Glucose and C-peptide Changes in the Peri-Onset Period of Type 1 Diabetes in the Diabetes Prevention Trial-Type 1

Jay M. Sosenko, MD, Jerry P. Palmer, MD, Lisa Rafkin-Mervis, MS CDE, Jeffrey P. Krischer, PhD, David Cuthbertson, MS, Della Matheson, RN, Jay S. Skyler, MD

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

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**Objective:** We examined metabolic changes in the period immediately following the diagnosis of type 1 diabetes (TID) and in the period leading up to its diagnosis in Diabetes Prevention Trial-1 (DPT-1) participants.

**Research Design and Methods:** The study included oral insulin trial participants and parenteral insulin trial controls (n=63) diagnosed with a 2-hr diabetic oral glucose tolerance test (OGTT) that was confirmed by another diabetic OGTT within three months. Differences in glucose and C-peptide levels between the OGTTs were assessed.

**Results:** Glucose levels increased at 90 (p=0.006) and 120 minutes (p<0.001) from the initial diabetic OGTT to the confirmatory diabetic OGTT (mean±SD interval: 5.5±2.8 weeks). Peak C-peptide levels fell substantially between the OGTTs (median change: -14.3%, p<0.001). Among the 55 individuals whose last non-diabetic OGTT was approximately six months prior to the initial diabetic OGTT, peak C-peptide levels decreased between these two OGTTs (median change: -14.0%, p=0.052). Among those same individuals the median change in peak C-peptide levels from the last normal OGTT to the confirmatory OGTT (interval: 7.5±1.3 months) was -23.8% (p<0.001). Median rates of change in peak C-peptide levels were 0.00 ng/ml/month (p=0.468, n=36) from approximately 12 months to 6 months before diagnosis, -0.10 ng/ml/month (p=0.059, n=55) from 6 months before diagnosis to diagnosis, and -0.43 ng/ml/month (p=0.002, n=63) from the initial diabetic OGTT to the confirmatory diabetic OGTT.

**Conclusion:** It appears that post-challenge C-peptide levels begin to decrease appreciably in the six months prior to diagnosis and decrease even more rapidly within three months after diagnosis.
Evidence suggests that there is progressive metabolic dysfunction prior to and following the diagnosis of type 1 diabetes (T1D). A considerable number of individuals who develop T1D appear to have a gradual metabolic deterioration (1-3) until within six months of diagnosis after which the deterioration becomes more rapid (4). Following diagnosis, there also appears to be a progressive loss of insulin secretion (5-8). However, evidence for this has been derived from studies performed within a clinical context. Individuals were assessed after being diagnosed by clinical presentation and after therapeutic measures were initiated. There are no studies that have followed changes in insulin secretion from before the diagnosis of T1D to immediately after its diagnosis in humans. Such information would be highly useful for gauging how quickly interventions should be implemented to delay or prevent the loss of insulin secretion in T1D. Interventions that are initiated before a substantial loss of insulin secretion occurs could be more efficacious.

The Diabetes Prevention Trial-1 (DPT-1) provides unique data for examining insulin secretion in the early stages of T1D (9,10). Oral glucose tolerance tests (OGTTs) were performed every six months for diagnostic surveillance, so that the diagnosis of T1D was captured very close to onset. Also, participants who had OGTTs in the diabetic range were confirmed for T1D with repeat OGTTs. These two features of the DPT-1 data were utilized to determine the rate and extent of metabolic deterioration that occurs in the peri-onset period of T1D.

**RESEARCH DESIGN AND METHODS**

**Subjects**—Sixty-three participants of the parenteral and oral insulin DPT-1 trials who were diagnosed with two consecutive diabetic 2-hr OGTTs (initial and confirmatory) are included in the analyses. Those in the intervention arm of the parenteral insulin trial (n=41) were excluded, since they received insulin (as per protocol) between the two diabetic OGTTs. Also excluded were those (n=8) whose interval between the two OGTTs was greater than three months. The algorithm for determining risk in DPT-1 has been described (9). The presence of islet cell autoantibodies was required for entry into both trials. Participants were considered to be at >50% 5-year risk and eligible for the parenteral insulin trial if either the first-phase insulin response on intravenous glucose tolerance testing was below a defined threshold and/or there were OGTT abnormalities. If those metabolic criteria were not present, but insulin autoantibodies were positive, the 5-year risk was considered to be 26-50% and participants were eligible for the oral insulin trial. There was no overall treatment effect in either trial.

