Fall in C-Peptide During First 4 Years From Diagnosis of Type 1 Diabetes: Variable Relation to Age, HbA$_{1C}$, and Insulin Dose

OBJECTIVE
We aimed to describe the natural history of residual insulin secretion in Type 1 Diabetes TrialNet participants over 4 years from diagnosis and relate this to previously reported alternative clinical measures reflecting β-cell secretory function.

RESEARCH DESIGN AND METHODS
Data from 407 subjects from 5 TrialNet intervention studies were analyzed. All subjects had baseline stimulated C-peptide values of ≥0.2 nmol/L from mixed-meal tolerance tests (MMTTs). During semiannual visits, C-peptide values from MMTTs, HbA$_{1C}$, and insulin doses were obtained.

RESULTS
The percentage of individuals with stimulated C-peptide of ≥0.2 nmol/L or detectable C-peptide of ≥0.017 nmol/L continued to diminish over 4 years; this was markedly influenced by age. At 4 years, only 5% maintained their baseline C-peptide secretion. The expected inverse relationships between C-peptide and HbA$_{1C}$ or insulin doses varied over time and with age. Combined clinical variables, such as insulin-dose adjusted HbA$_{1C}$ (IDA1A1C) and the relationship of IDA1A1C to C-peptide, also were influenced by age and time from diagnosis. Models using these clinical measures did not fully predict C-peptide responses. IDA1A1C ≤9 underestimated the number of individuals with stimulated C-peptide ≥0.2 nmol/L, especially in children.

CONCLUSIONS
Current trials of disease-modifying therapy for type 1 diabetes should continue to use C-peptide as a primary end point of β-cell secretory function. Longer duration of follow-up is likely to provide stronger evidence of the effect of disease-modifying therapy on preservation of β-cell function.

Type 1 Diabetes TrialNet is an international network established to conduct clinical trials to intervene in the type 1 diabetes disease process (1), either before diagnosis (prevention) or after clinical diagnosis (intervention), with the aim of preserving β-cell function.

Clinical trials to alter the course of β-cell destruction after diagnosis usually consider the amount of residual insulin secretion after 1 or 2 years of therapy as the primary outcome measure to assess effectiveness. Although other assessments have been used, insulin secretion is generally measured through stimulated
Subjects were monitored for up to 4 years after randomization with regularly scheduled MMTTs, as previously described. Subjects were monitored for the first 2 years under their original study protocol. Subsequently, subjects were enrolled in the TrialNet Long Term Investigative Follow-up (LIFT) protocol and monitored regularly with MMTT assessments as long as C-peptide was present.

**MMTT**

As previously described (2), data were collected from a 2-h MMTT begun before 10:00 A.M. after an overnight fast. MMTTs were begun only if the fasting glucose levels were between 70 and 200 mg/dL. Boost-HP (Nestle Health Care Nutrition, Inc.) was used at a dose of 6 mg/kg to a maximum of 360 ml.

C-peptide and HbA1c were measured in Northwest Lipid Laboratories at the University of Washington (Seattle, WA). C-peptide was measured by using a two-site immunoenzymometric assay performed on a Tosoh II 600 autoanalyzer. Samples with C-peptide ≥0.017 nmol/L were considered detectable. The C-peptide area under curve (AUC) was calculated using the trapezoidal method and then divided by the time period of the test. Insulin use was determined by averaging self-reported doses during a 3-day period the week before each MMTT visit.

**Maintenance of C-Peptide**

Subjects were classified as having maintained C-peptide over time if there was no change from baseline to each of the time points after baseline. To account for statistical variation in C-peptide measurements, we used three definitions of maintained C-peptide that are suggested by two published studies (2,11). The three definitions are similar in that they each consider no change or an increase from baseline to represent a positive response. They differ in the amount of decrease they allow for a subject to still be classified as having maintained C-peptide:

1. **“Percentage change” definition:** Follow-up C-peptide value of no more than 7.5% below baseline (one-half of the interassay coefficient of variation [CV] of the C-peptide assay used in the study) (11).
2. **“Intertest Variability” definition:** Change from baseline, either nonnegative or if negative, no more than 1 intertest SD bellow baseline (2).
3. **“CV” definition:** Change from baseline, either nonnegative or if negative, within the median CV from the MMTT/glucagon stimulation test study (2).

