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Persistent C-peptide levels and microvascular complications in childhood onset type 1 diabetes of long duration

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ABSTRACT

Aims: The aim was to determine if persistent c-peptide in long duration childhood onset (<17 years) type 1 diabetes (T1D) related to microvascular complications.
Methods: Pittsburgh Epidemiology of Diabetes Complications (EDC) participants (n = 185) had serum c-peptide levels measured by Mercodia ultra-sensitive ELISA at the 25-year follow-up exam. Microvascular complications between those with and without detectable c-peptide were compared.
Results: Eighteen (9.7%) participants had detectable median c-peptide levels of 3.8 (2.6, 12.2) pmol/L and did not differ from those without detectable levels. No differences in microalbuminuria, confirmed distal symmetric polyneuropathy, renal failure, or between those with one or more complications were found between the two groups. Proliferative retinopathy (PR) was marginally lower in those with detectable c-peptide (33.3% vs 55.1%, p = 0.08). However, those with c-peptide were somewhat less likely to have fasted for a full 8-h (66.7% vs. 84.9%, p = 0.09). Excluding those not fully fasted, PR no longer approached significance but macroalbuminuria became marginally lower in those with detectable levels (23.4% vs 0%, p = 0.07).
Conclusions: Low levels of c-peptide in T1D patients of long duration were detected but were not strongly related to microvascular complications.

1. Introduction

Type 1 diabetes has historically been defined as a disease of absolute insulin deficiency, in part due to standard clinical c-peptide assays that did not have an adequate sensitivity to detect very low levels.[1] Recent reports with more sensitive assays show low levels of c-peptide in many cases of type 1 diabetes,[2–4] suggesting incomplete beta cell destruction with residual insulin secretion. The presence of even low c-peptide levels has been associated with a lower risk of microvascular complications.[5–8]

The prevalence of detectable c-peptide levels in type 1 diabetes depends on multiple factors including age of diagnosis, diabetes duration, and method of c-peptide assessment. Davis et al reported that across a wide range of age of onset and duration (3–81 years), almost one out of three patients had detectable non-fasting c-peptide levels (above ≥17 pmol/L), with the frequency of detectable levels independently decreasing by age of onset, particularly with onset under the age of 18 years, and duration.[2] Using more sensitive c-peptide assays in type 1 diabetes of ≫5 years duration and a median age of diagnosis of 16 years, fasting c-peptide was detectable in two-thirds of participants in another population, with most levels in the very low and previously undetectable range compared to earlier assays.[3] In another type 1 diabetes study of 31–40 years duration, only one in 10 had detectable fasting c-peptide levels determined by an ultrasensitive assay with a lower limit of detection of 1.15 pmol/L, and these participants were incidentally noted to have no microvascular complications.[4] However, detectable c-peptide levels have also long been observed in the Joslin study of those with type 1 diabetes of 50 years or more duration.[9]

Lower incidences of retinopathy and abnormal albumin excretion rate after 7 years of follow-up were reported in Diabetes Control and Complications Trial (DCCT) participants who had higher levels of stimulated c-peptide at study entry.[5] Fasting concentrations of c-peptide showed weaker associations with these outcomes.[5] C-peptide assessments and outcomes in the DDCT excluded participants with ≥5 years duration at study entry due to non-measureable c-peptide with the assay which had a lower limit of detection of 30 pmol/L. In a clinical population of type 1 diabetes with a wide range of age of onset and duration, higher fasting c-peptide levels (above 10 pmol/L) measured with an ultrasensitive ELISA test were associated with a lower risk of

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microvascular complications.[6] Studies that used earlier versions of c-peptide assays with higher limits of detection have also suggested a lower risk of overall microvascular complications[7] and retinopathy.[8]

Ultrasensitive c-peptide assays now allow measurement of levels as low as 1.15 pmol/L, well below the range of standard assays, and may be particularly useful in type 1 diabetes of younger onset and longer duration, a population expected to have the lowest levels of c-peptide levels.[2,6] Using the prospectively followed cohort of childhood onset (≪17 years of age) participants with type 1 diabetes in the Pittsburgh Epidemiology of Diabetes Complications (EDC) study, we assessed whether detectable levels of c-peptide using an ultrasensitive ELISA method relate to their microvascular complication status assessed by a standardized protocol at a mean diabetes duration of 40 years.

