

Time to Peak Glucose and Peak C-peptide During the Progression to Type 1 Diabetes in the Diabetes Prevention Trial and TrialNet Cohorts

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Keywords: Type 1 diabetes, prediction, peak timing, glucose, C-peptide, OGTT

Abstract: 250/250

Manuscript: /4000

Tables: 3

This is the author's manuscript of the article published in final edited form as:

Voss, M. G., Cuthbertson, D. D., Cleves, M. M., Xu, P., Evans-Molina, C., Palmer, J. P., Redondo, M. J., Steck, A. K., Lundgren, M., Larsson, H., Moore, W. V., Atkinson, M. A., Sosenko, J. M., Ismail, H. M., & DPT-1 and TrialNet Study Groups. (2021). Time to Peak Glucose and Peak C-Peptide During the Progression to Type 1 Diabetes in the Diabetes Prevention Trial and TrialNet Cohorts. *Diabetes Care*, 44(10), 2329–2336. <https://doi.org/10.2337/dc21-0226>

Figures: 1

References: 31/40

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Abstract

Objective: To assess the progression of type 1 diabetes using time to peak glucose or C-peptide during oral glucose tolerance tests (OGTTs) in autoantibody positive (Ab+) relatives of people with type 1 diabetes.

Methods: We examined 2-hour OGTTs of participants in the Diabetes Prevention Trial Type 1 (DPT-1) and TrialNet Pathway to Prevention (PTP) studies. We included 706 DPT-1 participants (Mean±SD age: 13.84±9.53 years; BMI-Z-Score: 0.33±1.07; 56.1% male) and 3,720 PTP participants (age: 16.01±12.33 Years, BMI-Z-Score 0.66±1.3; 49.7% male). Log-rank testing and Cox regression analyses with adjustments (age, sex, race, BMI-Z-Score and peak Glucose/C-peptide levels, respectively) were performed.

Results: In each of DPT-1 and PTP, higher 5-year risk of diabetes development was seen in those with time to peak glucose >30 min and time to peak C-peptide >60 min (p<0.001 for all groups), before and after adjustments. In models examining strength of association with diabetes development, associations were greater for time to peak C-peptide versus peak C-peptide value (DPT-1: $X^2 = 25.76$ vs. $X^2 = 8.62$ and PTP: $X^2 = 149.19$ vs. $X^2 = 79.98$; all p<0.001). Changes in the percentage of individuals with delayed glucose and/or C-peptide peaks were noted over time.

Conclusions: In two independent at risk populations, we show that those with delayed OGTT peak times for glucose or C-peptide are at higher risk of diabetes development within 5 years, independent of peak levels. Moreover, time to peak C-peptide appears more predictive than the peak level, suggesting its potential use as a specific biomarker for diabetes progression.

Introduction

Significant and long-standing evidence exists to demonstrate progressive metabolic disturbances occurring in individuals prior to the clinical diagnosis of type 1 diabetes.¹⁻⁹ These disturbances result from chronic immune-mediated destruction of pancreatic β -cells with the endpoint being critical loss of β -cell mass and function presenting as type 1 diabetes.¹⁰ The 2-hour oral glucose tolerance test (OGTT) is a well-established clinical tool used to diagnose diabetes.¹¹⁻¹² Epidemiologic studies such as the Diabetes Prevention Trial (DPT-1) and TrialNet Pathway to Prevention (PTP) studies utilized longitudinal OGTT measurements to obtain a more complete picture of the metabolic changes leading up to the diagnosis of clinical type 1 diabetes.¹³⁻¹⁴

Peak glucose and C-peptide levels are known to become altered as individuals in these at-risk population studies progress to type 1 diabetes,¹⁵⁻²⁰ and a peak C-peptide at 120 mins during the 2-hr OGTT has been shown to be predictive of type 1 diabetes²⁰. Although the development of hyperglycemia in type 1 diabetes is driven by a reduction in β -cell mass, there is clear impairment in β -cell function evidenced by a loss in first phase insulin response early along with a compensatory delayed second phase response that is eventually lost as well. However, assessing the progression of type 1 diabetes by using both the timing of peak glucose or C-peptide levels at each time point post-glucose load during OGTTs has not been examined.

Recent studies in those at risk for type 2 diabetes, gestational diabetes, and pre-diabetes have examined whether glucose response curve patterns and/or the time to peak blood glucose levels are predictive of type 2 diabetes risk.²¹⁻²⁷ These studies, along with a recent study in an at risk type 1 diabetes population, suggest that individuals with earlier times to peak glucose and C-peptide levels are at lower risk of developing diabetes.²⁸ Additionally, with the advancements in

therapies slowing progression towards developing type 1 diabetes in high-risk individuals,²⁹⁻³¹ it will be important to identify the most predictive markers of progression for potential selection and monitoring in prevention trials. In this setting, identifying the most accurate markers of progression of type 1 diabetes, as well as having the ability to monitor shifts in disease progression, will be essential for appropriate counseling of those at risk with regard to choices in therapeutic interventions.

In this study, we examine OGTTs of participants in both the DPT-1 and PTP studies to determine whether the timing of the peak glucose and C-peptide levels during the 2-hr OGTT are independent predictors of progression to clinical type 1 diabetes. Our main objectives were to assess: 1) Risk of progression based on time to peak glucose and time to peak C-peptide during baseline 2-hr OGTTs, and 2) Change in the timing of the peak glucose/C-peptide from first to last non-diagnostic OGTT during the progression in those individuals who developed type 1 diabetes (Progressors) versus those who did not (Non-Progressors).

