Clinical Impact of Residual C-Peptide Secretion in Type 1 Diabetes on Glycemia and Microvascular Complications

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OBJECTIVE

To quantify the relationship of residual C-peptide secretion to glycemic outcomes and microvascular complications in type 1 diabetes.

RESEARCH DESIGN AND METHODS

C-peptide was measured in an untimed blood sample in the Scottish Diabetes Research Network Type 1 Bioresource (SDRNT1BIO) cohort of 6,076 people with type 1 diabetes monitored for an average of 5.2 years.

RESULTS

In regression models adjusted for age at onset and duration, effect sizes for C-peptide ≥200 vs. <5 pmol/L were as follows: insulin dose at baseline, 9% lower ($P = 2 \times 10^{-17}$); HbA_{1c} during follow-up, 4.9 mmol/mol lower ($P = 3 \times 10^{-13}$); hazard ratio for hospital admission for diabetic ketoacidosis during follow-up, 0.44 ($P = 0.0001$); odds ratio for incident retinopathy, 0.51 ($P = 0.0003$). Effects on the risk of serious hypoglycemic episodes were detectable at lower levels of C-peptide, and the form of the relationship was continuous down to the limit of detection (3 pmol/L). In regression models contrasting C-peptide 30 to <200 pmol/L with <5 pmol/L, the odds ratio for self-report of at least one serious hypoglycemic episode in the last year was 0.56 ($P = 6 \times 10^{-8}$), and the hazard ratio for hospital admission for hypoglycemia during follow-up was 0.52 ($P = 0.03$).

CONCLUSIONS

These results in a large representative cohort suggest that even minimal residual C-peptide secretion could have clinical benefit in type 1 diabetes, in contrast to a follow-up study of the Diabetes Control and Complications Trial (DCCT) intensively treated cohort where an effect on hypoglycemia was seen only at C-peptide levels ≥130 pmol/L. This has obvious implications for the design and evaluation of trials of interventions to preserve or restore pancreatic islet function in type 1 diabetes.

Persistent C-peptide secretion, reflecting some degree of endogenous β-cell function, is now recognized to be common in type 1 diabetes. We previously showed that this is under strong genetic influence and inversely associated with age at onset (1). It is important for people with diabetes and their clinicians to understand how such residual β-cell function relates to the heterogeneity of glycemic control and risk of acute and chronic complications in diabetes. Although C-peptide is now accepted by

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**A complete list of the SDRNT1BIO Investigators can be found in the supplementary material online.*

regulatory authorities as a primary end point in trials of interventions to preserve or restore β-cell function (2), there is not yet a consensus on what level of C-peptide secretion constitutes a clinically useful therapeutic effect. Current guidance is that "to provide convincing evidence of preserved endogenous beta-cell function, the trials should demonstrate a clinically meaningful reduction in mean daily insulin requirements accompanied by similar magnitude of glycemic control compared to the control arm (2)." Effects on clinical outcomes, such as hypoglycemia or retinopathy, may be more relevant to evaluating the benefits of preserving residual β-cell function. The development of highly sensitive assays with a lower limit of detection of <5 pmol/L has made it possible to investigate whether the relationships of these outcomes to C-peptide level is flat below some threshold level of C-peptide and whether these relationships are continuous down to very low levels of secretion. Recent studies using these sensitive C-peptide assays had small sample sizes or were limited to cross-sectional associations (3). The aim of this study was to examine the form of the relationship of residual C-peptide secretion to glycemic control and acute and chronic complications of diabetes in a prospective study of a large representative sample of adults with type 1 diabetes including a wide range of age at onset.

RESEARCH DESIGN AND METHODS

Scottish Diabetes Research Network Type 1 Bioresource

The Scottish Diabetes Research Network Type 1 Bioresource (SDRNT1BIO) is a cohort of 6,076 people clinically diagnosed with type 1 diabetes aged >16 years at recruitment. This cohort, described in detail previously, comprised one-third of all adults with type 1 diabetes in Scotland (4). Questionnaire data, clinical measurements, and samples obtained on the day of recruitment (2011–2013) were linked retrospectively and prospectively to the Scottish Care Information Diabetes Collaboration health record database and other nationally held electronic health records (5). The baseline questionnaire captured the daily dose of insulin at baseline and the prior year's history of hypoglycemic episodes that required assistance. It also included an item on awareness of hypoglycemia scored on a scale from 1 (always aware) to 7 (never aware), dichotomized to a score of ≥3 vs. ≤2 for this analysis.

Linkage to health records has captured all laboratory measurements of HbA_{1c}, drug prescriptions in primary care, grading of retinal photographs in the national screening program, and hospital admissions coded according to the ICD-10 classification for several years before recruitment and prospectively up to the end of 2018. There were 886 individuals who received a pump, 6 who received a continuous glucose monitoring, and 24 who received a flash glucose monitoring device at some time during follow-up. To model associations with HbA_{1c} during follow-up as a dependent variable, person-time intervals were right censored to exclude observations on individuals after the first date on which they received one of these devices.

