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

Michael G. Voss,[1] David D. Cuthbertson,[2] Mario M. Cleves,[2] Ping Xu,[2] Carmella Evans-Molina,[3] Jerry P. Palmer,[4] Maria J. Redondo,[5] Andrea K. Steck,[6] Markus Lundgren,[7] Helena Larsson,[7] Wayne V. Moore,[8] Mark A. Atkinson,[9] Jay M. Sosenko,[10] and Heba M. Ismail,[3] for the DPT-1 and TrialNet Study Groups*

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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 relatives of people with type 1 diabetes.

RESEARCH DESIGN AND METHODS

We examined 2-h 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, HOMA-insulin resistance, and peak glucose/C-peptide levels, respectively) were performed.

RESULTS

In each of DPT-1 and PTP, higher 5-year diabetes progression risk 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: $\chi^2 = 25.76$ vs. $\chi^2 = 8.62$; PTP: $\chi^2 = 149.19$ vs. $\chi^2 = 79.98$; 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.

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 end point being critical loss of β-cell mass and function

[1]Department of Medicine, Indiana University, School of Medicine, Indianapolis, IN
[2]Department of Pediatrics, Morsani College of Medicine, University of South Florida, Tampa, Florida
[3]Department of Pediatrics, Indiana University, School of Medicine, Indianapolis, IN
[4]Veterans Affairs Puget Sound Health Care System, Seattle, WA
[5]Texas Children's Hospital, Baylor College of Medicine, Houston, TX
[6]Barbara Davis Center for Diabetes, University of Colorado School of Medicine, Aurora, CO
[7]Unit for Pediatric Endocrinology, Department of Clinical Sciences Malmö, Lund University, Malmö, Sweden
[8]Division of Endocrinology and Diabetes, Children's Mercy Hospital, University of Missouri-Kansas City School of Medicine, Kansas City, MO
[9]Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL
[10]Division of Endocrinology, Diabetes, and Metabolism, University of Miami, Miami, FL

Corresponding author: Heba M. Ismail, heismail@iu.edu

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

presenting as type 1 diabetes (10). The 2-h 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, used 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 min during the 2-h OGTT has been shown to be predictive of type 1 diabetes (20). 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 the 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 prediabetes 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 toward 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 the DPT-1 and PTP studies to determine whether the timing of the peak glucose and C-peptide levels during the 2-h 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-h OGTTs, and *2*) change in the timing of the peak glucose/C-peptide from first to last nondiagnostic OGTT during the progression in those individuals who developed type 1 diabetes (progressors) versus those who did not (nonprogressors).

RESEARCH DESIGN AND 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-h OGTTs every 6–12 months to monitor for evidence of metabolic derangements up until the clinical diagnosis of type 1 diabetes as defined by the American Diabetes Association 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). Supplementary Fig. 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 five 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 the 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 6 months or annually, based on further risk stratification (14). Participants were required to have fasted overnight for at least 10 h 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 (1.75 g/kg; maximum, 75 g), and blood was drawn at 30-min intervals for up to 2 h, 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 h 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 American Diabetes Association criteria.

Plasma glucose levels were measured by standard glucose oxidase test. C-peptide levels were measured by a two-side immunoenzymometric assay performed

Table 1—Study participant demographics for DPT-1 and PTP populations at baseline

Selected demographic characteristics by study	DPT-1 (n = 706)	PTP (n = 3,720)	P value
Sex distribution			0.002
Male	396 (56.1)	1,842 (49.7)	
Female	310 (43.9)	1,867 (50.3)	
Racial/ethnic distribution			<0.001
White	639 (90.5)	2,979 (80.1)	
Black/African American	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, mean (SD), years	13.84 (9.53)	16.01 (12.33)	<0.001
BMI Z-score, mean (SD)	0.33 (1.07)	0.66 (1.31)	<0.001

Data are presented as n (%), unless indicated otherwise.

on a Tosoh 600 II analyzer (Tosoh Bioscience, South San Francisco, CA).

Statistical Analyses

Unpaired Student 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 (HRs) with 95% CIs examined the risk of developing type 1 diabetes. HRs were also subsequently adjusted for age, sex, race, peak glucose (or C-peptide) level, and BMI Z-score for age and sex, and HOMA-insulin resistance (HOMA-IR). A two-sided P value of <0.05 was used to define statistical significance. Statistical analyses were performed with Stata 15.1 software (StataCorp, 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 cutoffs for the timing of peak glucose and C-peptide. The threshold cutoffs for glucose and C-peptide were calculated independently. They were determined first by using Kaplan-Meir 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 (Supplementary Figs. 2 and 3) and later verified in PTP. For peak glucose, individuals were divided into those who had peak glucose levels occurring at 30 min (DPT-1: n = 372; PTP: n = 1,730) versus those with a peak glucose after 30 min (DPT-1: n = 334; PTP: n = 1,990). Similarly, we took the same cohorts within each trial's population and compared those with a peak C-peptide level at or before 60 min (DPT-1: n = 342; PTP: n = 1,697) to those with a peak C-peptide level after 60 min (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 IR