Methods

Participants

We analyzed data collected from participants in the DTP-1 and PTP studies. Institutional review board approval for both studies was obtained at participating sites along with written informed consent and assent as applicable. DPT-1 and the PTP studies follow participants who are first-, second- or third-degree relatives of individuals with type 1 diabetes and screened positive for at least one autoantibody (Ab) known to confer risk for type 1 diabetes, as has been previously described.¹³⁻¹⁴ We analyzed each study population separately to determine whether results were comparable across similar high risk (both Ab+ cohorts) yet distinct and different populations. A comparison of both cohorts is presented in Table 1. Participants in both studies

underwent serial 2-hr OGTTs every 6-12 months to monitor for evidence of metabolic derangements up until clinical diagnosis of type 1 diabetes as defined by the American Diabetes Association (ADA) diagnostic criteria.¹¹

We had data from a total of 6,292 participants enrolled in PTP. After including only participants with a complete OGTT at baseline and those with at least one additional complete follow-up OGTT, there were a total of 3,905 participants. Additional exclusion criteria were clinical diagnosis of diabetes at their initial screening visit (N = 181) or those who had a peak glucose or C-peptide level at the zero-time point (N = 4). Supplemental Figure 1 shows a schematic of PTP participants included in the present analyses. Data from 3,720 participants in PTP were included in our analysis for this study of which 908 (24.4%) were diagnosed with type 1 diabetes during follow-up. The same criteria as mentioned above were applied to the DPT-1 cohort leading to inclusion of 706 DPT-1 participants in our analyses. Only 5 participants were excluded due to a peak C-peptide or glucose occurring at the zero-time point, otherwise no participants in the DPT-1 cohort met any of the other exclusion criteria. In the DPT-1 cohort, 235 participants (33.2%) were diagnosed with type 1 diabetes during the study follow-up period.

OGTT Procedures

Baseline OGTTs were obtained at the initial study visit, which was used as participants' baseline visit for the study, with subsequent OGTTs at interval follow-up visits in both DPT-1 and PTP. DPT-1 participants had follow-up visits with OGTTs at 6-month intervals.¹³ Prior to 2012, PTP included OGTTs every 6 months, but after 2012 the follow-up intervals were either six months or annually based on further risk stratification.¹⁴ Participants were required to be fasting overnight for at least ten hours prior to each OGTT. After initial venous blood samples were obtained for baseline levels of plasma glucose and C-peptide, participants ingested an oral

glucose load (1.75g/kg; Max 75 grams) and blood was drawn at 30-minute (min) intervals for up to 2 hours, for a total of 5 time points. These samples were then analyzed for plasma glucose and C-peptide levels.

Those participants with a fasting glucose level of ≥ 126 mg/dL and/or a glucose level ≥ 200 mg/dL 2 hours after the oral glucose load underwent a confirmatory OGTT. If the confirmatory test again exceeded either of these thresholds, then the diagnosis of diabetes was made, and the participants were started on the appropriate therapy. If the confirmatory test did not meet criteria for diagnosis, then the participants remained in the study and continued with serial follow-up OGTTs. For both DPT-1 and PTP participants, the time of diagnosis was defined as the date of the first OGTT meeting diabetes criteria (if confirmed by a subsequent OGTT) or the date of clinical diagnosis according to the ADA criteria.

Plasma glucose levels were measured by standard glucose oxidase test. C-peptide levels were measured by a two-side immunoenzymometric assay performed on a Tosoh 600 II analyzer (Tosoh Bioscience, South San Francisco, CA, US).

Statistical Analyses

Unpaired Student's t-test and Pearson χ^2 were used for comparisons. Log-rank testing compared cumulative incidence curves for the development of type 1 diabetes. Univariate and multivariate Cox proportional hazard ratios (HR) with 95% confidence intervals (CI) examined the risk of developing type 1 diabetes. Hazard ratios were also subsequently adjusted for age, sex, race, peak glucose (or C-peptide) level, and BMI Z-score for age and sex. A two-sided p-value < 0.05 was utilized to define statistical significance. Statistical analyses were performed with Stata Software (Stata Version 15.1. Stata Corporation, College Station, TX).

Definition of Time to Peak Levels and Stratification of Study Participants

Individuals within DPT-1 and PTP were analyzed to determine the cut-offs for the timing of peak glucose and C-peptide. The threshold cut-offs for glucose and C-peptide were calculated independently. They were determined first by using Kaplan-Meier curves and log rank tests in the DPT-1 population to determine those cut-points that yielded the greatest significant division of the data. These thresholds were then verified in the PTP population. Stratification into groups was based on the initial distributions of Kaplan-Meier curves and log rank tests performed in the DPT-1 population, and later verified in PTP (See supplemental Figures 2 and 3 for DPT-1). For peak glucose, individuals were divided into those who had peak glucose levels occurring at 30 mins (DPT-1: N = 372; PTP: N = 1730) versus those with a peak glucose after 30 mins (DPT-1: N = 334; PTP: N = 1990). Similarly, we took the same cohorts within each trial's population and compared those with a peak C-peptide level at or before 60 mins (DPT-1: N = 342; PTP: N = 1,697) to those with a peak C-peptide level after 60 mins (DPT-1: N = 364; PTP: N = 2,023). Peak glucose and C-peptide values were taken at the time when the individual had the highest absolute value during the OGTT.