2. Methods

2.1. Study population

The EDC study is a prospective type 1 diabetes cohort, now in its 30th year of follow-up. Participants in EDC were diagnosed with childhood onset type 1 diabetes, at age ≪ 17 years, at Children's Hospital of Pittsburgh (CHP) between January 1, 1950 to May 31, 1980 and were living within 100 miles or 2.5 h from Pittsburgh at the time of recruitment. The original cohort of 658 participants has been described in detail elsewhere[10,11] and the CHP registry has been shown to be epidemiologically representative of community based type 1 diabetes.[12] In brief, baseline examinations were conducted 1986 through 1988 and used as baseline characteristics for this study. Participants were examined biennially for the first 10 years of the study and then again at 18 years and 25 years. By the 25-year exam, 187 had died and 67 had moved away (≫100 miles from Pittsburgh). Of 363 potentially eligible (alive, residing within 100 miles of Pittsburgh), 223 completed the 25-year exam. Participants who completed the 25-year exam but were not included in this report comprise those with pancreatic transplant (n = 12), inadequate venous access (n = 18) and insufficient amount of blood samples at the time of c-peptide analyses (n = 8). All procedures were approved by the Institutional Review Board and all participants provided informed consent.

2.2. Ascertainment of diabetes complications

Participants provided three timed urine samples (24 h, overnight, and 4-hour collections obtained over a two-week period) at each clinical examination. Urinary and serum albumin concentrations were measured using immunonephelometry[13] and albumin excretion rate (AER) was calculated for each sample. The urine assay had an intra-assay CV of 3% and an inter-assay CV of 9.5%. The serum assay had an intra-assay CV of 1.5% and an intra-assay CV of 3.4%. The AER (μg/min) in any given urine collection was determined as follows: urinary A/C (mg/mg) × calculated 24-hour creatinine excretion (μg) ÷ 1440 (min/24 h).

Microalbuminuria was defined as AER 30–300 mg/24 h (≥20 μg/min) and macroalbuminuria as AER ≫300 mg/24 h (≫200 μg/min) in at least two of the three timed urine collections. In the 10% of urine collections deemed inadequate based on creatinine excretion, as well as during the 25 year follow-up where AER was not assessed, albumin to creatinine ratio (DCA Vantage System) was used (microalbuminuria defined as 0.03–0.3 mg/mg, and macroalbuminuria, as ≫0.3 mg/mg).[13] Glomerular filtration rate was estimated using the Chronic Kidney Disease Epidemiology Collaboration creatinine equation,[14] and stage 3 kidney disease was defined as a value of ≪60 mL/min/1.73 m^2. End stage renal disease (ESRD) was defined as the start of dialysis or kidney transplant. Proliferative retinopathy was defined by 3-field stereoscopic fundus photographs read by the Fundus Photography Reading Center, University of Wisconsin, Madison and graded according to the ETDRS scale and/or history of laser therapy.[15,16] Confirmed distal symmetric polyneuropathy (CDSP) was defined using the DCCT Clinical Exam protocol[17] plus an abnormal age specific vibratory threshold (Vibraton II).

2.3. Risk factors

All participants completed questionnaires regarding demographic, medical history, health care and diabetes self-care information before each exam. An ever-smoker was one who smoked ≥100 cigarettes over his or her lifetime. Blood pressure readings were taken according to the Hypertension Detection and Follow-Up protocol,[18] and hypertension was defined as having blood pressure ≫140/90 or self-reported use of blood pressure–lowering therapy. For the first 18 months of the study, stable HbA_1 was measured by ion exchange chromatography (Isolab, Akron, OH) and for follow-up up to 10 years, automated HPLC (Diamat; BioRad, Hercules, CA) was performed. The two assays were highly correlated (r = 0.95; Diamat[HbA_1] = −0.18 + 1.00[Isolab HbA_1]. For assessments beyond 10 years, HbA_{1c} was measured with the DCA 2000 analyzer (Bayer, Tarrytown, NY). The DCA and Diamat assays were also highly correlated (r = 0.95). Both the original HbA_1 and subsequent HbA1c values were converted to DCCT-aligned HbA_{1c} using regression equations derived from duplicate assays (DCCT HbA_{1c} = 0.14 + 0.83[Diamat HbA_1]) and DCCT HbA_{1c} = (DCA HbA_{1c} − 1.13) / 0.81.[19] Cumulative glycemic exposure was calculated by multiplying the number of glycosylated hemoglobin units above normal at each cycle by the number of months between the midpoints of the preceding and succeeding cycle intervals as previously described.[20] Total, HDL, and LDL cholesterol and triglycerides were determined as previously described[21–24] and non-HDLc was calculated as total − HDL cholesterol.

