Trends of Earlier and Later Responses of C-peptide to Oral Glucose Challenges With Progression to Type 1 Diabetes in Diabetes Prevention Trial–Type 1 Participants

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

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

RESULTS — The 30–0 min C-peptide difference from OGTTs performed 2.0 years before diagnosis in progressors was lower than the 30–0 min C-peptide difference from OGTTs performed 2.0 years before the last visit in nonprogressors ($P < 0.01$) and remained lower over time. The 90–60 min C-peptide difference was positive at every OGTT before diagnosis in progressors, whereas it was negative at every OGTT before the last visit in nonprogressors ($P < 0.01$ at 2.0 years). The percentage whose peak C-peptide occurred at 120 min was higher in progressors at 2.0 years ($P < 0.05$); this persisted over time ($P < 0.001$ at 0.5 years). However, the peak C-peptide levels were only significantly lower at 0.5 years in progressors ($P < 0.01$). The timing of the peak C-peptide predicted type 1 diabetes ($P < 0.001$); peak C-peptide levels were less predictive ($P < 0.05$).

CONCLUSIONS — A decreased early C-peptide response to oral glucose and an increased later response occur at least 2 years before the diagnosis of type 1 diabetes.

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

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

RESEARCH DESIGN AND METHODS

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

Procedures

The interventions for the parenteral and oral insulin trials were recombinant human ultralente insulin and recombinant human insulin crystals, respectively. OGTTs were performed at 6-month (±3 month) intervals. For each OGTT, fasting samples were obtained before oral glucose administration (1.75 g/kg; maximum 75 g carbohydrate) and then at 30, 60, 90, and 120 min. If OGTTs were in the diabetic range, participants were asked to return for confirmation with another OGTT (unless contraindicated). The procedure for the intravenous glu-

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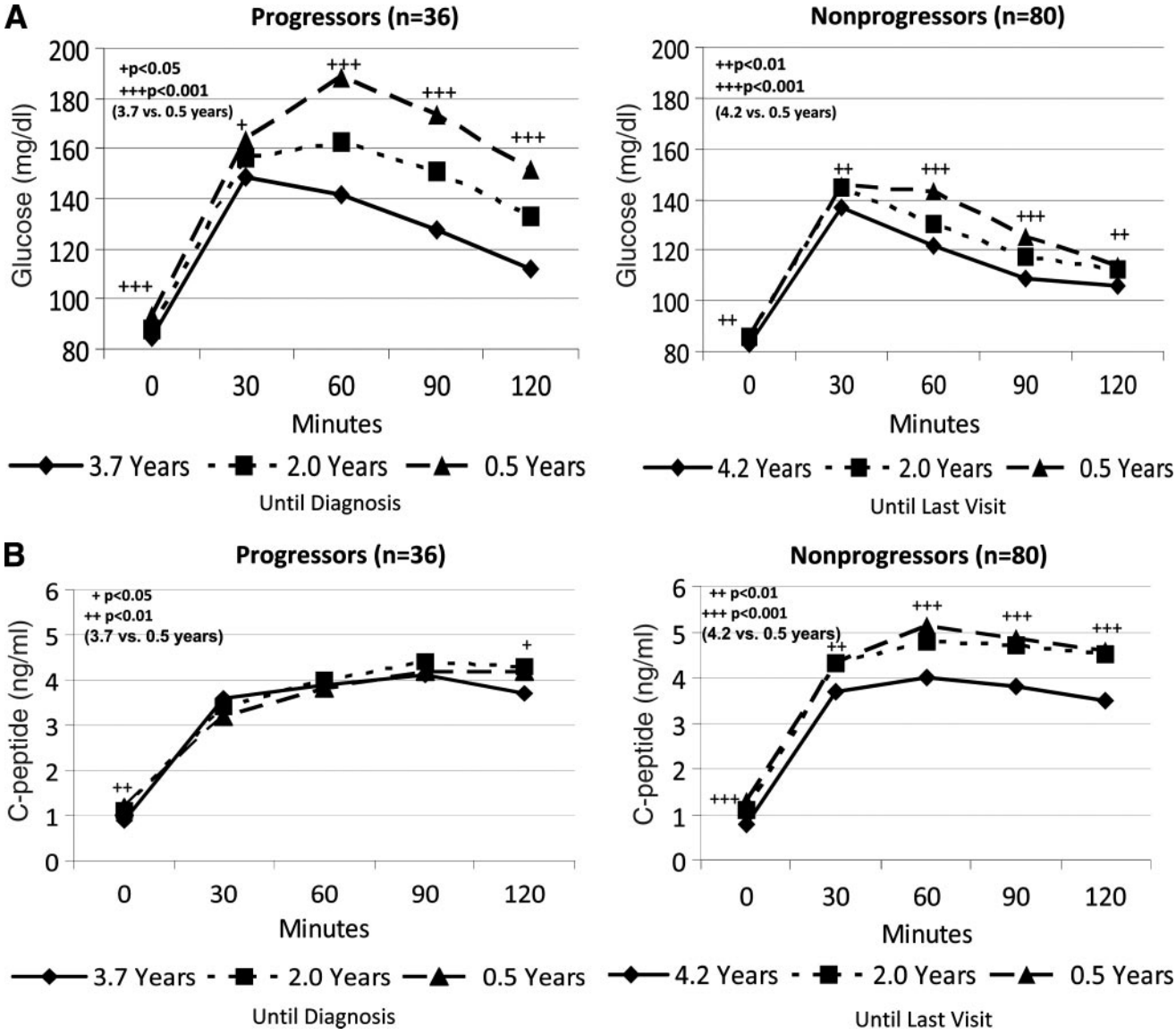

