Preservation of Beta Cell Function in Autoantibody Positive Youth with Diabetes

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Submitted 29 December 2008 and accepted 19 June 2009

This is an uncopyedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Association, publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes Care in print and online at http://care.diabetesjournals.org.
Objective: Determine the extent of beta cell function in youth with diabetes and GAD65 and/or IA2 autoantibodies.

Methods: Fasting C-peptide levels from 2789 GAD65 and/or IA2 autoantibody positive youth aged 1-23 years from the SEARCH for Diabetes in Youth study. Preserved beta cell function was defined based on cut points derived from the Diabetes Control and Complications Trial (fasting C-peptide \( \geq 0.23 \) ng/ml) and from the US adolescent population of the National Health and Nutrition Examination Survey (NHANES), 5th percentile for fasting C-peptide ( \( \geq 1.0 \) ng/ml). We compared the clinical characteristics between those with and without preserved beta cell function.

Results: Within the first year of diagnosis, 82.9% of youth had a fasting C-peptide \( \geq 0.23 \) ng/ml and 31.2% had values \( \geq 1.0 \) ng/ml. Among those with \( \geq 5 \) years of diabetes duration, 10.7% had preserved beta-cell function based on the DCCT cutoff and 1.0% were above the 5th percentile of the NHANES population.

Conclusion: Within the first year of diagnosis, four out of five youth with autoantibody positive diabetes have clinically significant amounts of residual beta cell function and about a third have fasting C-peptide levels above the 5th percentile of a healthy adolescent population. Even five years after diagnosis, one out of ten has fasting C-peptide above a clinically significant threshold. These findings have implications for clinical classification of youth with diabetes as well as clinical trials aimed at preserving beta-cell function after diabetes onset.
Immune mediated beta cell destruction, marked by the presence of diabetes autoantibodies, occurs prior to and continues after the clinical diagnosis of Type 1 diabetes (T1DM). This model has served as the foundation of pathophysiologic studies of the disease process and clinical studies designed to identify future risk for diabetes and to modify clinical course. The resultant perception is that most individuals with T1DM will have complete destruction of beta cells within a few years after diagnosis without a targeted intervention to sustain beta cell function.

Despite frequent use of this model in research and patient care, it represents only part of the picture. Data from placebo controlled populations in clinical intervention trials suggest that some individuals with T1DM will have persistent beta cell function years after diagnosis (1,2). The screening phase of the Diabetes Control and Complications Trial (DCCT) demonstrated that 48% of adults had significant C-peptide levels within 5 years of diagnosis, and 8% had significant C-peptide 5-15 years after diagnosis (3). Another study reported similar findings of 15% of individuals with measurable C-peptide 8-15 years after diagnosis (4). Multiple studies have reported that the loss of beta cell function after diagnosis is related to age of onset, as well as factors linked to autoimmunity such as autoantibodies (5-11). Nonetheless, there is a common belief that persistence of beta cell function is rare in young children with T1DM.

The SEARCH for Diabetes in Youth study, designed to determine the prevalence, incidence and characteristics of diabetes in US youth, provides an opportunity to examine the frequency of residual beta cell function in a population-based sample of GAD65 or IA2 positive youth with diabetes.

RESEARCH DESIGN AND METHODS

Data for this analysis derive from the SEARCH for Diabetes in Youth study as described previously (12). SEARCH is a population-based study conducted at six centers in the United States, including existing (prevalent) and newly diagnosed (incident) cases of diabetes in youth less than 20 years. Participants are asked to complete an initial survey and then invited to an in-person study visit. After informed consent, a brief exam was performed and blood samples were obtained.

Study population: Cases prevalent in 2001 and incident in 2002-2005 that participated in the in-person visit and had fasting C-peptide measured were eligible for this study (N=4529). The present analysis includes 2789 SEARCH participants who are also GAD65 and/or IA2 antibody positive. DNA samples were available for HLA analysis for 1968 (70.6%) of these individuals.

Measurements: Body mass index was normalized as standard deviation score (SDS or Z score) based on age and gender. Weight was classified according to gender specific percentiles on Center for Disease Control BMI-for-age growth charts as follows: underweight, < 15th percentile; healthy weight, 15th percentile to < 85th percentile; overweight, 85th to < 95th percentile; obese, ≥ 95th percentile.

