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Fasting plasma C-peptide, glucagon stimulated plasma C-peptide, and urinary C-peptide in relation to clinical type of diabetes

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Summary. Many patients with Type 2 (non-insulin-dependent) diabetes mellitus are treated with insulin in order to control hyperglycaemia. We studied fasting plasma C-peptide, glucagon stimulated plasma C-peptide, and 24 h urinary C-peptide in relation to clinical type of diabetes in 132 insulin treated diabetic subjects. Patients were classified clinically as Type 1 (insulin-dependent) diabetic subjects in the presence of at least two of the following criteria: 1) significant ketonuria, 2) insulin treatment started within one year after diagnosis, 3) age of diagnosis ≤ 40 years, and 4) weight below 110% of ideal weight of the same age and sex. Eighty patients were classified as Type 1 and 52 as Type 2 diabetic subjects. A second classification of patients into 6 C-peptide classes was then performed. Class I consisted of patients without islet B-cell function. Class II–VI had preserved islet B-cell function and were separated according to the 20%, 40%, 60% and 80% C-peptide percentiles. The two classifications of patients were compared by calculating the prevalence of clinical Type 1 and Type 2 diabetes in each of the C-peptide classes. This analysis showed that patients with a fasting plasma C-peptide value < 0.20 nmol/l, a glucagon stimulated plasma C-peptide value < 0.32 nmol/l, and a urinary C-peptide value < 3.1 nmol/l, or < 0.54 nmol/mmol creatinine/24 h, or < 5.4 nmol/24 h mainly were Type 1 diabetic patients; while patients with C-peptide levels above these values mainly were Type 2. At these limits the percentage, predictive value of positive tests as indicators of Type 2 diabetes were as follows: fasting C-peptide 83%, stimulated C-peptide 86%, and urinary C-peptide expressed as nmol/l 76%, as nmol/mmol creatinine/24 h 79%, and as nmol/24 h 78%. Similarly, the percentage predictive value of negative tests as indicators of Type 1 diabetes were as follows: fasting C-peptide 86%, stimulated C-peptide 88%, and urinary C-peptide expressed as nmol/l 79%, as nmol · mmol creatinine · 24 h 81%, and as nmol/24 h 80%. If patients without detectable C-peptide were excluded, the predictive value of negative tests were as follows: fasting C-peptide 81%, stimulated C-peptide 88%, urinary C-peptide expressed as nmol/l 61%, as nmol/mmol creatinine/24 h 69%, and as nmol/24 h 64%. In conclusion, post glucagon C-peptide gives a good distinction between Type 1 and Type 2 diabetes mellitus in insulin treated diabetes while 24 h urinary C-peptide gives a less sensitive distinction between the clinical types of diabetes.

Key words: Type 1 (insulin-dependent) diabetes mellitus, Type 2 (non-insulin-dependent) diabetes mellitus, islet B-cell function, plasma C-peptide, urinary C-peptide.

Classification of diabetes into Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes mellitus is clinical [1]. Patients with Type 1 diabetes are ketosis-prone and require insulin to sustain life due to a severe defect in islet B-cell function, while patients with Type 2 diabetes are non-ketosis-prone, often obese, and have defects in both insulin action and secretion. Furthermore, Type 2 diabetes is only rarely diagnosed before the age of 40 years [2]. The clinical classification of patients into Type 1 and Type 2 diabetes is supposed to reflect differences in islet B-cell function between the two types of diabetes. We, therefore, found it of interest to study estimates of islet B-cell function in patients with clinical Type 1 and Type 2 diabetes mellitus.