Figure 1—A: *Glucose curves from OGTTs at baseline and 2.0 and 0.5 years before diagnosis in the progressors, and at baseline and corresponding times before the last visit in the nonprogressors. In both the progressors and nonprogressors, glucose levels increased substantially at all OGTT time points (AUC glucose:* $P < 0.001$ *from baseline to 0.5 years in both groups). (Mean values are shown for the times before diagnosis or the last visit.)* B: *C-peptide curves from OGTTs at baseline and 2.0 and 0.5 years before diagnosis in the progressors, and at baseline and corresponding times before the last visit in the nonprogressors. In the nonprogressors, C-peptide levels increased at all OGTT time points (AUC C-peptide:* $P < 0.001$ *from baseline to 0.5 years). In the progressors, although the fasting and 120-min C-peptide levels were higher at 0.5 years than at baseline, there was no significant overall change (AUC C-peptide:* $P = 0.936$ *from baseline to 0.5 years). (Mean values are shown for the times before diagnosis or the last visit.)*

cose tolerance tests has been described elsewhere.

Laboratory measures

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

Data analysis

For group and paired comparisons, *t* tests and χ^2 tests were used. Spearman correlation was used to assess association. Cox proportional hazards regression was used for assessing type 1 diabetes associations over time. Glucose tolerance abnormalities were defined as follows: impaired fasting glucose = fasting glucose value 100–125 mg/dl; indeterminate = 30-, 60-, and/or 90-min glucose value ≥200 mg/dl; and impaired glucose tolerance = 2-h glucose value 140–199 mg/dl. The thresholds for diabetes were a fasting glucose value ≥126 mg/dl and/or a 2-h glucose value ≥200 mg/dl. The sum of C-peptide levels after 30 min was calcu-

