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Residual insulin production, glycaemic control and prevalence of microvascular lesions and polyneuropathy in long-term Type 1 (insulin-dependent) diabetes mellitus

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Summary. The aim of the present study was to evaluate the role of residual insulin production in long-term Type 1 (insulin-dependent) diabetes mellitus. Ninety-seven patients with a disease duration of 9-16 years and onset before the age of 30 years were studied. C-peptide excretion in 24-h urine samples was measured as an indicator of residual insulin production. Thirty-five patients (36%) excreted C-peptide ($\geq$ 0.2 nmol); as many as possible of them were carefully matched with a non-excretor patient with regard to age at onset of diabetes and disease duration. Twenty-nine pairs were obtained, and 22 of them agreed to participate in further investigations of glycaemic control and microangiopathic lesions. The patients who excreted C-peptide had significantly lower HbA_{1c} than the non-excretor group, $6.9 \pm 0.3\%$ vs $7.9 \pm 0.3\%$, ($p < 0.025$). Moderate-to-advanced background retinopathy was found in 2 patients in the excretor group and in 7 patients in the nonexcretor group. Microalbuminuria [ratio of albumin: creatinine (mg/l:mmol/l) ≥ 5] was found in 1 and in 5 patients, respectively, while proteinuria [ratio of protein: creatinine (mg/l:mmol/l $\times$ 10) ≥ 136] was found in 0 and in 4 patients, respectively. Microalbuminuria and/or proteinuria was found in 7 of the non-excretor group as compared to 1 in the excretor group ($p = 0.046$). When all the variables were taken into account, microalbuminuria and/or proteinuria and/or moderate-to-advanced background retinopathy was found in 3 of the excretor group compared to 11 of the non-excretor group ($p = 0.022$). Reduced sensory and motor nerve conduction velocities were common findings and occurred with the same frequency in the two groups. The data suggest that residual insulin production in long-term Type 1 diabetes is associated with a more satisfactory glycaemic control and a lower prevalence of early microangiopathic eye and kidney lesions.

Key words: C-peptide, insulin-dependent diabetes, albuminuria, proteinuria, haemoglobin A_{1c}.

Previous investigations suggest that there is an association between the incidence of retinopathy, nephropathy as well as neuropathy and the degree of glycaemic control in diabetes mellitus [1, 2]. Among the factors influencing glucose control, an important one seems to be the residual insulin production [3]. During the last decade several investigations have shown that many patients with Type 1 (insulin-dependent) diabetes mellitus have residual insulin production, which is detected by the analysis of C-peptide in plasma and/or in urine [3, 4]. Some investigators report that residual insulin production has been associated with a more satisfactory glucose control [3, 5], although others have failed to demonstrate such an association [6]. The importance of residual insulin production with regard to the prevention of microangiopathy has not been fully evaluated, but Eff et al. [7] found a lower prevalence of severe retinopathy in Type 1 patients with a low daily insulin requirement (≤ 0.50 IU/kg body weight) and detectable C-peptide levels in plasma after glucagon stimulation. However, there was no correlation between metabolic control and persistent insulin secretion. Furthermore, a recent study [8] has shown that patients with an unspecified type of diabetes and non-proliferative retinopathy had significantly higher C-peptide levels in plasma than those with proliferative or pre-proliferative diabetic retinopathy.

The present investigation was carried out to study whether residual insulin secretion in patients with long-standing Type 1 diabetes is associated with (1) a better glycaemic control and (2) a lower prevalence of polyneuropathy and microangiopathic eye and kidney lesions. Because of the marked increase in the incidence of microangiopathy at about 10-15 years after the diagnosis of diabetes [1], patients with this disease duration were studied.