Laboratory methods: Fasting samples were obtained under conditions of metabolic stability, defined as no episode of diabetic ketoacidosis during the previous month. Assays were performed at the Northwest Lipid Metabolism and Diabetes Research Laboratories, University of Washington.

Autoantibody testing was performed using a standardized assay protocol and a common serum calibrator developed by the NIDDK-sponsored standardization group. Results are expressed as DKU/mL. Based on analysis of 550 samples, the cut-off values for
positivity/negativity are 33 DKU/mL for GAD65, and 5 DKU/mL for IA2. The calculated specificity and sensitivity is 97 and 76 respectively for GAD65 and 99 and 64 respectively for IA2.

C-peptide was measured by a two-site immunoenzymetric assay (Tosoh Bioscience Inc., San Francisco, CA). The assay sensitivity is 0.05 ng/ml.

HbA1c levels were determined by an automated non-porous ion exchange high performance liquid chromatography system (G-7 Tosoh Biosciences, Inc.) with reference ranges of 4.2 – 5.9%.

HLA class II genotyping was performed by a commercially available LABType® SSO method (OneLambda, Los Angeles, CA).

**C-peptide categories:**

**DCCT** - Post-hoc analysis of DCCT data demonstrated that those with preserved C-peptide, defined as stimulated C-peptide value >0.6 ng/ml, had superior clinical outcomes, including less hypoglycemia and retinopathy, than those with lower C-peptide (14). Re-analysis of these data indicated that the corresponding fasting C-peptide was 0.23 ng/ml (personal communication Lachin, MgGee). Fasting C-peptide values ≥ 0.23 ng/ml are therefore considered clinically significant.

**Healthy adolescents** - The 5th and 50th percentile of fasting C-peptide in healthy adolescents, aged 12-19 years, who participated in the National Health and Nutrition Survey 1999-2002 (NHANES) were, respectively, 1.0 ng/ml and 1.9 ng/ml (15).

**Statistics:** Analyses used SAS for Windows software version 9.1 (SAS Institute Inc., Cary, NC). Chi-square or t-tests were used to evaluate relationships between preserved beta-cell function status and characteristics of interest. Two sets of regression models were run. The first included logistic regression models examining associations between variables of interest and preserved beta-cell function status, stratified by duration of diabetes (<1 year, 1-2 years, >2 years). Next, among participants with preserved C-peptide levels based on the DCCT cut-off, multiple linear regression models were used to identify variables significantly associated with fasting C-peptide levels. All logistic and linear regression models included the following covariates: age at diagnosis, gender, race/ethnicity, BMI z-score at time of visit, HbA1c, number of autoantibodies present, fasting plasma glucose and HLA genotype. Linear models were additionally adjusted for duration of disease.

**RESULTS**

The average age at diagnosis was 9.0 years: 16% (n=434) of the individuals were diagnosed when less than 5 years of age, 38% (n=1057) between ages 5 and 10, 37% (n=1028) between ages 11 and 15, and 10% (n=270) were 15-19 years of age at diagnosis. Average duration of diabetes was 3 years, with 33% (n=925) having less than 1 year of duration, 24% (n=661) having 1-2 years of duration, and 43% (n=1,202) having 2 or more years of duration.

Among individuals with duration of diabetes less than 1 year, 82.9% had a fasting C-peptide level ≥ 0.23 ng/ml, 31.2% had fasting C-peptide at or above the 5th percentile of the NHANES population, and 7.2% at or above the NHANES 50th percentile level. As expected, the proportion of individuals with preserved C-peptide diminished with increasing disease duration. This is illustrated in Figure 1 which shows the proportion with preserved C-peptide in quarterly intervals from time from diagnosis. Among those with five or more years of duration, 10.7% had preserved C-peptide at DCCT cut point, 1.0% at NHANES 5th percentile, and 0% at NHANES 50th percentile.
We then explored various characteristics of participants with and without preserved fasting C-peptide (Table 1). In the unadjusted analysis, the proportion of non-Hispanic whites who had preserved fasting C-peptide was smaller than that of other race/ethnicities. More individuals with preserved C-peptide had two antibodies and a significantly higher percentage of HLA DR15. In addition, those with preserved fasting C-peptide were older at diagnosis, had a shorter duration of diabetes, and a lower HbA1c at the time of the study visit. Those above the NHANES 5th percentile also had a higher BMI z-score than those below this level.