Assessment of β -cell function and insulin resistance:

We used the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) to assess insulin resistance in the PTP cohort. The HOMA-IR was calculated using the following: $\text{HOMA IR} = (\text{Fasting Insulin (mU/L)} * \text{Fasting Glucose (mg/dL)})/405$. The C-peptide Index was used as a measure of β -cell function. This was calculated using the change in C-peptide from 30 to 0 minutes (ng/mL) divided by the change in glucose from 30 to 0 minutes (mg/dL). Finally, the oral disposition index was used as a measure of β -cell function and the ability of the body to

dispose of the glucose load. The following formula was used to calculate the OGTT oral Disposition Index (oDI): $\text{oDI} = (1/\text{Fasting Insulin}) * \text{C-peptide Index}$.

Results

Baseline Characteristics

Baseline demographics for both the PTP (N=3720) and DPT-1 (N=706) study participants are shown in Table 1. Compared to the DPT-1 cohort, the PTP population included a higher proportion of male participants (56.1% vs. 49.7%). DPT-1 participants were younger (mean age \pm SD was 13.84 ± 9.53 vs. 16.01 ± 12.33) and had a lower BMI Z-Score (0.33 ± 1.07 vs. 0.66 ± 1.31). Both studies predominantly included Caucasian participants (90.5% in DPT-1 and 80.1% in PTP).

Progression of Type 1 Diabetes Based on Time-to-Peak Glucose and C-Peptide Levels

Figure 1 (A-D) depicts the cumulative incidence curves for type 1 diabetes development by time to peak glucose (at vs. after 30 min) and C-peptide levels (at or before 60 min vs. after). In both DPT-1 and PTP respectively, we found the 5-year risk estimate of type 1 diabetes progression with 95% CIs was significantly lower in those with a peak glucose at 30 min compared to those with a peak glucose after 30 min (DPT-1: 32.0% [26.2 – 38.8] vs. 59.6% [52.5 – 66.9]; $p < 0.001$); PTP: (15.1% [13.1 – 17.3] vs. 37.2% [34.7 – 39.8]; $p < 0.001$). Similarly, the 5-year risk estimate for type 1 diabetes development was significantly lower in those with a peak C-peptide level at or before 60 min compared to those with a peak C-peptide level after 60 min for both DPT-1 and PTP cohorts, respectively (DPT-1: 33.9% [27.8 – 41.0%] vs. 55.8% [49.0 – 62.8%]; $p < 0.001$; PTP: 16.7% [14.6 – 19.0%] vs. 35.5% [33.1 – 38.0%]; $p < 0.001$). Given the extended follow-up times available within the PTP study population, we also calculated the 10-year risk estimate of type 1 diabetes development. Lower risk of type 1

diabetes was again seen in those with peak glucose level at 30 min versus after (26.9% [23.4 – 30.9%] vs. 48.9% [45.4 – 52.6%]; $p < 0.001$) and peak C-peptide at or before 60 min versus after (24.9% [21.8-28.4%] vs. 50.4% [46.6-54.3%]; $p < 0.001$).

Risk of progression of type 1 diabetes was further assessed by calculating HRs with 95% CIs. Individuals with a peak glucose after 30 min versus a peak glucose at 30 min demonstrated significantly higher HRs in both DPT-1 and PTP (HR = 2.57 [1.97 – 3.36] and HR = 3.27 [2.77 – 3.85] respectively; both with $p < 0.001$). After adjusting for age, sex, race, BMI- Z-score, and peak glucose level, the higher risk of progression of type 1 diabetes remained statistically significant in both groups (DPT-1 adjusted HR = 2.30 [1.73 – 3.07] and PTP adjusted HR = 2.11 [1.75 – 2.53]; both with $p < 0.001$). Likewise, in both cohorts, those with a peak C-peptide level after 60 min compared to those with a peak C-peptide level at or before 60 min demonstrated significantly higher risk of type 1 diabetes development (DPT-1: HR = 1.89 [1.45 – 2.46] and PTP: HR = 2.67 [2.28 – 3.13]; both with $p < 0.001$). Again, the higher risk of progression to type 1 diabetes remained statistically significant after adjusting for age, sex, race, BMI Z-score, and peak C-peptide level in both study populations (DPT-1 adjusted HR = 2.11 [1.59 – 2.80] and PTP adjusted HR = 2.85 [2.41 – 3.37]; both with $p < 0.001$).

Strength of association between risk of progression of type 1 diabetes and time to glucose or C-peptide

We next evaluated the strength of the association between progression of type 1 diabetes and the time to peak glucose (at vs. after 30 min) or C-peptide (≤ 60 min vs. > 60 min) as well as compared to the absolute values of the peak glucose or C-peptide levels in both DPT-1 and PTP (Table 2). Interestingly, the time to peak C-peptide contributed significantly more to the model compared to the absolute value of the peak C-peptide in both DPT-1 ($X^2 = 25.76$ vs. $X^2 = 8.62$)

and PTP ($X^2 = 149.19$ vs. $X^2 = 79.98$) populations. When examining the glucose variables and the strength of their association with developing type 1 diabetes, the results differed. The absolute peak glucose level contributed more to the model compared to time to peak glucose in both DPT-1 ($X^2 = 48.67$ vs. $X^2 = 30.26$) and PTP ($X^2 = 818.92.19$ vs. $X^2 = 63.50$). . All differences in Table 2 were found to be statistically significant ($p \leq 0.01$).

