

# Clinical evolution of beta cell function in youth with diabetes: the SEARCH for Diabetes in Youth study

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## Abstract

**Aims/hypothesis** Few studies have explored the epidemiology of beta cell loss in youth with diabetes. This report describes the evolution and major determinants of beta cell function, assessed by fasting C-peptide (FCP), in the SEARCH for Diabetes in Youth study.

**Methods** Participants were 1,277 youth with diabetes (948 positive for diabetes autoantibodies [DAs] and 329 negative for DAs), diagnosed when aged <20 years, who were followed from a median of 8 months post diagnosis, for approximately 30 months. We modelled the relationship

between rate of change in log FCP and determinants of interest using repeated measures general linear models.

**Results** Among DA-positive youth, there was a progressive decline in beta cell function of 4% per month, independent of demographics (age, sex, race/ethnicity), genetic susceptibility to autoimmunity (HLA risk), HbA<sub>1c</sub> and BMI z score, or presence of insulin resistance. Among DA-negative youth, there was marked heterogeneity in beta cell loss, reflecting an aetiologically mixed group. This group likely includes youths with undetected autoimmunity (whose decline is similar to that of DA-positive youth) and youth with

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non-autoimmune, insulin-resistant diabetes, with limited decline (~0.7% per month).

**Conclusions/interpretation** SEARCH provides unique estimates of beta cell function decline in a large sample of youth with diabetes, indicating that autoimmunity is the major contributor. These data contribute to a better understanding of clinical evolution of beta cell function in youth with diabetes, provide strong support for the aetiological classification of diabetes type and may inform tertiary prevention efforts targeted at high-risk groups.

**Keywords** Beta cell function · Decline · Determinants · Epidemiology · Type 1 diabetes · Type 2 diabetes · Youth

### Abbreviations

DA	Diabetes autoantibodies
DPT-1	Diabetes Prevention Trial-Type 1
FCP	Fasting C-peptide
FPG	Fasting plasma glucose
GADA	Glutamic acid decarboxylase-65 autoantibody
HLA DR/DQ	Human leucocyte antigen DR/DQ genotypes
IA-2A	Insulinoma-associated-2 autoantibody
IQR	Interquartile range
IR	Insulin resistance
IS	Insulin sensitivity
NHW	Non-Hispanic white
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases

### Introduction

Diabetes mellitus is a spectrum of metabolic diseases with distinct aetiologies, characterised by a progressive decline in beta cell function and mass [1, 2]. An increasing body of evidence demonstrates the importance of preserving endogenous beta cell function in patients with diabetes, regardless of aetiology or presumed diabetes type [3, 4]. However, there is a paucity of data regarding the epidemiology of beta cell function, including the rate and major determinants of beta cell decline, especially among contemporary youth with diabetes [5]. Limited information has been provided by interventional studies in youth with autoimmune diabetes [6–9] and individuals with adult-onset diabetes [10]. Recent data suggest that there is a progressive deterioration of beta cell function both before and after the diagnosis of autoimmune diabetes [11, 12]; however, limited information is available regarding the factors that predict the rate of decline. Even less is known about the clinical evolution of beta cell function in youth without autoimmune diabetes. Moreover, most previous studies were designed to explore the effects of specific interventions, were usually much smaller and often included selected participants, making it difficult

to draw conclusions applicable to the general population of youth with diabetes. Such data may be relevant for the aetiological classification of diabetes type.

SEARCH for Diabetes in Youth is a population-based, multicentre and multi-ethnic study of paediatric diabetes in the USA. With the aim of rigorously characterising the epidemiology of diabetes in youth, this study has carefully collected detailed clinical and pathophysiological data on a large number of youth with all types of diabetes. Moreover, SEARCH has recently developed operational definitions of diabetes types, as recommended by the ADA expert committee, using an aetiological approach based on assessment of autoimmunity and insulin resistance (IR), in which four aetiological subgroups were defined [13]. The purpose of this manuscript is to describe the presentation, short-term clinical evolution and major determinants of decline in beta cell function, as measured by fasting C-peptide (FCP), in a general population of youth with diabetes, using data from the prospective cohort of the SEARCH for Diabetes in Youth study.