We used the HOMA-IR to assess IR. The HOMA-IR was calculated using the following: HOMA-IR = (fasting insulin [mU/L] * fasting glucose [mg/dL])/405. For the PTP analysis, fasting insulin levels were readily available from the OGTT samples and data. However, for DPT-1, the HOMA-IR was calculated from the baseline insulin levels obtained during intravenous glucose tolerance tests (IVGTTs) and not the baseline OGTTs because insulin was not captured from OGTTs in DPT-1. The baseline IVGTTs and baseline OGTTs were typically collected within 1 month or so of each other. 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 min (ng/mL) divided by the change in glucose from 30 to 0 min (mg/dL).

Data Resource and Availability

The data were analyzed or generated during the study and are available on request from the authors.

RESULTS

Baseline Characteristics

Baseline demographics for both the PTP (n = 3,720) and DPT-1 (n = 706) study participants are provided in Table 1. Compared with 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 ± SD age 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% CI was significantly lower in those with a peak glucose at 30 min compared with 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 with those with a peak C-peptide level after 60 min for both the 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 levels 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

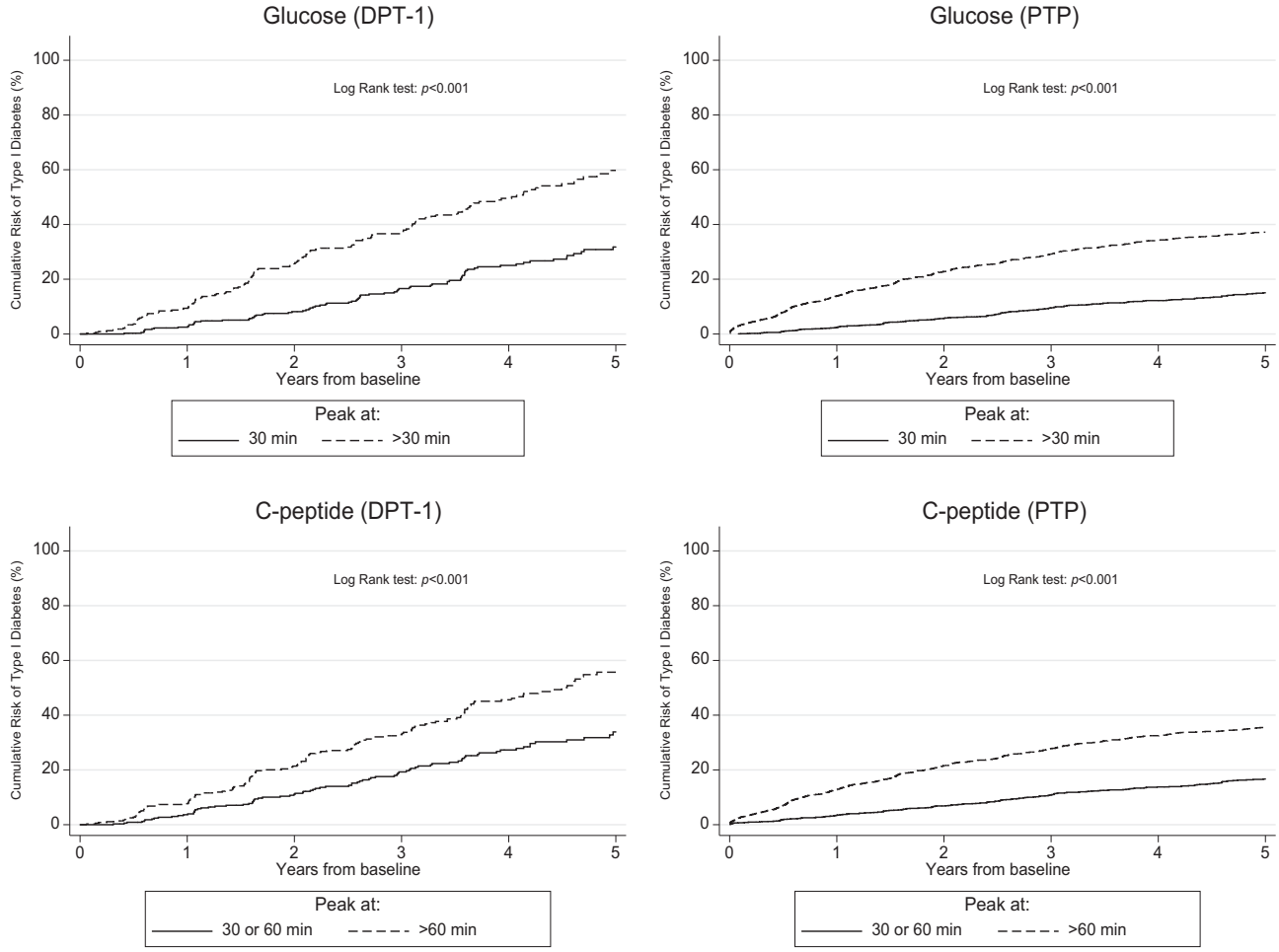

Figure 1—Cumulative incidence curves for type 1 diabetes in DPT-1 and PTP, based on time to peak glucose (*A* and *B*) or peak C-peptide (*C* and *D*) levels during 2-h OGTTs ($P < 0.001$ for all). Dashed lines indicate peak glucose after 30 min (*A* and *B*) or peak C-peptide after 60 min (*C* and *D*). Solid lines indicate peak glucose at 30 min (*A* and *B*) or peak C-peptide at 60 min (*C* and *D*). TN, TrialNet.

[2.77–3.85] respectively; both with $P < 0.001$). After adjusting for age, sex, race, BMI Z-score, HOMA-IR, and peak glucose level, the higher risk of progression of type 1 diabetes remained statistically significant in both groups (DPT-1–adjusted HR 2.32 [1.74– 3.10] and PTP-adjusted HR 3.73 [3.09–4.50]; both with $P < 0.001$). Likewise, in both cohorts, those with a peak C-peptide level after 60 min compared with 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, HOMA-IR, and peak C-peptide level in both study populations (DPT-1–adjusted HR 1.96 [1.48–2.58] and PTP-adjusted HR 2.94 [2.45–3.52]; 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 the progression of type 1 diabetes and the time to peak glucose (at vs. after 30 min) or C-peptide ($\leq$60 min vs. $>$60 min) as well as compared with the absolute values of the peak glucose or C-peptide levels in both DPT-1 and PTP (Table 2). Interestingly, the time to peak C-peptide contributed significantly more to the model compared with the absolute value of the peak C-peptide in both the DPT-1 (χ^2 = 25.76 vs. χ^2 = 8.62) and PTP (χ^2 = 149.19 vs. χ^2 = 79.98) populations. When examining the glucose variables and the strength