Table 1. Characteristics of 97 Type 1 (insulin-dependent) diabetic patients with disease duration between 9-16 years analyzed for urinary C-peptide excretion. A value of ≥0.2 nmol/24 h was considered as significant C-peptide excretion

	C-peptide excretor group	C-peptide non-excretor group	*p* value
Sex (M/F)	19/16	23/39	NS
Duration of diabetes (years)	11.3±0.5	13.4±0.3	$p<0.001$
Age of onset (years)	20.0±1.0	16.0±0.9	$p<0.01$
Body mass index (kg/m^2)	22.0±0.3	22.5±0.3	NS
Insulin dose (U/kg)	0.64±0.04	0.75±0.02	$p<0.01$

Table 2. Characteristics of two patient groups with and without C-peptide excretion carefully matched for age at onset and diabetes duration

	C-peptide excretor group	C-peptide non-excretor group
Sex (M/F)	10/12	12/10
Duration of diabetes (years)	11.7±0.4	12.0±0.4
Age at onset (years)	20.8±1.0	20.6±1.4
Body mass index (kg/m^2)	21.6±0.3	22.1±0.5
Insulin dose, 5 years after diagnosis (U/kg)	0.59±0.07	0.79±0.06[a]
Insulin dose (U/kg)	0.67±0.04	0.72±0.03

[a] $p<0.05$

Subjects and methods

Subjects

All patients with Type 1 diabetes who regularly attended our outpatient department were invited by letter to participate in the study if they fulfilled the following criteria: (1) onset of diabetes before the age of 30 years; (2) duration of diabetes 9-16 years; (3) body mass index <25 kg/m^2.

Of 108 patients who fulfilled the criteria, 97 (90%) agreed to participate and supplied aliquots from a 24-h urine collection. Thirty-five (36%) of them had a significant C-peptide excretion of ≥ 0.2 nmol. A comparison between the groups with and without C-peptide excretion showed that the disease duration was significantly shorter ($p<0.001$), the age at debut was higher and the insulin dose/kg/d significantly lower ($p<0.01$) in the excretor group. The body mass index was about the same (Table 1).

Each of the patients who excreted C-peptide was carefully matched with a non-excretor subject. This matching procedure was performed by an independent physician. It was possible to find match pairs for 29 of the 35 excretor patients that differed ≤5 years on age at onset of disease and who did not differ more than 2 years in disease duration. Of the 29 pairs obtained, 22 (Table 2) consented to participate in the additional investigations. In 2 of the C-peptide excretor patients insulin was started one year after diagnosis; these patients were initially given glibenclamide and a special diet. In 1 of the patients in the non-excretor group treatment with insulin was started within 2 years after diagnosis. This patient also initially had been given glibenclamide and a special diet. In all other patients the insulin treatment was initiated within 6 months after diagnosis. The study was reviewed and approved by the hospital Ethics Committee.

Methods

Data from patient records. The insulin dose after a disease duration of 5 years was noted (U/kg). The number of cigarettes consumed per day and the corresponding quantity of tobacco were recorded.

Determination of C-peptide. The concentrations of C-peptide in plasma and urine were measured in duplicate samples by a radioimmunological technique [9] using antibody M1230. Synthetic human C-peptide was used as the standard and ^{125}I-tyrosylated C-peptide as the tracer. The detection limit in the assay is 0.05 nmol/l. The intra-assay variation was 6% and the inter-assay variation 20% at 0.06 nmol/l. C-peptide in the plasma was assayed after precipitation with polyethylene glycol (25%) to avoid falsely elevated values due to cross-reactions with proinsulin bound to insulin antibodies. The plasma samples were diluted 1:5 with an 0.04 mol/l phosphate buffer (pH 7.4) containing 6% (w/v) human albumin (Behring Institute, Behringwerke, Marburg, FRG). Urine was collected over a 24 h period for measurement of C-peptide. Urine specimens were analysed after dilution 1:5-1:100 with a 0.04 mol/l phosphate buffer (pH 7.4) containing 0.1% human albumin. All C-peptide samples were frozen and stored at −18 °C until analysis within 3 months. Nearly all samples were analysed in the same assay.

HbA_{1c}. HbA_{1c} was determined by specific ion exchange chromatography at 22 °C [10] using commercially available microcolumns (Bio Rad, Richmond, Calif, USA). The normal upper limit at our laboratory is 5.6%. The intra-assay and inter-assay variations are 3% and 4%, respectively.