### Methods

**Overview** Data for these analyses derive from the SEARCH for Diabetes in Youth prospective cohort study. A detailed description of SEARCH study methods has been published elsewhere [14]. Briefly, SEARCH has been conducting population-based ascertainment of youth and young adults with newly diagnosed (incident) diabetes diagnosed when <20 years of age, starting in 2002 and continuing through to the present. SEARCH recruited participants from four geographically defined populations in the USA (Ohio, Colorado, South Carolina and Washington) and also Indian Health Service beneficiaries from several American Indian populations and enrollees in several managed healthcare plans in California and Hawaii. Participants with newly diagnosed diabetes in 2002–2005 were invited to participate in a baseline research visit (median duration of diabetes 8 months, interquartile range [IQR] 4.5–14.5 months) and two follow-up exams centred around 12 and 24 months after their baseline visit. At each research visit fasting blood samples were obtained from metabolically stable participants (defined as no episode of diabetic ketoacidosis during the previous month), physical measurements were conducted and questionnaires were administered. The study was reviewed and approved by the local Institutional Review Board(s) that had jurisdiction over the local study population and all participants provided informed consent and/or assent.

**Data collection** Study visits occurred after an 8 h overnight fast. Participants did not take diabetes medications on the morning of the visit and long-acting insulin was administered on the evening before the visit and then discontinued.

Fasting blood samples were used to analyse diabetes autoantibodies (DAs), HbA<sub>1c</sub>, lipids and FCP and fasting plasma glucose (FPG). Assays were performed at the Northwest Lipid Metabolism and Diabetes Research Laboratories, University of Washington, the Central Laboratory for SEARCH. Glutamic acid decarboxylase-65 (GADA) and insulinoma-associated-2 (IA-2A) autoantibodies were analysed using a standardised protocol and a common serum calibrator developed by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-sponsored harmonisation group [15]. The cut-off values for positivity were 33 NIDDKU/ml for GADA and 5 NIDDKU/ml for IA-2A [15]. HbA<sub>1c</sub> was measured in whole blood with an automated nonporous ion-exchange HPLC system (model G-7; Tosoh Bioscience, San Francisco, CA, USA). Measurements of plasma cholesterol, triacylglycerols and HDL-cholesterol were performed on a Roche Modular-P autoanalyzer (Roche Diagnostics, Indianapolis, IN, USA). Levels of FCP were determined by a two-site immunoenzymetric assay (Tosoh 1800; Tosoh Bioscience) with a sensitivity of 0.05 ng/ml. Human leucocyte antigen class II genotyping (HLA DR/DQ) was performed with a PCR-based sequence-specific oligonucleotide probe system in the laboratories of S. Gaur (University of Washington, Seattle, WA, USA) and H. Erlich (Roche Molecular Systems, Pleasanton, CA, USA) on all consenting participants [16].

Weight and height were measured using standardised procedures and used to calculate BMI. Waist circumference was measured using the National Health and Nutrition Examination Survey (NHANES) III protocol [17].

Insulin sensitivity (IS) was estimated using the following equation:  $\log_e IS = 4.64725 - 0.02032 (\text{waist, cm}) - 0.09779 (\text{HbA}_{1c}, \%) - 0.00235 (\text{triacylglycerol, mg/dl})$ ; to convert triacylglycerol values from mg/dl to mmol/l, multiply by 0.0113). This equation was developed and validated in a previous study [18] using direct measurements of glucose disposal rate from euglycaemic–hyperinsulinaemic clamps. IR was defined as an IS value below the 25th percentile in the general US youth population (<8.15), as previously described [13].

Data on self-reported race and ethnicity were collected using 2000 US Census questions [19] and classified as Hispanic, non-Hispanic white (NHW), non-Hispanic black, American Indian and Asian/Pacific islander.

**Determinants of interest** Autoimmunity was defined by positive titres for either GADA or IA-2A at baseline. Because many participants were treated with insulin, positive insulin autoantibodies were not used. Genetic susceptibility to autoimmunity was defined based on HLA DR/DQ. HLA genotypes were categorised as susceptible (DR3/4, DR4/4, DR4/8, DR4/1, DR 4/13, DR 3/3, DR 3/9, DR 4/9 and DR 9/9 genotypes) and not susceptible, including

neutral and protective genotypes [13], as recommended by the Type 1 Diabetes Genetic Consortium, with modifications for the multi-ethnic population [16]. For these analyses race/ethnicity was categorised as NHW and other than non-Hispanic white, including all other racial/ethnic groups. BMI z scores from measured height and weight at baseline visit were derived for age and sex using the US Centers for Disease Control and Prevention National Center for Health Statistics growth charts [20]. Finally, SEARCH participants were also classified according to baseline aetiological category of diabetes type in four mutually exclusive groups as previously described [13]: autoimmune and insulin sensitive, autoimmune and insulin resistant, non-autoimmune and insulin sensitive, non-autoimmune and insulin resistant.