**Procedures**—Participants in the parenteral insulin trial intervention group received recombinant human ultralente insulin, while those in the oral insulin trial intervention group received recombinant human insulin crystals. OGTTs were performed at 6 month (±3 months) intervals in both trials. All study treatments were to be suspended for three days prior to the OGTT. The dose of oral glucose was 1.75 g per kilogram (maximum, 75 g of carbohydrate). Samples were obtained for plasma glucose and C-peptide measurements in the fasting state and at 30, 60, 90 and 120 minutes. Insulin measurements were not obtained; there was concern over the formation of insulin autoantibodies. Individuals with glucose values in the diabetic range at a routine visit were asked to return for confirmation by an OGTT within 60 days (some returned beyond 60 days), unless this was clinically contraindicated. Participants were to continue the same study regimen they
had been using prior to the initial diabetic OGTT. The age at the first of the diabetic OGTTs was considered the age at diagnosis. The thresholds for diabetes were fasting glucose values $\geq 126$ mg/dl and/or 2-hr glucose values $\geq 200$ mg/dl.

**Laboratory Measures**—Plasma glucose levels were measured by the glucose oxidase method. C-peptide levels 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**—The statistical significance of percent change against a null hypothesis of no change was assessed with signed rank tests. Pearson correlations and linear regression were utilized to assess associations. Values for rates of change in peak C-peptide were obtained by dividing the difference in peak C-peptide values for an interval by the length of the interval. OGTT areas under the curve (AUC) were calculated with the trapezoidal rule. Designated time intervals prior to diagnosis were within ±3 months. SAS 9.1.3 was used for the analyses. All p-values are 2-sided.

**RESULTS**

Sixty-three DPT-1 participants (51% female) are included in the analyses. All had a complete OGTT in the diabetic range that was confirmed by a second complete OGTT within an interval of 3 months. Of these, 31 were in the parenteral insulin trial and 32 were in the oral insulin trial (15 in the intervention group). The mean±SD age at the first diabetic OGTT was 13.2±6.9 years. The mean interval between the diabetic OGTTs was 5.5±2.8 weeks.

Table 1 shows glucose levels for the initial and confirmatory OGTTs. There was a tendency for glucose levels to increase between the first diabetic to the confirmatory diabetic OGTT with statistically significant increases at 90 (p=0.006), and 120 minutes (p<0.001), and for the AUC glucose (p=0.016). Figure 1A shows the corresponding percent changes.

Table 2 shows the C-peptide levels for the initial and confirmatory OGTTs. There were significant declines in C-peptide levels at each post-challenge time point, and for AUC and peak C-peptide values (p<0.01 for all). Figure 1B shows the corresponding percent changes. The median percent change in peak C-peptide levels was -14.3% (p<0.001). There was less of a decline in fasting C-peptide levels (-6.7%, p=0.416). When the fasting C-peptide/fasting glucose and the AUC C-peptide/AUC glucose ratios were examined, percent changes were appreciable for both the former (-10.3%, p=0.046) and the latter (-16.7%, p<0.001).

The change in AUC glucose values between the two diabetic OGTTs was positively associated with the length of the interval between them (r=0.32, p=0.011), whereas there was an inverse correlation of change of peak C-peptide levels with that interval (r=-0.31, p=0.014). Thus, the fall in peak C-peptide levels increased with longer intervals. A scatterplot for the association of the change in peak C-peptide levels between the OGTTs and the interval between the diabetic OGTTs (with the removal of an outlier) is shown in Figure 2. The correlation was almost identical (r=-0.31, p=0.016) with the outlier excluded. With an allowance for the peak C-peptide levels from the first diabetic OGTT, the slope for the association of change in peak C-peptide levels with the interval between the diabetic OGTTs was -0.56 ng/ml/month.