Risk of progression of type 1 diabetes based on time to peak glucose or C-peptide after stratifying by age

We subsequently asked whether the time to peak glucose and C-peptide remain strong predictors of progression of type 1 diabetes after stratifying the PTP population by age (age < 18 vs. ≥ 18). The PTP population was chosen for this analysis given the greater age range in the population and larger number of participants. We compared HRs with 95% CIs for risk for clinical type 1 diabetes development both before and after adjusting for peak glucose (or C-peptide) levels, sex, race, and BMI Z-score. In both age groups (age less than 18 and those 18 years or older), we again found HRs (before and after adjustments) demonstrating significantly higher risk for progression of type 1 diabetes in those with a peak glucose level after 30 min and those with a peak C-peptide level after 60 min. The calculated HRs both before and after adjustments did not vary between the two age groups and the overall risk for type 1 diabetes remained high while maintaining statistical significance ($p \leq 0.01$ for all), Supplemental Table 1.

Risk for progression of type 1 diabetes based on time to peak glucose or C-peptide after stratifying by number of autoantibodies

We further asked whether the time to peak glucose and C-peptide, respectively, remain strong predictors of progression of type 1 diabetes after stratifying the PTP population based on single versus multiple autoantibody positive (Ab+) status at baseline. We evaluated the risk of

progression of type 1 diabetes by calculating HRs with 95% CIs both before and after adjusting for peak glucose (or C-peptide) levels, age, sex, race, and BMI Z-score. The calculated HRs were not statistically different between those with single versus multiple autoantibodies at baseline. Overall, those with a peak glucose level after 30 min and C-peptide level after 60 min continued to demonstrate higher risk of progression of type 1 diabetes both before and after adjustments. All associations were found to be significant ($p \leq 0.01$) and are shown in Supplemental Table 2.

Change in frequency of OGTTs with delayed times to peak glucose/C-peptide from first to last OGTT by progressor status

Lastly, we explored if the proportion of individuals with delayed peak C-peptide and/or glucose changed over time. Specifically, whether there were changes in the frequency of those with a time to peak glucose after 30 min or time to peak C-peptide after 60 min from baseline OGTT to last OGTT in each study cohort. We compared changes in frequencies between those who developed type 1 diabetes (Progressors) during follow-up versus those individuals who did not (Non-Progressors). It is important to note that in the Progressors, we classified the last OGTT as the last “Non-diagnostic” OGTT or stated differently the last OGTT prior to type 1 diabetes diagnosis. For Non-Progressors the last available OGTT was used.

At baseline, Progressors in each of the DPT-1 and PTP cohorts had a higher frequency of late peak glucose and C-peptide levels during the OGTT. This further increased at the last OGTT, with statistically significant increases in all measures except for the late peak C-peptide increase in DPT-1, where the increase was not statistically significant. In addition, there were significant differences in the distribution of those with a peak glucose > 30 min and peak C-peptide > 60 min at baseline and at last OGTT in Progressors compared to Non-Progressors in

both DPT-1 and PTP (all with $p < 0.001$), Table 3. Additional analysis to compare racial differences in distribution showed no significant difference in time to peak for either glucose or C-peptide by racial groups in both DPT-1 and the PTP cohort.

Further, since insulin resistance could further burden the β -cells and impact the timing of the peak, we assessed the change in BMI-z from first to last OGTT as well as assessed the HOMA-IR (as a measure of insulin resistance) by progressor as well as timing of the peak status and found that there were minimal and often inconsistent differences (Supplemental Table 3).

Assessment of β -cell function and insulin resistance by the timing of peak

We further assessed whether the timing of peak glucose or C-peptide was indicative of β -cell function, insulin resistance and insulin secretion in the face of insulin resistance. We found that the C-peptide index was significantly higher in those with a peak C-peptide occurring at or before 60 mins, Supplemental Table 4. Whereas when assessing these measures by timing of peak glucose, both the C-peptide index and the oral disposition indices were significantly lower among those with a peak glucose that occurred after 30 mins. However, there were no significant differences in HOMA-IR measures by timing of peak, Supplemental Table 4.

Discussion

Our findings demonstrate that within a high-risk population for type 1 diabetes (relatives of individuals with type 1 diabetes who are Ab+), those individuals with delayed times to peak glucose or C-peptide in a 2-hr OGTT are at even higher risk for progression of type 1 diabetes. Specifically, individuals with a peak glucose level after 30 minutes progress to clinical diabetes development faster than those with a peak glucose level at 30 minutes. Similarly, those with a peak C-peptide level after 60 minutes are also at higher risk of progression to clinical diabetes

compared to those with a peak C-peptide level at or before 60 minutes. Our study shows that this risk of progression is independent of age, sex, race, BMI Z-score, and the number of autoantibodies. Our results were confirmed in two similar, yet distinct populations (DPT-1 and PTP), further validating the utility of time to peak glucose and C-peptide in the prediction of type 1 diabetes progression.

When assessing the strength of association of these variables with risk of type 1 diabetes there were a few significant and interesting findings. First, the time to peak C-peptide level contributed significantly more to the prediction model when compared to the absolute level of the peak C-peptide in both DPT-1 and PTP. This is in agreement with prior work by Sosenko et al [20], where the 120 min peak C-peptide was found to be a stronger predictor of type 1 diabetes development than the peak C-peptide level. However, this study did not analyze other time points as in our study and did not include both level and timing in a prediction model as performed in our study. Further, the study did not assess the time to peak glucose levels. However, these findings further confirm and validate our findings. On the other hand, and although there was a significant association with the timing of glucose peak to development of diabetes, the association was stronger for the peak glucose level. This would appear to make physiologic sense, since as individuals progress to diabetes, their C-peptide (insulin) levels decrease as their glucose levels increase. It is not entirely clear why there was such a noticeable difference in the strength of peak glucose level association for PTP vs. DPT-1. This may be due to the smaller sample size in DPT-1 and perhaps a less homogeneous population in PTP. Nonetheless, there was an increase seen in both cohorts.

