

# Fasting Plasma C-Peptide and Micro- and Macrovascular Complications in a Large Clinic-Based Cohort of Type 1 Diabetic Patients

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**OBJECTIVE** — A protective effect of residual  $\beta$ -cell function on microvascular complications of type 1 diabetes has been suggested. Our aim was to retrospectively evaluate the association of fasting plasma C-peptide values with micro- and macrovascular complications.

**RESEARCH DESIGN AND METHODS** — We recruited a clinic-based cohort of 471 type 1 diabetic patients born after 1945 and cared for in the period 1994–2004. Centralized measurements and standardized procedures of ascertainment of micro- and macrovascular complications were employed. Individual cumulative averages of A1C up to 2007 were calculated.

**RESULTS** — Residual  $\beta$ -cell secretion was detected even many years after diabetes diagnosis. In multivariate linear regression analysis, fasting plasma C-peptide values were positively associated with age at diagnosis ( $\beta = 0.02$ ;  $P < 0.0001$ ) and triglycerides ( $\beta = 0.20$ ;  $P = 0.05$ ) and inversely associated with diabetes duration ( $\beta = -0.03$ ;  $P < 0.0001$ ) and HDL cholesterol ( $\beta = -0.006$ ;  $P = 0.03$ ). The final model explained 21% of fasting C-peptide variability. With respect to fasting C-peptide values in the lowest tertile ( $<0.06$  nmol/l), higher values were associated with lower prevalence of microvascular complications (odds ratio [OR] 0.59 [95% CI 0.37–0.94]) independently of age, sex, diabetes duration, individual cumulative A1C average during the study period, hypertension, and cardiovascular diseases. No association was evident with macrovascular complications (0.77 [0.38–1.58]).

**CONCLUSIONS** — Our study shows an independent protective effect of residual  $\beta$ -cell function on the development of microvascular complications in type 1 diabetes, suggesting the potential beneficial effect of treatment that allows the preservation of even modest  $\beta$ -cell function over time.

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Type 1 diabetes is due to chronic autoimmune destruction of insulin-producing  $\beta$ -cells. Few studies, however, have assessed changes in insulin secretion over time in affected people and its relationship with diabetes complications (1–4). C-peptide has been widely accepted as the most appropriate measure of residual  $\beta$ -cell function because it is secreted on a basis equimolar to insulin

and, unlike the latter, is not removed in the first pass through the liver (5).

Studies have pointed out relationships of residual  $\beta$ -cell function with age at diagnosis (6,7), markers of  $\beta$ -cell autoimmunity (8,9), and glycemic control (10). The Diabetes Control and Complications Trial (4) has pointed out the benefits of higher and sustained levels of C-peptide secretion; indeed, in the inten-

sively treated group, even modest residual  $\beta$ -cell secretion was associated with decreased incidence of microvascular complications and hypoglycemia (4). The next frontier for treating diabetes is now considered to be the preservation of  $\beta$ -cell function by reducing the autoimmune stimulus directed at pancreatic islets (11–14). However, at present, it has been suggested that a potential beneficial effect on micro- and macrovascular complications might be achieved by administering C-peptide in type 1 diabetic patients, although clinical evidence is still quite limited (15–17). Experimental studies are consistent regarding the biological effect of C-peptide on  $\text{Na}^+/\text{K}^+$ -ATPase, endothelial nitric oxide (NO) synthase activities, and nuclear factor- $\kappa\text{B}$  activation of endothelial cells that under high-glucose conditions are subject to progressive dysfunction (15,18–19). The aims of this study were to describe residual  $\beta$ -cell secretion of C-peptide and its effect on micro- and macrovascular complications, in particular, independent of glycemic control and other risk factors.

## RESEARCH DESIGN AND METHODS

Out of a large clinic-based cohort of 1,024 type 1 diabetic patients cared for at the academic diabetes clinic at S. Giovanni Battista Hospital in Turin, Italy, we recruited all born in or after 1945 who had been examined at least once during the period 1994–2004 ( $n = 573$ ). A diagnosis of type 1 diabetes was based on permanent insulin treatment within 6 months of the initial diagnosis.