Prospective rates of serious hypoglycemia (ICD-10 codes E15, E16.0, E16.1, and E16.2) and diabetic ketoacidosis (ICD-10 codes E10.1, E10.11, E11.1, E12.1, E13.1, and E14.1) were based on hospital discharge diagnoses and deaths out of hospital. There were 596 admissions for hypoglycemia, 2,348 admissions for ketoacidosis, and 28 out-of-hospital deaths from ketoacidosis during follow-up. The 90 admissions with a primary diagnosis of "diabetic coma" (codes E10.0, E11.0, E12.0, E13.0, and E14.0) and no associated diagnostic code for ketoacidosis or hypoglycemia were excluded from analysis. Retinopathy was defined as any grade of retinopathy (codes R1 to R4) or maculopathy (M1 to M2) in either eye in the national screening program (6). Incident retinopathy was calculated in those free of retinopathy (coded R0 and M0) at baseline. Estimated glomerular filtration rate (eGFR) was calculated from serial serum creatinine values using the Chronic Kidney Disease (CKD) Epidemiology Collaboration equation (7).

Ethics Approval and Consent to Participate

The study was conducted in accordance with the ethical principles in the Declaration of Helsinki and was approved by the Tayside Research Ethics Committee (reference 10/S1402/43). Informed consent was obtained from all participants.

C-Peptide Levels

Nonfasting serum samples were obtained at the recruitment visit in 5,928 of those clinically diagnosed as type 1. The median time from sampling to freezing at −80°C was 2 h 15 min (interquartile range 1 h 30 min–3 h 10 min). As previously reported (1), glucose measured in these samples was >5 mmol/L in 88% of individuals, and C-peptide was not related to glucose level in a regression model. C-peptide measurements on these samples were undertaken at the Exeter Clinical Laboratory using the sandwich electrochemiluminescence immunoassay on a Roche Modular E170 platform, with a lower limit of detection of C-peptide of 3 pmol/L (8). The assay was calibrated using Roche C-peptide CalSet calibration material (Roche Diagnostics, Mannheim, Germany), traceable to World Health Organization International Reference Reagent for C-peptide of human insulin for immunoassay (International Reference Reagent code 84/510).

Autoantibodies to GAD65, tyrosine phosphorylase-related protein 2 (IA2), and zinc transporter 8 (ZnT8) were also measured at the Exeter Laboratory, which participates in the Diabetes Antibody Standardization Program (9). As described elsewhere (1), the 203 individuals with C-peptide >600 pmol/L who were negative for all three autoantibodies were classified as "possible type 2." The remaining 5,726 were classified as type 1, and the analyses reported here are restricted to this category.

Statistical Analysis

For initial analyses of the effects of C-peptide levels, we modeled C-peptide as a categoric variable with four levels (<5, 5 to <30, 30 to <200, and ≥200 pmol/L) corresponding to categories used in previous studies to allow comparison with these studies. The dependence of outcomes recorded at baseline on C-peptide levels was modeled with linear regression for continuous variables (daily insulin dose and HbA_{1c} levels) and logistic regression for binary outcomes (any severe hypoglycemic episode in the past year, hypoglycemia unawareness, and any retinopathy, eGFR ≤60 mL/min/1.73 m^2). For HbA_{1c} levels during follow-up, a linear mixed model was fitted with the R function lme4::lmer, encoding individuals as random effects and covariates as fixed effects. For incident retinopathy and CKD stage 3 during follow-up, logistic regression models were specified. For rates of hypoglycemia and ketoacidosis during

Table 1—Cohort characteristics by baseline C-peptide level

Covariate	<5 pmol/L (n = 3,571)	5 to <30 pmol/L (n = 697)	30 to <200 pmol/L (n = 803)	≥200 pmol/L (n = 661)	All (N = 5,732)
Baseline					
Sex (female), %	45.3	45.2	40.3	39.5	43.9
Age (years)	46.2 (35.0, 56.5)	45.0 (32.4, 54.8)	42.1 (31.0, 52.5)	41.7 (30.1, 52.5)	45.0 (33.2, 55.4)
Age at onset (years)	17.0 (9.7, 27.1)	20.3 (12.7, 31.2)	25.9 (18.1, 35.6)	31.7 (23.3, 43.2)	20.8 (11.9, 31.2)
Diabetes duration (years)	25.4 (17.2, 34.7)	18.8 (11.3, 30.4)	11.2 (6.1, 21.5)	4.8 (1.9, 12.4)	20.9 (11.5, 31.6)
BMI (kg/m^2)	26.4 (23.8, 29.6)	26.0 (23.5, 28.9)	26.1 (23.5, 29.1)	26.5 (23.3, 30.2)	26.3 (23.7, 29.5)
Daily insulin dose (units)	50.0 (38.0, 66.0)	51.0 (37.0, 66.0)	50.0 (38.8, 68.0)	38.0 (25.0, 56.0)	49.5 (36.0, 65.0)
HbA_{1c} (mmol/mol)	70.0 (62.0, 80.0)	70.0 (61.0, 80.0)	71.0 (62.0, 83.0)	64.0 (54.0, 77.9)	69.0 (61.0, 80.0)
HbA_{1c} (%)	8.6 (7.8, 9.5)	8.6 (7.7, 9.5)	8.6 (7.8, 9.7)	8.0 (7.1, 9.3)	8.5 (7.7, 9.5)
Serious hypoglycemic episode in last year, %	32.1	24.0	17.6	12.7	26.8
Any prior retinopathy, %	44.1	33.1	21.9	14.2	36.3
eGFR <60 mL/min/1.73 m^2, %	7.1	5.6	4.5	4.9	6.3
Prospective					
Final HbA_{1c} (mmol/mol)	69.2 (61.0, 80.0)	69.5 (61.0, 80.0)	70.8 (61.0, 83.0)	64.0 (54.0, 77.6)	69.0 (60.0, 80.0)
Person-years of follow-up	18,838	3,629	4,248	3,490	30,206
Hypoglycemia admissions, n	391	60	75	70	596
At least one hypoglycemia admission, %	5.7	3.9	3.0	2.3	4.7
DKA admissions, n	1,601	248	316	231	2,396
At least one DKA admission, %	10.7	10.5	12.1	7.0	10.4
Incident eGFR <60 mL/min/1.73 m^2, %	10.5	8.9	6.9	6.9	9.4
Incident retinopathy, %	18.7	16.5	12.4	7.4	15.6

