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C-peptide measurement in the differentiation of Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes mellitus

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Summary. To determine whether individual subjects with Type 1 (insulin-dependent) diabetes or Type 2 (non-insulin-dependent) diabetes, who are treated with insulin, could be reliably distinguished, C-peptide concentrations and urinary C-peptide excretion were measured in 10 Caucasoids and 10 Pima Indians. All the subjects had developed diabetes before 21 years of age and were receiving insulin treatment. Fasting C-peptide concentrations were significantly higher in the Pima Indians (0.73 ± 0.17 versus 0.02 ± 0.01 nmol/l in Caucasoids; $p < 0.001$), but there were slight overlaps in individual values. Urinary C-peptide excretion, an index of 24-h-insulin excretion, was also higher in the Pima Indian group (27.6 ± 1.85 versus 0.72 ± 0.18 pmol/min in Caucasoids; $p < 0.001$) and there was no overlap in the individual values between the groups. The Pima Indians with early onset diabetes have been previously shown to have Type 2 diabetes, and the Caucasoids with an early onset are most likely to have Type 1 diabetes. These results suggest that distinction between these two major types of diabetes can be made effectively by using C-peptide measurements provided that overt renal disease is absent. This differentiation between insulin-treated patients will be useful for a variety of research applications and possibly in making clinical management decisions.

Key words: Type 1 diabetes, Type 2 diabetes, plasma C-peptide, urinary C-peptide Pima Indian.

Heterogeneity in diabetes mellitus is well recognized [1, 2] as is reflected in the recent classification of the National Diabetes Data Group and World Health Organization [3, 4]. Many patients with Type 2 diabetes are treated with insulin to control hyperglycaemia, and although they are not prone to spontaneous ketosis, ketoacidosis can occur in Type 2 diabetes with severe intercurrent illness or other stress. Conversely, many patients with well controlled Type 1 diabetes, who receive insulin therapy regularly, may never experience an episode of ketoacidosis. Thus, in some insulin-treated patients, unless insulin is withdrawn, it may not be possible to establish the type of diabetes by clinical history alone. This is particularly true in adolescents and young adults who receive insulin treatment from the time of diagnosis of diabetes, as well as in older patients who may have received insulin treatment for many years.

The present study was undertaken to determine whether it was possible to discriminate between Types 1 and 2 diabetes among subjects who had insulin treatment from the time of diagnosis of diabetes by examining plasma C-peptide concentrations, fasting and in response to glucose and arginine, and 24 h-urinary C-peptide excretion.

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Subjects and methods

Subjects

Ten Caucasoids and ten Pima Indians with the onset of diabetes before age 21 years were admitted to the Clinical Research Section for investigation. The study was approved by the National Institutes of Health Clinical Research Committee, and each subject gave written consent to participate in the study.

The subjects were teenagers or young adults, who had been treated with insulin. They included a group of Caucasoids, who were assumed almost certainly to have Type 1 diabetes, and Pima Indians who, on the basis of previous evidence, were believed to have Type 2 diabetes [5]. The clinical characteristics of the individual subjects are given in Table 1. The Pima participants were either full-blooded Pima Indians or a combination of Pima/Papago. The Papago tribe is closely related to the Pima Indians and also has a high prevalence of Type 2 diabetes.

Study design

After a 12-h overnight fast, subjects were given their usual insulin dose and 30 min later received a 100 g carbohydrate load (Koladex-Custom Laboratories, Baltimore, Maryland). Venous blood, for determination of plasma glucose and C-peptide concentrations, was drawn at -30, 0, 30, 60, 90, 120, and 180 min. On day 2 insulin was withheld and after an overnight fast, arginine monohydrochloride ($5.0\ mg \cdot kg^{-1} \cdot min^{-1}$) was infused intravenously for 40 min. Venous blood for measurement of glucose and C-peptide concentrations was withdrawn from the contralateral forearm at $-20, -10, 0, 5, 10, 20, 30$, and 40 min. A 24-h urine collection for measurement of C-peptide, creatinine and protein was obtained. Blood for HLA typing and islet cell antibodies was also collected.