We compared frequencies of individuals with delayed times to peak glucose/C-peptide levels at baseline and at follow-up OGTTs. The overall frequency of individuals with a time to

peak glucose > 30 min or C-peptide > 60 min was significantly higher both at baseline and at the last non-diagnostic OGTT in the Progressors, compared to Non-Progressors. Additionally, within the Progressors in PTP, there was a significantly higher increase in the frequency of those with a later peak glucose or C-peptide at the last OGTT compared to the baseline OGTT. The same was true for DPT-1, except that the increase was not statistically significant with regards to the late C-peptide in Progressors but was highly significant when compared to the Non-Progressors. The latter may be again due to the fact that the sample size became much smaller when looking at Progressors within DPT-1. These findings are clinically relevant in that they demonstrate a shift in the time to peak glucose/C-peptide as individuals progress towards clinical type 1 diabetes. This observation would allow for monitoring of the effects of intervention therapies in prevention trials. Additionally, given the recent staging mechanism for type 1 diabetes development³², our findings may serve to be used as a novel stage-specific biomarker for progression from one stage to another.

Finally, we aimed to assess whether the timing of peak glucose or C-peptide was indicative of β -cell function, insulin resistance and β -cell function in the face of insulin resistance. We found no differences in insulin resistance in the cohort. Meanwhile, the C-peptide index was significantly higher in those with a peak C-peptide at or before 60 mins while in those with a peak glucose occurring after 30 mins, both the C-peptide index and the oral disposition were significantly lower.

Peak glucose and C-peptide levels are known predictors of type 1 diabetes development.¹⁸⁻²¹ Further, our results are consistent with what has been published in the type 2 diabetes literature. Indeed, the time to glucose peak,^{24, 25, 26} the 1-hr peak,²⁷ and the overall glucose trajectories^{22,23} have been shown to be more reproducible and stronger prognostic factors

for risk of type 2 diabetes than the 2-hr OGTT glucose in adults. These studies further support our findings that those who are autoantibody positive and have delayed time to peak glucose/C-peptide levels during standard OGTTs are at higher risk of progression of type 1 diabetes regardless of other baseline characteristics.

Our results are consistent with expected physiologic changes seen during the progression to T1D. With worsening β -cell insulin secretory defects, manifesting as loss of early insulin secretion in the first 30-minutes post-glucose load, we expect a delayed C-peptide as well as delayed glucose peaks during the progression. We have previously demonstrated that, based on the glucose response curve and among those with a monophasic or inverted U-shaped glucose response curve²⁸, those with delayed C-peptide peaks appear to have later glucose peaks and are at higher risk for progression to T1D.

The natural history of β -cell decline would appear to be that of loss of early-phase insulin secretion, accompanied or followed by delayed, compensatory increased insulin secretion in response to a glucose load and that this compensatory late phase insulin would gradually decline with worsening glucose tolerance and eventually development of diabetes. These results therefore become critical in identifying those at risk of progression as well as those with earlier peaks to best determine timing of intervention and prevention strategies as well as restore this early phase insulin response among those at risk.

The ability to analyze the OGTT data sets from both the DPT-1 and PTP cohorts is a major strength of this study. Evaluating these two unique at-risk populations separately with inter-cohort comparisons yielded similar results. This further validates our findings and suggests our results can be applied broadly within this special at-risk population of individuals despite the apparent heterogeneity of these cohorts. It is also worth mentioning that the observed increased

risk is maintained throughout an extended follow-up period (up to 5-years in DPT-1 and 10-years in PTP). We believe that this data can indeed alter screening and clinical practices. Currently, clinicians typically assess the 0- and 120-minute time points for evaluation of glucose tolerance without assessment of other interval time points or C-peptide values. Therefore, results from this study allow for better assessment of risk of progression by using data from interval time points that appear to be more indicative of metabolic changes and declining β -cell function. This can be applied to those at risk of progression to T1D based on our results, as well as those at risk for T2D based on published data, therefore allowing for earlier intervention and reversal strategies. Further, based on our results, it is perhaps sufficient and more cost-effective to perform a 1-hour OGTT to determine risk as the lower risk group peaked at 30 mins for C-peptide and before 60 minutes for glucose.

There were a few limitations to our study including the limited number of time points in the OGTTs. It is certainly conceivable our results may have differed slightly with more frequent time points at shorter time intervals. In addition, we were unable to assess other factors that are widely recognized to contribute to the timing of those peaks such as incretin hormone response and levels which likely play a role in the pattern of insulin secretion and peak timing. Lastly, our findings may not be generalizable to other populations. Further studies are needed to evaluate whether earlier time points may be better predictors of progression of type 1 diabetes as utilizing earlier time points would reduce the burden of testing for future individuals and may be more accurate predictors.

Conclusions

Our study shows that within two distinct and high-risk populations of autoantibody positive relatives of individuals with type 1 diabetes, that individuals with delayed times to peak

glucose and C-peptide levels are at even higher risk of progression to type 1 diabetes.

Importantly, we have also shown that the number of autoantibodies and age, as well as other characteristics, do not significantly affect these observations. Time to peak C-peptide appears more predictive than the peak level, suggesting its potential use as a specific biomarker for prediction of type 1 diabetes progression and for potential inclusion into and monitoring of prevention trials.