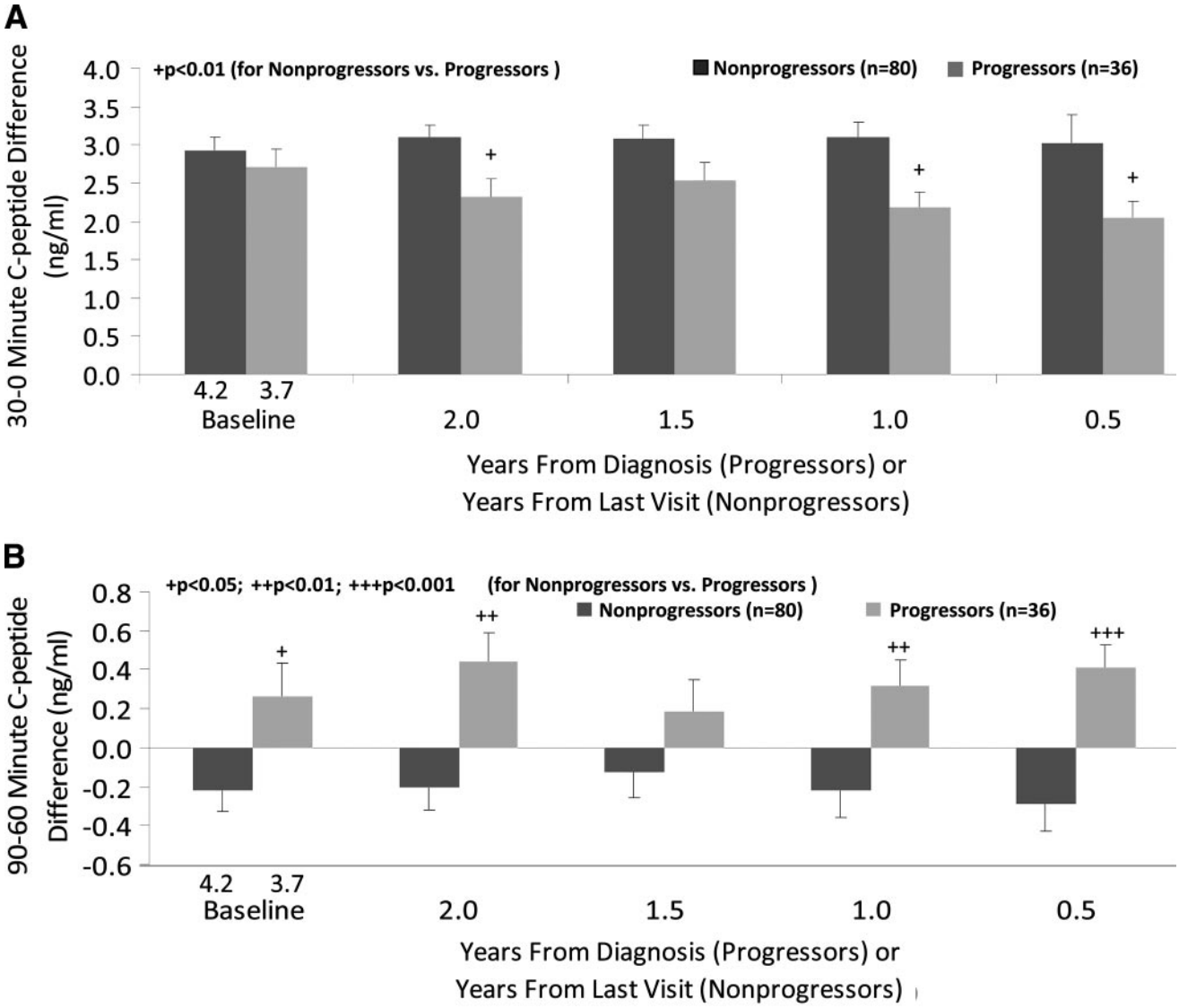

Figure 2—A: *The difference (mean ± SE) in C-peptide levels from 0 to 30 min (the 30–0 min C-peptide difference) according to the times before diagnosis (progressors) or the times before the last visit (nonprogressors). The 30–0 min C-peptide difference was consistently lower in the progressors than in the nonprogressors. (Mean values are shown for the times before diagnosis or the last visit.)* B: *The difference (mean ± SE) in C-peptide levels from 60 to 90 min (the 90–60 min C-peptide difference) according to the times before diagnosis (progressors) or the times before the last visit (nonprogressors). At every time before diagnosis, the 90–60 min C-peptide difference was positive in the progressors, whereas at every time before the last visit, it was negative in the nonprogressors. (Mean values are shown for the times before diagnosis or the last visit.)*

lated by subtracting the 30-min values from the 60-, 90-, and 120-min values and adding the sum of each of the differences. The first-phase insulin response was defined as the sum of insulin levels at the 1st and 3rd min of the intravenous glucose tolerance test. The trapezoidal rule was used to calculate OGTT AUC. The SAS 9.1.3 version was used for the analyses. All *P* values are two-sided.