**Study participants** The eligible study population consisted of all SEARCH participants who were diagnosed in 2002–2005, who had a baseline visit with non-missing FCP, DA and demographic data, plus at least another follow-up visit with non-missing FCP. There were 2,763 individuals that had a baseline SEARCH visit and 1,895 participants had non-missing FCP, DA and demographic data at baseline. An additional sample of 629 were dropped because they did not have at least one follow-up visit with non-missing FCP, resulting in 1,277 SEARCH individuals contributing to this analysis. Of the 1,277 participants included in these analyses, 634 contributed two visits (baseline plus one follow-up) and 643 contributed three visits (baseline plus two follow-up visits). There were 948 DA-positive participants (487 with double and 461 with single antibody positivity) and 329 DA-negative participants.

**Statistical analyses** Statistical analyses were performed using SAS software (version 9.2; SAS Institute, Cary, NC, USA), with  $p < 0.05$  indicating significance. Comparisons of baseline participant characteristics by baseline DA status were examined using  $\chi^2$  tests,  $t$  tests and Wilcoxon tests. Beta cell function was estimated using FCP concentration [21].

Using all baseline and follow-up data, we used mixed effects regression analysis to derive predicted FCP levels over time, based on fitted models that included up to three observed measures of FCP for each participant. These models adjust for the correlation between repeated observations in the same individual and have the advantage of handling a varying number of observations within individuals, thereby allowing for inclusion of the maximum number of data points [22]. Further, this method allows for a random intercept and slope in disease duration to account for within-individual dependence. These models incorporate duration of diabetes (actual time in months) as a continuous time-varying covariate and explore significant associations (statistical interactions) between determinants of interest

(baseline DA status, age at diagnosis, sex, race/ethnicity, HLA DR/DQ susceptibility status, baseline BMI *z* score and HbA<sub>1c</sub>) and time (duration of diabetes) on FCP levels. All models are also adjusted for site- and time-varying FPG levels measured at each FCP measurement. Since baseline DA status emerged as the main determinant of FCP decline all analyses were stratified by baseline DA status. For DA-positive individuals, the effect of double vs single DA positivity at baseline on FCP decline over time was also explored, adjusting for all the covariates mentioned above. Age at diagnosis was modelled both continuously and categorically (<10 and ≥10 years, for illustrative purposes) with very similar results. To account for a skewed distribution of FCP levels, values were log-transformed for these analyses. Predicted FCP rate of change was expressed as per cent change in FCP level per month with 95% CIs.

Finally, we were also interested in modelling the rate of change in FCP levels as a function of baseline aetiological category of diabetes type. Therefore, a separate model was built to explore the association between the four-level aetiological diabetes type variable (autoimmune and insulin sensitive, autoimmune and insulin resistant, non-autoimmune and insulin sensitive, non-autoimmune and insulin resistant) and log-transformed FCP over time. This analysis excluded 66 participants due to missing data on variables that are part of the IS equation.

## Results

Baseline characteristics of study participants, stratified by baseline DA status, are shown in Table 1. As expected, DA-positive participants were younger at diagnosis, more likely to be of male sex and NHW race/ethnicity, more likely to have susceptible HLA DR-DQ genotypes and had lower FCP levels and BMI *z* scores, but higher baseline FPG levels, compared with those who were DA negative. Table 2 presents the number of individuals participating at each of the three SEARCH visits, their average diabetes duration at each visit and mean FCP and FPG levels, by DA status.

Table 3 presents the associations between baseline FCP levels and determinants of interest, from multivariable analyses stratified by baseline DA status. In both DA-positive and DA-negative participants, younger age at diagnosis, NHW race/ethnicity, higher baseline HbA<sub>1c</sub> and lower baseline BMI *z* score were independently associated with significantly lower baseline FCP levels. In DA-positive participants only, male sex was also associated with lower baseline FCP levels. HLA DR/DQ susceptibility status was not significantly associated with FCP levels at baseline in either group.

