over time (p<0.001 at 0.5 years). However, the peak C-peptide levels were only significantly lower at 0.5 years in progressors (p<0.01). The timing of the peak C-peptide predicted T1D (p<0.001); peak C-peptide levels were less predictive (p<0.05).

Conclusion. A decreased early C-peptide response to oral glucose and an increased later response occur at least two years before the diagnosis of T1D.
Studies indicate that type 1 diabetes (T1D) develops over a period of years (1-5). Immunologic damage and destruction of β-cells results in ongoing metabolic deterioration which continues even after diagnosis. It appears that there can be an increase in glucose levels for at least two years before diagnosis. This increase is rather gradual initially, but becomes more rapid as onset approaches. Despite the increase in glucose with progression, overall measures of C-peptide from oral glucose tolerance testing (OGTT), such as the area under the curve (AUC) C-peptide and the peak C-peptide, change relatively little until close to diagnosis (2,6).

It is quite possible, however, that overall measures of C-peptide fail to discern more subtle changes that occur with progression to T1D. The partitioning of C-peptide responses according to the time after an oral glucose challenge could yield a better understanding of changes in insulin secretion over time. Thus, we have utilized the serial oral glucose tolerance tests from the Diabetes Prevention Trial-Type 1 (DPT-1) (7,8) to examine changes in earlier and later C-peptide responses to an oral glucose challenge with progression to T1D.

**RESEARCH DESIGN AND METHODS**

**Subjects.** There were 504 participants of the parenteral and oral insulin DPT-1 trials included in the analysis. For certain analyses, subgroups of that cohort were studied according to specific criteria. All DPT-1 participants were islet cell autoantibody (ICA) positive relatives of T1D patients. Estimated 5-year risks of >50% and 26-50% were required for entry into the parenteral and oral insulin trials, respectively. A >50% 5-year risk estimate was based on a first-phase insulin response (FPIR) from an intravenous glucose tolerance test (IVGTT) below a defined threshold and/or the presence of an OGTT abnormality other than diabetes. If those metabolic criteria were not present, but there were insulin autoantibodies, individuals were characterized as having a 26-50% 5-year risk. There was no overall effect from the intervention in either trial.

**Procedures.** The interventions for the parenteral and oral insulin trials were recombinant human ultralente insulin and recombinant human insulin crystals, respectively. OGTTs were performed at 6 month (±3 month) intervals. For each OGTT, fasting samples were obtained before oral glucose administration (1.75 g per kilogram; maximum, 75 g of carbohydrate) and then at 30, 60, 90 and 120 minutes. If OGTTs were in the diabetic range, participants were asked to return for confirmation with another OGTT (unless contraindicated). The procedure for the IVGTTs has been described elsewhere.

**Laboratory Measures.** Methodologies for assessing autoantibody positivity in DPT-1 have been described (9). These included measurements of ICAs by indirect immunofluorescence and insulin autoantibodies by competitive fluid-phase radioassay. Plasma glucose was measured by the glucose oxidase method. Insulin and C-peptide were measured by radioimmunoassay. The interassay coefficient of variation for the C-peptide assay was 6.9% in a reference pool with relatively high values and 7.8% in a reference pool with relatively low values. Fasting C-peptide values in the undetectable range (<0.2 ng/ml) were assigned a value of 0.1 ng/ml for the analyses.

**Data Analysis.** For group and paired comparisons, t-tests and chi-square tests were utilized. Spearman correlation was utilized to assess association. Cox proportional hazards regression was utilized for assessing T1D associations over time. Glucose tolerance abnormalities were defined as: impaired fasting glucose=fasting glucose value 100-125 mg/dl; indeterminate=30, 60, and/or 90-
minute glucose value ≥200 mg/dl; impaired glucose tolerance=2-hr glucose value 140-199 mg/dl. The thresholds for diabetes were a fasting glucose value ≥126 mg/dl and/or a 2-hr glucose value ≥200 mg/dl. The sum of C-peptide levels after 30 minutes was calculated by subtracting the 30 minute values from the 60, 90, and 120 minute values and adding the sum of the differences. The FPIR was defined as the sum of insulin levels at the 1st and 3rd minutes of the IVGTT. The trapezoidal rule was used to calculate OGTT areas under the curve. The SAS 9.1.3 version was used for the analyses. All p-values are 2-sided.