Glucagon test. At 07.30 hours, after an 8-10 h fast and before the injection of insulin, a teflon catheter was placed in an antecubital vein. After a short period of rest, 1 mg glucagon (Novo, Bagsvaerd, Denmark) was injected intravenously [11]. Blood samples for determination of C-peptide were taken before and 6 min after the injection.

Determination of urinary protein excretion. Urine was collected between 07.30 a.m. and 14.00 p.m. during the visit to the hospital. The urine was immediately frozen and stored at −18 °C pending analysis.

Determination of the total protein concentration was made using the Biuret method. This method was found to detect protein concentrations ≥100 mg/l. A protein/creatinine index >136 (mg/l: mmol/l×10), using the method described by Shaw et al. [12], was considered to be pathological; it corresponded to an excretion of about 100 µg/min.

Determination of urinary albumin excretion. In the same urine samples albumin and IgG concentrations were measured by a modification of the method described by Blom and Hjörne [13]. Rabbit anti-human albumin and anti-human IgG were obtained from DAKO-Immunoglobulins, Copenhagen, Denmark, and stabilized albumin from Behring Institute, Behringwerke, Marburg, FRG. A standard human plasma was used as the standard for IgG. Urine samples were diluted in 0.05 molar sodium phosphate buffer with 0.1 molar NaCl. The antisera were diluted 1-40 in the same buffer with 20 g/l polyethylene glycol, allowed to stand at room temperature for at least 30 min and filtered (0.45 µm) immediately before use. The sample (5 µl) and antiserum (40 µl) were mixed in a centrifugal analyzer, and the increasing turbidity during the first 4 min after mixing was determined. The sensitivity of this method allows quantitative determinations of both albumin and IgG down to 1-2 mg/l. The refer-

Table 3. Glycaemic control and signs of incipient nephropathy and/or moderate-to-advanced retinopathy in the patient groups with and without C-peptide excretion

	C-peptide excretor group ($n=22$)	C-peptide non-excretor group ($n=22$)	p value
HbA_{1c} (%)	6.9±0.3	7.9±0.3	$p<0.025$
fB-glucose (mmol/l)	9.0±0.9	10.7±1.0	NS
Retinopathy (no. of patients)	2	7	NS
Protein/creatinine index >136 (no. of patients)	0	4	NS
Albumin/creatinine ratio ≥5 (no. of patients)	1	5	NS
Proteinuria and/or albuminuria (no. of patients)	1	7	$p=0.046$
Retinopathy and/or proteinuria and/or albuminuria (no. of patients)	3	11	$p=0.022$
Polyneuropathy (no. of patients)	15	12	NS
Electroneurography (ENeG) index	0.79±0.50	0.73±0.49	NS

ence values obtained from more than 400 healthy persons are less than 30 mg/l for albumin and less than 10 mg/l for IgG. An albumin/creatinine index ≥5 (mg/l:mmol/l) was considered to be significant microalbuminuria, and corresponds to an overnight albumin excretion rate of about 50 μg/min [14].

Determinations of beta$_2$-microglobulin, lysozyme (in serum and urine) and glomerular filtration rate. Determination of beta$_2$-microglobuline was performed using a radioimmunoassay (Beta$_2$-micro-RIA 100, Pharmacia, Uppsala, Sweden).

Measurements of lysozyme in urine and serum were made by Osserman's method [15]. The glomerular filtration rate was assessed by the clearance of ^{51}Cr-EDTA as described by Granérus and Aurell [16].