Continuous variables are presented as median (interquartile range) and categorical variables as indicated. DKA, diabetic ketoacidosis.

follow-up, based on counts of events in each person-time interval, a generalized linear mixed model with Poisson likelihood was specified.

Form of Relationship of Outcome to C-Peptide Level

A key objective of this study was to investigate the form of the relationships of each outcome to C-peptide to determine for each outcome at what level of C-peptide the effects on that outcome become detectable. Such a relationship might be a continuous curve or flat below some threshold level of C-peptide and sloping above this threshold. To find what form of relationship best fits the data, it is necessary to specify a family of models that can represent a wide range of possible curve shapes. A widely used approach to this type of problem, introduced by Royston and Altman (10), is to fit fractional polynomial regression models. Although a two-term fractional polynomial can represent functions of diverse shape, in practice, the results depend critically on the arbitrary choice of the threshold P value used to determine which terms should be retained. To overcome this, we defined a related class of models which we denote as a "mixture of fractional powers." This class of models specifies a finite mixture of generalized linear models encoding different power transformations of the covariate as Box-Cox transforms, each scaled to zero mean and unit variance. The models were specified with a uniform prior over 12 evenly spaced values of the power transform parameter used in the Box-Cox transform, with upper and lower values set to encompass the range of values with nonzero posterior probability on the basis of a preliminary sampling run. The posterior distribution of model parameters was used to generate a posterior predictive distribution for the outcome variable over a grid of values of C-peptide level, averaged over the discrete distribution of the power transform parameter, with all other covariates held at their mean values and random effects set to zero. The functional relationships shown are based on the mean and interquartile range of this posterior predictive distribution at each value of C-peptide.

To model repeated observations of HbA_{1c}, admissions for hypoglycemia and admissions for ketoacidosis we used mixed models as described in the Supplementary Material.

Data and Resource Availability

In accordance with governance requirements, a data access committee oversees applications for access to individual-level data as described elsewhere (4). All code used to generate this manuscript will be made freely available with this paper.

RESULTS

The distribution of C-peptide levels by age at onset and duration and the method used to exclude possible cases of type 2 diabetes have been described in detail previously (1). The distribution of characteristics at baseline and during follow-up by C-peptide category at baseline is reported in Table 1. The average

Table 2—Regression models of relationship of glycemic outcomes to C-peptide adjusted for covariates

	Log_{10} insulin dose		HbA_{1c} baseline		HbA_{1c} prospective		Unawareness of hypoglycemia		At least one hypoglycemic event last year		Rate of hypoglycemia		Rate of ketoacidosis	
	Slope (95% CI)	*P* value	Slope (95% CI)	*P* value	Slope (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Sex (female)	−0.017 (−0.021, −0.012)	1×10^{-13}	2.434 (1.583, 3.286)	2×10^{-8}	2.376 (1.611, 3.141)	1×10^{-9}	1.28 (1.14, 1.45)	6×10^{-5}	0.95 (0.84, 1.07)	0.4	0.67 (0.47, 0.96)	0.03	1.38 (1.11, 1.71)	0.004
Age at onset (years)	−0.001 (−0.001, −0.001)	8×10^{-16}	−0.077 (−0.112, −0.043)	1×10^{-5}	−0.092 (−0.122, −0.062)	3×10^{-9}	1.00 (1.00, 1.01)	0.2	0.99 (0.98, 0.99)	3×10^{-5}	1.03 (1.02, 1.05)	4×10^{-6}	1.00 (0.99, 1.01)	0.7
Duration (years)	−0.001 (−0.001, −0.001)	3×10^{-14}	−0.200 (−0.238, −0.162)	8×10^{-25}	−0.270 (−0.298, −0.243)	3×10^{-83}	1.02 (1.01, 1.02)	8×10^{-11}	1.01 (1.01, 1.02)	3×10^{-6}	1.05 (1.03, 1.07)	2×10^{-10}	0.98 (0.97, 0.99)	8×10^{-5}
BMI (kg/m^2)	0.001 (0.001, 0.002)	2×10^{-9}	−0.130 (−0.223, −0.037)	0.006	−0.466 (−0.511, −0.421)	3×10^{-91}	0.97 (0.96, 0.99)	4×10^{-5}	0.96 (0.95, 0.98)	1×10^{-7}	0.90 (0.87, 0.93)	7×10^{-11}	0.90 (0.88, 0.91)	6×10^{-28}
HbA_{1c} (mmol/mol)	—	—	—	—	—	—	—	—	1.00 (1.00, 1.01)	0.1	0.98 (0.97, 0.99)	1×10^{-8}	1.01 (1.01, 1.02)	2×10^{-18}
C-peptide 5–<30 pmol/L	−0.001 (−0.007, 0.006)	0.9	0.084 (−1.247, 1.416)	0.9	−0.932 (−2.133, 0.269)	0.1	0.92 (0.76, 1.11)	0.4	0.73 (0.60, 0.89)	0.001	0.56 (0.30, 1.02)	0.06	0.94 (0.63, 1.39)	0.7
C-peptide 30–<200 pmol/L	−0.003 (−0.010, 0.004)	0.5	0.433 (−0.894, 1.759)	0.5	−0.286 (−1.455, 0.883)	0.6	0.67 (0.54, 0.82)	1×10^{-4}	0.56 (0.46, 0.69)	6×10^{-8}	0.52 (0.29, 0.93)	0.03	0.95 (0.68, 1.34)	0.8
C-peptide ≥200 pmol/L	−0.041 (−0.048, −0.033)	1×10^{-23}	−6.415 (−7.938, −4.892)	2×10^{-16}	−4.922 (−6.244, −3.600)	3×10^{-13}	0.79 (0.62, 1.00)	0.05	0.47 (0.36, 0.61)	2×10^{-8}	0.35 (0.16, 0.76)	0.008	0.44 (0.29, 0.67)	1×10^{-4}