RESULTS

Five-hundred and four DPT-1 participants less than 15 years of age were included in the overall study cohort (n=504; 9.2±3.1 years; 59% male). Of these individuals, data were analyzed for sequential glucose and C-peptide levels in 36 (9.0±3.1 years; 58% male) who progressed to T1D (progressors) and 80 (9.0±3.3 years; 65% male) who did not progress to T1D (non-progressors). The progressors had OGTTs performed every 6±3 months for at least 2 years prior to diagnosis, whereas the non-progressors had OGTTs performed every 6±3 months for at least 2 years prior to the last visit. The progressors and non-progressors all had normal glucose tolerance at baseline.

Glucose and C-peptide curves from OGTTs performed at baseline, and at 2.0 and 0.5 years prior to diagnosis in the progressors, or at baseline and at 2.0 and 0.5 years prior to the last visit in the non-progressors are shown in Figure 1. Glucose levels (Figure 1A) increased significantly from baseline to 0.5 years at all OGTT time points in both the progressors and non-progressors, but to a greater extent in the progressors. The increase in the AUC glucose from baseline to 0.5 years was highly significant for both (p<0.001).

C-peptide levels (Figure 1B) increased significantly at each OGTT time point from baseline to 0.5 years in the non-progressors (p<0.001 for the AUC C-peptide from baseline to 0.5 years). However, the change in C-peptide levels from baseline to 0.5 years in the progressors varied according to the OGTT time point. Fasting and 120 minute C-peptide levels increased from baseline to 0.5 years (p<0.01 and p<0.05, respectively) in the progressors, but there was no significant change at the other OGTT time points, nor in the AUC C-peptide (p=0.936).

We compared the 30-0 minute C-peptide difference at baseline and every 6 months for 2 years prior to diagnosis in the progressors with the 30-0 minute C-peptide difference at baseline and at corresponding times prior to the last visit in the non-progressors. [Among the 504 participants at baseline, there was a positive correlation between the 30-0 minute C-peptide difference and the FPIR (r=0.50, p<0.001).] The 30-0 minute C-peptide difference (Figure 2A) was similar at baseline; however, by 2.0 years that difference was lower in the progressors (p<0.01). Among the progressors, the 30-0 minute C-peptide difference declined from baseline to 0.5 years before diagnosis (p<0.01).

Whereas the 90-60 minute C-peptide difference (Figure 2B) decreased (i.e., the 90 minute value was less than the 60 minute value) at all times prior to the last visit in the non-progressors, it increased at all times prior to diagnosis in the progressors. This contrast was not only significant at 2.0 (p<0.01), 1.0 (p<0.01), and 0.5 years (p<0.001), but also at baseline (p<0.05).

As an overall measure of later C-peptide responsiveness to oral glucose, we utilized the sum of each of the C-peptide differences of the 30 minute value subtracted from the values at 60, 90 and 120 minutes (C-peptide sum after 30 minutes). Figure 3 shows that those values were significantly higher in the progressors than in the non-progressors at corresponding time points from 2.0 years to
1.0 year. Values were also higher, but not significantly so, at baseline and at 0.5 years. Among the progressors, the C-peptide sum after 30 minutes increased significantly from baseline to all subsequent time points (p<0.05 from baseline to 2.0 years and to 0.5 years; p<0.01 from baseline to 1.5 years and to 1.0 year). Consistent with the above findings, the timing of the peak C-peptide was delayed in the progressors. The percentage of those with the peak C-peptide occurring at 120 minutes was significantly higher in the progressors at 2.0 years (39% vs. 21%, p<0.05). By 0.5 years the peak C-peptide occurred at 120 minutes in 56% of the progressors compared with 18% of the non-progressors (p<0.001). Actual peak C-peptide levels did not differ between the progressors and non-progressors until 0.5 years (p<0.01). Among the full cohort of 504 at baseline, the occurrence of the peak C-peptide at 120 minutes and the 90-60 minute C-peptide difference (above vs. below 0) were both highly predictive of T1D with and without age as a covariate (p<0.001); the peak C-peptide level was somewhat predictive (p=0.028), but not with age in the model. Figure 4 shows C-peptide changes in the progressors who had OGTTs 0.5 years before diagnosis and at diagnosis (n=66), and in the non-progressors who had OGTTs 0.5 years before the last visit and at the last visit (n=192). The 30-0 minute C-peptide difference (Figure 4A) declined considerably in the progressors (p<0.001). The C-peptide sum after 30 minutes declined somewhat at diagnosis, but not below levels in the non-progressors at the last visit (Figure 4B). Even at diagnosis the delay in the peak C-peptide persisted. The peak C-peptide occurred at 120 minutes in 52% of the progressors at diagnosis compared with 23% of the non-progressors at their last visit (p<0.001).