Figure 2 presents the results of the multiple logistic regression models with the dichotomous outcome of preserved fasting C-peptide status based on the DCCT definition stratified by diabetes duration. Variables found to be independently associated with preserved beta-cell function in all the models included older age at diagnosis and lower HbA1c at the time of study visit. Race/ethnicity other than non-Hispanic White was also significant for those participants with diabetes duration 1-2 years and \( \geq \) 2 years; higher current BMI-z score was also significant only for those participants with duration of 1-2 years (Figure 2).

Among those with preserved fasting C-peptide (DCCT definition), older age at diagnosis, higher current BMI-z score, race/ethnicity other than non-Hispanic White, female gender, lower HbA1c, and shorter duration of diabetes were independently related to higher fasting C-peptide levels. The association of age at diagnosis and diabetes duration with fasting C-peptide is illustrated in Figure 3, demonstrating that lower fasting C-peptide is seen in participants diagnosed at a younger age and those with longer duration of disease. The relationship between weight categories at time of study visit and prevalence of preserved beta cell function at various times from diagnosis is shown in Figure 4. While a greater percentage of obese/overweight as compared to underweight subjects had preserved function, the association was significant (p<0.0001) only in those less than 2 years of duration (p=0.48; 0.41; 0.58 for 2-4, 4-6, and 6+ years from diagnosis respectively).

**CONCLUSIONS**

This study finds that about four out of five youth with antibody positive diabetes have clinically significant amounts of residual beta cell function within the first year after diagnosis. In adjusted regression analyses of individuals within one year of diagnosis only age at diagnosis and HbA1c were identified as significant variables in whether or not C-peptide was preserved. Race/ethnicity was additionally important in those further from diagnosis. While it is known that beta cell function continues to decline after diabetes diagnosis, our data also indicate that as many as one out of ten youth have preserved beta cell function even five years after diagnosis.

Despite evidence to the contrary, the belief persists that youth with T1DM have little insulin secretion at diagnosis and that insulin secretion rapidly disappears after diagnosis. Classification schemes define T1DM as a state of absolute insulin deficiency and type 2 diabetes as a state of insulin resistance combined with inadequate insulin secretion (16). As such, health care providers have used C-peptide measurements clinically to establish type of diabetes and to select therapies. While this analysis does not address the question of whether there is a C-peptide cut-point value that could distinguish different types of diabetes, the data demonstrate that many youth with antibody positive diabetes have C-peptide levels within the range of the normal population. Specifically, within one year of diagnosis, almost a third of subjects had C-peptide values that exceeded the 5th percentile and
approximately one in fourteen (7%) exceeded the 50\textsuperscript{th} percentile for healthy adolescents.

Most (3,14,17-21), but not all (22), reports document that even a small amount of residual beta cell function is of clinical significance. Using a level of C-peptide associated with clinical significance in the DCCT, we find that 82.9\% of participants with diabetes duration of less than one year exceed this threshold. The frequency of clinically significant beta cell function is lower among youth with longer duration of disease (53.3\% for duration of one to two years and 18.6\% for four to five years). Because the DCCT cut point has been suggested as an endpoint for clinical trials designed to preserve beta cell function, we suggest caution when interpreting data from non-randomized trials or short-term pilot studies because our data show that endogenous C-peptide is present years after diagnosis in a substantial portion of children.

The data in the present study are similar to that reported by the DCCT, where 33\% of the 466 adolescents tested less than 5 years from diagnosis had residual beta cell function. Because the DCCT population only included individuals over age 13 years, many pediatricians felt that preservation of C-peptide was unlikely in younger children. Among SEARCH participants, we found a profound impact of advancing age at diagnosis on preservation of C-peptide production. The association was linear throughout the complete range of age of diagnosis (from one to 20 years) suggesting that insulin resistance associated with puberty does not account for this finding. Others have demonstrated an effect of age on C-peptide in individuals at risk for T1DM (23).