Figure 1 shows predicted FCP levels over time among youth with positive DA at baseline, by age at diagnosis of

**Table 1** Characteristics of study participants, according to baseline DA status

Characteristic	DA positive	DA negative	<i>p</i> value <sup>a</sup>
Number	948	329	
Age at diagnosis (years)	9.6 (±3.9)	12.0 (±3.8)	<0.0001
Age at diagnosis <10 years	436 (46.0)	73 (22.2)	<0.0001
Sex			
Female	460 (48.5)	175 (53.2)	0.1
Male	488 (51.5)	154 (46.8)	
Race/ethnicity			
NHW	730 (77.0)	151 (45.9)	<0.0001
Hispanic	104 (11.0)	55 (16.7)	
Non-Hispanic black	83 (8.8)	77 (23.4)	
Asian/Pacific Islander	29 (3.1)	32 (9.7)	
American Indian	2 (0.2)	14 (4.3)	
HLA DR-DQ group			
Susceptible	422 (47.1)	52 (17.5)	<0.0001
Not susceptible	474 (52.9)	245 (82.5)	
FCP (nmol/l), median (IQR)	0.17 (0.3)	0.62 (1.0)	<0.0001
HbA <sub>1c</sub> (%)	7.6 (1.4)	7.3 (1.8)	0.0010
HbA <sub>1c</sub> (mmol/mol)	59.65	56.28	
BMI <i>z</i> score	0.6 (1.0)	1.4 (1.2)	<0.0001
FPG (mmol/l), mean (SD)	9.16 (4.09)	8.12 (3.89)	<0.0001

Data are mean (±SD) or *n* (%) except where stated otherwise

<sup>a</sup>*p* value from  $\chi^2$  test (categorical) or *t* test (continuous) or Wilcoxon test (FCP only)

diabetes (stratified as <10 and ≥10 years). The model showed that age at diagnosis, sex, race/ethnicity, HLA risk, baseline HbA<sub>1c</sub> and BMI *z* score were not associated with FCP decline over time (see electronic supplementary material [ESM] Table 1). The predicted rate of decline in FCP levels among youth positive for DA (shown in Fig. 1 for an average baseline HbA<sub>1c</sub> of 8%, BMI *Z*-score of 0.6 and an average time-varying FPG of 9.2 mmol/l) was 4.1% (95% CI 3.9, 4.3) per month, regardless of age at diagnosis, sex, race/ethnicity and HLA risk status. When baseline DA positivity status was added to the model, presence of two DAs vs one DA was the only variable significantly associated with faster decline in FCP levels (*p*<0.0001): estimated 4.4% (95% CI 4.1, 4.7) per month in those with double DA positivity vs 3.7% (95% CI 3.4, 4.0) per month in those with single DA positivity (ESM Table 2).

Figure 2 shows predicted FCP levels over time among youth with negative DA at baseline, by age at diagnosis of diabetes (<10 and ≥10 years). The model showed that age at diagnosis, race/ethnicity, HLA risk status, baseline HbA<sub>1c</sub> and BMI *z* score were independently associated with FCP decline over time (ESM Table 3). Predicted rates of decline are shown in Fig. 2 for an average baseline HbA<sub>1c</sub> of 7.5%, BMI *z* score of 1.3 and an average time-varying FPG of

**Table 2** Number of participants at each visit, their average diabetes duration and measured FCP and FPG levels, by DA status

Variable	DA positive			DA negative		
	Visit 1	Visit 2	Visit 3	Visit 1	Visit 2	Visit 3
Number	948	729	705	329	248	238
Duration (months), median (IQR)	8 (9)	23 (10)	35 (11)	10 (11)	24 (12)	37 (12)
FCP (nmol/l), median (IQR)	0.17 (0.27)	0.07 (0.13)	0.05 (0.08)	0.62 (1.0)	0.57 (1.11)	0.61 (1.10)
Glucose (mmol/l), mean (SD)	9.16 ( $\pm$ 47.09)	10.64 ( $\pm$ 4.52)	11.09 ( $\pm$ 4.73)	8.12 ( $\pm$ 3.89)	9.18 ( $\pm$ 4.37)	9.9 ( $\pm$ 4.59)

Data are median (IQR) or mean ( $\pm$ SD)

