Glucose Excursions between States of Glycemia with Progression to Type 1 Diabetes in the Diabetes Prevention Trial-Type 1 (DPT-1)

Running Title: Glucose Excursions Prior to Type 1 Diabetes

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**Objective.** We characterized fluctuations between states of glycemia in progressors to type 1 diabetes (T1D), and studied whether those fluctuations are related to the early C-peptide response to oral glucose.

**Research Design and Methods.** Sequential oral glucose tolerance tests (OGTTs) from differing states of glycemia were compared within individuals for glucose and C-peptide. Dysglycemic OGTTs were compared with normal OGTTs, while transient diabetic OGTTs were compared with subsequent non-diabetic OGTTs, and with OGTTs performed at diagnosis.

**Results.** Of 135 progressors with $\geq 4$ OGTTs, 30 (22%) went from normal to dysglycemic OGTTs at least twice. Area under the curve (AUC) glucose values from the second normal OGTT were higher ($p<0.001$) than values from the first normal OGTT. Among 98 progressors whose dysglycemic and normal OGTTs were synchronized for the time before diagnosis, despite higher glucose levels ($p<0.01$ at all time points) in the dysglycemic OGTTs, 30-0 minute C-peptide difference values changed little. Likewise, 30-0 minute C-peptide difference values did not differ between transient diabetic OGTTs and subsequent (within 3 months) non-diabetic OGTTs in 55 progressors. In contrast, as glucose levels increased overall from the first to last OGTTs before diagnosis ($p<0.001$ at every time point, $n=207$), 30-0 minute C-peptide difference values decreased ($p<0.001$).

**Conclusions.** Glucose levels fluctuate widely as they gradually increase overall with progression to T1D. As glucose levels increase, the early C-peptide response declines. In contrast, glucose fluctuations are not related to the early C-peptide response. This suggests that changes in insulin sensitivity underlie the glucose fluctuations.

Glucose levels can fluctuate substantially during the progression to T1D (1). In this report we examine glucose variability and explore its basis by comparing the C-peptide response to oral glucose between states of glycemia. The findings provide additional insights into the metabolic progression to T1D.

**RESEARCH DESIGN AND METHODS**

**Subjects.** Diabetes Prevention Trial-Type 1 (DPT-1) participants and procedures have been described in detail (2,3). All were islet cell autoantibody (ICA) positive. Those analyzed for this report participated in either the parenteral insulin (2) or oral insulin (3) trials.

**Procedures.** The interventions for the parenteral and oral trials were recombinant human ultralente insulin and recombinant human insulin crystals, respectively. Visit intervals were 6 months. Diagnoses of T1D were frequently determined from the OGTTs at routine visits. OGTTs in the diabetic range were confirmed with another OGTT (unless symptoms occurred or there was marked fasting hyperglycemia).

**Laboratory Measures.** 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 <0.2 ng/ml were assigned a value of 0.1 ng/ml for the analyses. OGTTs included in the analysis
had complete glucose and C-peptide measurements at all time points.

**Data Analysis.** Dysglycemia was defined as any of the following: 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 a diabetic OGTT were a fasting glucose value ≥126 mg/dl and/or a 2-hr glucose value ≥200 mg/dl. The 30-0 minute C-peptide difference, defined as the fasting C-peptide value subtracted from the 30 minute C-peptide value, was used to indicate the early C-peptide response. Since the early C-peptide response diminishes with progression to T1D (4), it was necessary to take into account the influence of the time from diagnosis that the dysglycemic and normal OGTTs were performed. Thus, OGTTs were paired according to: the first normal OGTT to the subsequent last dysglycemic OGTT; the first dysglycemic OGTT to the subsequent last normal OGTT; and a normal OGTT and a dysglycemic OGTT that were synchronized to nearly the same time (on average) before diagnosis. The synchronization was performed by pairing the first dysglycemic OGTT with the last normal OGTT (both after randomization), as long as those OGTTs were within two years of each other. A transient diabetic OGTT was defined as an OGTT in the diabetic range that was followed by an OGTT that was not in the diabetic range. If a participant had more than one transient OGTT, the first was used for the analysis.