Funding:

We acknowledge the support of the Type 1 Diabetes TrialNet Study Group, which identified study participants and provided samples and follow-up data for this study. The Type 1 Diabetes TrialNet Study Group is a clinical trials network funded by the National Institutes of Health (NIH) through the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Allergy and Infectious Diseases, and The Eunice Kennedy Shriver National Institute of Child Health and Human Development, through the cooperative agreements U01 DK061010, U01 DK061034, U01 DK061042, U01 DK061058, U01 DK085453, U01 DK085461, U01 DK085465, U01 DK085466, U01 DK085476, U01 DK085499, U01 DK085504, U01 DK085509, U01 DK103180, U01 DK103153, U01 DK103266, U01 DK103282, U01 DK106984, U01 DK106994, U01 DK107013, U01 DK107014, UC4 DK106993, UC4 DK11700901, U01 DK 106693-02, and the JDRF. The contents of this article are solely the responsibility of the authors and do not necessarily represent the official views of the NIH or the JDRF.

This work was also made possible with support from Grant Numbers, KL2TR002530 (A Carroll, PI), and UL1TR002529 (A. Shekhar, PI) from the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical and Translational Sciences Award.

Acknowledgments:

Members of the Type 1 Diabetes TrialNet Study Group and TrialNet Affiliate Centers are listed in the online only.

Data Availability: The data was analyzed or generated during the study and is available on request from the authors.

Contribution statement:

HMI, PX and JMS conceptualized the study. MG, HMI, MC, PX, DDC analyzed and interpreted the data, and wrote the manuscript. CEM, JPP, MJR, AKS, ML, HL, WVM and MAA contributed to the design, interpreted the data and reviewed/edited the manuscript.

Duality of Interest: The authors declare that there is no duality of interest associated with this manuscript.

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Tables and Figures**Table 1: Study participant demographics for both DPT-1 and PTP populations at baseline.**

Selected Demographic Characteristics by Study			
	DPT-1	PTP	p-value
	N=706	N=3720	
Sex Distribution			0.002
Male	396 (56.1%)	1842 (49.7%)	
Female	310 (43.9%)	1867 (50.3%)	
Racial Distribution			<0.001
White	639 (90.5%)	2979 (80.1%)	
Black/AA	9 (1.3%)	92 (2.5%)	
Hispanic	31 (4.4%)	366 (9.8%)	
Other	12 (1.7%)	132 (3.5%)	
Unknown	15 (2.1%)	151 (4.1%)	
Age at Baseline	13.84 (9.53)	16.01 (12.33)	<0.001
BMI Z-Score	0.33 (1.07)	0.66 (1.31)	<0.001

Table 2: Strength of association with development of type 1 diabetes, comparing the time to peak glucose or C-peptide levels versus the magnitude of the glucose or C-peptide peak level.

DPT-1		
	χ^2	<i>p</i> -value
Time to Peak C-peptide	25.76	<0.001
Peak C-peptide Levels	8.62	0.003
Time to Peak Glucose	30.26	<0.001
Peak Glucose Levels	48.67	<0.001
PTP		
	χ^2	<i>p</i> -value
Time to Peak C-peptide	149.19	<0.001
Peak C-peptide Levels	79.98	<0.001
Time to Peak Glucose	63.50	<0.001
Peak Glucose Levels	818.92	<0.001

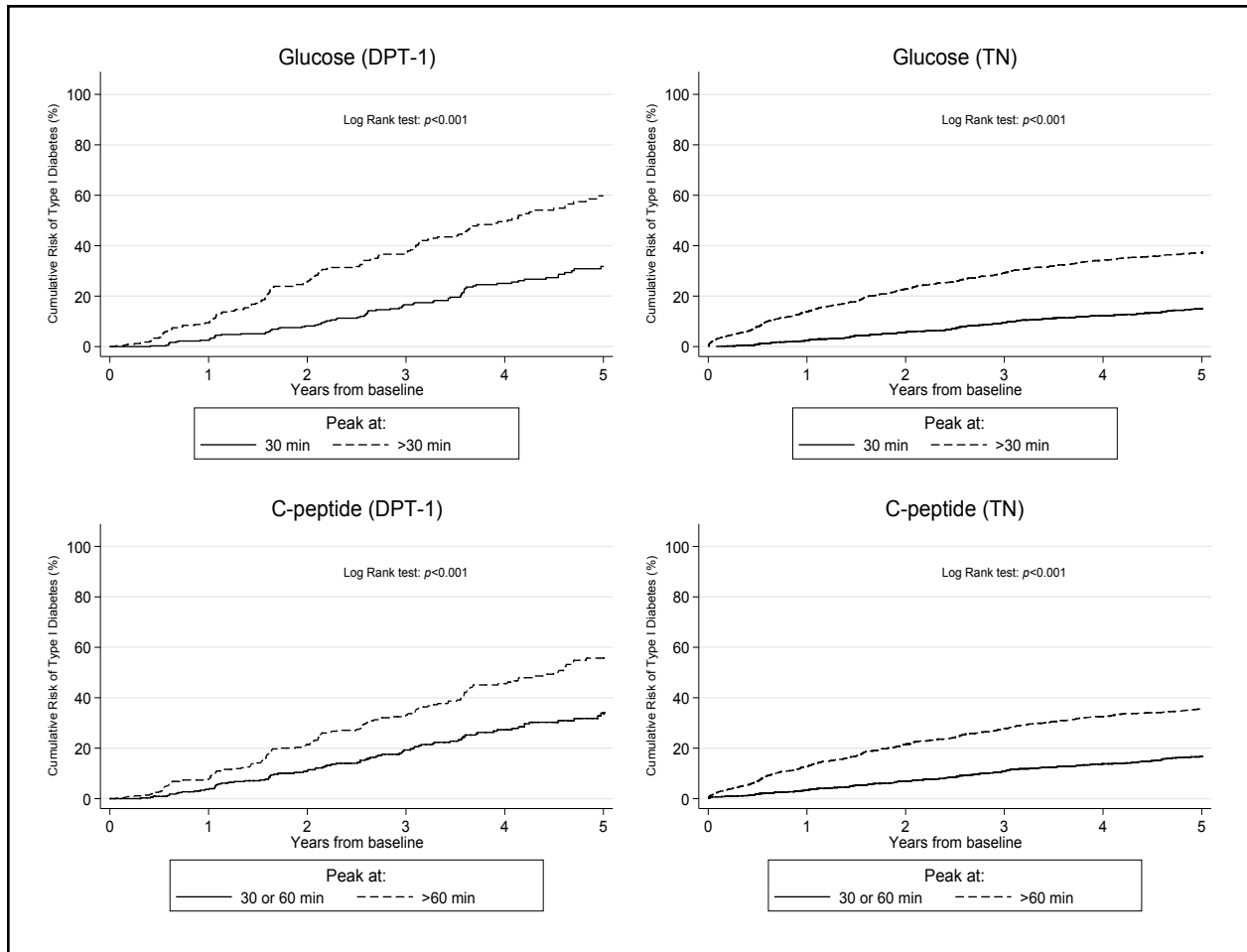
Table 3: Change in frequency of time to peak glucose > 30 mins or time to peak C-peptide > 60 mins among non-progressors and progressors in PTP and DPT-1, respectively.