HR, hazard ratio; OR, odds ratio.

Table 3—Regression models for each outcome by duration of diabetes

	C-peptide level			
Duration	5 to <30 pmol/L	30 to <200 pmol/L	≥200 pmol/L	*P* value
Number of individuals				
<10	148	360	453	—
10–19	219	216	123	—
≥20	330	227	85	
Log_{10} insulin dose				
<10	−0.009 ± 0.009	−0.006 ± 0.007	−0.046 ± 0.008	2×10^{-10}
10–19	0.008 ± 0.007	−0.007 ± 0.007	0.005 ± 0.009	0.3
≥20	−0.005 ± 0.005	0.01 ± 0.005	0.024 ± 0.009	0.008
HbA_{1c} baseline				
<10	−0.409 ± 1.97	−0.002 ± 1.628	−8.709 ± 1.751	2×10^{-9}
10–19	0.65 ± 1.257	−0.468 ± 1.294	−2.167 ± 1.627	0.5
≥20	−0.618 ± 0.866	0.82 ± 1.034	0.117 ± 1.659	0.7
HbA_{1c} prospective				
<10	−2.497 ± 1.758	−0.783 ± 1.399	−5.746 ± 1.364	2×10^{-5}
10–19	−0.112 ± 1.122	0.835 ± 1.133	−1.046 ± 1.426	0.7
≥20	−0.628 ± 0.799	0.119 ± 0.945	0.584 ± 1.502	0.8
Unawareness of hypoglycemia (log odds ratios)				
<10	−0.264 ± 0.25	−0.803 ± 0.217	−0.42 ± 0.227	0.003
10–19	−0.268 ± 0.189	−0.466 ± 0.211	−0.431 ± 0.272	0.06
≥20	0.02 ± 0.128	−0.3 ± 0.165	−1.429 ± 0.381	7×10^{-4}
At least one serious hypoglycemic event in last year (log odds ratios)				
<10	−0.237 ± 0.258	−0.725 ± 0.232	−0.7 ± 0.263	0.008
10–19	−0.769 ± 0.202	−0.495 ± 0.201	−0.208 ± 0.246	4×10^{-4}
≥20	−0.108 ± 0.129	−0.334 ± 0.163	−0.456 ± 0.275	0.08
Any hypoglycemia admission during follow-up (log odds ratios)				
<10	−0.367 ± 0.51	−0.574 ± 0.441	−1.423 ± 0.599	0.1
10–19	−1.389 ± 0.604	−0.545 ± 0.426	−0.88 ± 0.619	0.05
≥20	−0.08 ± 0.254	−0.958 ± 0.426	0.259 ± 0.418	0.1
Any ketoacidosis admission during follow-up (log odds ratios)				
<10	0.052 ± 0.258	−0.294 ± 0.224	−1.126 ± 0.273	8×10^{-5}
10–19	−0.334 ± 0.234	−0.345 ± 0.251	−0.715 ± 0.367	0.1
≥20	−0.256 ± 0.225	−0.17 ± 0.259	−0.702 ± 0.524	0.4

The coefficients (±SE) are for C-peptide categories with <5 pmol/L as the reference category, and the covariates are the same as in Table 2. Summary *P* values are for the null hypothesis that all three coefficients are zero.

duration of follow-up was 5.2 years. With >600 individuals in in each category of C-peptide levels, 596 admissions for hypoglycemia and 2,396 admissions for ketoacidosis during 29,768 person-years of follow-up, the numbers of individuals and events were adequate for the form of relationship of these outcomes to C-peptide to be examined. Insulin dose information was missing for 1,552 participants. The mean number of HbA_{1c} measures during follow-up was 9.3. As reported in Table 1 and described previously (1), persistent C-peptide secretion was associated with short duration and late age at onset. Because the associations of C-peptide level with clinical outcomes are heavily confounded by duration and age at onset, we have not given *P* values for these crude associations in Table 1. To examine the independent associations of clinical outcomes to C-peptide levels, it is necessary to adjust for these covariates in regression models as in Tables 2 and 5. Even in Table 3 which gives results stratified by duration, we have adjusted for duration within each stratum.