Ophthalmological examination. Each patient was examined with ophthalmoscopy, biomicroscopy, fundus photography and fluorescein angiography. The ophtalmologist responsible for this part of the study was not aware of whether the patients had residual insulin secretion or not, thus minimizing the methodological and statistical errors. The fundus area examined was 60° × 45°. None of the patients had proliferative retinopathy. The diagnosis of retinopathy was based on the presence of (1) microaneurysms, (2) intraretinal microvascular abnormalities (IRMA), (3) haemorrhages, (4) leakage spots, (5) and hard and/or soft exudates. The score for each item used was "0" = no signs of retinopathy, "1" = 1-3 signs of retinopathy, "2" = 4-10 signs of retinopathy, "3" >10 signs of retinopathy. The presence of microaneurysms, IRMAs and leakage spots was determined with fluorescein angiography. Haemorrhages and exudates were easier to evaluate by means of biomicroscopy or colour photographs. The method which gave the highest score for each item in the most affected eye was used. The total score was summarized. Theoretically it was possible to obtain a maximum total score of 15. The patients were placed in one of the following groups according to their total score:

Total score 0 = no signs of retinopathy
Total score 1-5 = slight non-proliferative retinopathy
Total score 6-10 = moderate non-proliferative retinopathy
Total score 11-15 = advanced non-proliferative retinopathy

Neurophysiological examination. Electroneurography (ENeG) was performed with conventional neurophysiological technique on the median, peroneal and sural nerves on one side. To obtain a measure of the degree of polyneuropathy, an index based on the various nerve conduction parameters was used. The index was calculated by dividing the mean deviation from normal, in SD, for 10 parameters by 10 (i.e. the number of parameters) [17]. A significant deviation from normal (±2 SD) corresponds to an index of ±0.63.

The autonomic nervous system was examined by recording R-R variations in the ECG, with the subject resting in the supine position. R-R variations relative to mean R-R intervals (in percent) were calculated during 60 s of normal breathing and during 60 s of deep breathing [18].

Blood pressure. The blood pressure was automatically determined by the oscillometric method (DINAMAP™, Model 845XT, Critikon Inc., Tampa, Fla, USA) after at least 5 min rest in the supine position and after 5 min in the standing position. Hypertension was defined as a diastolic pressure ≥90 mmHg.

Statistical analysis

For statistical analysis the paired and unpaired Student's t-tests, Mann Whitney non-parametric test, Fishers' exact test for four-field analysis and chi^2 analysis were used. Values are given as mean± SEM.

Results

C-peptide in urine and plasma

The mean urinary C-peptide excretion in the excretor group was 1.4±0.4 nmol/24 h (range 0.2-5.5 nmol). The normal value in our laboratory is 22±4 nmol/24 h.

Plasma C-peptide (≥0.05 nmol/l) in the fasting state was detected in 7 of the patients with urinary C-peptide excretion. A further 3 of the excretor patients attained detectable plasma levels 6 min after the administration of 1 mg glucagon intravenously. Thus, plasma C-peptide in the fasting state and/or after glucagon stimulation was detected in 10 of the C-peptide excretor but in none of the non-excretor group ($p<0.005$).

HbA_{1c} and blood glucose (Table 3)

The mean haemoglobin A_{1c} level was significantly lower in the C-peptide excretor group than in those without C-peptide excretion (6.9±0.3% vs 7.9±0.3%) ($p<0.025$). The fasting blood glucose levels were similar.

Data from patient records (Table 2)

The daily insulin dose given at 5 years after diagnosis was significantly lower in the C-peptide excretor group

Table 4. Some serum and urine protein levels and glomerular filtration rate in Type 1 diabetic patients with and without C-peptide excretion

	C-peptide excretor group ($n=22$)	C-peptide non-excretor group ($n=22$)	p value
s-albumin (g/l)	43±1	41±1	NS
s-creatinine (μmol/l)	80±2 (48–107)	75±3 (30–92)	NS
^{51}Cr-EDTA clearance (ml/min/1.73 m^2)	114±4	114±5	NS
s-beta$_2$-microglobulin (mg/l)	1.4±0.1	1.7±0.1	$p<0.01$
s-lysozyme (μg/ml)	1.1±0.1	1.2±0.2	NS
s-IgG (g/l)	10±1	11±1	NS
u-beta$_2$ microglobulin (μg/l)	108±28	157±54	NS
u-lysozyme (no. patients ≥0.5) (μg/ml)	1	0	NS
u-IgG (no. of patients)	2	4	NS

than in the non-excretor group (0.59±0.07 vs 0.79±0.06, U/kg/d) ($p<0.05$). In the former group 6 patients smoked ≥10 cigarettes/day as compared to 12 in the non-excretor group, (NS).