2.4. Measurement of C-peptide

Serum specimens were obtained upon arrival the morning of the 25-year exam in 185 participants who were instructed to fast overnight. Participants who reported a history of pancreas transplantation (n = 12) were excluded from these analyses. Serum c-peptide levels were measured in duplicate by Mercodia ultra-sensitive ELISA (Uppsala, Sweden) using the ultrasensitive ELISA kits (10-1141-01) which have a lower detection limit of 1.15 pmol/L and an intra-assay coefficient of variation (CV) of 4.5%.

2.5. Statistical analysis

Data were summarized as means and standard deviations or medians with interquartile ranges for continuous variables and as frequencies and percentages for categorical variables. Two-sample *t*-tests were used to compare risk factors between the groups with and without detectable c-peptide levels for normally distributed continuous variables whereas the Wilcoxon rank sum test was used for non-normally distributed variables. The chi-square or Fisher's exact test, as appropriate, was used for categorical variables. A p-value of ≪0.05 was considered statistically significant. Multivariable logistic regression models were used to assess the cross-sectional association between c-peptide status and the presence of microvascular complications. All statistical analyses were performed using SPSS version 24 (IBM Corp, Armonk, NY, USA).

3. Results

3.1. C-peptide levels

A total of 18 (9.7%) of 185 participants had detectable c-peptide levels at their 25-year exam. The median c-peptide level was 3.8 pmol/L an interquartile range of 2.6–12.2 pmol/L. The majority (11/18, 61.1%) of the participants had levels of 1.15–5.0 pmol/L. Those with c-peptide available at 25 years (n = 197) compared to those not (n = 166) were of similar age (25.9 ± 7.3 vs 25.7 ± 7.2 years), age of diabetes onset (8.4 ± 4.1 vs 8.2 ± 4.1 years), and duration (17.5 ± 6.6

vs 17.5 ± 7.2 years) at baseline, but differed in baseline HbA1c (8.5 ± 1.3 vs 8.8 ± 1.7%, p = 0.04).

3.2. Demographic characteristics

Men and women were equally represented in the two groups (50.9% female in non-detectable group and 50.0% in detectable, p = 0.94), and age of onset was comparable but lower in the non-detectable group (8.1 ± 4.1 years vs 9.7 ± 4.2 years in detectable group, p = 0.12). At baseline (Table 1), participants with and without subsequently detectable c-peptide levels did not differ in demographics of age, duration of diabetes, BMI, or WHR. Likewise, the two groups did not differ in clinical measures of HbA_{1c}, insulin dose, blood pressure, pulse, hypertension, estimated GFR (eGFR), or AER parameters. Total cholesterol and non-HDL cholesterol levels were lower in participants with detectable c-peptide levels. Median self-reported total alcohol intake was higher in those with detectable c-peptide levels.

At the 25-year exam (Table 1), there were no differences in measured demographic or clinical variables and cholesterol and total alcohol intake were comparable between the two groups. There was no difference in fasting blood glucose levels between those with or without detectable c-peptide (198 ± 95 vs 170 ± 71 mg/dl, p = 0.13 respectively). Insulin pump use, which was not reported at baseline as it was rare at that time, was non-significantly lower in those with detectable c-peptide levels at the 25-year follow-up (p = 0.07) even though mean HbA_{1c} levels were identical between the two groups at 7.8%. Among pump users, HbA1c levels were lower at the 25 year exam (7.4 ± 0.9% vs 8.2 ± 1.5%, p ≪ 0.001), but HbA1c months did not differ between those with and without pump use (1129 ± 478 months vs 1247 ± 548 months, p = 0.13).