7.3 mmol/l. The fastest rate of decline was predicted in youth of NHW race/ethnicity who had susceptible HLA DR/DQ genotypes (4.0% [95% CI 3.1, 4.9] per month in youth diagnosed at <10 years of age and 3.2% [95% CI 2.4, 3.9] in those diagnosed at age  $\geq$ 10 years). These estimates are very similar to those predicted for youth who were DA positive at baseline, suggesting undetected autoimmunity in this group of SEARCH participants. Similarly, rates of decline that were consistently elevated were predicted among youth with susceptible HLA DR/DQ genotypes who were of other racial/ethnic groups than NHW (2.9% [95% CI 1.9, 4.0] per month among those with age at diagnosis <10 years and 2.1% [95% CI 1.2, 2.9] per month among those with older age at diagnosis). These data also suggest an undetected autoimmune process, given the presence of susceptible HLA markers. The slowest rates of decline were predicted among DA-negative youth of minority racial/ethnic groups who did not have susceptible HLA DR/DQ genotypes (1.4% [95% CI 0.6, 2.1] per month in those diagnosed at <10 years of age and 0.5% [95% CI 0.1, 1.0] per month in those diagnosed at an age older than 10 years). These participants seem to belong to a different aetiological group characterised by absence of DA and genetic markers of autoimmunity and also by a preponderance of minority racial/ethnic backgrounds. Interestingly, the group of NHW participants without susceptible HLA genotypes who were diagnosed at an age older than 10 years had an intermediate predicted rate of

change, 2.5% (95% CI 1.7, 3.2) per month, likely marking an aetiologically mixed group of individuals with and without autoimmune diabetes.

Finally, predicted rates of decline in FCP levels by baseline aetiological category of diabetes type are presented in Table 4. Overall, baseline category of diabetes type was significantly associated with FCP decline ( $p < 0.0001$ ). The fastest rates of decline were predicted among youth with autoimmunity, regardless of whether they were insulin sensitive (4.0% [95% CI 3.8, 4.3] per month) or insulin resistant (4.2% (95% CI 3.8, 4.7) per month). An intermediate rate of decline (2.4% [95% CI 1.9, 3.0] per month) was predicted for youth without evidence of autoimmunity who were insulin sensitive, while the predicted rate of decline among youth with non-autoimmune, insulin-resistant diabetes was significantly slower (0.7% [95% CI 0.3, 1.2] per month).

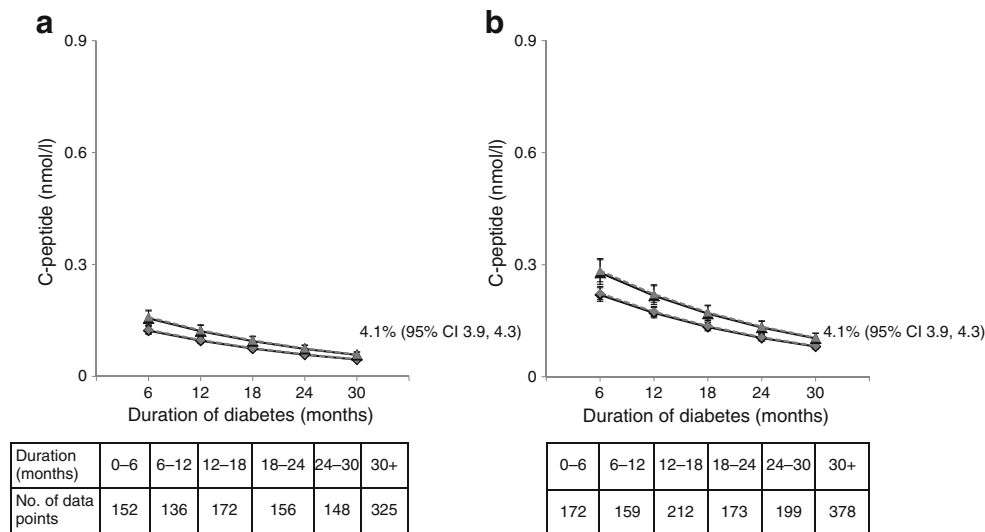
## Discussion

Our study provides unique quantitative estimates of decline in beta cell function over the first 30 months following diagnosis of diabetes in a large, diverse sample of youth in the USA. SEARCH provides strong evidence that autoimmunity is the main driver of beta cell loss even after diabetes diagnosis. Among DA-positive youth, there is a progressive decline in beta cell function, independent of demographic