The patients at S. Giovanni Battista Hospital are generally cared for three or four times per year by diabetologists, with centralized measurements, updated clinical information, and annual assessments of complications according to standardized procedures. For all patients, data regarding the presence of micro- and macrovascular complications at the last clinical assessment were retrieved from clinical records up to 2007, as well as data concerning BMI, blood pressure, and plasma values of fasting glucose, lipids,

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and creatinine. Hypertension was defined as either blood pressure  $\geq 140/90$  mmHg or current antihypertensive treatment. Cardiovascular disease was defined as physician-diagnosed myocardial infarction, angina, coronary artery bypass graft, stroke, or arterial disease of lower limbs or epiaortic arterial trunks. Screening for diabetic nephropathy was performed measuring the albumin-to-creatinine ratio from the first morning urine collection and confirming abnormal results with albumin excretion rate from overnight urine collection. Screening for diabetic retinopathy was performed locally by trained diabetologists and ophthalmologists, who also performed laser therapy when indicated. Distal symmetrical polyneuropathy was diagnosed on the basis of presence of one or more neuropathic symptoms, absence of two or more ankle or knee reflexes, and abnormal vibration perception threshold, measured by biothesiometers on the right big toe and on the right medial malleolus. Autonomic neuropathy was defined as a loss of heart rate variability with an R-R ratio  $< 1.04$  or postural hypotension with a fall in systolic blood pressure  $\geq 20$  mmHg. Residual  $\beta$ -cell function was assessed at the initial visit at the clinics in prevalent cases or at disease stabilization in incident cases, by measuring fasting plasma C-peptide (normal values 0.36–1.17 nmol/l; Diagnostics Product Corporation, Los Angeles, CA). Of the 573 individuals of the study base, plasma C-peptide values were available in 471 (82.2%). The median lag time between C-peptide measurement and last clinical assessment of complications was 4.5 years (interquartile range 2.1–6.8).

Cumulative individual averages of A1C over the observational study period (1994–2007) were calculated. Markers of  $\beta$ -cell autoimmunity (GAD antibodies [GADAs] and islet cell antibodies [ICAs]) were assessed at the same time as C-peptide plasma levels. GADAs were measured by a radioligand assay using human recombinant GAD65 as the antigen (Medipan Diagnostica, Selchow, Germany); immunocomplexes were precipitated with protein A, according to the method of Schmidli. GADA values  $> 0.9$  units/ml were considered positive. Anti-ICAs were assayed by indirect immunofluorescence on frozen sections of pancreata of human blood group O with fluorescein isothiocyanate-conjugated rabbit antibodies. ICA positivity was expressed in Juvenile Diabetes Foundation units (JDF-U) by a standard curve based on the international

**Table 1—Characteristics of the clinic-based cohort of 471 individuals with type 1 diabetes by C-peptide tertile**

	C-peptide tertile (nmol/l)			P
	<0.06	0.06–0.10	0.11–2.76	
n	201	148	122	—
Male	111 (55.2)	79 (53.4)	75 (61.5)	0.38
Age (years)	38.2 $\pm$ 10.5	38.6 $\pm$ 9.4	39.1 $\pm$ 9.7	0.79
Diabetes duration (years)	13.9 (7.5–19.8)	11.1 (4.9–19.2)	4.0 (0.8–8.5)	0.001
<10	38 (23.0)	43 (26.1)	84 (50.9)	<0.0001
10–19	79 (48.8)	58 (35.8)	25 (15.4)	
20–29	56 (55.4)	34 (33.7)	11 (10.9)	
$\geq 30$	28 (65.1)	13 (30.2)	2 (4.7)	
BMI (kg/m <sup>2</sup> )	24.1 $\pm$ 3.4	24.5 $\pm$ 3.6	24.5 $\pm$ 4.1	0.50
Systolic blood pressure (mmHg)	123.1 $\pm$ 15.8	121.9 $\pm$ 15.2	120.3 $\pm$ 14.9	0.29
Diastolic blood pressure (mmHg)	74.4 $\pm$ 9.9	74.4 $\pm$ 9.1	75.1 $\pm$ 8.9	0.74
Glucose (mmol/l)	12.4 $\pm$ 5.8	12.9 $\pm$ 5.2	12.1 $\pm$ 5.3	0.51
A1C (%)	8.2 $\pm$ 1.3	8.2 $\pm$ 1.3	8.0 $\pm$ 1.4	0.44
Positivity for autoimmunity	119 (65.4)	98 (72.1)	102 (85.7)	0.001
Total cholesterol (mmol/l)	5.20 $\pm$ 1.00	5.28 $\pm$ 1.05	5.00 $\pm$ 1.18	0.10
HDL cholesterol (mmol/l)	1.56 $\pm$ 0.47	1.57 $\pm$ 0.47	1.41 $\pm$ 0.45	0.005
LDL cholesterol (mmol/l)	3.14 $\pm$ 0.83	3.18 $\pm$ 0.85	2.96 $\pm$ 0.96	0.12
Triglycerides (mmol/l)	0.96	1.01	1.11	0.05