C-Peptide Association With Insulin Dose

In a linear regression model adjusting for sex, BMI, age at diagnosis, and diabetes duration, daily insulin dose was lower in those with C-peptide ≥200 pmol/L than in those with C-peptide <5 pmol/L (ratio of geometric means 0.91, $P = 2 \times 10^{-17}$) as reported in Table 2.

Stratification of the cohort by duration (Table 3) showed that this effect was restricted to those with duration of diabetes of <10 years. In a mixture of fractional powers model, the form of the relationship to log C-peptide was concave downward with slope steepest at higher levels of C-peptide (Fig. 1). The size of this effect was modest: predicted daily insulin dose was only ~10% lower at a C-peptide level of 200 pmol/L than in those with C-peptide at or below the detection limit.

C-Peptide Association With HbA_{1c}

For both baseline HbA_{1c} and time-updated HbA_{1c} as a dependent variable in a linear mixed model, C-peptide levels >200 pmol/L were significantly associated with lower HbA_{1c} adjusted for sex, BMI, age at diagnosis, and diabetes duration (Table 2). In the subset of individuals with nonmissing insulin dose at baseline, further adjustment for insulin dose changed the coefficient for C-peptide >200 pmol/L only slightly (−4.3 without adjustment, −3.6 mmol/mol with adjustment). Stratifying by duration (Table 3) showed that this effect was strongest in those with duration <10 years. In a mixture of fractional powers model to explore the form of the relationship of HbA_{1c} during follow-up to log C-peptide, the curve was concave downward with no evidence of a threshold effect, but the relationship to levels in the range 3–200 pmol/L was weak: the predicted HbA_{1c} at 200 pmol/L was only 1 mmol/mol lower than the predicted value at the detection limit (Fig. 1).

In a model with C-peptide levels as the outcome variable, adjusted for sex, age at diagnosis, duration, and BMI, HbA_{1c} levels above the baseline category of <48 mmol/L were associated with lower C-peptide (Table 4).

C-Peptide Association With Hypoglycemic Episodes

Among the participants, 27% reported at least one hypoglycemic episode serious enough to have required help during the year before baseline, and 5% had at least one hospital admission for hypoglycemia during follow-up (Table 1). In comparison with those whose C-peptide was <5 pmol/L, C-peptide levels of 30–200 pmol/L were associated with lower rates of self-reported unawareness of hypoglycemia: in a regression model adjusted for age at onset and duration, the odds ratio was 0.67 ($P = 10^{-4}$). In regression models, covariates associated with increased risk of self-reported serious hypoglycemic episodes were later age at diagnosis, longer duration, lower HbA_{1c}, and lower BMI. Adjusting for these covariates, the