**Table 3** Determinants of baseline FCP levels from multivariate analyses, stratified by baseline DA status

Determinant	DA positive		DA negative	
	Effect, % (95% CI) <sup>a</sup>	<i>p</i> value	Effect, % (95% CI) <sup>a</sup>	<i>p</i> value
Age at diagnosis (<10 vs $\geq$ 10 years)	44 (38, 49)	<0.0001	57 (47, 65)	<0.0001
Sex (male vs female)	11 (2, 19)	0.0185		0.1
Race (NHW vs others)	19 (9, 28)	0.0004	30 (15, 42)	0.0002
HLA (susceptible vs not susceptible)		0.7		0.5
Baseline HbA <sub>1c</sub> (per 1% increase)	19 (15, 22)	<0.0001	7 (1, 13)	0.0170
Baseline BMI <i>z</i> score (per 1 unit decrease)	17 (13, 21)	<0.0001	37 (32, 42)	<0.0001

<sup>a</sup> Percentage by which baseline FCP level is lower

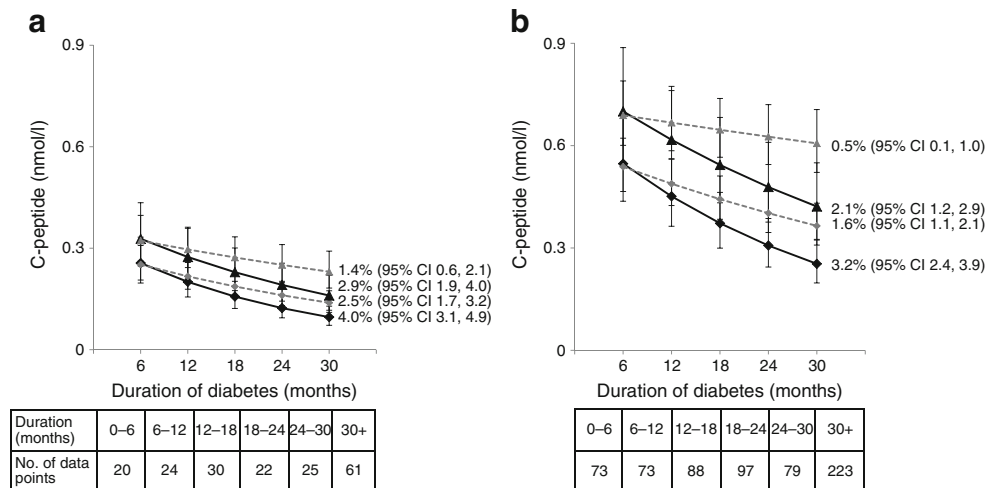


**Fig. 1** Estimated mean FCP levels and rate of monthly decline among youth with positive DA status at baseline; **(a)** age at diagnosis of diabetes <10; **(b)** age at diagnosis ≥10. Black symbols, HLA susceptible; grey symbols, HLA not susceptible; diamonds, NHW race/ethnicity; triangles, other race. Multivariate mixed model predicting log-transformed FCP over time based on diabetes duration, age at diagnosis of diabetes, sex, race/ethnicity, HLA risk group, baseline

HbA<sub>1c</sub>, BMI z score, site and time-varying fasting glucose (FPG). Estimates and rate of decline (95% CIs) are shown for an average baseline HbA<sub>1c</sub> of 8% (63.93 mmol/mol), BMI z score of 0.6 and an average time-dependent FPG of 9.2 mmol/l. The data under the graph present the number of FCP data points available in various time intervals

factors, genetic susceptibility to autoimmunity (HLA risk), HbA<sub>1c</sub> levels and BMI z score. Among DA-negative youth, there is marked heterogeneity in beta cell loss, reflecting an aetiologically mixed group. Our data contribute to a better understanding of the clinical progression of diabetes in youth in the first years following diagnosis.