**Statistical Analysis**

Subjects were divided into one of three prespecified age cohorts based on age at time of randomization: <12 years, 12–17 years, and ≥18 years. Associations between the covariates and the C-peptide AUC and C-peptide peak were assessed using Spearman correlation coefficients for continuous factors and ANOVA for categorical factors. C-peptide AUCs were calculated using the trapezoidal method; areas were then divided by the time period of the test (120 min). Kaplan-Meier analyses were used to assess time to C-peptide ≤0.20 nmol/L and time to undetectable C-peptide (≤0.017 nmol/L) across the period of 0 to 48 months. Simple least-squares regressions adjusted for baseline C-peptide were used to assess relationships between C-peptide and various factors. Similar analyses were used to assess the relationship between HbA1c and various factors, adjusting for baseline HbA1c. A significance of 0.05 was used in all tests. Adjustments were not made for multiple testing. SAS software was used for all analyses.

Because the analyses combined data from placebo-treated subjects from all studies with data from actively treated subjects from “negative” TrialNet intervention studies to increase the number of observations, we first investigated whether inclusion of the actively treated subjects affected the multivariate analyses by using a variable to indicate treatment in the active studies. The results were not significant at 0.05. The Levene test for homogeneity of variance and graphical comparisons were used to assess the appropriateness of combining the five studies into one analysis. The test was not significant (P = 0.67), and the graphical comparisons did not uncover any appreciable differences in variability.

**RESULTS**

C-Peptide Change Over Time

Demographic and baseline characteristics of the 407 subjects included in this analysis are reported in Supplementary Table 1. Overall, 84%, 63%, 41%, and
31% of individuals had peak stimulated C-peptide values \( \geq 0.2 \text{ nmol/L} \) at the end of 1, 2, 3, and 4 years of follow-up, respectively. The corresponding percentages of individuals with detectable stimulated C-peptide values \( (\geq 0.017 \text{ nmol/L}) \) over time were 98%, 92%, 83%, and 69% at 1, 2, 3, and 4 years (Supplementary Fig. 1). The percentages differed markedly by age, as shown in Fig. 1.

We then evaluated C-peptide preservation over time, using the classification of subsets as having maintained C-peptide or not, separately for each of the three definitions introduced in the RESEARCH DESIGN AND METHODS. When the baseline was compared with year 1, 2, 3, and 4 values, 78 (21%), 37 (12%), 7 (6%), and 3 (5%) subjects maintained C-peptide as defined by the CV definition, respectively, with similar values for other definitions (as reported in Supplementary Table 2).

**Relationship of C-Peptide With Clinical Variables HbA1c and Insulin Use Varies by Age and Over Time**

We evaluated the C-peptide (mean AUC) and the relationship of C-peptide with HbA1c, and insulin use over time by age category. As expected, mean C-peptide

![Figure 1](attachment:image.png)

**Figure 1**—Probabilities (Prob.) of subjects having peak stimulated C-peptide \( \leq 0.2 \text{ nmol/L} \) (A) and undetectable (Undet.) stimulated C-peptide (B) over time by age. Subjects were divided into three predefined age-groups (<12, 12–17, and \( \geq 18 \) years). C-peptide diminished over the 4 years from diagnosis and was affected by age.
values decreased in all age-groups over 4 years (Fig. 2A), although the rate of fall was less in adults. The relationship between C-peptide and HbA1c (Fig. 2B) or insulin use in units/kg (Fig. 2C) also differed by age. The relationship between C-peptide and the clinical variables of HbA1c and insulin use changed over time in all three age-groups.

To further explore relationships of clinical factors with C-peptide over time, we evaluated regression models using age as a continuous and categorical (data not shown) variable (age <18 or ≥18), sex, HbA1c, insulin dose, and baseline stimulated C-peptide (0 months). Two points are noteworthy from this analysis. At each time period, the R² for these models ranged from 0.44 to 0.58, indicating that the above factors do not fully explain C-peptide concentrations (Table 1). Moreover, the effect of age, HbA1c, insulin dose, and baseline stimulated C-peptide on the outcome varied over time, with only baseline C-peptide and HbA1c remaining significantly associated with stimulated C-peptide at 4 years. Similarly, regression models including age, C-peptide, insulin dose, and HbA1c at baseline had very weak ability to predict HbA1c (R² from 0.16 to 0.33) at any time point (Table 1).