Paired and unpaired t-tests, and the Wilcoxon Rank Sum Test were used to assess differences. Non-parametric testing was utilized particularly in analyses that involved diabetic range OGTTs because of their glucose distributions. 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**

There were 258 progressors to T1D in the DPT-1 trials, of whom 207 (mean±SD: 11.4±7.8 years; 58% male) were studied. All had a baseline OGTT and at least one OGTT during follow-up. Of the 135 progressors from both the parenteral and oral insulin trials with a minimum of 4 OGTTs, 30 (22%) had an alternating OGTT pattern (not necessarily consecutive) of normal, dysglycemic, normal again, and then dysglycemic again over their course of progression to T1D. Area under the curve (AUC) glucose values from those OGTTs are shown in Figure 1. As expected, there were significant increases in AUC glucose values when the DYSOGTTs were compared to their prior NLOGTTs (p<0.001 for each difference). AUC glucose values from the second NLOGTTs were significantly higher than those from the first NLOGTTs (p<0.001). Also, AUC glucose values from the second DYSOGTTs were significantly higher than those from the first DYSOGTTs (p<0.01).

We assessed whether the state of glycemia was related to the early insulin response to an oral glucose challenge. For this purpose, we used the 30-0 minute C-peptide difference as a measure of early insulin secretion (4). Table 1 shows early C-peptide response values according to pairings of DYSOGTTs and NLOGTTs (see Research Design and Methods). The early C-peptide response was substantially lower (p<0.001) in the DYSOGTTs (p<0.01 for both the parenteral and oral trials separately) when they occurred after the NLOGTTs (NL→DYSGLY; n=146). However, when the DYSOGTTs preceded the NLOGTTs (DYSGLY→NL; n=70), the early C-peptide response from the DYSOGTTs did not differ significantly from that of the NLOGTTs (nor when the trials were analyzed separately). In fact, the early C-peptide response tended to be higher in the
DYSOGTTs. When the DYSOGTTs and the NLOGTTs were synchronized for the time before diagnosis (SYNCH; n=98), the early C-peptide response was similar between the NLOGTTs and the DYSOGTTs (also when the trials were analyzed separately). Thus, when the time to diagnosis was minimized as a factor, the early C-peptide response did not vary between the normal and dysglycemic states. In addition, no significant difference was found between the normal and dysglycemic states in the ratio of the C-peptide response over the glucose response from 0 to 30 minutes.

We examined the entire glucose and C-peptide curves from the OGTTs among SYNCH, the group whose DYSOGTTs and NLOGTTs were synchronized to the same time before diagnosis (Figure 2). As expected, glucose levels (Figure 2A) from the DYSOGTTs were significantly higher (p<0.01) at every OGTT time point. The differences were especially apparent from 60-120 minutes. Even though glucose levels were higher from the DYSOGTTs, C-peptide levels (Figure 2B) were also significantly higher in the fasting state (p<0.05), at 90 minutes (p<0.05), and at 120 minutes (p<0.001). The sum of the differences between the 30 minute C-peptide value and the subsequent values during the OGTT was significantly higher in the DYSOGTTs than in the NLOGTTs (3.20±3.47 ng/ml vs. 2.14±3.55 ng/ml, p=0.008). Body mass index (BMI) values did not differ between DYSOGTTs and NLOGTTs in 49 paired measurements concurrent with the paired DYSOGTTs and NLOGTTs (19.9±5.2 kg/m² vs. 19.6±4.9 kg/m², respectively).

Among 60 progressors with TDOGTTs, we analyzed data from 55 who had a non-diabetic OGTT (NDOGTT) within three months (mean±SD: 36±16 days) of the TDOGTT. Glucose levels (Figure 1A-Online Appendix available at http://diabetes.diabeteesjournals.org) were higher in the TDOGTTs at the later time points (p<0.01 at 60 minutes; p<0.001 at both 90 and 120 minutes). Despite those higher glucose levels in the TDOGTTs, C-peptide levels (Figure 1B-Online Appendix) were similar at all time points, except for higher 120 minute C-peptide levels (p<0.01) in the TDOGTTs.