Table 1. Individual characteristics and C-peptide results of subjects studied

	Sex	Age at examination (years)	Duration of diabetes (years)	Body mass Index (kg/m^2)	History[a] of keto-acidosis	HLA-B8 antigen	Islet cell antibodies	Basal blood glucose (mmol/l)	C-peptide	
									Basal plasma (nmol/l)	Urinary excretion (pmol/min)
Caucasoids	M	19	5	20.8	+	+	+	3.1	0.13	4.5
	M	20	6	21.1	−	+	−	11.6	0.03	1.6
	M	23	8	22.9	−	+	+	4.7	0.02	0.0
	F	24	10	20.9	+	−	NT	5.8	0.00	0.3
	M	24	11	23.0	+	+	+	3.6	0.00	0.0
	F	30	14	18.0	−	−	+	10.4	0.00	0.0
	F	29	16	21.5	−	−	NT	3.1	Renal failure	
	F	22	18	21.5	−	+	NT	13.9	0.00	0.0
	F	27	18	21.3	−	+	+	5.0	0.00	0.1
	F	29	25	20.3	+	+	NT	9.2	0.00	0.0
Mean ± SEM		24.2 ± 0.4	13.1 ± 0.7	20.9 ± 0.4				7.0 ± 1.3	0.02 ± 0.01	0.7 ± 0.18
Pima Indians	F	17	0.5	25.2	−	−	NT	10.5	0.54	30.2
	M	14	2	28.3	+	−	NT	14.5	1.74	56.2
	M	23	4	26.2	−	−	−	14.1	0.16	28.8
	F	25	5	27.0	+	−	−	5.8	1.29	39.0
	M	27	6	23.5	+	−	−	12.9	0.77	20.2
	F	19	7	25.1	−	−	−	13.3	0.31	11.3
	F	28	7	30.9	−	−	−	13.3	0.88	40.1
	M	37	20	21.1	+	−	−	9.3	0.44	8.2
	F	42	23	29.6	−	−	−	14.3	0.46	14.5
	F	37	27	30.8	−	−	−	6.0	Renal failure	
Mean ± SEM		27.9 ± 1.0	10.2 ± 1.0	26.3 ± 0.9[e]		[c]	[b]	11.4 ± 0.8[c]	0.73 ± 0.17[d]	27.6 ± 1.85[d]

+ = present; NT = Not tested; [a] with blood pH ≤ 7.2 or serum bicarbonate ≤ 12 mmol/l; [b] $p < 0.05$; [c] $p < 0.01$; [d] $p < 0.001$

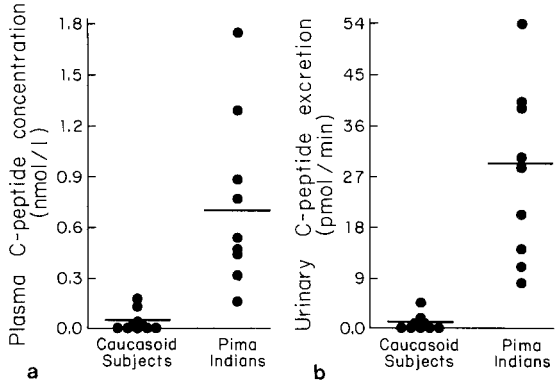

Fig. 1. A and B Individual C-peptide levels in the nine Caucasoid and nine Pima Indian subjects. The horizontal bars represent the mean value of each group. **A** Fasting plasma concentrations; $p < 0.01$. **B** Urinary excretion rates; $p < 0.005$

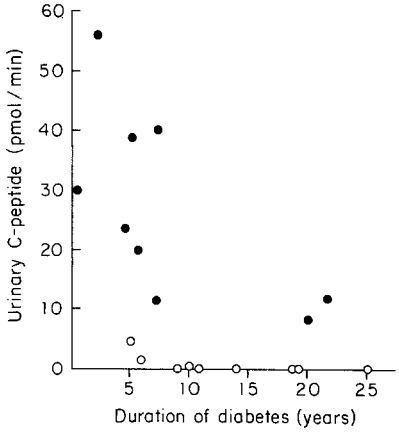

Fig. 2. Urinary C-peptide excretion rates in the nine Pima (●) and nine Caucasoid (○) subjects according to duration of diabetes

Methods

Samples for plasma glucose determinations were collected in tubes containing sodium fluoride, stored at −20 °C and were analyzed in duplicate using the glucose oxidase method on a glucose analyzer (Beckman Instruments, Anaheim, California). Plasma for C-peptide determinations was stored at −20 °C and measured using a non-equilibrium ethanol precipitation radioimmunoassay [6]. The sensitivity of this assay is 0.02 pmol/ml with an intra- and interassay variation of 5.8% and 9.6%, respectively. Urine for C-peptide measurements was centrifuged at 2500 *g* for 30 min to remove particulate Matter and the pH adjusted to 7.0 with NaOH before analysis [7]. HLA typing was performed using standard microlymphocytotoxicity techniques for A and B loci antigens only [8]. Islet cell cytoplasmic antibodies were measured by the method of Bottazzo et al. [9].