In addition, at the 25-year follow-up, overall HbA_{1c} exposure determined by HbA1c month did not differ between groups (1200 ± 509 months in the non-detectable and 1038 ± 572 months in the non-detectable group, p = 0.22). No difference in severe hypoglycemia (ever experiencing loss of consciousness, hospitalization, requiring assistance of another person) was noted between the two groups (68.7% in detectable, 60.0% in undetectable; p = 0.56) at the 25-year follow-up. In correlations between c-peptide and clinical variables, borderline associations between c-peptide and the albumin to creatinine ratio (0.48, p = 0.06) and estimated GFR (−0.45, p = 0.06) were observed with no significant correlations between c-peptide and any other clinical variables.

3.3. C-peptide and microvascular complications

At baseline, complication prevalence did not differ by subsequent (25 year) c-peptide status (Table 2). At the 25-year follow-up exam, the overall prevalence of one or more complications (proliferative retinopathy, macroalbuminuria and/or confirmed distal symmetrical polyneuropathy) was lower, but not significantly, in those with detectable c-peptide (61.1% vs 77.2%, p = 0.15). Individually, proliferative retinopathy (p = 0.08), macroalbuminuria (p = 0.13), and confirmed distal symmetrical polyneuropathy (p = 0.27) were lower in those with detectable c-peptide, though non-significantly.

Those with detectable c-peptide were somewhat less likely to have fully fasted for 8-h (66.7% vs 84.9%, p = 0.09). Fasting blood glucose (Table 3) was lower in those fully-fasted (9.3 ± 4.0 mmol/l) compared to those incompletely fasted (11.0 ± 4.6 mmol/l, p = 0.035), but fasting blood glucose levels were ≥5.56 in the majority of participants in both groups (87% fully fasted and 89% completely fasted, p = 1.0). All other clinical variables were comparable between groups, including HbA1c (7.8 ± 1.2% in fully fasted vs 7.9 ± 1.7% incompletely fasted, p = 0.52). The most common reason for non-fasting status was self-treatment of overnight or morning hypoglycemia prior to the clinic visit. When analyses were repeated excluding those who were not fully fasted for 8 h (Table 2), there were no differences in the prevalence of proliferative retinopathy and confirmed distal symmetrical polyneuropathy by c-peptide status (p = 0.55 and p = 0.75 respectively) although the difference for macroalbuminuria prevalence approached significance (p = 0.07). Controlling for diabetes duration further minimized these differences.

4. Discussion

In this study of participants with childhood onset (≪17 years of age) type 1 diabetes with a mean duration of over 40 years, 9.7% (n = 18) had fasting c-peptide levels detectable with an ultrasensitive assay. While most of the detectable levels were very low, those with detectable c-peptide had a marginally lower prevalence of proliferative retinopathy and nephropathy in the group as a whole. When excluding

Table 1
Participant characteristics at baseline (1986–1988) and 25-year follow-up (2011–2013) by detectable c-peptide status at the 25-year follow-up exam.