**Relationship of C-Peptide and IDAA1C Over Time and by Age**

IDAA1C ≤ 9 has been reported as indicative of “remission” (4). We first examined the effect of age and time on IDAA1C. As shown in Fig. 3, less than 80% of individuals met this definition at baseline, and by 2 years, less than 10% of children and 50% of adults remained with IDAA1C ≤ 9. We then explored the correlation between stimulated C-peptide and IDAA1C over time by age. As reported in Table 1, we observed negative correlations between stimulated C-peptide and IDAA1C. The correlations changed over time and differed by age. At 4 years, the significant negative correlation was maintained in adult participants but was lost in young children. Importantly, using IDAA1C ≤ 9 as a surrogate of measure of residual β-cell function significantly underestimated the number of subjects with stimulated C-peptide ≥ 0.2 nmol/L, especially in children.

**CONCLUSIONS**

We report in this study that in individuals with type 1 diabetes assessed longitudinally, neither HbA1c, nor insulin use, nor a combination of both using IDAA1C, are reliable surrogates of insulin secretion compared with C-peptide responses to MMTT stimulation. Although age continues to affect the rate of fall of C-peptide up to 4 years from diagnosis, only a small percentage of subjects of any age maintain stable β-cell function at 4 years.

Understanding the natural history of residual β-cell function change after onset of type 1 diabetes is needed to test treatments designed to alter the disease process and preserve residual β-cell function. In the Diabetes Control and Complications Trial (DCCT), maintaining β-cell insulin secretion, as assessed by residual C-peptide production, demonstrated benefits for clinical diabetes management and also delayed and/or prevented complications of the disease (12–14). Evidence from islet transplantation studies demonstrated that attainment of endogenous C-peptide after islet transplantation correlates with glycemic control, including restoring hypoglycemia awareness, preventing recurrent severe hypoglycemia, and decreasing glycemic variability (15–17).

This report extends our previous analysis describing changes in MMTT-stimulated C-peptide in subjects who enrolled in Type 1 Diabetes TrialNet studies within 3 months of diagnosis by describing changes up to 4 years after diagnosis. Our previous report (18) included data from only three intervention trials (6,7,9), whereas our current analysis includes data from five intervention trials (6–10) with double the number of
participants. We demonstrated more rapid C-peptide decline during the first year compared with the subsequent 2 to 4 years, and only a few subjects of any age maintained β-cell function at 4 years. We extend our previous report by confirming the effect of age on residual β-cell function out to 4 years from diagnosis. Although limited to those enrolled in TrialNet clinical trials, our findings of residual β-cell function changes over time and the age effect on insulin secretion at diagnosis are comparable with a recent cross-sectional cohort study report of the prevalence of detectable C-peptide in those with long-standing disease (19).

Individuals living with other autoimmune diseases, such as rheumatoid arthritis, have seen a recent transformation in care from symptomatic treatment to disease-modifying treatment (20). This is the same goal we wish to achieve in type 1 diabetes, transitioning from treating hyperglycemia to treating with agents that alter the disease course. These efforts involve clinical trials before the onset of clinical disease (prevention studies) and also trials soon after diagnosis (intervention trials) aimed to preserve β-cell function. The first trials using immune therapies to alter the disease course considered the aim of prolonging the honeymoon period after diagnosis, defined as taking individuals off of insulin. Indeed, an early trial of cyclosporine (21) reported 24.1% of treated subjects were off insulin (in complete remission) vs. 5.8% of the placebo group at 9 months. Some studies have still reported this end point. Subsequent studies looked toward a direct measure of the biological effect of these interventions and used C-peptide, usually in response to MMTT stimulation, as the primary end point of this end point. Subsequent studies aimed to alter the disease course.