Microalbuminuria and proteinuria (Table 3)

In the excretor group 1 patient had microalbuminuria and none had proteinuria. In the non-excretor group 5 patients had microalbuminuria and 4 had proteinuria. Only 1 patient in the C-peptide excretor group had either microalbuminuria or proteinuria, whereas 7 patients in the non-C-peptide excretor group had either microalbuminuria and/or proteinuria ($p=0.046$). The patients with microalbuminuria had an excretion of 46 to 208 μg/min. The long-term glycaemic control was different in patients with and without albuminuria and/or proteinuria (fasting blood glucose: 10.0±0.8 mmol/l vs 9.3±1.3, mmol/l, respectively, NS; HbA_{1c} 8.4±0.5% vs 7.2±0.2%, respectively, $p<0.025$, Mann-Whitney). Six of the 8 patients with microalbuminuria and/or proteinuria were smokers as compared to 12 of 36 who were nonsmokers ($p=0.048$).

Beta$_2$-microglobulin, glomerular filtration rate and other findings (Table 4)

The s-beta$_2$-microglobulin level was significantly higher ($p<0.01$) in the C-peptide non-excretor group than in the excretor group, although all values were within normal limits. The urinary excretions of beta$_2$-microglobulin were similar in the two study groups. There was a positive correlation in the 44 patients studied between 1/beta$_2$-microglobulin ratio and ^{51}Cr-EDTA clearance ($r=0.37$, $p<0.02$).

Retinopathy (Table 3)

In the C-peptide excretor group 2 patients had moderate or advanced non-proliferative retinopathy (score >5). None of them had microalbuminuria and/or proteinuria. In the group without C-peptide excretion 7 patients had moderate-to-advanced non-proliferative retinopathy. Of these, 3 had microalbuminuria and/or proteinuria. There was no correlation between retinopathic lesions and disease duration in the groups.

Thus, in the C-peptide excretor group 3 patients had microalbuminuria and/or proteinuria and/or moderate-to-advanced non-proliferative retinopathy, as compared to 11 of the non-C-peptide excretor group ($p=0.022$). A subgroup analysis of normotensive patients showed that the tendency for low frequency of microangiopathic lesions remained in the excretor group. Only 3 of 20 normotensive patients had lesions compared to 7 of 17 normotensive in the non-excretor group ($0.05<p<0.10$).

Polyneuropathy (Table 3)

Impaired nerve conduction was common both in the C-peptide excretor group (15 patients, 68%) and in the C-peptide excretor group (12 patients, 55%) ($p>0.5$). Among 14 patients with microvascular lesions 11 had an abnormally low ENeG index, whereas in the group of 30 patients without microvascular lesions 16 patients had polyneuropathy (NS).

Autonomic neuropathy was found in 40% of the C-peptide excretor group compared to 52% of the non-C-peptide excretor group ($p>0.5$).

To exclude the possibility that patients with polyneuropathy mainly due to axonal dysfunction were not excluded by our ENeG index, the patients with and without microvascular lesions were compared with reference to sensory nerve action potential amplitude only. No difference between the groups was found.

Blood pressure

In the C-peptide excretor group 2 patients had a diastolic pressure ≥90 mm Hg. In the non-C-peptide excretor group 5 patients had a diastolic pressure ≥90 mm Hg and 2 of them were on regular anti-hypertensive treatment (furosemide and bendroflumethiazide).

Discussion

Urinary C-peptide excretion in relation to pancreatic C-peptide release seems to fairly constant between individuals [19]. Therefore, urinary C-peptide excretion

can serve as a tool for assessment of insulin production. This study shows that, of our 22 patients with urinary C-peptide, residual insulin production was detected in only 10 by analysing the plasma in the fasting state and/or after glucagon (1 mg intravenous) stimulation [11].

The results of this study confirm earlier reports that residual insulin secretion can be detected many years after the diagnosis of diabetes mellitus [4, 20]. In the present study our finding that age at clinical onset was higher in the C-peptide excretor group than in the non-excretor group agrees with the findings in an earlier study [21].