	Baseline (1986–1988)			Follow-up (2011–2013)		
	Non-detectable (n = 167)	Detectable (n = 18)	p-Value	Non-detectable (n = 167)	Detectable (n = 18)	p-Value
Age (years)	25.7 (7.3)	26.5 (8.8)	0.69	51.1 (7.4)	51.8 (8.8)	0.70
Duration (years)	17.6 (6.6)	16.7 (7.2)	0.61	43.0 (6.7)	42.1 (7.4)	0.61
BMI (k/m^2)	23.5 (3.1)	22.6 (3.5)	0.28	28.4 (5.2)	26.7 (4.9)	0.17
WHR	0.82 (0.06)	0.82 (0.04)	0.96	0.88 (0.10)	0.87 (0.05)	0.62
HbA1c (%)	8.5 (1.4)	8.1 (1.1)	0.22	7.8 (1.2)	7.8 (1.4)	0.91
Insulin per body weight (U/kg)	0.78 (0.64, 0.94)	0.68 (0.61, 0.89)	0.29	0.56 (0.39, 0.71)	0.53 (0.46, 0.60)	0.60
SBP (mm Hg)	108.9 (11.6)	111.8 (14.7)	0.32	116.3 (16.5)	117.6 (16.7)	0.74
DBP (mm Hg)	70.6 (9.5)	69.2 (9.6)	0.55	65.6 (9.3)	64.9 (8.9)	0.77
Pulse (beats/min)	76 (70, 82)	76 (74, 88)	0.35	76 (68, 80)	68 (64, 80)	0.18
HTN medications (%)	5.0 (8)	0 (0)	1.00	27.3 (44)	37.5 (6)	0.39
HTN (%)	7.8 (13)	5.6 (1)	1.00	32.5 (52)	43.7 (7)	0.36
Total cholesterol (mmol/l)	4.64 (0.86)	4.10 (0.74)	0.013	4.68 (0.96)	4.42 (0.90)	0.27
HDL cholesterol (mmol/l)	1.43 (0.31)	1.41 (0.34)	0.78	1.59 (0.50)	1.51 (0.54)	0.51
Non-HDL cholesterol (mmol/l)	3.21 (0.84)	2.70 (0.66)	0.014	3.09 (0.95)	2.91 (0.83)	0.44
Lipid medications (%)	0.62 (1)	0 (0)	1.00	47.8 (77)	37.5 (6)	0.43
eGFR ($ml/min/1.73\ m^2$)	110.1 (24.8)	117.3 (23.9)	0.24	79.3 (21.5)	88.2 (19.9)	0.09
AER (μg/min)	10.0 (6.5, 24.3)	9.9 (5.5, 20.3)	0.32			
A/C ratio[a] (μg/mg)				12.0 (6.9, 38.4)	8.7 (6.0, 20.8)	0.41
ACE/ARB (%)	2.5 (4)	0 (0)	1.00	34.2 (55)	43.7 (7)	0.44
Total alcohol intake (g/d)	1.0 (0, 4.0)	4.5 (1.0, 15.0)	0.035	0 (0, 3.0)	2.0 (0, 6.0)	0.08
Pump use (%)				55.3 (89)	31.2 (5)	0.07
Statin use (%)				46.0 (74)	37.5 (6)	0.52

[a] If a 24 h sample was not available, an overnight or spot urine sample was used. AER was not assessed at the 25 year follow-up.

Table 2
Microvascular complication prevalence at baseline and 25 year follow-up exam by c-peptide status at the 25-year exam.

	Baseline (1986–1988)			Follow-up (2011–2013) All participants			Follow-up (2011–2013) Full 8-hour fast		
	Non-detectable (n = 167)	Detectable (n = 18)	p-Value	Non-detectable (n = 167)	Detectable (n = 18)	p-Value	Non-detectable (n = 141)	Detectable (n = 12)	p-Value
Proliferative retinopathy, % (n)	18.7 (31/166)	5.9 (1/17)	0.31	55.1 (92/167)	33.3 (6/18)	0.08	53.9% (76/141)	41.7% (5/12)	0.55
Microalbuminuria, % (n)	18.6 (31/167)	22.2 (4/18)	0.76	57.4 (96/167)	55.6 (10/18)	0.88	57.4% (81/141)	50.0% (6/12)	0.62
Macroalbuminuria, % (n)	11.4 (19/167)	5.6 (1/18)	0.69	22.8 (38/167)	5.6 (1/18)	0.13*	23.4% (33/141)	0 (0/12)	0.07*
Stage 3 kidney disease, % (n)	1.2 (2/165)	0 (0/18)	1.00	21.0 (35/167)	11.1 (2/18)	0.26*	1.5% (2/137)	0 (0/12)	1.00*
Renal failure, % (n)	0 (0/167)	0 (0/18)	–	4.2 (7/167)	0 (0/18)	0.48*	4.3% (6/141)	0 (0/12)	1.00
Confirmed distal symmetric polyneuropathy (CDSP), % (n)	18.0 (30/167)	11.1 (2/18)	0.74	63.3 (105/166)	50 (9/18)	0.27	65.7% (92/140)	58.3% (7/12)	0.75*

* Fisher's exact test p-value.

those who could not adhere to an 8-hour fast, a marginally lower difference remained only for the prevalence of macroalbuminuria. These differences were no longer present after controlling for duration of diabetes.