CONCLUSIONS

The data suggest that the early C-peptide response to oral glucose is decreased for at least two years before the diagnosis of T1D, and is especially decreased as diagnosis approaches. Although the early C-peptide response to the glucose challenge declines, C-peptide levels increase at later time points. This was evident in the interval from 60 to 90 minutes. The C-peptide increase in that interval in the progressors contrasted with the decline in the non-progressors. It appears that the increase in C-peptide levels from 60 to 90 minutes can occur even three years before diagnosis. This prolonged increase in C-peptide levels after the glucose challenge is also manifested by a delayed peak C-peptide at least two years before diagnosis.

It is possible that the continuing increase in C-peptide levels after 30 minutes in progressors occurs as a result of the deficient early C-peptide response. However, this later C-peptide response still does not prevent glucose levels from rising as is evident in Figure 1. The decrease in the C-peptide sum after 30 minutes at diagnosis suggests that the later response is also failing by that time.

There is little longitudinal data available regarding the metabolic progression to T1D. Although we have previously examined changes in C-peptide and glucose indexes with progression to T1D (4), data pertaining to the timing of the C-peptide response prior to the diagnosis of T1D has not been reported, nor has the prediction of T1D by the timing of the C-peptide response been reported. A decreased early insulin response was a risk factor for progression to type 2 diabetes (T2D) in Pima Indians. However, in contrast with our findings for T1D, a decreased (rather than increased) later insulin response was predictive of T2D in Pima Indians with impaired glucose tolerance (10).

The decreased early C-peptide response together with the increased later C-peptide response to oral glucose that we observed in the “pre-diabetic” state of T1D is similar to
the abnormal insulin responses to oral glucose in patients already diagnosed with T2D (11,12). This suggests that there could be some commonality between the disorders in the progression of metabolic abnormalities.

The analysis was limited to children, since the non-progressors were appreciably older than the progressors in the full DPT-1 cohort. By restricting the analysis to younger individuals, differences in progression due to an age effect were minimized. Also, among those who developed T1D, pathogenetic heterogeneity related to age was lessened. The numbers were insufficient to specifically examine the older onset age group.

Since a number of the non-progressors would probably ultimately develop T1D, and thus were not metabolically normal, differences between progressors and a normal reference group could be even more substantial. It is of interest that there was such a marked increment in C-peptide over time in the non-progressors. This could represent typical changes with aging, early pathogenetic changes, or both. The pattern of change suggests increasing insulin resistance over time.

The findings in this report help to explain why such measures as peak C-peptide and AUC C-peptide values provide relatively little information with regard to the prediction of T1D and its natural history. Those indexes change little with progression because the deficient early C-peptide response to the oral glucose challenge is somewhat balanced by a continuing, compensatory response until close to diagnosis. Thus, the peak C-peptide and AUC C-peptide indexes fail to detect the substantial changes in the β-cells that are occurring years before diagnosis. It is evident that OGTTs can yield appreciably more prediction and natural history information when they are partitioned according to the time after the glucose challenge.