Of the 63 individuals included in the analysis, 55 had an OGTT approximately six months prior to the initial diabetic OGTT. The median percent change for the peak C-
The percent change in the peak C-peptide from the last non-diabetic OGTT to the second diabetic OGTT (mean±SD interval: 7.5±1.3 months) was -23.8% (p<0.001). The AUC C-peptide/AUC glucose percent change was even more marked (-45.7%, p<0.001) in that interval.

Figure 3 shows the median rates of change in peak C-peptide levels over intervals in the peri-onset period. The values were obtained by dividing the difference in peak C-peptide values for an interval by the length of the interval. There was minimal change (0.00 ng/ml/month, p= 0.468, n=36) in peak C-peptide from approximately 12 months to 6 months prior to diagnosis. There was a greater rate of decline in peak C-peptide levels from 6 months before diagnosis to diagnosis (-0.10 ng/ml/month, p=0.059, n=55), and an even greater rate of decline from diagnosis to within 3 months after diagnosis (-0.43 ng/ml/month, p=0.002, n=63).

CONCLUSIONS

The data in this report show that, on average, C-peptide levels decreased substantially in the interval from diagnosis to within three months after diagnosis. These changes occurred even with glucose levels still in a range associated with minimal or no symptoms.

We previously examined metabolic progression prior to diagnosis in DPT-1 participants (4). In that report peak C-peptide levels were consistent from approximately 30 to 6 months before diagnosis, after which levels declined. This report extends observations to the post-diagnostic period and suggests that there is an acceleration of post-challenge C-peptide loss once glucose levels are in the diabetic range. The median decline of -23.8% in peak C-peptide levels from the last non-diabetic OGTT to the confirmatory OGTT indicates that there is a marked loss of insulin secretion in the peri-onset period. The extent to which this loss is reversible cannot be determined from the data.

Estimates for the rate of change of peak C-peptide levels in the post-diagnostic period were obtained in two ways. In one approach (Figure 2) a regression analysis was utilized, whereas in the other approach (Figure 3) the estimate was derived from an analysis based on rate of change calculated for each individual. The rate of decline was substantial with either approach.

Glucose levels seem to have been maintained relative to the decline in C-peptide levels after diagnosis. This suggests the possibility that compensatory mechanisms for glucose homeostasis are at play, such as an increase in insulin sensitivity. Since C-peptide levels are only indicative of insulin secretion, it is also possible that a slowing of insulin degradation could have contributed to the maintenance of glucose levels.

For calculations of the rate of change in peak C-peptide levels, it was assumed that the rate of change was constant throughout the interval. This assumption is of particular importance in the interval from six months before diagnosis to diagnosis, since one cannot discern from the data the pattern of C-peptide decline within that period. Thus, the rate of decrease in C-peptide may be more rapid closer to diagnosis and similar to the rate of decline in C-peptide after diagnosis. Also, it should be emphasized that the average change provides an overall picture; individual patterns of change vary considerably.

Participation in the DPT-1 trials could have influenced the findings. However, we excluded those on parenteral insulin from the analyses and there was no overall effect from either insulin intervention. Knowledge of the results of the first diabetic OGTT could have resulted in lifestyle changes (11) or perhaps even have caused some to attempt to lower glucose levels with medication. Still, it
is doubtful that such interventions would explain the large degree of C-peptide loss.

There are no prior studies that have examined metabolic changes from before diagnosis to after diagnosis with OGTT surveillance. Also, no studies have assessed metabolic changes in newly diagnosed individuals as close to the onset of T1D. C-peptide levels appear to be much lower when T1D is clinically diagnosed (12-14) than when it is diagnosed through OGTT surveillance. It is important to emphasize that of all individuals diagnosed with T1D in DPT-1, 75% were asymptomatic (9). How our observations relate to the rate of decline of insulin secretion in symptomatic, clinically diagnosed patients is unknown. Studies of clinically diagnosed patients suggest that there is a progressive loss of insulin secretion which can be decreased by effective glucose control (15,16).