Our findings extend those of prior studies of residual c-peptide to a large group of closely followed childhood onset type 1 diabetes of long duration. While c-peptide levels have been detected in significant numbers of patients with type 1 diabetes, studies suggest that levels are least likely to persist in those of younger onset and longer duration,[2,6] although detectable c-peptide levels have been previously identified in ≫67% of Medalists with a mean diabetes duration of 56 years.[9] Unlike prior studies which included patients with a wide age range at diabetes onset,[2,3,5] all of our participants were diagnosed at ≪17 years of age, a subset of patients less likely to have persistent c-peptide production. Our finding of detectable levels in 9.7% of people with a mean diabetes duration of over 40 years is consistent with prior reports of detectable levels in 10% of cases of type 1 diabetes of 31–40 years duration in a small subset of subjects using the same ultrasensitive assay.[4]

Prior studies have shown a relationship between c-peptide levels and protection from microvascular complications.[7] In a clinical population, c-peptide levels of ≫10 pmol/L were associated with protection from microvascular complications.[6] The levels of c-peptide detected in our study were very low, with 14 of 18 (78%) below 10 pmol/L. Studies of the DCCT cohort who had diabetes of shorter duration used stimulation tests to assess c-peptide production which may have revealed a greater prevalence of persistent c-peptide production than seen in our cohort,[5] although most were hyperglycemic in our study at the time of testing as a stimulus for c-peptide production. Although an 83% agreement between fasting and stimulated c-peptide results has been reported,[5] future studies in long-duration T1D could include a c-peptide stimulation test to capture the c-peptide producers who were potentially missed by fasting levels only. The tendency for a higher reported rate of non-fasting in those with detectable c-peptide levels suggests that those who were not fully fasted may have had a stimulus for c-peptide secretion that those who were fully fasted did not have.

Table 3
Participant characteristics at 25-year follow-up (2011–2013) by fasting status at the 25-year follow-up exam.

	Full 8-hour fast (n = 153)	Incomplete fast (n = 31)	p-Value
Age (years)	51.3 (7.4)	50.7 (8.4)	0.69
Duration (years)	42.7 (6.7)	43.5 (7.5)	0.57
BMI (k/m^2)	28.6 (5.3)	27.0 (4.5)	0.11
WHR	0.88 (0.10)	0.86 (0.08)	0.16
Fasting blood glucose, mmol/l	9.3 (4.0)	11.0 (4.6)	0.035
HbA1c (%)	7.8 (1.2)	7.9 (1.6)	0.52
Insulin per body weight (U/kg)	0.55 (0.38, 0.69)	0.54 (0.47, 0.61)	0.32
SBP (mm Hg)	117 (17)	115 (15)	0.57
DBP (mm Hg)	66 (9)	64 (10)	0.19
Pulse (beats/min)	76 (65, 80)	72 (64, 80)	0.80
HTN (%)	35.3 (52)	24.1 (7)	0.24
Total cholesterol (mmol/l)	4.62 (0.98)	4.73 (0.91)	0.62
HDL cholesterol (mmol/l)	1.57 (0.49) 9	1.62 (0.54)	0.66
Non-HDL cholesterol (mmol/l)	2.64 (0.80)	2.66 (0.72)	0.87
Lipid medications (%)	47.6 (70)	43.3 (13)	0.43
eGFR (ml/min/1.73 m^2)	79.6 (21.8)	82.2 (20.1)	0.54
A/C ratio* (μg/mg)	10.2 (5.7, 24.0)	8.3 (5.3, 17.2)	0.56
ACE/ARB (%, n)	35.3 (52)	33.3 (10)	0.83
Total alcohol intake (g/d)	0 (0, 3.0)	1.0 (0, 6.0)	0.59
Pump use (%)	55.8 (82)	40.0 (12)	0.11
Statin use (%)	46.3 (68)	40.0 (12)	0.53

* Albumin to creatinine ratio.

The impact of c-peptide detection on microvascular complications has been particularly noted with assessments of stimulated c-peptide levels in the DCCT.[5] Use of an ultrasensitive ELISA in the EDC allowed for detection of c-peptide levels which were much lower (1.15 pmol/L) than levels detectable with the radioimmunoassay (30 pmol/L) used for DCCT/EDIC participants.[5] Differences in the two cohorts, e.g., older mean age of onset in DCCT/EDIC of 26–28 years compared to 8.3 years in the EDC, shorter duration of diabetes, and exclusion of individuals with ≫5 years duration in the DCCT/EDIC c-peptide analyses, may account for the observed differences in c-peptide concentrations.[5] Another study using an earlier c-peptide assay with a lower limit of detection of 17 pmol/L, found that 19% of c-peptide negative participants had detectable stimulated c-peptide levels in response to a mixed meal tolerance test stimulus, while 5% had undetectable stimulated levels despite detectable non-fasting levels.[2] In comparison, the Mercodia assay used in this study had a lower limit of detection of 1.15 pmol/L and the majority of detectable c-peptide levels in our study were below 10 pmol/L, so the increased sensitivity of the Mercodia assay may have allowed detection of lower c-peptide levels that prior assays would have missed.