The C-peptide response to glucagon was introduced for more than a decade ago as a measure of pancreatic responsiveness in Type 1 diabetes mellitus [3]. The test has now become widely used in patients with Type 1 diabetes [4–9] but also in patients with Type 2 diabetes mellitus [8–11]. The test has also been used in attempts to discriminate between Type 2 diabetic patients with and without insulin requirement in order to control hyperglycaemia [8, 9]. However, only Hoekstra et al. have evaluated the test in discrimination between Type 1 and Type 2 diabetes [12]. They discontinued insulin therapy in 11 obese insulin treated diabetic subjects. The two C-peptide non-responders had to resume insulin due to a large increase in ketone body levels

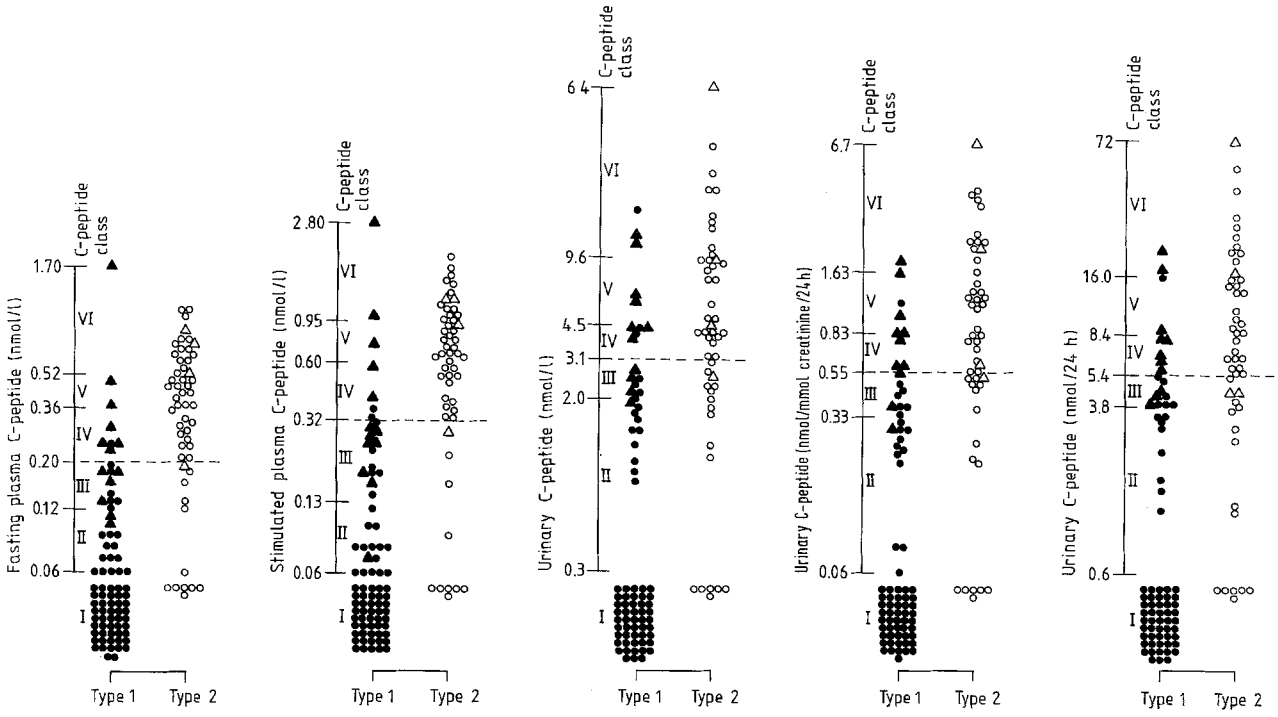

Fig. 1. Fasting plasma C-peptide, glucagon stimulated plasma C-peptide, and the mean of three 24 h urinary C-peptide values expressed as nmol/l, nmol/mmol creatinine/24 h, or nmol/24 h in relation to clinical type of diabetes in insulin treated diabetic subjects marked according to duration of diabetes. Patients were divided into six C-peptide classes. Class I and II were separated according to the "effective" detection limit of C-peptide in plasma or urine. Class II–VI were separated according to the 20%, 40%, 60%, and 80% C-peptide percentiles for patients with C-peptide values above "effective" detection limit. Figures on the ordinate indicate the limits between the six C-peptide classes.