There are several possible reasons why some patients retain residual insulin secretion for many years. The report by Hoogwerf et al. [22] that HLA-DR 4 positive patients had higher C-peptide values than DR 4 negative patients suggests an association with the HLA-DR 4 phenotype. The more rapid loss of C-peptide in association with islet cell antibodies [23] suggests that the severity of the associated immunological response in Type 1 diabetic patients may be of importance.

The HbA_{1c} was significantly lower among the C-peptide excretor group. That residual insulin production has been of clinical significance for their glycaemic control is supported by our finding that the excretor group had a lower insulin requirement 5 years after the diagnosis was made.

There were significantly more patients with microalbuminuria and/or proteinuria in the group without C-peptide excretion as compared to the excretor group. That these patients had, as reflected by HbA_{1c}, better glycaemic control shows the importance of maintaining satisfactory glycaemic control. The number of smokers among these patients was significantly greater. This may indicate that smoking is a contributory factor in the development of kidney lesions in diabetes [24]. Retinopathy was slightly more frequent, but not significantly so, in the non-excretor group. However, when the signs of microvascular lesions were considered together, it was evident that the non-excretor group had a higher prevalence of late complications than the excretor group. Our findings are in line with a previous report showing a lower prevalence of retinopathy in patients with a lower insulin requirement and better glycaemic control [1] and with a report by Eff et al. [7], showing that patients with a lower insulin requirement and persistent residual insulin secretion have a lower prevalence of retinopathy. The higher prevalence of microalbuminuria and/or proteinuria and/or retinopathy in our non-excretor group, as compared to the excretor group, suggests that these patients are at a higher risk for progression to advanced microvascular complications.

The reason for the higher levels of serum $beta_2$-microglobulin in the group without residual insulin secretion is not clear. It may be due to an increased cell turnover in their vessels [25] or to a decrease in renal catabolism of serum $beta_2$-microglobulin [26]. There was no difference in the ^{51}Cr-EDTA clearance between the patients with or without residual insulin secretion, indicating that the increase in serum beta-microglobulin was not due to a decrease in the glomerular filtration rate [25].

Hypertensive diastolic blood pressure (≥ 90 mm Hg) was noted in 7 of the patients, and 2 of them had microalbuminuria and/or proteinuria. These 2 had regular treatment during less than 3 years for hypertension, whereas the other hypertensive patients had not been previously diagnosed.

Neurophysiological signs of polyneuropathy were found in more than 50% of our patients, a frequency which is in good agreement with an earlier study [1]. However, we found no difference between the C-peptide excretor and the non-excretor groups regarding either the frequency or the degree of polyneuropathy. This was probably not due to the fact that our ENeG index is more sensitive to a reduction in the conduction velocity than to a diminished amplitude of the sensory nerve action potentials. The C-peptide excretor and non-excretor groups did not differ if we considered only the amplitude as a measure of the neuropathy. Unlike Young et al. [27], we found no relation between deteriorated motor, sensory and autonomic nerve functions and glycaemic control, and no association between microvascular lesions and neuropathy; however, like Young, we did find a relation between microvascular lesions and HbA_{1c} levels.

The overall results of this study indicate that urinary C-peptide is a more sensitive method than plasma measurements of detecting residual insulin secretion. The results also suggest that patients with residual insulin secretion are less prone to develop microvascular late complications. The better glycaemic control in this group emphasizes the importance of the glycaemic control. For an evaluation of the prognosis after clinical onset of Type 1 diabetes regarding the development of microangiopathy, it may be of interest to assess residual insulin production during the course of the disease. It should be noted that the present study is cross-sectional and, like the report by Viberti et al. [28], diagnosis of microalbuminuria is based on analyses of only one single urine sample; it should also the noted that the microangiopathic lesions described are early and may be partly reversible. Prospective studies are needed to clarify more definitely whether residual insulin production in long-term Type 1 diabetes has a preventive effect against diabetic late complications.

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