Statistical methods

The fasting C-peptide concentrations were taken as the mean of the −30 and 0 min samples for each subject. The mean fasting plasma and urinary concentrations of C-peptide were compared using Student's t-test. A Fisher exact test for 2 × 2 tables was utilized to analyze the HLA and islet cell antibody data and Pearson correlation coefficients were used to examine relationships between C-peptide concentrations, excretion rates, obesity and duration of disease within each group of subjects [10]. Analysis of variance with repeated measures was used to test for differences from baseline in responses to oral glucose tolerance and arginine infusion tests [10].

Results

The age of onset of diabetes was somewhat lower and the duration of diabetes slightly longer in the Caucasoids compared with the Pima Indians. All the Pima had at least one parent with diabetes, whereas none of the Caucasoids had a parent or other first degree rela-

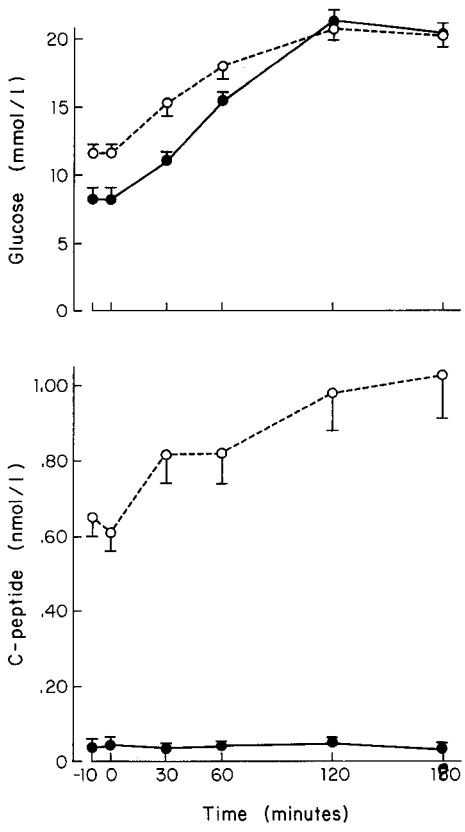

Fig. 3. Mean ± SEM blood glucose (top panel) and plasma C-peptide (bottom panel) concentrations before and in response to a 100-g oral glucose load in the Pima Indians (- - O - -) and Caucasoids (- - ● - -). Mean plasma C-peptide concentrations were higher than the basal level at 120 and 180 min ($p < 0.01$) in the Pima Indians, but showed no significant change in the Caucasoids

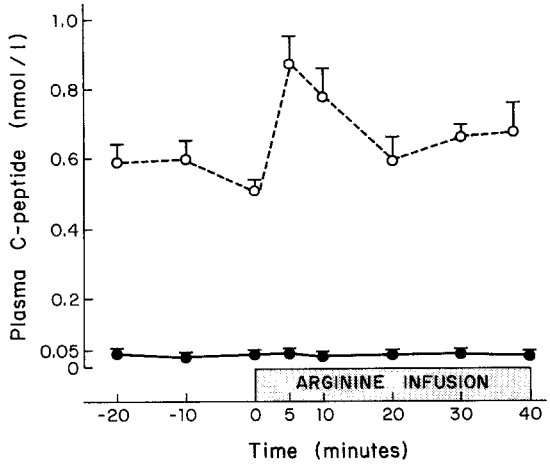

Fig. 4. Mean ± SEM plasma C-peptide concentrations before and during an arginine infusion (5.0 mg · kg^{-1} · min^{-1}) in the Pima Indians (- - O - -) and the Caucasoid (- - ● - -) subjects. A significant response occurred in the Pima group at 5 and 10 min ($p < 0.01$)

tive with the disease. The Pima group were more obese than the Caucasoids ($p < 0.001$), and their mean fasting plasma glucose levels were higher (11.4 ± 0.8 mmol/l in Pima and 6.9 ± 1.3 mmol/l in Caucasoids). In both groups, four of the ten subjects had a history of ketoacidosis, although in the Pima the episodes had been associated with a combination of prolonged insulin withdrawal (≥ 30 days) and alcohol ingestion in three patients or soft tissue infection in one, indicating that they were not insulin-dependent. Seven of the Caucasoids were HLA-B8 positive, whereas none of the Pima had HLA-B8 (or BW15) ($p < 0.05$ Table 1). Five of the six Caucasoids had islet cell antibodies, whereas none were found in the Pima ($p < 0.05$). For analysis of C-peptide concentrations and excretion, results were not included from one Caucasoid and one Pima subject as they had evidence of nephropathy with impaired creatinine clearance which has been shown to suppress urinary C-peptide excretion [11, 12].