Prior studies reported c-peptide levels to be related to better glycemic control.[5,6] We did not find this in our study as those with and without detectable c-peptide had similar glycemic control, possibly due to the very low clinically insignificant levels of c-peptide present. Glycemic control based on 25-year follow-up HbA1c was better among pump users, even though HbA1c months did not differ. Because insulin pump users had better glycemic control, more frequent use of the insulin pump in the undetectable c-peptide group might have also resulted in the comparable glycemic control between the groups. This suggests that overall glycemic control, rather than the low c-peptide levels detected in this group, is critical for the prevention of microvascular complications.[25] C-peptide levels had a borderline direct association with the albumin and creatinine ratio and a borderline inverse association with estimated GFR. While these associations did not reach significance and the c-peptide levels were very low, the clearance of c-peptide may have been reduced in cases of early renal dysfunction. There were no cases of macroalbuminuria observed in those with detectable c-peptide. The DCCT reported a lower risk of hypoglycemia with residual

c-peptide after 5 years of follow-up,[5] but we could not confirm a relationship between severe hypoglycemia and the low levels of residual c-peptide production after a longer follow-up in our study.

While c-peptide status did not impact clinical variables at 25-years of follow-up in our study, those with detectable c-peptide had lower total cholesterol, non-HDL cholesterol, and higher alcohol intake at baseline. Perhaps those with persistent c-peptide production would have had even higher levels of c-peptide if measured at baseline, potentially resulting in improved regulation of lipids and perhaps less alcohol related hypoglycemia[26] allowing for greater alcohol intake.

The strengths of this study are the long duration of diabetes, the sensitivity of the assay, and the well-characterized complication status of the EDC population. The limitations of this study are the small sample size, which could have affected the significance of the observed associations, and the cross-sectional design. The sample of 18 participants with detectable c-peptide levels only allowed for detection of a 30% difference in complications between groups. A larger sample size might have allowed detection of a smaller difference in complications rates. The sample size was further reduced in analyses excluding those not fully fasted. The clinical variability in fasting status noted above has not been reported in prior c-peptide studies and may have served as a stimulus for c-peptide production that fully fasted participants did not have.

5. Conclusions

In conclusion, after decades of childhood onset type 1 diabetes, c-peptide levels remain detectable in a very low range in a subset of patients when assessed with an ultrasensitive assay but these levels are not associated with a reduction in microvascular complications. The clinical implications of these findings are that our data provide little support for a role of very small amounts of residual c peptide secretion on the prevalence of microvascular complications.

Author contributions

Katherine Williams was responsible for literature search, figures, data analysis and interpretation, writing and revision. Dorothy Becker provided concept, study design, c-peptide analyses, data interpretation, writing and revision. Trevor Orchard was responsible for concept, study design, data collection, data analysis and interpretation, writing and revision. Tina Costacou was responsible for literature search, study design, data collection, data analysis, data interpretation, writing and revision.