FIGURE LEGENDS

Figure 1A
Shown are glucose curves from OGTTs at baseline, and 2.0 and 0.5 years before diagnosis in the progressors, and at baseline and corresponding times prior to the last visit in the non-progressors. The points represent mean±SD glucose values. In both the progressors and non-progressors, glucose levels increased substantially at all OGTT time points (AUC glucose: p<0.001 from baseline to 0.5 years in both groups). (Mean values are shown for the time prior to diagnosis or to the last visit.)

Figure 1B
Shown are C-peptide curves from OGTTs at baseline, and 2.0 and 0.5 years before diagnosis in the progressors, and at baseline and corresponding times prior to the last visit in the non-progressors. The points represent mean±SD C-peptide values. In the non-progressors, C-peptide levels increased at all OGTT time points, (AUC C-peptide: p<0.001 from baseline to 0.5 years). In the progressors, although the fasting and 120 minute C-peptide levels were higher at 0.5 years than at baseline, there was no significant overall change (AUC C-peptide: p=0.936 from baseline to 0.5 years). (Mean values are shown for the time prior to diagnosis or to the last visit.)

Figure 2A
Shown is the difference (mean±SEM) in C-peptide levels from 0 to 30 minutes (the 30-0 minute C-peptide difference) according to the time before diagnosis (progressors) or the time before the last visit (non-progressors). The 30-0 minute C-peptide difference was consistently lower in the
progressors than in the non-progressors. (Mean values are shown for the time prior to diagnosis or to the last visit.)

**Figure 2B**
Shown is the difference (mean±SEM) in C-peptide levels from 60 to 90 minutes (the 90-60 minute C-peptide difference) according to the time before diagnosis (progressors) or the time before the last visit (non-progressors). At every time prior to diagnosis the 90-60 minute C-peptide difference was positive in the progressors, whereas at every time prior to the last visit it was negative in the non-progressors. (Mean values are shown for the time prior to diagnosis or to the last visit.)

**Figure 3**
Shown is the C-peptide sum after 30 minutes (mean±SEM) according to the time before diagnosis (progressors) or the time before the last visit (non-progressors). The values were higher in the progressors from baseline to 0.5 years. (Mean values are shown for the time prior to diagnosis or to the last visit.)

**Figure 4A**
Shown is the 30-0 minute C-peptide difference (mean±SEM) in the progressors who had OGTTs 0.5 years before diagnosis and at diagnosis, and in the non-progressors who had OGTTs 0.5 years before the last visit and at the last visit. The 30-0 minute C-peptide difference declined considerably in the progressors.

**Figure 4B**
Shown is the C-peptide sum after 30 minutes (mean±SEM) in the progressors who had OGTTs 0.5 years before diagnosis and at diagnosis, and in the non-progressors who had OGTTs 0.5 years before the last visit and at the last visit. The C-peptide sum after 30 minutes declined, but did not fall below that in the non-progressors.
REFERENCES


Figure 1A

Progressors (n=36)

Non-Progressors (n=80)

Glucose (mg/dl)

Minutes

3.7 Years • 2.0 Years • 0.5 Years

Until Diagnosis

Figure 1B

Progressors (n=36)

Non-Progressors (n=80)

C-peptide (mg/ml)

Minutes

3.7 Years • 2.0 Years • 0.5 Years

Until Diagnosis
Figure 2A

(30-0 Minutes)

- Non-Progressors (n=80)  
- Progressors (n=36)

+ p<0.01 (for Non-Progressors vs. Progressors)

Figure 2B

(90-60 Minutes)

- Non-Progressors (n=80)  
- Progressors (n=36)

+ p<0.05; ++ p<0.01; +++ p<0.001 (for Non-Progressors vs. Progressors)
Figure 3

![Figure 3](image)

Figure 4

![Figure 4](image)
Figure 4B

+ \( p < 0.01 \) (for Non-Progressors vs. Progressors)

Non-Progressors \( (n=192) \)

Progressors \( (n=66) \)

60-120 Minute C-peptide Sum
(From 30 Minute Value)

0.5 Years from Diagnosis (Progressors) or Last Visit (Non-Progressors)

At Diagnosis (Progressors) or Last Visit (Non-Progressors)