In youth with autoimmune diabetes there was an estimated decline in beta cell function of ~4% per month, or approximately 40% per year. Similarly, in a series of 204 consecutive patients with type 1 diabetes, Snorgaard et al [23] reported that FCP levels (on average 0.5 ng/ml at diagnosis) declined in a homogeneous manner at a rate of 0.24 ng/ml



**Fig. 2** Estimated mean FCP levels and rate of monthly decline among youth with negative DA status at baseline; **(a)** age at diagnosis of diabetes <10; **(b)** age at diagnosis ≥10. Black symbols, HLA susceptible; grey symbols, HLA not susceptible; diamonds, NHW race/ethnicity; triangles, other race. Multivariate mixed model predicting log-transformed FCP over time based on diabetes duration, age at diagnosis of diabetes, sex, race/ethnicity, HLA risk group, baseline

HbA<sub>1c</sub>, BMI z score, site and time-dependent fasting glucose (FPG) levels. Estimates and rates of decline (95% CIs) are shown for an average baseline HbA<sub>1c</sub> of 7.5% (58.47 mmol/mol) and BMI z score of 1.3 and an average time-dependent FPG of 7.3 mmol/l. The data under the graph present the number of FCP data points available in various time intervals

**Table 4** Baseline FCP levels and estimated monthly decline in FCP, according to baseline aetiological category of diabetes<sup>a</sup>

Aetiological category	<i>n</i>	Baseline FCP (nmol/l), median (IQR)	Estimated monthly decline, % (95% CI)
Autoimmune + insulin sensitive	688	0.17 (0.22)	4.0 (3.8, 4.3)
Autoimmune + insulin resistant	212	0.27 (0.42)	4.2 (3.8, 4.7)
Non-autoimmune + insulin sensitive	122	0.22 (0.33)	2.4 (1.9, 3.0)
Non-autoimmune + insulin resistant	189	1.13 (0.95)	0.7 (0.3, 1.2)

<sup>a</sup> This analysis includes a slightly smaller number of SEARCH participants due to missing data on variables that are part of the IS equation Overall  $p < 0.0001$ ; adjusted for time-dependent FPG levels

annually. Other studies have estimated the decline in beta cell function during the first year after diagnosis of type 1 diabetes, with variable results. Most of the recent studies are based on data provided by the control arm of intervention studies, include small numbers of highly selected participants and use stimulated C-peptide as the measure of beta cell function. While some report modest reduction in beta cell function at 1 year [24, 25], others report a 50% decline in the first year post-diagnosis [1, 26–28], consistent with our data in an unselected population.

There are limited data on factors influencing beta cell function in youth with autoimmune diabetes [12, 29–35]. We found that younger age, male sex, NHW race/ethnicity, higher HbA<sub>1c</sub> and lower BMI *z* score were each associated with lower FCP levels at baseline (Table 3). The Diabetes Prevention Trial-Type 1 (DPT-1) study has demonstrated that C-peptide levels at diagnosis are much higher in individuals diagnosed through surveillance of those at high risk [36] compared with those diagnosed in the community, with signs and symptoms of hyperglycaemia. Our findings that higher HbA<sub>1c</sub> and lower BMI *z* score are associated with lower baseline FCP levels are consistent with DPT-1 findings, suggesting a more severe or longer preclinical course in those with less residual beta cell function at presentation. Consistent with our data, several reports have shown that younger age is associated with lower C-peptide levels at diagnosis with type 1 diabetes [8, 9, 36]. This may suggest a more aggressive preclinical course or an overall lower beta cell mass in younger children [6], while in older patients greater IR may lead to a clinical presentation at an earlier preclinical stage of disease [21, 37].

Although age, sex, race/ethnicity, HbA<sub>1c</sub> and BMI *z* score were each associated with lower FCP levels at baseline (Table 3), none of them were significantly associated with the subsequent rate of decline (Fig. 1). Based on our data, on average, residual beta cell function at any point in time is a function of baseline C-peptide levels and disease duration. Others have also reported that baseline C-peptide level is the strongest predictor of decline in beta cell function in youth [21] and young adults [38] with autoimmune diabetes.