Mean fasting plasma C-peptide levels were significantly different between the groups (Pima 0.73 ± 0.17 nmol/l; Caucasoids 0.02 ± 0.01 nmol/l; $p < 0.01$), with only one subject in the Pima group falling into the range observed in the Caucasoids, and vice versa (Fig. 1 a). Twenty-four hour urinary C-peptide excretion, however, showed no overlapping values (Fig. 1 b) (Pima range 8.2–56.2 pmol/min and Caucasoids 0.00–4.46 pmol/min; mean ± SEM 27.6 ± 1.9 and 0.7 ± 0.2 pmol/min, respectively; $p < 0.0001$). Both fasting C-peptide and urinary C-peptide excretions were positively correlated with body mass index in the Pima Indians ($r = 0.56$ $p < 0.05$ and 0.77; $p < 0.05$, respectively), but non-significant negative relationships were found in the Caucasoids. The fasting plasma C-peptide levels showed a correlation with the urinary C-peptide excretion (Pima $r = 0.81$; Caucasoids 0.97; $p < 0.01$).

Urinary excretion of C-peptide tended to be lower with increasing duration of diabetes in both groups (Pima $r = -0.65$, $p \simeq 0.05$; Caucasoids −0.58, $p > 0.05$; Fig 2), but the levels in the Pima Indians were higher than in the Caucasoid group regardless of the duration of diabetes.

The blood glucose and C-peptide concentrations, measured during an oral glucose tolerance test (Fig. 3), showed that while the fasting glucose levels were lower among the Caucasoids than among the Pima Indians, the glucose levels were similar during the second and third hours of the test. The C-peptide concentrations, however, remained low and did not change in response to the change in glucose concentration among the Caucasoids, whereas among the Pima Indians there were increments in seven of the ten subjects, resulting in a significant increase in mean C-peptide concentration ($p < 0.01$).

In response to arginine infusion (Fig. 4), mean C-peptide concentrations in the Pima Indians were significantly higher 5 and 10 min after the start of infusion ($p < 0.01$), but did not change in the Caucasoids. Neither group showed a significant change in blood glucose in response to the arginine infusion.

Discussion

Determination of urinary excretion rates of C-peptide resulted in complete differentiation between two small groups of Caucasoid and Pima Indian insulin-treated

young adult diabetic patients with Type 1 and Type 2 diabetes, respectively. Measurement of the fasting serum C-peptide concentrations also led to a substantial degree of discrimination between the two groups, but there was some overlap; whereas with excretion rates the lowest level observed in the Pima group (8.2 pmol/min) was almost twice that of the highest seen in the Caucasoid group. Thus, fasting C-peptide concentrations in sera, and to a greater extent urinary C-peptide measurements, appear useful to differentiate between Type 1 and Type 2 diabetes.

The conclusion that C-peptide excretion is useful in distinguishing between Type 1 and Type 2 diabetes depends on the likelihood that the Caucasoids and Pima, respectively, do indeed have these different types of diabetes. The Causasoids were selected because it was believed that the vast majority of these patients, who had no first degree relatives with the diabetes and an age of onset below 20 years, on clinical grounds, would have Type 1 diabetes. In contrast, among the Pima Indians, even those with diabetes with an early onset do not appear to be insulin dependent [5]. The differences in the types of diabetes were reflected in this study in differences between the groups in degree of obesity, the frequency of islet cell antibody detection, the frequency of a parental (or other family) history of diabetes, that the majority of the Caucasoids had an HLA phenotype associated with Type 1 diabetes and that in the Pima Indians ketoacidosis had developed only under stress and never spontaneously. These findings agree with earlier studies indicating that the Pima Indians appear to have exclusively Type 2 diabetes as determined from the absence of islet cell antibodies in those of recent onset [13], and of their other clinical characteristics at onset and the clinical course of the disease [5].

That the diabetic subjects could be partitioned into two separate groups lends additional support to the concept of different aetiological entities in diabetes as defined in the National Diabetes Data Group and World Health Organization classification [3, 4]. Prior to the availability of the C-peptide assay, objective laboratory definition of these two diseases in individual subjects was not possible in many who had received insulin treatment, as residual insulin secretion or plasma insulin concentrations could not be measured because of the presence of insulin antibodies. The determination of C-peptide concentrations in urine and blood now permits an accurate characterization of insulin secretion of many such patients [7, 12, 14] and facilitates the classification of insulin-treated subjects into the Type 1 and Type 2 classes. This categorization is necessary for studies of the genetics of the two conditions, as well as in other research. Such measurements may also have clinical applications in determining whether or not it is safe to withdraw insulin treatment, or in determining its necessity where distinction is difficult on clinical grounds alone. The C-peptide results in the present study also emphasize the characteristic of insulinopenia as being pathognomonic of Type 1 diabetes and add further to the evidence that diabetes in the Pima Indians, even when it has its onset at an early age, is not characterized by insulinopenia and is of the non-insulin-dependent type.