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

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

C-peptide levels (Fig. 1*B*) increased significantly at each OGTT time point from baseline to 0.5 years in the nonprogressors ($P < 0.001$ for the AUC C-peptide from baseline to 0.5 years). However, the change in C-peptide levels

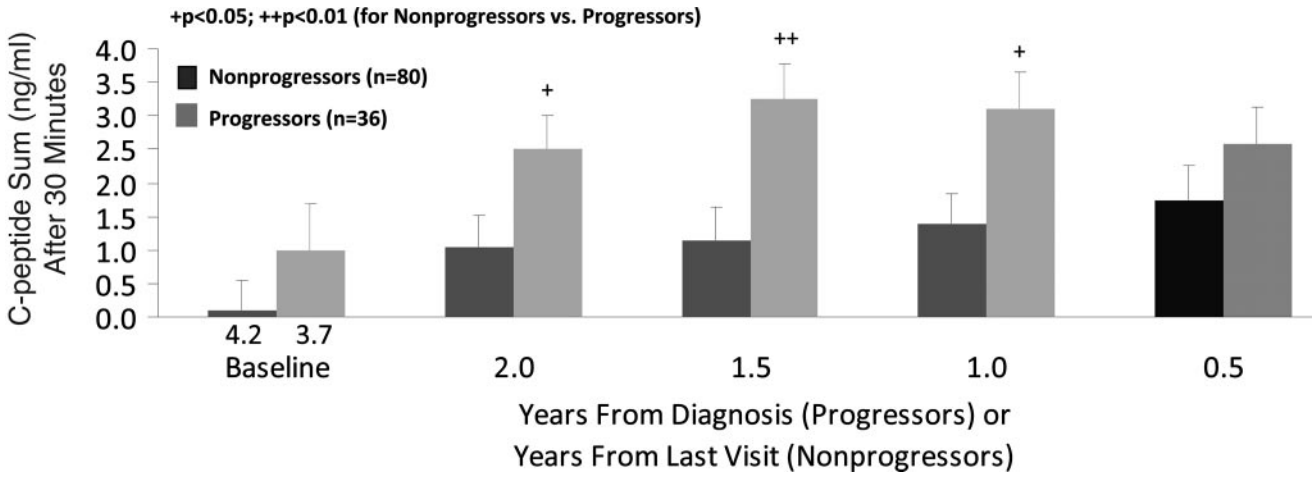

Figure 3—*The C-peptide sum after 30 min (mean ± SE) according to the times before diagnosis (progressors) or the times before the last visit (nonprogressors). The values were higher in the progressors from baseline to 0.5 years. (Mean values are shown for the times before diagnosis or the last visit.)*

from baseline to 0.5 years in the progressors varied according to the OGTT time point. Fasting and 120-min C-peptide levels increased from baseline to 0.5 years ($P < 0.01$ and $P < 0.05$, respectively) in the progressors, but there was no significant change at the other OGTT time points nor in the AUC C-peptide ($P = 0.936$).

We compared the 30−0 min C-peptide difference at baseline and every 6 months for 2 years before diagnosis in the progressors with the 30−0 min C-peptide difference at baseline and at corresponding times before the last visit in the nonprogressors. (Among the 504 participants at baseline, there was a positive correlation between the 30−0 min C-peptide difference and the first-phase insulin response [$r = 0.50$, $P < 0.001$]). The 30−0 min C-peptide difference (Fig. 2*A*) was similar at baseline; however, by 2.0 years, that difference was lower in the progressors ($P < 0.01$). Among the progressors, the 30−0 min C-peptide difference declined from baseline to 0.5 years before diagnosis ($P < 0.01$).

Whereas the 90−60 min C-peptide difference (Fig. 2*B*) was negative (i.e., the 90-min value was less than the 60-min value) at all times before the last visit in the nonprogressors, it was positive at all times before diagnosis in the progressors. This contrast was not only significant at 2.0 ($P < 0.01$), 1.0 ($P < 0.01$), and 0.5 years ($P < 0.001$), but also at baseline ($P < 0.05$).