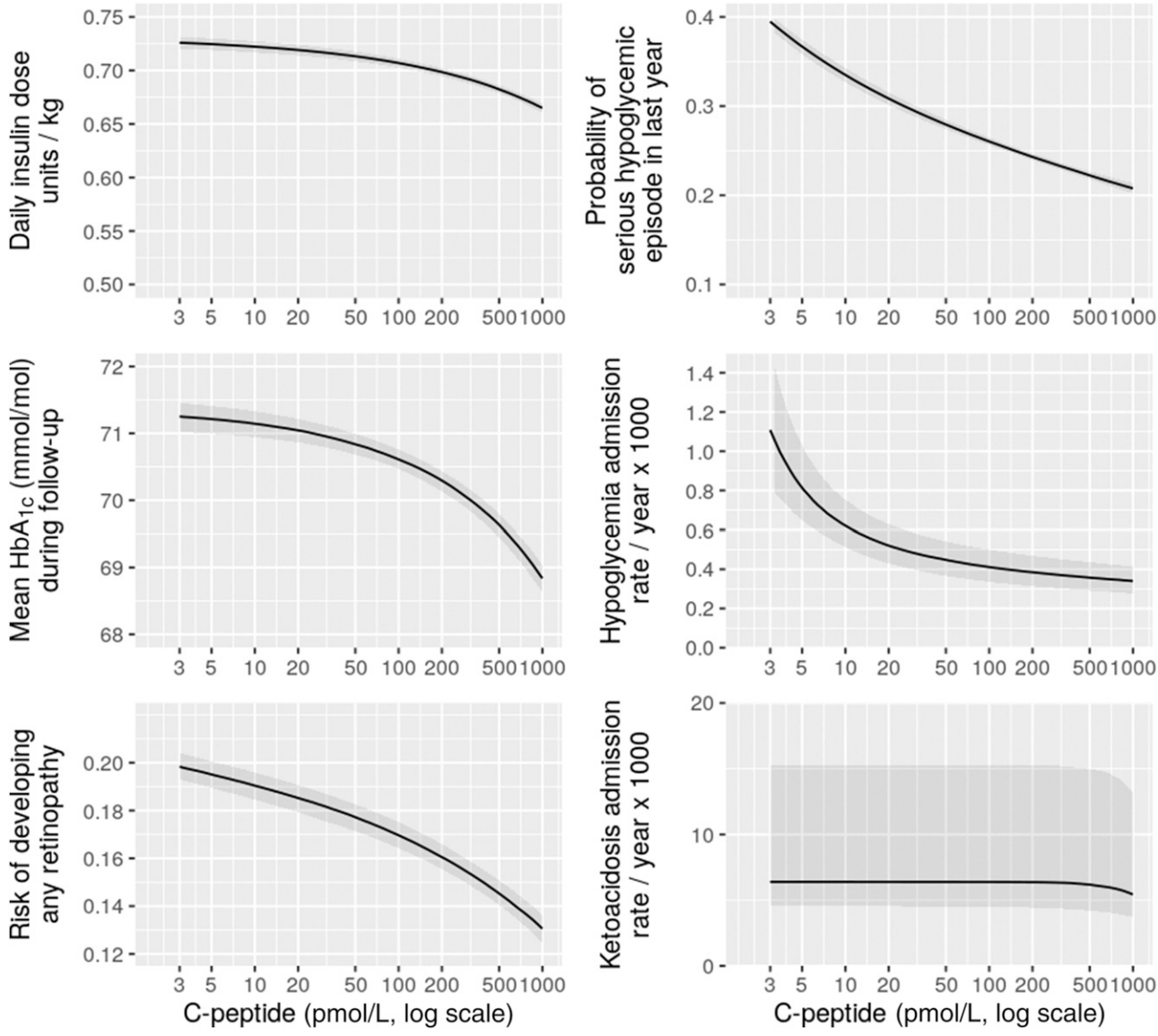

Figure 1—Posterior predicted medians and interquartile range (as shaded ribbon) based on fractional powers mixture models for effect of C-peptide level at baseline. *Left column*: Daily insulin dose, HbA_{1c} during follow-up, and development of any retinopathy during follow-up. *Right column*: Self-reported serious hypoglycemic episode in the last year, admissions for hypoglycemia, and admissions for diabetic ketoacidosis.

odds ratios of a serious hypoglycemic episode in the last year and the hazard ratios for prospective admissions were progressively lower across each category of C-peptide compared with undetectable C-peptide (Table 2).

To exclude the possibility that measurement of unstimulated C-peptide might have misclassified those with high residual C-peptide secretory capacity as having low levels, we compared the associations of C-peptide level with at least one serious hypoglycemic episode in the last year before and after restricting to those with glucose levels of at least 8 mmol/L in the same sample as that used for C-peptide measurement. With C-peptide <5 pmol/L as the baseline category, the log odds ratios associated with levels 5 to <30 pmol/L, 30 to <200 pmol/L, and ≥200 pmol/L were −0.31, −0.58, and −0.75 in the full cohort and −0.39, −0.52, and −0.63 in those with glucose levels of at least 8 mmol/L.

Table 3 shows that the strength of associations of serious hypoglycemia in the last year or during follow-up with C-peptide level were as strong or stronger in those with diabetes duration of 10–19 years as in those with duration of <10 years). In those in whom the daily dose was available, adjustment in the model for daily dose at baseline did not change the size of the effect of C-peptide level >5 mmol/L on the odds of at least one admission for hypoglycemia during follow-up: the unadjusted odds ratio was 0.52, and the adjusted odds ratio was 0.52. In a mixture of fractional powers model, the form of the relationship of hypoglycemia admission rates during follow-up to C-peptide levels at baseline was convex downward, with the steepest reduction in risk of hypoglycemia occurring between 5 and 20 pmol/L (Fig. 1).

C-Peptide Association With Diabetic Ketoacidosis

In regression models adjusting for covariates, the rate of ketoacidosis admissions and deaths was lower in those with C-peptide ≥200 pmol/L than in those with levels <5 pmol/L but not in those

Table 4—Linear regression of $\log_{10}$ C-peptide at baseline on HbA_{1c} at baseline

	Slope (95% CI)	*P* value
Sex	−0.062 (−0.106, −0.017)	0.007
Age at diagnosis	0.013 (0.011, 0.015)	9×10^{-45}
Duration (years)	−0.030 (−0.032, −0.028)	1×10^{-202}
BMI (kg/m^2)	0.008 (0.003, 0.013)	9×10^{-4}
HbA_{1c} 54–58 mmol/mol	−0.204 (−0.301, −0.108)	3×10^{-5}
HbA_{1c} 59–75 mmol/mol	−0.354 (−0.429, −0.279)	2×10^{-20}
HbA_{1c} ≥76 mmol/mol	−0.366 (−0.443, −0.290)	1×10^{-20}

with levels between 5 and 200 pmol/L (Table 2). In a mixture of fractional powers model, the form of the relationship was consistent with a threshold effect. The relation of ketoacidosis risk to C-peptide level was nearly flat in those with C-peptide <200 pmol/L but declined steeply with C-peptide level above this threshold (Fig. 1). In this model, the posterior predictive distribution of admission rates for ketoacidosis is right skewed, so that the mean is above the interquartile range, but the form of the relationship with C-peptide level is similar for the mean and the 25th and 75th percentiles.