Data are means  $\pm$  SD, n (%), or geometric mean (25th–75th percentile) unless otherwise indicated.

JDF-U reference sera sample. An ICA  $\geq 5$  JDF-U was considered positive. Both sensitivity and specificity were 100% at the 4th GADA and 13th ICA proficiency program of the Research Institute for Children, New Orleans, Louisiana (Laboratory no. 13).

### Statistical analysis

Data are shown as means  $\pm$  SD, except for nonnormally distributed variables (triglycerides and C-peptide), which were analyzed after a logarithmic transformation and are referred to in tables as geometric mean (interquartile range). Pearson correlations were performed. Multiple linear regression analysis was performed to assess variables independently associated with fasting plasma C-peptide. We then performed logistic regression analysis in order to study variables independently associated with microvascular (retinopathy, micro- and macroalbuminuria, and diabetic neuropathy) and macrovascular complications (myocardial infarction, angina, coronary artery bypass graft, stroke, and peripheral arteriopathy). The independent role of C-peptide was examined using tertiles of its distribution (<0.06, 0.06–0.10, and 0.11–2.76 nmol/l). Odds ratios (ORs) in the second and third tertile were similar and were aggregated as the reference category in the final analysis and compared with the lowest tertile. The variables assessed in the models were age, mean cumulative A1C, gap between mea-

surement of C-peptide and last clinical assessment, positivity of markers of  $\beta$ -cell autoimmunity (ICA and GADA), BMI, micro- and macrovascular complications, lipids, blood pressure, smoking, and treatment with ACE inhibitors or sartans. We compared nested models using both the backward and forward strategy (20); two models are nested if both contain the same predictors and one has at least one additional predictor. In the final analysis, we included variables that were significantly associated with the independent variable on a likelihood ratio test basis or that modified ORs for other variables included (20). All analyses were performed using Stata 10 (StataCorp, College Station, TX).

**RESULTS**— Mean diabetes duration in the 471 individuals recruited for the present analyses was  $15.8 \pm 10.2$  years (range 5.2–39). As shown in Table 1, characteristics of individuals by C-peptide tertiles were similar apart from diabetes duration and plasma triglycerides, which were significantly higher in those in the lowest tertile, and HDL cholesterol values, which were significantly lower in those in the lowest tertile. Residual  $\beta$ -cell secretion was detected even many years after diabetes diagnosis. Indeed, almost 50% of people with C-peptide values in the upper tertile had

Table 2—Characteristics of the clinic-based cohort of 471 individuals with type 1 diabetes by diabetes duration

	Duration of diabetes (years)				P
	<10	10–19	20–29	≥30	
n	165	162	101	43	—
Male	104 (63.0)	91 (56.2)	52 (51.5)	18 (41.9)	0.05
Age (years)	34.2 ± 9.7	37.3 ± 9.0	41.6 ± 8.4	47.5 ± 6.1	<0.001
C-peptide (nmol/l)	0.12 (0.08–0.27)	0.05 (0.02–0.10)	0.04 (0.02–0.10)	0.03 (0.01–0.10)	<0.0001
Retinopathy					
Background	17 (10.3)	48 (29.6)	45 (44.6)	23 (55.5)	<0.0001
Preproliferating	0 (0)	5 (3.1)	5 (5.0)	0 (0)	
Proliferating	0 (0)	12 (7.4)	15 (14.9)	7 (16.3)	
Diabetic nephropathy	2 (1.2)	12 (7.4)	15 (14.9)	7 (16.3)	<0.0001
Autonomic or peripheral neuropathy	25 (15.1)	55 (33.0)	53 (52.5)	32 (74.4)	<0.0001
All microvascular complications	37 (22.4)	90 (55.6)	76 (75.3)	39 (90.7)	<0.0001
Cardiovascular disease	2 (1.2)	5 (3.1)	16 (15.8)	8 (18.6)	<0.0001

Data are means ± SD, n (%), or geometric mean (25th–75th percentile) unless otherwise indicated.