PTP	Time to Peak Glucose > 30 minutes			Time to Peak C-peptide > 60 minutes		
	First OGTT	Last OGTT	p-value [^]	First OGTT	Last OGTT	p-value [^]
Non-Progressors (N = 2,812)	47.2%	49.8%	0.015	48.7%	50.8%	0.037
Progressors (N = 908)	73.1%	86.9%	<0.001	71.9%	80.8%	<0.001
p-value#	<0.001	<0.001		<0.001	<0.001	
DPT-1	Time to Peak Glucose > 30 minutes			Time to Peak C-peptide > 60 minutes		
	First OGTT	Last OGTT	p-value [^]	First OGTT	Last OGTT	p-value [^]
Non-Progressors (N = 471)	39.3%	53.5%	<0.001	46.3%	53.3%	0.020
Progressors (N = 235)	63.4%	87.7%	<0.001	62.1%	66.4%	0.322
p-value#	<0.001	<0.001		<0.001	0.001	

p-value based on Chi-Square Test

[^] p-value based on McNemer's Test

Figure 1: Cumulative incidence curves for type 1 diabetes in both DPT-1 and PTP, based on time to peak glucose (A, and B) or peak C-peptide (C, and D) levels during 2-hr OGTTs, ($p < 0.001$ for all). Dashed lines indicate (A, and B) peak glucose after 30 minutes or (C, and D) peak C-peptide after 60 minutes. Solid lines indicate (A, and B) peak glucose at 30 minutes or (C, and D) peak C-peptide at 60 minutes.



Supplemental Tables and Figures:

Supplemental Table 1: Unadjusted and adjusted Hazard Ratios (HRs) for the association between risk of developing type 1 diabetes and time to peak C-peptide/peak glucose at baseline in the PTP cohort stratified by age less than 18 and those 18 years or older.

PTP (Age < 18 years); N =2758						
	<i>Unadjusted</i>			<i>Adjusted*</i>		
	HR	95% CI	p-value	HR	95% CI	p-value
Time to Peak C-peptide (> 60 vs ≤ 60 mins)	2.85	2.40, 3.37	<0.001	2.77	2.31, 3.31	<0.001
Time to Peak Glucose (> 30 vs at 30 mins)	3.45	2.90, 4.10	<0.001	2.12	1.75, 2.57	<0.001
PTP (Age ≥ 18 years); N =959**						
	HR	95% CI	p-value	HR	95% CI	p-value
Time to Peak C-peptide (> 60 vs ≤ 60 mins)	3.46	2.12, 5.65	<0.001	3.54	2.11, 5.92	<0.001
Time to Peak Glucose (> 30 vs at 30 mins)	5.10	2.92, 8.91	<0.001	2.48	1.33, 4.61	0.004
* C-peptide adjusted for peak C-peptide level, age, sex, race and BMI z-score. Glucose adjusted for peak glucose level, age, sex, race and BMI z-score.						
**Age was missing in 3.						

Supplemental Table 2: Unadjusted and adjusted HRs for association between risk of developing type 1 diabetes and time to peak C-peptide/peak glucose at baseline in the PTP cohort stratified by number of antibody status (single versus multiple).

PTP (Single Ab+)						
	<i>Unadjusted</i>			<i>Adjusted*</i>		
	HR	95% CI	p-value	HR	95% CI	p-value
Time to Peak C-peptide (> 60 vs ≤ 60 min)	5.19	2.30, 11.71	<0.001	6.09	2.49, 14.89	<0.001
Time to Peak Glucose (> 30 vs at 30 min)	5.21	2.31, 11.75	<0.001	4.07	1.60, 10.37	0.003
PTP (Multiple Ab+)						
	HR	95% CI	p-value	HR	95% CI	p-value
Time to Peak C-peptide (> 60 vs ≤ 60 min)	2.51	2.13, 2.96	<0.001	2.59	2.18, 3.07	<0.001
Time to Peak Glucose (> 30 vs at 30 min)	3.15	2.66, 3.73	<0.001	1.93	1.60, 2.32	<0.001
* C-peptide adjusted for peak C-peptide level, age, gender, race and BMI-for-age z score Z-Score. Glucose adjusted for peak glucose level, age, gender, race and BMI-for-age z score Z-Score.						