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References

1. Faustman DL. Why were we wrong for so long? The pancreas of type 1 diabetic patients commonly functions for decades. Diabetologia 2014;57:1-3, https://doi.org/10.1007/s00125-013-3104-9.
2. Davis AK, DuBose SN, Haller MJ, et al. Prevalence of detectable c-peptide according to age at diagnosis and duration of type 1 diabetes. Diabetes Care 2015;38:476-81, https://doi.org/10.2337/dc14-1952.
3. Oram RA, Jones AG, Besser REJ, et al. The majority of patients with long-duration type 1 diabetes are insulin microsecretors and have functioning beta cells. Diabetologia 2014;57:187-91, https://doi.org/10.1007/s00125-013-3067-x.
4. Wang L, Lovejoy NF, Faustman DL. Persistence of prolonged C-peptide production in type 1 diabetes as measured with an ultrasensitive C-peptide assay. Diabetes Care 2012;35:465-70, https://doi.org/10.2337/dc11-1236.
5. Lachin JM, McGee P, Palmer JP. Impact of c-peptide preservation on metabolic and clinical outcomes in the diabetes control and complications trial. Diabetes 2014;63:739-48, https://doi.org/10.2337/db13-0881.
6. Kuhtreiber WM, Washer SLL, Hsu E, et al. Low levels of C-peptide have clinical significance for established Type 1 diabetes. Diabet Med 2015;32:1346-53, https://doi.org/10.1111/dme.12850.
7. Panero F, Novelli G, Zucco C, et al. Fasting plasma c-peptide and micro- and macrovascular complications in a large clinic-based cohort of type 1 diabetic patients. Diabetes Care 2009;32:301-5, https://doi.org/10.2337/dc08-1241.
8. Nakanishi K, Watanabe C. Rate of beta-cell destruction in type 1 diabetes influences the development of diabetic retinopathy: protective effect of residual beta-cell function for more than 10 years. J Clin Endocrinol Metab 2008;93:4759-66, https://doi.org/10.1210/jc.2008-1209.
9. Keenan HA, Sun JK, Levine J, et al. Residual insulin production and pancreatic β-cell turnover after 50 years of diabetes: Joslin medalist study. Diabetes 2010;59:2846-53, https://doi.org/10.2337/db10-0676.
10. Orchard TJ, Dorman JS, Maser RE, et al. Factors associated with avoidance of severe complications after 25 yr of IDDM. Pittsburgh epidemiology of diabetes complications study I. Diabetes Care 1990;13:741-7.
11. Orchard TJ, Dorman JS, Maser RE, et al. Prevalence of complications in IDDM by sex and duration: Pittsburgh Epidemiology of Diabetes Complications Study II. Diabetes 1990;39:1116-24.
12. Wagener DK, Sacks JM, LaPorte RE. MacGregor. The Pittsburgh study of insulin-dependent diabetes mellitus. Risk for diabetes among relatives of IDDM. Diabetes 1982;31:136-44.
13. Ellis D, Coonrod BA, Dorman JS, et al. Choice of urine sample predictive of microalbuminuria in patients with insulin-dependent diabetes mellitus. Am J Kidney Dis 1989;13:321-8.
14. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604-12.
15. Early Treatment of Diabetic Retinopathy Study Coordinating Center. *Manual of operations*. Baltimore, MD: University of Maryland School of Medicine. 1980.
16. Conway BN, Miller RG, Klein R, Orchard TJ. Prediction of proliferative diabetic retinopathy with hemoglobin level. Arch Ophthalmol 2009, https://doi.org/10.1001/archophthalmol.2009.274.
17. Feldman EL, Stevens MJ, Thomas PK, Brown MB, Canal N, Greene DA. A practical two-step quantitative clinical and electrophysiological assessment for the diagnosis and staging of diabetic neuropathy. Diabetes Care 1994, https://doi.org/10.2337/diacare.17.11.1281.
18. Group HD and FPC. The hypertension detection and follow-up program. Prev Med 1976;5:207-15.
19. Prince CT, Becker DJ, Costacou T, Miller RG, Orchard TJ. Changes in glycaemic control and risk of coronary artery disease in type 1 diabetes mellitus: findings from the Pittsburgh Epidemiology of Diabetes Complications Study (EDC). Diabetologia 2007;50:2280-8.
20. Orchard TJ, Forrest KY-Z, Ellis D, Becker DJ. Cumulative glycemic exposure and microvascular complications in insulin-dependent diabetes mellitus. The glycemic threshold revisited. Arch Intern Med 1997, https://doi.org/10.1001/archinte.1997.00440370091009.
21. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470-5.
22. Warnick GR, Albers JJ. Heparin-Mn2+ quantitation of high-density-lipoprotein cholesterol: an ultrafiltration procedure for lipemic samples. Clin Chem 1978;24:900-4.
23. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 1973;19:476-82.
24. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
25. Diabetes Control and Complications Trial Research Group. Nathan DM, Genuth S, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993, https://doi.org/10.1056/NEJM199309303291401.
26. Turner BC, Jenkins E, Kerr D, Sherwin RS, Cavan DA. The effect of evening alcohol consumption on next-morning glucose control in type 1 diabetes. Diabetes Care 2001, https://doi.org/10.2337/diacare.24.11.1888.