The marked rate of decline of C-peptide levels in the peri-onset period provides a strong rationale for developing early interventions to prevent or delay the progression to T1D. Moreover, the data suggest that post-diagnostic interventions should be developed for application as close to the diagnosis of T1D as possible.
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Table 1. Glucose (mg/dl) Values+ of Initial and Confirmatory Diabetic OGTTs (n=63)

|                    | First OGTT             | Confirmatory OGTT         | p-value* |
|--------------------|------------------------|---------------------------|----------|
| Glucose Fasting    | 106 (91,115)           | 107 (98,119)              | 0.117    |
| Glucose 30 minutes | 195 (168,217)          | 194 (170,216)             | 0.760    |
| Glucose 60 minutes | 241 (208,267)          | 254 (222,283)             | 0.089    |
| Glucose 90 minutes | 253 (234,284)          | 279 (238,310)             | 0.006    |
| Glucose 120 minutes| 246 (212,280)          | 283 (243,332)             | <0.001   |
| Glucose AUC (2-hr) | 25.6 (24.0,28.2)       | 27.5 (24.2,31.1)          | 0.016    |

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+ Median values are shown. Values at the 25th and 75th percentiles are in parentheses.

Table 2. C-peptide (ng/ml) Values+ of Initial and Confirmatory Diabetic OGTTs (n=63)

|                    | First OGTT             | Confirmatory OGTT         | p-value |
|--------------------|------------------------|---------------------------|---------|
| C-peptide Fastings | 1.5 (0.7,2.1)          | 1.2 (0.8,1.7)             | 0.054   |
| C-peptide 30 minutes| 2.6 (1.9,4.0)         | 2.2 (1.6,3.5)             | 0.001   |
| C-peptide 60 minutes| 3.1 (2.1,4.5)         | 2.7 (1.9,3.8)             | <0.001  |
| C-peptide 90 minutes| 3.6 (2.3,5.3)         | 3.0 (2.1,4.3)             | 0.001   |
| C-peptide 120 minutes| 3.5 (2.5,5.5)         | 3.2 (2.1,5.0)             | 0.004   |
| C-peptide Peak     | 3.8 (2.7,5.9)          | 3.2 (2.2,5.0)             | <0.001  |
| C-peptide AUC (2-hr)| 350 (249,501)         | 309 (212,443)             | <0.001  |

+ Median values are shown. Values at the 25th and 75th percentiles are in parentheses.
FIGURE LEGENDS

**Figure 1A: Percent Changes in Glucose Indices after Diagnosis**
Shown are the medians for the percent changes of glucose indices from the initial diabetic OGTT to the confirmatory diabetic OGTT. Glucose levels tended to increase especially at the later time points of the OGTT.

**Figure 1B: Percent Changes in C-peptide Indices after Diagnosis**
Shown are the medians for the percent changes of C-peptide indices from the initial diabetic OGTT to the confirmatory diabetic OGTT. With the exception of the fasting C-peptide, there was more than a 10% median decline for all of the indices.

**Figure 2: Association between Change in Peak C-peptide and Time after Diagnosis**
Shown is the scatterplot for the association between the change in peak C-peptide levels and the time after diagnosis. The amount of decline becomes more substantial with increasing time after diagnosis. [An outlier was removed with a change in peak C-peptide of -8.8 ng/ml and a time after diagnosis of 8.0 weeks (r=-0.31, p= 0.014 with the outlier included).] When an allowance was made for the peak C-peptide at the first diabetic OGTT, the slope for the difference in peak C-peptide vs. time after diagnosis was -0.56 ng/ml/month.

**Figure 3: Rates of Change in Peak C-peptide in Peri-Onset Period**
Shown is the rates of change of peak C-peptide levels according to intervals prior to and after diagnosis. C-peptide levels changed minimally between approximately 12 months and 6 months prior to diagnosis. There was a decline in the 6 months prior to diagnosis that was more substantial in the period following diagnosis.
Figure 1A: Percent Changes in Glucose Indices after Diagnosis

Figure 1B: Percent Changes in C-peptide Indices after Diagnosis
Figure 2: Association between Change in Peak C-peptide and Time after Diagnosis

![Graph showing the association between change in peak C-peptide and time after diagnosis. The correlation coefficient (r) is -0.31, p = 0.016, n=62.]

Figure 3: Rates of Change in Peak C-peptide in Peri-Onset Period

![Graph showing the rates of change in peak C-peptide in the peri-onset period. The median rate of change is significantly different between 12 months to 6 months (p = 0.059) and 6 months to diagnosis (p = 0.002).]

Peri-Onset Changes in Type 1 Diabetes