of their association with developing type 1 diabetes, the results differed. The absolute peak glucose level contributed more to the model compared with time to peak glucose in both DPT-1 (χ^2 = 48.67 vs. χ^2 = 30.26) and PTP (χ^2 = 818.92.19 vs. χ^2 = 63.50). All of the differences in Table 2 were statistically significant ($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. $\geq$18). The PTP population was chosen for this analysis given the greater age range in the population and the larger number of participants. We compared

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

	χ^2	*P* value
DPT-1		
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		
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

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, HOMA-IR, and the BMI Z-score. In both age-groups (age <18 and ≥18 years), we again found HRs (before and after adjustments) demonstrated 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) (Supplementary Table 1).

Risk for Progression of Type 1 Diabetes Based on Time to Peak Glucose or C-Peptide After Stratifying by Number of Abs

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 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, BMI Z-score, and HOMA-IR. The calculated HRs were not statistically different between those with single versus multiple Abs 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 significant ($P \leq 0.01$) and are shown in Supplementary 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 whether 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 the baseline OGTT to the 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 (nonprogressors). It is important to note that in the progressors, we classified the last OGTT as the last "nondiagnostic" OGTT or, stated differently, the last OGTT prior to type 1 diabetes diagnosis. For nonprogressors, 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 the last OGTT in progressors compared with nonprogressors 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 glucose or C-peptide by racial groups in the DPT-1 and PTP cohorts.

Assessment of β-Cell Function and IR by the Timing of Peak

Since IR could further burden the β-cells and impact the timing of the peak, we assessed the change in BMI Z-score from the first to the last OGTT and assessed the HOMA-IR (as a measure of IR) by progressors as well as the timing of the peak status and found that there were minimal and often inconsistent differences (Supplementary Table 3).

We then assessed whether the timing of peak glucose or C-peptide was indicative of overall β-cell function or IR, regardless of progressor status. We found that the C-peptide index was significantly higher in those with a peak C-peptide occurring at or before 60 min (Supplementary Table 4). Whereas when assessing these measures by timing of peak glucose, the C-peptide index was significantly lower among those with a peak glucose that occurred after 30 min. However, there were no significant differences in HOMA-IR measures by timing of peak (Supplementary Table 4).