▲ Type 1 (insulin-dependent) diabetic subjects with diabetes duration ≤ 2 years

● Type 1 diabetic subjects with diabetes duration > 2 years

△ Type 2 (non-insulin-dependent) diabetic subjects with diabetes duration ≤ 2 years

○ Type 2 diabetic subjects with diabetes duration > 2 years

while no ketoacidosis developed in the 9 C-peptide responders. The authors concluded, that measuring C-peptide after glucagon is a simple test that may be a discriminative method to establish insulin dependency in obese diabetic patients treated with insulin.

Many patients with Type 2 diabetes mellitus are treated with insulin in order to control hyperglycaemia [2]. We, therefore, expected insulin treated diabetic subjects followed at the outpatient clinic, medical department, Fredericia Hospital Fredericia, Denmark, to be a mixture of patients with Type 1 and Type 2 diabetes mellitus. Among 132 patients of this population, fasting plasma C-peptide, glucagon stimulated plasma C-peptide, and 24 h urinary C-peptide were related to the clinical type of diabetes.

Subjects and methods

Patients

Fredericia Hospital serves a population of approximately 50,000. From a study of prescriptions, it appeared that approximately 80% of insulin treated diabetic subjects in the area are followed at the clinic. All 178 insulin treated diabetic subjects aged 18 years or more, with a duration of diabetes for more than six months and attending the clinic were invited to participate in the study. Thirty-five patients were not interested. Eleven patients with a plasma creatinine above 150 μmol/l were excluded; 132 patients entered the study. Nine patients were not able to collect the 24 h urinary samples.

Each patient was classified according to clinical type. Type 1 diabetes was considered in the presence of at least two of the following criteria: 1. significant ketonuria (more than + at ketostix corresponding to a urinary acetoacetate concentration above 4 mmol/l) at diagnosis or any time after diagnosis, 2. insulin treatment initiated within one year after diagnosis, 3. age at diagnosis ≤ 40 years, and 4. weight ≤ 110% of the ideal weight for the same sex and age (13). Type 2 diabetes was considered in the abscence of such criteria or in the presence of only one criterion. Using these criteria, 80 patients were considered Type 1 while 52 were considered Type 2 diabetic subjects. A second classification of patients into 6 C-peptide classes was then performed. Class 1 consisted of patients without detectable C-peptide in plasma (< 0.06 nmol/l) or urinary C-peptide values < 0.3 nmol/l, < 0.06 nmol/mmol creatinine/24 h, or < 0.6 nmol/24 h. These urinary C-peptide values ("effective" detection limits) correspond to a plasma C-peptide concentration < 0.06 nmol/1 [14]. Classes II–VI consisted of patients with increasing C-peptide levels above the "effective" detection limit separated according to the 20%, 40%, 60% and 80% percentiles.

The two different classifications could then be matched by calculating the prevalence of Type 1 and Type 2 diabetes in each of the C-peptide classes. Furthermore, predictive values (see methods section) of positive tests as indicators of Type 2 diabetes and of negative tests as indicators of Type 1 diabetes could be calculated at the C-peptide limits separating the 6 C-peptide classes.

All patients were treated with intermediately acting insulin alone or in combination with regular insulin given once or twice daily. The