As an overall measure of later C-peptide responsiveness to oral glucose, we used the sum of each of the C-peptide differences of the 30-min value subtracted from the values at 60, 90, and 120 min (C-peptide sum after 30 min). Figure 3 shows that those values were significantly higher in the progressors than in the nonprogressors at corresponding time points from 2.0 years to 1.0 year. Values were also higher, but not significantly so, at baseline and at 0.5 years. Among the progressors, the C-peptide sum after 30 min increased significantly from baseline to all subsequent time points ($P < 0.05$ from baseline to 2.0 years and to 0.5 years; $P < 0.01$ from baseline to 1.5 years and to 1.0 year).

Consistent with the above findings, the timing of the peak C-peptide was delayed in the progressors. The percentage of individuals with the peak C-peptide occurring at 120 min was significantly higher in the progressors at 2.0 years (39 vs. 21%, $P < 0.05$). By 0.5 years, the peak C-peptide occurred at 120 min in 56% of the progressors compared with 18% of the nonprogressors ($P < 0.001$). Actual peak C-peptide levels did not differ between the progressors and nonprogressors until 0.5 years ($P < 0.01$). Among the full cohort of 504 at baseline, the occurrence of the peak C-peptide at 120 min and the 90−60 min C-peptide difference (above versus below 0) were both highly predictive of type 1 diabetes with and without age as a covariate ($P < 0.001$) in proportional hazards models; the peak C-peptide level was somewhat predictive ($P = 0.028$), but not with age in the model.

Figure 4 shows C-peptide changes in the progressors who had OGTTs 0.5 years before diagnosis and at diagnosis ($n = 66$), and in the nonprogressors who had OGTTs 0.5 years before the last visit and at the last visit ($n = 192$). The 30−0 min C-peptide difference (Fig. 4*A*) declined considerably in the progressors ($P < 0.001$). The C-peptide sum after 30 min declined somewhat at diagnosis, but not below levels in the nonprogressors at the last visit (Fig. 4*B*). Even at diagnosis, the delay in the peak C-peptide persisted. The peak C-peptide occurred at 120 min in 52% of the progressors at diagnosis compared with 23% of the nonprogressors at their last visit ($P < 0.001$).

CONCLUSIONS — The data suggest that the early C-peptide response to oral glucose can be decreased for at least 2 years before the diagnosis of type 1 diabetes and especially as diagnosis approaches. Although the early C-peptide response to the glucose challenge declines, C-peptide levels increase at later time points. This was evident in the interval from 60 to 90 min. The C-peptide increase in that interval in the progressors contrasted with the decline in the nonprogressors. It appears that the increase in C-peptide levels from 60 to 90 min can occur even 3 years before diagnosis. This prolonged increase in C-peptide levels after the glucose challenge is also manifested by a delayed peak C-peptide at least 2 years before diagnosis.