CONCLUSIONS

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-h OGTT are at even higher risk for progression of type 1 diabetes. Specifically, individuals with a peak glucose level after 30 min progress to clinical diabetes development faster than those with a peak glucose level at 30 min. Similarly, those with a peak C-peptide level after 60 min are also at higher risk of progression to clinical diabetes compared with those with a peak C-peptide level at or before 60 min. Our study shows that this risk of progression is independent of age, sex, race, BMI Z-score, HOMA-IR, and the number of Abs. 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

Table 3—Change in frequency of time to peak glucose >30 min or time to peak C-peptide >60 min among nonprogressors and progressors in PTP and DPT-1

	Time to peak glucose >30 min			Time to peak C-peptide >60 min		
	First OGTT	Last OGTT	*P* value^	First OGTT	Last OGTT	*P* value^
PTP						
Nonprogressors (*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						
Nonprogressors (*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 χ^2 test. ^*P* value based on the McNemer test.

significant and interesting findings. First, the time to the peak C-peptide level contributed significantly more to the prediction model when compared with 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, their study did not analyze other time points as in this study and did not include both level and timing in a prediction model as performed in this study. Further, their 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, because 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 versus 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 nondiagnostic OGTT in the progressors, compared with nonprogressors. 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 with 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 compared with the nonprogressors. 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 toward 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 development (32), our findings may serve to be used as a novel stage-specific biomarker for progression from one stage to another. This is particularly true since the calculated HRs were not statistically different between those with single versus multiple Abs at baseline (Supplementary Table 2).

Finally, we aimed to assess whether the timing of peak glucose or C-peptide was indicative of β-cell function, IR, and β-cell function in the face of IR. No significant differences were found in HOMA-IR between the groups, and adjustments for baseline HOMA-IR did not alter the regression analyses results. Meanwhile, the C-peptide index was significantly higher in those with a peak C-peptide at or before 60 min, while in those with a peak glucose occurring after 30 min, the C-peptide index was significantly lower. To assess β-cell function in the face of IR, we calculated the oral disposition index (oDI) using each of HOMA-IR and C-peptide index as well as 1/fasting insulin and C-peptide index in two separate regression models. We found that in both cases, the relationship was not hyperbolic (Supplementary Fig. 4), therefore suggesting that the oDI cannot be calculated based on these methods. Therefore, we could not answer the question whether the time to peak glucose/C-peptide was indicative of β-cell function in the face of IR by calculating oDI as a simple product of the C-peptide index and HOMA-IR or 1/fasting insulin. However, it is quite possible that the use of the insulinogenic index in the model could have allowed for the calculation of the oDI as a simple product. However, insulin levels were not collected at other time points during the OGTT. Nonetheless, given that there were no differences in IR as measured by HOMA-IR, the C-peptide index differences suggest that the timing of peak glucose and C-peptide are, thus far, indicative of β-cell function.

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 (24–26), the 1-h 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-h OGTT glucose in adults. Additionally, we previously demonstrated that based on the glucose response curve shape and among those with a

monophasic or inverted U-shaped glucose response curve (28), those with delayed C-peptide peaks appear to have later glucose peaks and are at higher risk for progression to type 1 diabetes. These studies further support our findings that those who are Ab+ 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 also consistent with expected physiologic changes seen during the progression of type 1 diabetes. The natural history of β-cell decline is that with worsening β-cell insulin secretory defects, manifesting as loss of early insulin secretion in the first 30-min postglucose load, we expect a delayed compensatory C-peptide response as well as delayed glucose peaks. This is followed by progressive worsening glucose tolerance and eventually, development of clinical diabetes. These results, therefore, become critical in identifying those at risk for 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 of the DPT-1 and PTP cohorts is a major strength of this study. Evaluating these two unique at-risk populations separately with intercohort 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 these data can indeed alter screening and clinical practices. Currently, clinicians typically assess the 0- and 120-min 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 the 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 for progression to type 1 diabetes based on our results as well as those at risk for type 2 diabetes 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-h OGTT to determine risk as the lower-risk group peaked at 30 min for glucose and at or before 60 min for C-peptide.

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 responses 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, because using earlier time points would reduce the burden of testing for future individuals and may be more accurate predictors.

Conclusion

Our study shows that within two distinct and high-risk populations of Ab+ 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 Abs 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.

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Author Contributions. M.G.V., D.D.C., M.M.C., P.X., and H.M.I. analyzed and interpreted the data, and wrote the manuscript. P.X., J.M.S., and H.M.I. conceptualized the study. C.E.-M., J.P.P., M.J.R., A.K.S., M.L., H.L., W.V.M., and M.A.A. contributed to the design, interpreted the data, and reviewed and edited the manuscript. D.D.C. and H.M.I. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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