Relationship of C-Peptide to Retinopathy and Nephropathy

In regression models adjusted for covariates, rates of prevalent and incident retinopathy were lower in those with C-peptide ≥30 pmol/L than in those with levels <5 pmol/L (Table 5). In a mixture of fractional powers model, the form of the relationship of incident retinopathy to C-peptide levels at baseline was approximately linear, with no evidence of a threshold effect (Fig. 1).

The unadjusted baseline prevalence of eGFR <60 mL/min/1.73 m^2 (CKD stage 3) was slightly lower with higher C-peptide category (Table 1), but the strong relationships of eGFR to other covariates including age and sex means that adjusting for these in regression models is important. Prevalence of CKD stage 3 at baseline was positively associated with C-peptide level >200 pmol/L, but incident CKD stage 3 was not related to C-peptide level in a model that included eGFR at baseline as a covariate (Table 5).

CONCLUSIONS

We have shown that even low levels of residual C-peptide secretion are associated with lower risk of serious hypoglycemic episodes, after adjusting for covariates including duration of diabetes. In those with C-peptide between 30 and 200 pmol/L, the frequency of at least one hypoglycemic episode in the previous year that was serious enough to require help is approximately halved from a risk of 32% in those with C-peptide <5 pmol/L. The size of this effect is enough to be clinically significant and does not differ much between those with relatively short duration and those with longer duration. The inverse relationship of serious hypoglycemia with C-peptide secretion appears to be continuous down to near the lower limit of detection of the assay. The association was not explained by effects of residual C-peptide secretion on insulin dose or HbA_{1c} level. Incident diabetic retinopathy also showed a strong inverse relationship with C-peptide down to the lower limit of detection. In contrast, associations with baseline insulin dose and with HbA_{1c}, although consistent with linear effects, were detectable only at C-peptide levels of at least 200 pmol/L. The interpretation of the association between HbA_{1c} and C-peptide is complicated by the effects of improved glycemic control on residual β-cell capacity, demonstrated in the Diabetes Control and Complications Trial (DCCT) (11). An inverse association between HbA_{1c} and C-peptide levels could thus be attributable to a causal relationship in either direction or both directions. Effects on incident ketoacidosis were nonlinear and detectable only in those with C-peptide ≥200 pmol/L.

Strengths of our study include the large sample size covering a wide range of duration and age at onset of type 1 diabetes, prospective recording of outcomes, and the use of a highly sensitive assay. As we previously reported (1) (Table 1) in this cohort, 46 of 660 people with duration <5 years had undetectable C-peptide, and 1,003 of 3,762 people with duration >15 years had detectable C-peptide. Thus, we are not reliant on extrapolation to infer relationships that include these regions of the distribution.

To model the relationship of counts of events such as hypoglycemia admissions, we have used novel statistical methods to take account of the overdispersed distribution of counts that is generated by individual variation in susceptibility. To investigate the form of the relationship of each outcome to C-peptide levels, we have used methods that average over an ensemble of possible models rather than forcing an arbitrary choice of a single model. Limitations of our study include the reliance on a single untimed blood sample for measurement of C-peptide rather than a profile of C-peptide levels during a mixed-meal tolerance test. However, conducting stimulated C-peptide studies at sufficient scale to have power to detect effects on complications is expensive; studies that have used this approach are much smaller than ours. Furthermore, nonfasting untimed serum C-peptide levels in people with type 1 diabetes have been shown to be highly correlated with C-peptide levels after a mixed meal (12). If use of serum rather than plasma with an inhibitory of glycolysis had led to underestimation of glucose levels, this would only strengthen the argument that glucose levels in most individuals were high enough to evoke whatever residual capacity for insulin secretion was present. Misclassification of people with high stimulated C-peptide levels as having low levels would affect all outcomes equally. Our results, however, show that it is only for hypoglycemia and retinopathy that protective effects at low levels of C-peptide are detected: for insulin dose, HbA_{1c}, and ketoacidosis, effects are detectable only at levels >200 pmol/L.

These results help to resolve some of the uncertainties and inconsistencies in earlier studies of this topic. In the DCCT, initial analyses focused on predefined

Table 5—Logistic regression models of prevalent and incident CKD stage 3 (eGFR <60 mL/min/1.73 m^2) and prevalent and incident retinopathy on baseline covariates

	CKD stage 3 at baseline		CKD stage 3 during follow-up		Retinopathy at baseline		Retinopathy during follow-up	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Sex (female)	1.60 (1.26, 2.02)	9×10^{-5}	1.19 (0.96, 1.49)	0.1	0.81 (0.72, 0.92)	0.001	0.73 (0.61, 0.89)	0.001
Age at onset (years)	1.07 (1.06, 1.08)	6×10^{-40}	1.03 (1.02, 1.04)	1×10^{-7}	1.00 (1.00, 1.01)	0.07	0.99 (0.98, 0.99)	4×10^{-4}
Duration (years)	1.12 (1.10, 1.13)	2×10^{-82}	1.05 (1.03, 1.06)	2×10^{-14}	1.08 (1.08, 1.09)	8×10^{-148}	1.01 (1.01, 1.02)	0.002
HbA_{1c} (mmol/mol)	1.02 (1.01, 1.03)	3×10^{-6}	1.03 (1.02, 1.04)	4×10^{-15}	1.02 (1.02, 1.03)	3×10^{-27}	1.03 (1.03, 1.04)	2×10^{-33}
eGFR (mL/min/1.73 m^2)	—	—	0.91 (0.91, 0.92)	1×10^{-86}	—	—	—	—
C-peptide 5 to <30 pmol/L	1.01 (0.69, 1.47)	1	1.00 (0.71, 1.42)	1	0.83 (0.69, 1.01)	0.06	0.91 (0.69, 1.21)	0.5
C-peptide 30 to <200 pmol/L	1.19 (0.80, 1.77)	0.4	0.94 (0.65, 1.35)	0.7	0.69 (0.56, 0.85)	4×10^{-4}	0.66 (0.50, 0.89)	0.005
C-peptide ≥200 pmol/L	1.95 (1.25, 3.05)	0.003	1.19 (0.78, 1.82)	0.4	0.66 (0.51, 0.86)	0.002	0.51 (0.35, 0.74)	3×10^{-4}