One of our most novel findings is that markers of obesity and IR do not significantly influence the decline in FCP levels after diagnosis of autoimmune diabetes. Investigators and clinicians have previously described a group of youth with autoimmunity and obesity as having ‘hybrid’ or ‘double’ diabetes [39], suggesting a mixed aetiology and, likely, a more aggressive clinical evolution. The aetiological classification of diabetes type proposed by SEARCH [13] and findings from other recent studies [40] provide evidence that rather than being a distinct aetiological category this group represents the upper tail of the distribution of IR and obesity among youth with autoimmune diabetes. We now provide novel evidence that the rate of FCP decline in youth with autoimmune, insulin-resistant diabetes is not significantly different from that observed in youth with autoimmune, insulin-sensitive diabetes (4.2% [95% CI 3.8, 4.7] per month vs 4.0% [95% CI 3.8, 4.3] per month,  $p = 0.5$ , Table 4). This was also suggested by baseline BMI *z* score not being significantly associated with decline in FCP levels in DA-positive youth. These findings lend additional support to the notion that type 1 diabetes is a distinct aetiological category and that the main driver of beta cell loss is autoimmunity, regardless of prevailing IR that may occur simultaneously. These findings also have important clinical implications indicating the importance of a correct diagnosis of autoimmune type 1 diabetes in obese youth, and thus adequate insulin treatment.

Less is known about the clinical history of beta cell function in youth with non-autoimmune diabetes. Although small studies suggested that youth with type 2 diabetes have some degree of beta cell dysfunction at diagnosis [41, 42], there are no large prospective studies of the natural evolution and determinants of beta cell function in youth with type 2 diabetes. The estimated rate of decline among SEARCH youth who had non-autoimmune, insulin-resistant diabetes was 0.7% per month, or approximately 8% per year, in the first 30 months following diagnosis (Table 4). This is consistent with studies in adults showing a 7% decline annually [3, 43]. However, a recent study has suggested no signs of progressive beta cell damage during 20 years of prospective follow-up of autoantibody-negative, adult-onset diabetes [44], which contrasts with the generally

held view of progressive beta cell loss in type 2 diabetes. There is an obvious need for long-term follow-up studies of individuals with youth-onset, non-autoimmune, type 2 diabetes.

Our study has some limitations and unique strengths. Because SEARCH is a large epidemiological study, we used fasting, rather than stimulated, C-peptide levels to assess baseline and change in beta cell function over time. FCP level is easy to obtain and correlates well with stimulated C-peptide [45–47], although it may be insufficient to detect subtle effects of therapy. In addition, we used a standard protocol to assess beta cell function, with all measurements conducted in the fasting state, in the absence of hypo- or hyperglycaemia, which are known to affect FCP levels [48]. Only a fraction of participants had FCP measures in the first 6 months after diagnosis and, therefore, during this period the rate of decline in FCP may have been estimated with less precision. Only two autoantibodies were measured in SEARCH. Thus, individuals who may have autoimmune markers other than GADA or IA-2A were misclassified as having non-autoimmune diabetes. However, the predicted rates of change in FCP levels based on models that included other characteristics, such as HLA DR/DQ susceptibility genotypes, race/ethnicity and age at diagnosis, identified groups of participants with likely undetected autoimmune diabetes. In addition, SEARCH is planning to assess ZnT8 autoantibodies in stored samples once assays are standardised. We also do not have follow-up data on all SEARCH participants. However, minimal differences existed between those who did ( $n=1,277$ ) and did not participate ( $n=629$ ) in the follow-up assessment. In particular, the proportion of DA-positive and DA-negative participants, the racial/ethnic distribution, the mean age and FCP levels according to DA status were not substantially different. Finally, our data are not the result of a randomised clinical trial, which is much better suited to address specific questions regarding the role of glycaemic control or that of specific therapies, on beta cell decline in youth with diabetes. Our study also has very important strengths. This is a unique cohort of youth with diabetes ascertained without regard to presumed diabetes type, family history or related factors, such as age, treatment or race/ethnicity, which has permitted us to accurately quantify progression of beta cell damage in a large sample of youth with diabetes.

In summary, SEARCH provides unique quantitative estimates of beta cell function decline over the first 30 months following diagnosis in a large sample of youth with diabetes in the USA. Our data provide strong evidence that autoimmunity is the main driver of beta cell loss even after diabetes diagnosis. We also provide novel evidence that markers of obesity and IR do not influence the decline in beta cell function in youth with autoimmune diabetes. Finally, youth with non-autoimmune, insulin-resistant diabetes have a

much slower progression of beta cell dysfunction. These data contribute to a better understanding of clinical evolution of beta cell function in youth with diabetes, provide strong support for the aetiological classification of diabetes type and may inform tertiary prevention efforts targeted at high-risk groups.

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