People with single Abs are thought to be at less risk and might be older, it's likely the delayed peak identify those more like T1D or within the single ab.

HR: multiple Abs risk is high at baseline, RR

Supplemental Table 3: Change in HOMA-IR and BMI-z from first to last OGTT by progressor status as well as by timing of peak status:

Change from 1st to (Last OGTT)	Non-Progressors	Progressors	P-Value
<u>By Progression Status</u>			
HOMA-IR	0.204 (1.737)	0.310 (1.533)	0.110
BMI Z-Score	0.081 (0.673)	0.061 (0.556)	0.395
<u>By Glucose Peak and Progression Status</u>			
Glucose Peak =30 mins			
HOMA-IR	0.193 (1.607)	0.259 (2.003)	0.663
BMI Z-Score	0.062 (0.710)	0.149 (0.702)	0.089
Glucose Peak >30 mins			
HOMA-IR	0.217 (1.875)	0.327 (1.335)	0.161
BMI Z-Score	0.102 (0.629)	0.028 (0.487)	0.006
<u>By C-Peptide Peak and Progression Status</u>			
C-peptide Peak ≤60 mins			
HOMA-IR:	0.243 (1.602)	0.297 (1.384)	0.612
BMI Z-Score	0.071 (0.630)	0.138 (0.721)	0.187
C-peptide Peak > 60 mins			
HOMA-IR	0.163 (1.870)	0.315 (1.588)	0.081
BMI Z-Score	0.091 (0.717)	0.030 (0.471)	0.029

HOMA IR = [Fasting Insulin (μU/L) * Fasting Glucose (mg/dL)] / 405
 Values represent mean (±SD)

Supplemental Table 4: Comparison of measures of β -cell function and insulin resistance

	Glucose Peak =30 mins	Glucose Peak >30 mins	p-value
HOMA-IR	1.739 (1.589)	1.798 (1.728)	0.305
C-peptide Index	0.092 (0.096)	0.068 (0.212)	<0.001
oDI	0.017 (0.026)	0.013 (0.047)	<0.001

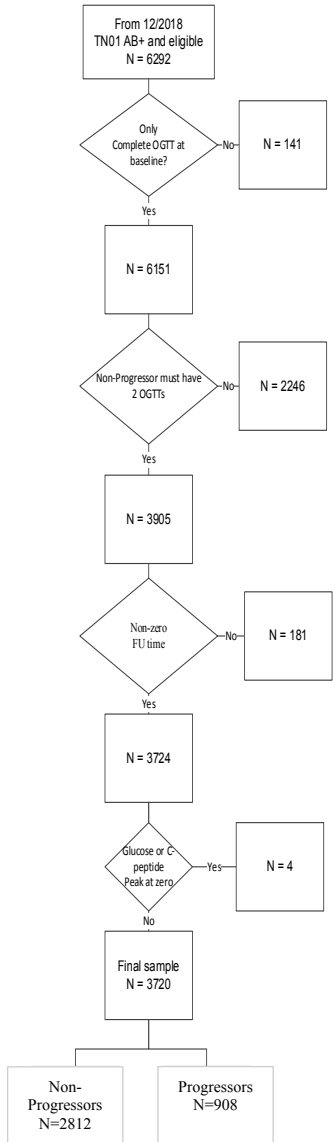
	C-peptide Peak \leq60 mins	C-peptide Peak >60 mins	p-value
HOMA-IR	1.729 (1.418)	1.805 (1.849)	0.180
C-peptide Index	0.097 (0.228)	0.065 (0.090)	<0.001
oDI	0.016 (0.044)	0.014 (0.033)	0.133

C-peptide Index = (Change in C-peptide 30 – 0 minutes (ng/mL))/(Change in Glucose 30 – 0 minutes (mg/dL))

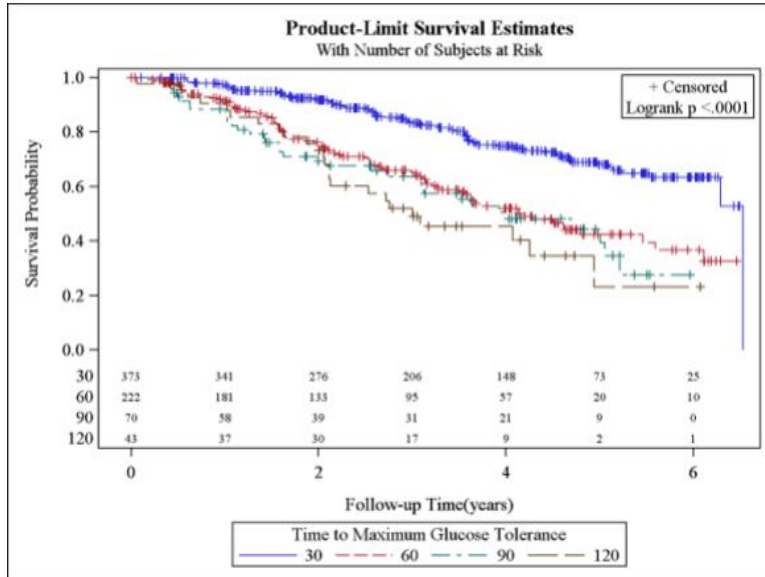
OGTT Disposition Index (oDI) = (1/Fasting Insulin) * C-peptide Index

Values represent the mean (\pm SD)

Supplemental Figure 1: PTP Sample selection



Supplemental Figure 2: Type 1 Diabetes Free curve by timing of peak glucose in the DPT-1 cohort.



Supplemental Figure 3: Type 1 Diabetes Free curve by timing of peak C-peptide in the DPT-1 cohort.