Several reasons have been suggested for preserved beta cell function. First, increased awareness of the symptoms of T1DM and improved screening and diagnostic tools may have resulted in diagnosis at an earlier point in the autoimmune destruction of the beta cells. Thus, the individuals may have more beta cell reserve at diagnosis. Second, aggressive treatment at diagnosis, with rapid and tight control of hyperglycemia, may result in improved beta cell function (24). Third, increased insulin resistance associated with the epidemic of obesity may have created a greater strain on the declining beta cell function resulting in diagnosis at a time when the individuals have more beta cell function than individuals in the past (25). Consistent with this concept, among those with preserved beta cell function, BMI z-score was an important variable in determining the fasting C-peptide level. However, the impact of BMI on whether or not C-peptide was preserved was less clear. As evident in figure 4, even many years from diagnosis, about 10\% of normal weight individuals had preserved function, suggesting that other, as yet, uncharacterized factors contribute to heterogeneity in disease progression.

This study also emphasizes the relationship between HbA1c and C-peptide, but provides no insight as to whether better glucose control results in preserved function or whether preserved function allows for better HbA1c. Prospectively conducted trials with clinical data such as insulin use, carbohydrate consumption, and exercise may be helpful to address this question.

This paper documents the frequency of preserved beta cell function in a population-based racially/ethnically diverse cohort of antibody positive youth with diabetes. Though the fasting C-peptide concentration in antibody positive youth with diabetes is often below normal, these data suggest that clinically significant amounts may persist in some individuals for some time even among subjects with more than one autoantibody. Further, these data indicate that there is a marked relationship between age at diagnosis, race/ethnicity, and residual C-peptide. Differences in pathophysiology
between non-Hispanic White youth and youth of other races/ethnicities, may be present. Incorporating age and race/ethnicity into assessments of residual beta cell function may provide a better picture of the natural history of disease both before and after diagnosis and a more accurate assessment of the effectiveness of interventions designed to prevent beta cell destruction.

ACKNOWLEDGMENTS

The authors thank John Lachin, SCD and Paula Friedenberg McGee, MS of the George Washington University Biostatistics Center for their analysis of the DCCT data to determine the fasting C-peptide cut-point used to define preserved C-peptide.

The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention and the National Institute of Diabetes and Digestive and Kidney Diseases.

Grant Support: SEARCH for Diabetes in Youth is funded by the Centers for Disease Control and Prevention (PA number 00097 and DP-05-069) and supported by the National Institutes of Health. Kaiser Permanente Southern California (U01 DP000246); University of Colorado Health Sciences Center (U01 DP000247); Pacific Health Research Institute (U01 DP000245); Children’s Hospital Medical Center (Cincinnati) (U01 DP000248); University of North Carolina (U01 DP000254); University of Washington School of Medicine (U01 DP000244); Wake Forest University School of Medicine (U01 DP000250).

The authors acknowledge the involvement of General Clinical Research Centers (GCRC) at the following institutions: Medical Center of South Carolina (Grant Number M01 RR01070); Cincinnati Children’s Hospital (Grant Number M01 RR08084); Children’s Hospital and Regional Medical Center and the University of Washington School of Medicine (Grant Number M01RR00037 and M01RR001271); Colorado Pediatric General Clinical Research Center (Grant Number M01 RR00069).
REFERENCES
**TABLE 1:** Characteristics of 2789 SEARCH antibody positive participants by preserved beta-cell function status as defined by fasting C-peptide levels.