Similar to the findings from the comparison between the DYSOGTTs and the NLOGTTs, the early C-peptide response did not significantly differ between the TDOGTTs and the NDOGTTs. Also, there was no significant difference in the ratio of the C-peptide response over the glucose response from 0 to 30 minutes.

Of the 55 TDOGTTs analyzed above, 38 also had a subsequent OGTT at the time of diagnosis (DOGTT). The mean±SD difference in time from the TDOGTTs to the DOGTTs was 0.9±0.8 years. Glucose levels (Figure 1A-Online Appendix) were significantly higher at every time point in the DOGTTs than in the TDOGTTs, especially post-challenge (p<0.001 for all ≥30 minutes). C-peptide levels (Figure 1B-Online Appendix) were significantly lower in the DOGTTs at every post-challenge time point (p<0.01) after 30 minutes. The early C-peptide response was also significantly lower in the DOGTTs (p<0.001) than in the TDOGTTs.

Figure 3 shows the C-peptide response relative to the glucose response from the fasting state to 30 minutes in the serial TDOGTTs, NDOGTTs and DOGTTs from those 38 progressors. The C-peptide response relative to the glucose response was greater in both the TDOGTTs (p<0.001) and the NDOGTTs (p<0.017) than in the DOGTTs. There was no significant difference between the TDOGTTs and the NDOGTTs.

In the 207 progressors who had two OGTTs, glucose levels increased overall from the first (mean±SD: 2.8±1.4 years before diagnosis) to the last OGTTs (0.6±0.5 years before
diagnosis) at every time point. In contrast with the lack of change in the early C-peptide response between states of glycemia, the early C-peptide response declined markedly from the first OGTT to the last OGTT (mean±SD: 2.38±1.25 ng/dl to 1.87±1.11 ng/dl, p<0.001).

**DISCUSSION**

We have previously shown that on average glucose levels increase over time with progression to T1D (5). However, the data in this report suggest that within the individual, glucose levels do not increase in a simple, linear manner; rather there are wide fluctuations that occur on a background of gradually increasing glucose levels. The overall picture can perhaps best be described as a kind of ratcheting, as is evident in Figure 1. The second normal OGTT did not have the same degree of “normalcy” as the first normal OGTT. The data indicate that this pattern extends even into the higher ranges of glycemia as the onset of T1D approaches. There appear to be at least two distinct patterns of change in glucose levels during the course of progression to T1D, each occurring through a different mechanism. In one pattern, glucose levels increase over time as the early C-peptide response decreases. This pattern was evident when the first and last OGTTs were compared. The data suggest that the increasing glucose is at least in part attributable to a decline in early insulin secretion.

The second pattern, characterized by wide fluctuations of glucose levels, contrasts with the first pattern in that the excursions into the higher glucose range do not appear to be associated with a decrease in the early C-peptide response. The early C-peptide response was similar between the DYSOGTTs and NLOGTTs when they were synchronized to the time before diagnosis. Also, the early C-peptide response did not differ between the TDOGTTs and their subsequent NDOGTTs. Moreover, there were no significant differences in the ratio of the C-peptide response over the glucose response from 0 to 30 minutes between the DYSOGTTs and the NLOGTTs, and between the TDOGTTs and the NDOGTTs. Thus, two separate analyses at different ranges of glycemia were consistent in showing a lack of association between glucose fluctuations and the early C-peptide response.

The data appear to indicate that differences in the early C-peptide response between DYSOGTTs and NLOGTTs are a function of the time before diagnosis when the OGTT is performed. The fact that the early C-peptide response tended to be higher in the OGTT that came first, and was independent of the state of glycemia, is consistent with the decline in the early C-peptide response with progression to T1D.

Since glucose excursions were not related to the early C-peptide response, variation in glucose sensitivity could have been a factor. Although BMI values were not significantly higher when they were associated with the DYSOGTTs, the higher later OGTT C-peptide values in the DYSOGTTs is consistent with insulin data in non-obese adults with impaired glucose tolerance (6), and adults with diabetes (6,7). Data from other studies lend some support to the view that insulin resistance could be involved in the pathogenesis of T1D (8-12). Interestingly, it appears that increased insulin sensitivity could contribute to the remissions that occur following the diagnosis of T1D (13,14).