OR, odds ratio.

categories of C-peptide (13). In a later study of 412 individuals in the intensive treatment group, where C-peptide was modeled as a continuous variable down to the limit of detection of the assay used (30 pmol/L) (14), an inverse relationship with serious hypoglycemia was detectable only above levels of ~130 pmol/L. Our results showing a continuous inverse association of serious hypoglycemia with C-peptide down to a limit of detection of 3 pmol/L differ markedly from the findings of the DCCT and a follow-up study of 98 individuals in which no association between self-reported severe hypoglycemia and C-peptide at baseline was found (15). Our results, however, are based on a representative population sample much larger than either of these two studies and are consistent with a recent small (n = 157) cross-sectional study using the same assay that we have used, in which self-reported hypoglycemic episodes were one-third lower in participants with postprandial C-peptide levels >20 pmol/L than in those with levels below this cutoff (3).

The strong association between residual β-cell function and hypoglycemia that we observed is consistent with a study of a case series of 12 recipients of islet cell transplants after an average follow-up time of 18 months, in which the rate of severe hypoglycemic events was reduced 12-fold even though the reductions in insulin dose and HbA_{1c} levels were only modest (16). In another case series at 3 years posttransplant with preservation of only low levels of C-peptide secretion, there remained a profound reduction in hypoglycemic events (17). This is consistent with the results of the Inducing Remission in New-Onset T1D with Alefacept (T1DAL) Trial, where alefacept therapy preserved C-peptide and where a strong continuous relationship between hypoglycemia and C-peptide levels was observed with no threshold regardless of whether such levels were achieved by the treatment or not (18).

Our results for HbA_{1c} and insulin dose are more consistent with the DCCT findings, in which inverse relationships with log C-peptide were detectable above levels of 80 pmol/L (14). Our finding that the associations of HbA_{1c} and insulin dose with C-peptide levels are stronger early in the course of diabetes rather than later is consistent with earlier studies. In 407 TrialNet participants, HbA_{1c} and insulin dose were strongly inversely associated with C-peptide up to 4 years from diagnosis (19). In the DCCT, effects on dose and HbA_{1c} were only apparent >80 pmol/L. Oram et al. (20) did not find any relationship of insulin dose or HbA_{1c} with C-peptide in 925 people with type 1 diabetes duration of at least 5 years.

In contrast to hypoglycemia, we found no appreciable protective effect of residual C-peptide against diabetic ketoacidosis unless C-peptide levels were at least 200 pmol/L. Previous studies reported significant associations with diabetic ketoacidosis but did not explore the level at which such effects became detectable (21,22).

We found a strong relationship of retinopathy with C-peptide, consistent with follow-up of the intensive treatment arm of the DCCT (14). We did not detect any association of incident kidney disease with C-peptide levels. The interpretation of any association between diabetic kidney disease and C-peptide levels is complicated by reverse causation. Because approximately half of C-peptide produced is removed by the kidneys (23), blood levels of C-peptide can be elevated in people with type 1 diabetes who have kidney disease (24). It is possible that this could mask a protective effect, leading to the nearly flat relationship of prevalent stage 3 CKD and the apparently nonmonotonic relationship of microalbuminuria to C-peptide levels in these analyses.

It is interesting to speculate why effects of residual C-peptide secretion on hypoglycemia and retinopathy are so much more easily detectable than effects on HbA_{1c}, insulin dose, and ketoacidosis. In healthy humans a decrease in intraislet insulin is a signal for the glucagon response to hypoglycemia (25). It is possible that a minimal residual capacity for C-peptide secretion is enough to maintain this signaling, even though in

people with established type 1 diabetes there is no relationship between stimulated C-peptide and glucagon levels during a mixed-meal test (26). It is well established that acute reductions in blood glucose impair vision and worsen retinopathy, although this is usually considered transient. This raises the possibility that the relationship of incident retinopathy to residual C-peptide secretion might be mediated through reduction in the frequency and severity of hypoglycemia.

Conclusion

These results support the contention that assessment of the clinical benefit of an intervention that preserves or restores C-peptide secretion should be based not on its effect on insulin dose requirements or glycemic control but on its effect on the rate of serious hypoglycemic events and the risk of retinopathy. Even if such interventions do not appreciably reduce insulin requirement, they may still profoundly improve quality of life for people with type 1 diabetes.

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