<table>
<thead>
<tr>
<th></th>
<th>ENTIRE COHORT</th>
<th>DCCT</th>
<th>NHANES 5th</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-cell Function</td>
<td>β-cell Function</td>
<td>β-cell Function</td>
</tr>
<tr>
<td></td>
<td>Preserved (FCP ≥ 0.23 ng/ml)</td>
<td>Not-Preserved (FCP &lt; 0.23 ng/ml)</td>
<td>Preserved (FCP ≥ 1.0 ng/ml)</td>
</tr>
<tr>
<td>N (%)</td>
<td>2789</td>
<td>1329 (47.7)</td>
<td>1460 (52.4)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1385 (49.7)</td>
<td>682 (49.2)</td>
<td>703 (50.8)</td>
</tr>
<tr>
<td>Female</td>
<td>1404 (50.3)</td>
<td>647 (46.1)</td>
<td>757 (53.9)</td>
</tr>
<tr>
<td>Race/Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>2103 (75.4)</td>
<td>9781 (46.5)</td>
<td>1125 (53.5)</td>
</tr>
<tr>
<td>Non-White</td>
<td>686 (24.6)</td>
<td>351 (51.2)</td>
<td>335 (48.8)</td>
</tr>
<tr>
<td>HLA DR *</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>03/04</td>
<td>1781 (90.5)</td>
<td>716 (40.2)</td>
<td>1065 (59.8)</td>
</tr>
<tr>
<td>15</td>
<td>35 (1.8)</td>
<td>23 (65.7)</td>
<td>12 (34.3)</td>
</tr>
<tr>
<td>Other</td>
<td>152 (7.7)</td>
<td>62 (40.8)</td>
<td>90 (59.2)</td>
</tr>
<tr>
<td>Number of autoantibodies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two</td>
<td>1172 (42.0)</td>
<td>647 (55.2)</td>
<td>525 (44.8)</td>
</tr>
<tr>
<td>One</td>
<td>1617 (58.0)</td>
<td>682 (42.2)</td>
<td>935 (57.8)</td>
</tr>
<tr>
<td>[Mean (Range)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>9.0 (0-19)</td>
<td>10.5 (1-19)</td>
<td>7.6 (0-18)</td>
</tr>
<tr>
<td>Age at visit (years)</td>
<td>12.4 (1.5-22.7)</td>
<td>12.3 (1.9-22.5)</td>
<td>12.5 (1.5-22.7)</td>
</tr>
<tr>
<td>Duration of diabetes (months)</td>
<td>35.8 (0-213)</td>
<td>16.1 (0-210)</td>
<td>53.7 (0-213)</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>0.6 (-4.2-4.7)</td>
<td>0.7 (-4.2-3.1)</td>
<td>0.6 (-3.3-4.7)</td>
</tr>
<tr>
<td>HbA1c %</td>
<td>8.1 (3.1-17.9)</td>
<td>7.7 (3.1-17.3)</td>
<td>8.5 (5.3-17.9)</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>191.2 (42-658)</td>
<td>170.8 (42-518)</td>
<td>209.8 (43-658)</td>
</tr>
</tbody>
</table>

For the Column “Entire Cohort”, percentages total to 100 vertically within each variable (gender, race/ethnicity, HLA, autoantibody number). For the remaining columns, the percentages total to 100 horizontally between preserved and non-preserved for each definition. All continuous variables were different between those with preserved and not-preserved C-peptide (T-test; p<0.05), except age at visit and BMI z-score for DCCT cut-off. Race/Ethnicity, number of autoantibodies, and HLA DR showed a significant association with preserved status; (chi-square; at p<0.05).

* HLA was tested on a subset of subjects (N=1968)
Figure 1
Percent of participants with preserved C-peptide by duration of diabetes in three month intervals according to:
DCCT definition (fasting C-peptide < 0.23 ng/ml)(closed diamond)
NHANES 5th percentile definition (fasting C-peptide < 1.0 ng/ml)( closed square)
NHANES 50th percentile definition (fasting C-peptide <1.9 ng/ml) (closed triangle)
N= number of participants in each three month interval

Figure 2
Odds ratios for having preserved Cpeptide according to DCCT definition for individuals less than 1 year (open diamond), 1-2 years (closed square) , and more than 2 years (closed circle) from diagnosis. Incremental units are glucose 1 mg/dl; age 1 year; BMI 1; HbA1c 1%.

Figure 3
Fasting C-peptide by duration and age at diagnosis among those with preserved C-peptide by DCCT definition. Cells with less than 5 subjects are not reported.

Figure 4
Percent of participants with preserved C-peptide by duration of diabetes according to DCCT definition (fasting C-peptide < 0.23 ng/ml) stratified by BMI classification (black bar = obese; dark grey bar = overweight; light grey bar = normal weight; white bar = underweight).
FIGURE 1

[Graph showing percentage preserved over duration of diabetes in months, with data points for DCCT, NHANES 5th, and NHANES 50th.

N = 120 48 72 24 12 6 0

FIGURE 2

[Graph showing glucose levels over time with different symbols for age at diagnosis, BMI z-score, race, gender, and antibody number.

Glucose  Age at diagnosis  BMI z-score  Race (other vs white)  Gender (F vs M)  HbA1c  Antibody number (1 vs 2)
Cells with <5 subjects are not reported