The DOGTTs had much higher glucose levels, and much lower C-peptide levels and early C-peptide responses than did the TDOGTTs. Thus, β-cell function is much more impaired when an OGTT is diagnostic of T1D than when it is transiently in the diabetic range. However, those with TDOGTTs represent a potential high risk target population for T1D prevention trials. Excursions into higher glucose ranges could exacerbate the loss of β-cell function through
factors such as glucotoxicity (15). Therefore, it seems reasonable to consider interventions that would decrease glucose variability, and perhaps ultimately, preserve β-cell function.

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Table 1. The Early C-peptide (ng/ml) Response (30-0 Minutes) According to the Sequence of Normal OGTT (NLOGTT) and Dysglycemic OGTT (DYSOGTT) Pairs before Diagnosis

|                | NL→DYSGLY (N=146) | DYSGLY→NL(N=70) | SYNCH**(N=98) |
|----------------|-------------------|-----------------|--------------|
|                | NLOGTT | DYSOGTT | NLOGTT | DYSOGTT | NLOGTT | DYSOGTT |
| 30-0 Minutes* | 2.45±1.25 | 2.01±1.64* | 1.96±1.11 | 2.25±2.00 | 2.24±1.38 | 2.27±1.87 |
| Years to Diabetes* | 2.97±1.32 | 0.63±0.45 | 1.31±0.81 | 2.90±1.22 | 1.66±0.83 | 1.72±0.97 |

*Mean±SD
**OGTTs synchronized to the time before diagnosis
+p<0.001 vs. NLOGTT

FIGURE LEGENDS

Figure 1. Shown is a sequence of alternating normal and dysglycemic OGTTs in progressors to T1D. Each point represents the mean AUC glucose from the OGTTs. The mean time before diagnosis is shown for each of the OGTTs. There were significant increases in the AUC glucose from each of the normal OGTTs to their subsequent respective dysglycemic OGTTs. There were also significant increases from the first normal OGTTs to the second normal OGTTs, and from the first dysglycemic OGTTs to the second dysglycemic OGTTs.

Figure 2. Shown are glucose (A) and C-peptide (B) values (mean±SD) for paired normal and dysglycemic OGTTs that were synchronized on average to the time before diagnosis in progressors to T1D. As expected, the glucose values from the dysglycemic OGTTs were substantially higher at every time point. Even though glucose levels were higher from the dysglycemic OGTTs, C-peptide values were also significantly higher in the fasting state, and at 90 and 120 minutes.

Figure 3. Shown are C-peptide responses in relation to glucose responses from 0 to 30 minutes in the TDOGTTs, the subsequent NDOGTTs (within 3 months) and the DOGTTs in progressors to T1D. The ratio of the C-peptide response over the glucose response from 0 to 30 minutes was significantly higher in both the TDOGTTs and the NDOGTTs than in the DOGTTs. (Median values are shown.)
Glucose Excursions Prior to Type 1 Diabetes

Figure 1

NGT = Normal Glucose Tolerance
DG = Dysglycemia

AUCGlucose (mg/dl/120 min)

Years Before Diagnosis

n=30
Figure 2

A

**Figure 2A**

![Graph showing glucose excursion in mg/dL over time for DYSOGTT and NLOGTT](image)

- **Y-axis:** Glucose (mg/dL)
- **X-axis:** Minutes

- **Legend:**
  - DYSOGTT (blue line)
  - NLOGTT (red line)

- **Statistical Notations:**
  - +p<0.01
  - ++p<0.001

- **Sample Size:** n=98

B

**Figure 2B**

![Graph showing C-peptide excursion (ng/ml) over time for DYSOGTT and NLOGTT](image)

- **Y-axis:** C-peptide (ng/ml)
- **X-axis:** Minutes

- **Legend:**
  - DYSOGTT (blue line)
  - NLOGTT (red line)

- **Statistical Notations:**
  - +p<0.05
  - ++p<0.001

- **Sample Size:** n=98
Figure 3

C-peptide (ng/ml)

Glucose (mg/dL)

+ p<0.01
++ p<0.001
(vs. DOGTT)

Fasting

n=38

30 Minutes