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

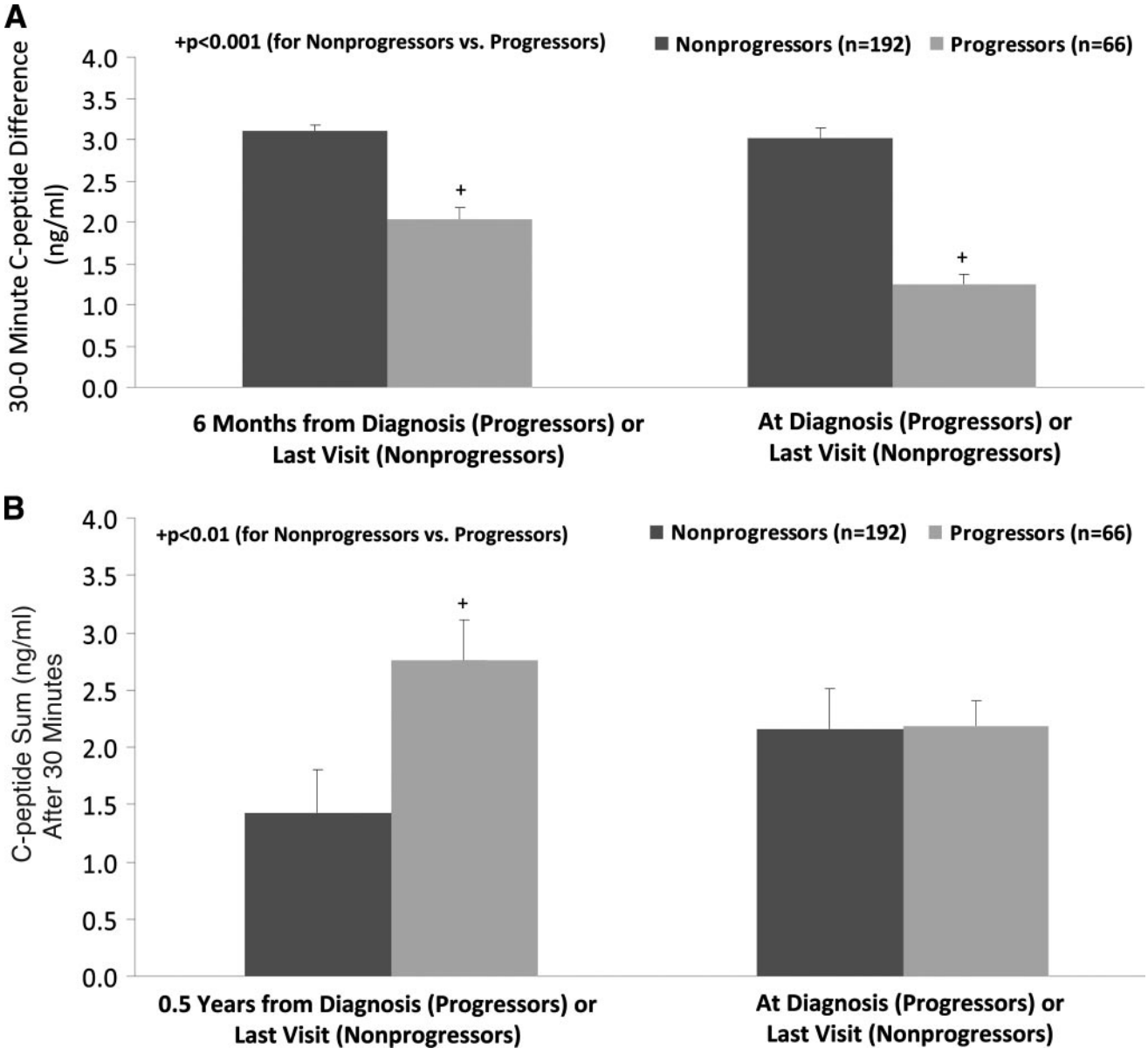

Figure 4—A*: The 30–0 min C-peptide difference (mean ± SE) in the progressors who had OGTTs 0.5 years before diagnosis and at diagnosis and in the nonprogressors who had OGTTs 0.5 years before the last visit and at the last visit. The 30–0 min C-peptide difference declined considerably in the progressors.* B*: The C-peptide sum after 30 min (mean ± SE) in the progressors who had OGTTs 0.5 years before diagnosis and at diagnosis and in the nonprogressors who had OGTTs 0.5 years before the last visit and at the last visit. The C-peptide sum after 30 min declined, but did not fall below that in the nonprogressors.*

suggests that the later response is also failing by that time.

There are few longitudinal data available regarding the metabolic progression to type 1 diabetes. Although we have previously examined changes in C-peptide and glucose indexes with progression to type 1 diabetes (4), data pertaining to the timing of the C-peptide response before the diagnosis of type 1 diabetes has not been reported, nor has the prediction of type 1 diabetes by the timing of the C-peptide response been reported. A decreased early insulin response was a risk factor for progression to type 2 diabetes in Pima Indians. However, in contrast to our findings for type 1 diabetes, a decreased (rather than increased) later insulin response was predictive of type 2 diabetes in Pima Indians with impaired glucose tolerance (10).