≥10 years of diabetes duration. In contrast, no differences between tertiles were found in BMI, blood pressure, glycemic control, or LDL cholesterol. In the whole cohort, frequency of positivity of markers of  $\beta$ -cells autoimmunity (ICA and GADA) was high (73.0%); frequency, however, was higher in individuals with C-peptide values in the upper tertile than in those with values in the lower tertile (85.7 vs. 65.4%).

In univariate analysis, fasting C-peptide values were positively correlated with age at diagnosis ( $r = 0.32$ ;  $P < 0.0001$ ), triglycerides ( $r = 0.14$ ;  $P = 0.0002$ ), and BMI ( $r = 0.11$ ;  $P = 0.006$ ) and negatively with diabetes duration ( $r = -0.34$ ;  $P < 0.0001$ ) and HDL cholesterol ( $r = -0.16$ ;  $P < 0.0001$ ), whereas no significant correlations were found with age, A1C, blood pressure, or total or LDL cholesterol. In multivariate linear regression analysis, fasting plasma C-peptide values were positively associated with age at diagnosis ( $\beta = 0.02$ ;  $P < 0.0001$ ) and BMI ( $\beta = 0.03$ ;  $P = 0.02$ ) and inversely associated with duration of disease ( $\beta = -0.03$ ;  $P < 0.0001$ ) independently of positivity for markers of  $\beta$ -cell autoimmunity (ICA and GADA) ( $\beta = 0.12$ ;  $P = 0.27$ ). After inclusion of HDL cholesterol ( $\beta = -0.006$ ;  $P = 0.03$ ) and triglycerides ( $\beta = 0.20$ ;  $P = 0.05$ ), however, the association with BMI was no longer significant; indeed, BMI was significantly correlated with both HDL cholesterol ( $r = -0.15$ ;  $P < 0.0001$ ) and triglycerides ( $r = 0.28$ ;  $P < 0.0001$ ). The final model explained 21% of fasting C-peptide variability.

Table 2 shows the characteristics of the cohort by diabetes duration. Age was

higher in individuals with longer diabetes duration, whereas fasting C-peptide levels were significantly lower. Prevalence of both micro- and macrovascular complications was high, particularly in individuals with longer diabetes duration: at the final clinical assessment, 90% of the subgroup with duration ≥30 years had at least one microvascular complication and 21.7% had one macrovascular complication.

We then performed logistic regression analyses of variables associated with micro- or macrovascular complications at the last clinical assessment (Table 3). Regarding microvascular complications, we showed that with respect to C-peptide values in the lowest tertile (<0.06 nmol/l) higher values conferred a protective effect (0.59 [0.37–0.94]). This finding was independent of age, sex, diabetes duration, individual cumulative A1C average during the study period, hypertension, and cardiovascular diseases; all of these variables correlated with significantly increased likelihood of microvascular complications, as shown by point estimates of ORs and lower limits of CIs, both exceeding 1. Further adjustment for either lag time between diabetes C-peptide evaluation and final clinical assessment or treatment with ACE-inhibitors/sartans did not modify observed associations.

With regard to macrovascular complications, no independent associations with either fasting plasma C-peptide values (OR 0.77 [CI 95% 0.38–1.58]) or mean cumulative A1C (0.93 [0.57–1.50]) were found, whereas other well-known risk factors (hypertension, LDL cholesterol, and microvascular complications) provided significant OR values.

**CONCLUSIONS**— Results of our clinic-based study pointed out that 1) people with type 1 diabetes and higher fasting C-peptide values had lower prevalence of microvascular complications independently of duration of diabetes, individual cumulative average of A1C, and other risk factors, whereas no similar association was found with macrovascular complications; 2) residual  $\beta$ -cell secretion was detected even many years after diabetes diagnosis; and 3) 90% of individuals with diabetes duration ≥30 years had at least one microvascular complication.