Table 1—Regression models of analysis of predictors for stimulated C-peptide or HbA1c over time and correlation between peak C-peptide and IDAA1C over time by age

| Duration of diabetes (years) | Peak C-peptide ≥0.2 nmol/L | HbA1c ≤9% (95% CI) | IDAA1C* ≤9% (95% CI) | Correlation coefficient (P value) |
|-----------------------------|-----------------------------|------------------|----------------------|----------------------------------|
| At 1 year                   |                             |                  |                      |                                  |
| <12                         | 71.2 (62.1, 79.5)            | 29.0 (21.1, 37.3)| −0.55 (<0.0001)      |
| 12–17                       | 85.7 (79.4, 90.2)            | 29.7 (23.0, 36.7)| −0.69 (<0.0001)      |
| ≥18                         | 94.7 (88.5, 97.6)            | 63.7 (53.9, 71.9)| −0.68 (<0.0001)      |
| At 2 years                  |                             |                  |                      |                                  |
| <12                         | 42.8 (33.4, 51.8)            | 9.8 (5.2, 16.0)  | −0.24 (0.03)         |
| 12–17                       | 65.3 (57.4, 72.1)            | 13.2 (8.6, 18.8)| −0.45 (<0.0001)      |
| ≥18                         | 80.5 (71.6, 86.8)            | 52.9 (43.0, 61.8)| −0.65 (<0.0001)      |
| At 3 years                  |                             |                  |                      |                                  |
| <12                         | 26.9 (17.6, 37.1)            | —                | −0.41 (0.05)         |
| 12–17                       | 36.4 (27.9, 45.0)            | 6.3 (3.0, 11.2)  | −0.48 (0.003)        |
| ≥18                         | 64.0 (52.6, 73.4)            | 39.1 (28.8, 49.2)| −0.72 (<0.0001)      |
| At 4 years                  |                             |                  |                      |                                  |
| <12                         | 9.6 (3.1, 20.5)              | —                | −0.06(0.85)          |
| 12–17                       | 27.0 (18.5, 36.1)            | 2.7 (0.8, 6.8)   | −0.34(0.18)          |
| ≥18                         | 61.2 (48.9, 71.4)            | 20.4 (9.2, 34.8) | −0.70 (0.003)        |

*IDAA1C = HbA1c (percentage) + 4 × (insulin dose [in units/kg/day]).
and practical issues with these end points. For example, with tight glycemic control as standard of care, investigators work to achieve this goal for all trial participants, aiming to have placebo and treatment groups have equal HbA1c. Other measures of glycemic control are too infrequently seen in persons with new-onset disease to serve as clinical trial end points (e.g., severe hypoglycemia or diabetic ketoacidosis) or are not collected consistently by study participants (home glucose monitoring), thus leading to potential bias. In contrast to rigid meals and limited types of insulin and dosing schedules that were the standard of care in the 1980s, there is now wide variability in insulin delivery methods and insulin types used. The units/kg of insulin use may vary considerably according to physician and patient choices, including whether one is using continuous subcutaneous insulin infusion or injections, the amount of carbohydrate consumed, and the amount of exercise. Thus, using insulin use in units/kg is unlikely to strongly correlate with residual insulin secretion in multicenter clinical trials without limiting the other known behavioral factors.

We observed only weak relationships between the biological measure of interest (stimulated insulin secretion) and the clinical measures (HbA1c and/or insulin use), whether alone or in combination. Moreover, we found that time influences this relationship, making them poor outcome measures for evaluating the effect of therapy on preservation of β-cell function. We highlight the well-known effect of age not only on C-peptide over time but now also on the relationship of C-peptide to other clinical variables of HbA1c and insulin dose by age.

The aim of disease-modifying therapies in type 1 diabetes is to prevent the clinical presence of disease altogether. An interim goal would be a markedly less intensive needs for insulin administration and glucose monitoring without hypoglycemia or risk of long-term complications. A necessary step to these end points in those after clinical diagnosis is to first demonstrate that a new therapy can robustly and safely preserve β-cell function. Most clinical trials of disease-modifying therapies to date have used 1 and 2 years as the primary end point to determine the effects of therapy. In addition to reemphasizing that clinical trials aiming to preserve β-cell function should use measures of insulin secretion as the outcome measure, the data presented here suggest that continued follow-up of such individuals could provide additional evidence of the effect of therapy because few individuals, particularly children, have significant insulin secretion after 4 years, and <5% of all individuals of any age maintain their baseline C-peptide levels at that time point.

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