The decreased early C-peptide response together with the increased later C-peptide response to oral glucose that we observed in the "pre-diabetic" state of type 1 diabetes is similar to the abnormal insulin responses to oral glucose in patients already diagnosed with type 2 diabetes (11,12). This suggests that there could be some commonality between the disorders in the progression of metabolic abnormalities.

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

Because a number of the nonprogressors would probably ultimately develop type 1 diabetes, and thus were not metabolically normal, differences between progressors and a normal reference group could be even more substantial. It is of interest that there was such a marked increment in C-peptide over time in the nonprogressors. This could represent typical changes with aging, early pathogenetic changes, or both. The pattern of change suggests increasing insulin resistance over time.

The findings in this report help to explain why such measures as peak C-peptide and AUC C-peptide values provide relatively little information with regard to the prediction of type 1 diabetes and to its natural history. Those indexes change little with progression because the deficient early C-peptide response to the oral glucose challenge is somewhat balanced by a continuing compensatory response until close to diagnosis. Thus, the peak C-peptide and AUC C-peptide indexes fail to detect the substantial changes in the β-cells that are occurring years before diagnosis. It is evident that OGTTs can yield appreciably more prediction and natural history information when they are partitioned according to the time after the glucose challenge.

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References

1. Sherry NA, Tsai EB, Herold KC. Natural history of β-cell function in type 1 diabetes. Diabetes 2005;54 (Suppl. 2):S32–S39
2. Sosenko JM, Palmer JP, Greenbaum CJ, Mahon J, Cowie C, Krischer JP, Chase HP, White NH, Buckingham B, Herold KC, Cuthbertson D, Skyler JS. Patterns of metabolic progression to type 1 diabetes in the Diabetes Prevention Trial–Type 1. Diabetes Care 2006;29:643–649
3. Skyler JS. Prediction and prevention of type 1 diabetes. Clin Pharm Ther 2007; 81:768–771
4. Eisenbarth GS. Update in type 1 diabetes. J Clin Endocrinol Metab 2007;92:2403–2407
5. Sosenko JM, Palmer JP, Greenbaum CJ, Mahon J, Cowie C, Krischer JP, Chase HP, White NH, Buckingham B, Herold KC, Cuthbertson D, Skyler JS, Diabetes Prevention Trial–Type 1 Study Group. Increasing the accuracy of oral glucose tolerance testing and extending its application to individuals with normal glucose tolerance for the prediction of type 1 diabetes. Diabetes Care 2007;30: 38–42
6. Sosenko JM, Palmer JP, Rafkin-Mervis L, Krischer JP, Cuthbertson D, Matheson D, Skyler JS. Glucose and C-peptide changes in the peri-onset period of type 1 diabetes in the Diabetes Prevention Trial–Type 1. Diabetes Care 2008;31:2188–2192
7. Diabetes Prevention Trial–Type 1 Diabetes Study Group. Effects of insulin in relatives of patients with type 1 diabetes mellitus. N Engl J Med 2002;346:1685–1691
8. Skyler JS, Krischer JP, Wolfsdorf J, Cowie C, Palmer JP, Greenbaum C, Cuthbertson D, Rafkin-Mervis LE, Chase HP, Leschek E. Effects of oral insulin in relatives of patients with type 1 diabetes. Diabetes Care 2005;28:1068–1076
9. Yu L, Cuthbertson DD, Eisenbarth GS, Krischer JP. Diabetes Prevention Trial 1: Prevalence of GAD and ICA512 (IA-2) autoantibodies by relationship to proband. Ann N Y Acad Sci 2002;958:254–258
10. Nagi DK, Knowler WC, Charles MA, Liu QZ, Hanson RL, McCance DR, Pettitt DJ, Bennett PH. Early and late insulin response as predictors of NIDDM in Pima Indians with impaired glucose tolerance. Diabetologia 1995;38:187–192
11. Yalow RS, Glick SM, Roth J, Berson SA. Plasma insulin and growth hormone levels in obesity and diabetes. Ann N Y Acad Sci 1965;131:357–373
12. Bagdade JD, Bierman EL, Porte D Jr. The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects. J Clin Invest 1967;41:1549–1557