Our study extended previous knowledge (1–3), based on a large cohort of patients cared for in a single health care structure, with centralized measurements of all examined variables and standardized procedures of complications assessment. A study conducted on 97 type 1 diabetic individuals showed that measurable C-peptide levels in urine were significantly associated with lower A1C and lower prevalence of retinopathy and microalbuminuria (1). In another study recruiting 160 diabetic individuals, higher levels of urinary C-peptide excretion were associated with lower prevalence of diabetic retinopathy (2), whereas contrary findings were reported in a study including 62 subjects only (3). Our results, although based on an observational study design, are consistent with results obtained from the Diabetes Control and Complications Trial, showing that preservation of even small residual pancreatic secretion confers a lower risk for microvascular complications independently of glycemic control (4). Indeed, among intensively treated patients, the risk of de-

Table 3—Logistic regression of variables associated with either microvascular or macrovascular complications in a cohort of 471 people with type 1 diabetes

	OR (95% CI)*
Microvascular complications	
Duration of diabetes (years)	1.12 (1.08–1.15)
Cumulative average of A1C (%)	1.20 (1.01–1.41)
Cardiovascular disease	
No	1.00
Yes	4.69 (1.01–21.84)
Hypertension (mmHg)	
No	1.00
Yes	1.51 (0.95–2.40)
Fasting C-peptide (nmol/l)	
<0.06	1.00
≥0.06	0.59 (0.37–0.94)
Macrovascular complications	
Duration of diabetes (years)	1.05 (0.99–1.11)
Hypertension (mmHg)	
No	1.00
Yes	3.90 (1.40–10.89)
Microvascular complications	
No	1.00
Yes	9.66 (1.18–78.86)
LDL cholesterol (mmol/l)	1.90 (1.12–3.22)
Mean A1C (%)	0.93 (0.57–1.50)
C-peptide (nmol/l)	
<0.06	1.00
≥0.06	0.77 (0.38–1.58)

\*Adjusted for age, sex, and all other variables in the model.

veloping diabetic retinopathy was 3.2 times higher in those with immeasurable fasting plasma C-peptide than in those with higher residual pancreatic secretion, whereas data from conventionally treated patients did not reach statistical significance (4).

Studies conducted over the last 15–20 years have shown that strict glycaemic control after diabetes diagnosis can prevent both micro- and macrovascular complications, and the concept of metabolic memory has been emphasized (21). In our study, the protective effect of fasting C-peptide values was independent of cumulative individual averages of A1C during the study period, suggesting that the effect could be directly due to the biological properties of C-peptide. Indeed, recent trials have suggested that plasma C-peptide levels might have a beneficial effect on both diabetic nephropathy and neuropathy, but evidence is still quite limited (16–17). On a molecular level, C-peptide would interact with its own membrane receptor and activate two enzymes scarcely represented in type 1 diabetic people:  $\text{Na}^+/\text{K}^+$ -ATPase and endothelial NO synthase (15).

Limitations and strengths of our study should be taken into account. First, our results are based on a prevalence cohort, so results might have been affected by survival bias. However, the mortality rate in this relatively young cohort is quite low, thus ruling out a major effect of survival bias on our results. Second, the retrospective study design did not allow us to examine the role of both C-peptide and glycaemic control after diabetes onset; the centralized assessment of laboratory data, however, allowed us to include in the analyses both retrospective and prospective data over a long period of time. Third, C-peptide levels were obtained at variable times both from time of diagnosis and from the clinical outcome. Diabetes duration is associated with both increased prevalence of complications and lower fasting C-peptide levels. Our results were virtually unmodified in multivariate analyses after further adjustment for diabetes duration; however, residual confounding effects on our data cannot be ruled out. Strengths of the study are the large number of examined people, the centralized measurements and standardized procedures employed over time, and the high

level of C-peptide measurements available in the cohort.

In conclusion, our study, based on a large cohort of type 1 diabetic people, identifies the protective effect (–41%) of plasma C-peptide levels >0.06 nmol/l on microvascular complications, independent of diabetes duration, glycaemic control, and other confounders. Prospective studies are needed to confirm the hypothesis that early therapeutic interventions aimed at preserving even small residual  $\beta$ -cell secretion may modify the natural development of diabetes.

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