To some extent the differences in C-peptide concentrations and excretion observed between the Caucasoids and Pima Indians in this study might be the result of differences in fasting blood glucose levels between the groups. The lower fasting glucose levels in the Caucasoids could possibly have resulted in a greater degree of suppression of C-peptide secretion than in the Pima, in whom the levels of both fasting glucose and C-peptide were higher. This is an improbable explanation for the differences observed, because when blood glucose levels during the oral glucose tolerance test in the two groups became equivalent, and exceeded 10 mmol/l, a concentration which appears to stimulate maximally C-peptide secretion [15], mean C-peptide levels became even higher in the Pima Indian group, but remained low and unchanged in the Caucasoids. Likewise, in response to arginine infusion, no C-peptide response was seen among the Caucasoids, suggesting that the fasting level may have been reflecting maximum secretory capacity, rather than partial suppression. Indeed, we may speculate that the greater discriminating power of the urinary measurement may reflect the extent to which C-peptide secretion was stimulated in Type 2 diabetes by food intake, etc., during the collection period, whereas in Type 1 diabetes the pancreas appeared to be refractory even to oral glucose and arginine stimulation.

The urinary C-peptide measurement appears to be a better measure than the fasting serum level for making the distinction between Type 1 and Type 2 diabetes as it reflects 24-h insulin output, except in renal failure when C-peptide excretion is reduced and serum levels may be increased [12]. However, the collection of a 24-h urine sample can be difficult, particularly in epidemiological studies. As there is a high correlation between the fasting C-peptide level and the 24-h excretion, in practice the majority of patients can probably be distinguished on the basis of the serum measurement alone, although as reported previously by Welborn et al. [16], there will remain a minority of diabetic patients where this is not possible. In these subjects urinary C-peptide excretion appears to be helpful, and while 24-h excretion was determined in this study, it has recently been suggested that a 4-h urine collection may be sufficient [17].

If renal failure is present, the fasting C-peptide level in blood may be elevated. If this is suspected the renal clearance of C-peptide can be determined, and if decreased, both the serum and urinary concentrations should be considered unreliable as indices of insulin secretion. In the present study, C-peptide clearance was calculated among the Pima Indian group to exclude the possibility that the higher fasting C-peptide levels could be explained by diminished renal clearance. The results indicated that in all but one subject, already excluded as she had renal failure on the basis of other criteria, reduced clearance was not responsible for the higher plas-

ma levels observed. Similarly one Caucasoid was excluded because of evidence of renal failure.

The use of glucose or arginine stimulation before C-peptide measurement did not increase the ability to discriminate between Type 1 and Type 2 diabetes in this study although evidence of increased secretion in response to these stimuli did occur in some, but not all of the Type 2 diabetic subjects.

Although the sample size was small, and in the Caucasoid group the range of measurements narrow, there was a negative correlation between duration of diabetes and C-peptide excretion in both the Type 1 and Type 2 groups suggesting a fall in insulin secretion with increasing duration (Fig. 2). Decreases in fasting serum C-peptide levels and responses to insulin secretogogues with increasing duration of diabetes have been reported previously [16, 18] and the urinary excretion data reported here show similar trends. These findings suggest that insulin output falls with increasing disease duration in both Type 2 and Type 1 diabetes, although longitudinal data are needed to validate the observation. In the present study, the levels of C-peptide excretion in Type 1 diabetes of less than 5 years duration were not measured, although other reports indicate that in Type 1 diabetes of recent onset urinary excretion of C-peptide may often be below 10 pmol/min [19]. The levels seen in our Type 2 diabetic patients with a duration of 5 years or less were at least twice as high. Nevertheless, in the absence of data from this study on Type 1 diabetes of less than five years duration, the relative merits of fasting and urinary C-peptide excretion data to discriminate between Type 1 and Type 2 diabetes of short duration cannot be determined.

We conclude that among patients with early onset diabetes mellitus, who have been treated with insulin, C-peptide measurements in fasting blood or in 24-h urine collections can be used to categorize subjects without renal failure into insulin-dependent and non-insulin-dependent groups even after many years of diabetes. Application of such measurements could facilitate research into these diseases, and may sometimes help in selecting appropriate clinical management and treatment.

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