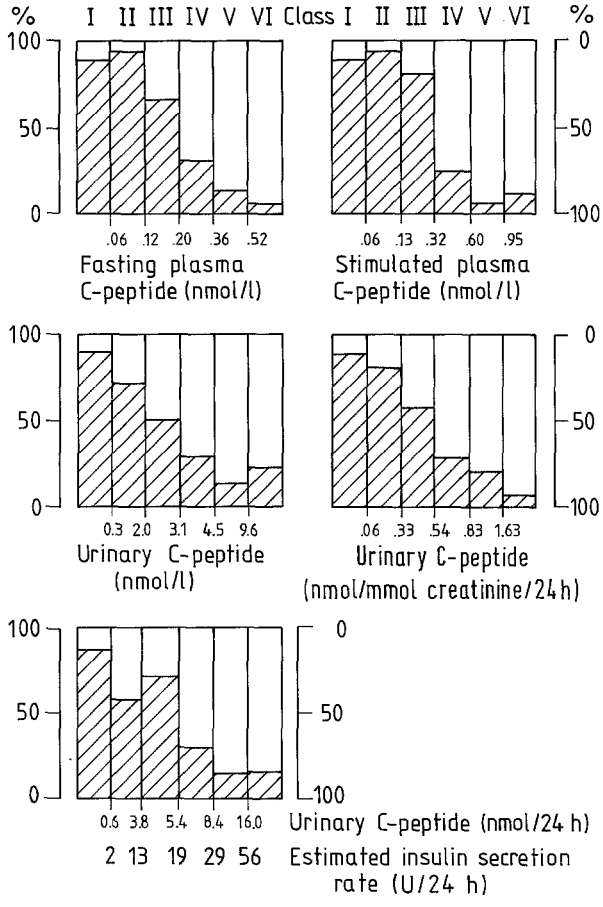

Fig. 2. Prevalence of Type 1 ▨ and Type 2 □ diabetes in C-peptide classes

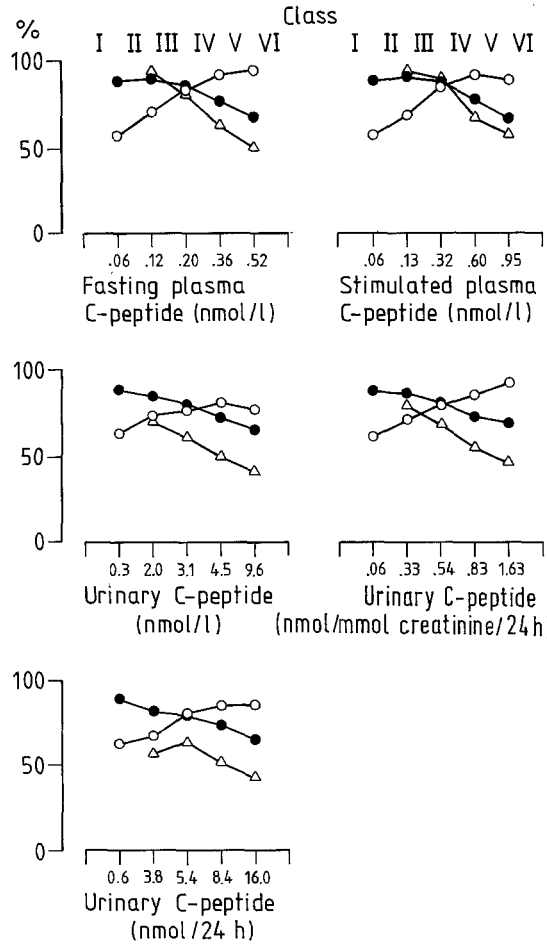

Fig. 3. Predictive values of positive tests in the detection of Type 2 diabetes (○—○) and of negative tests in the detection of Type 1 diabetes (●—●) at the limits separating C-peptide class I-VI. Predictive values of negative tests are also shown when patients belonging to class I are excluded (△—△)

last insulin injection was given either in the morning or late in the afternoon on the day preceeding the investigation. Patients were studied after an overnight 10–12 h fast.

Methods

Plasma C-peptide was measured before and 6 min after intravenous injection of 1 mg of glucagon. The same morning the patients completed the third consecutive 24-h urinary collection.

Twenty-four h urinary samples were collected at home in plastic containers. Patients were instructed to keep the samples under refrigeration during the collection period. After volume measurement an aliquot of the urine was adjusted to pH 7.0–7.5 with 5 mmol/1 NaOH and kept frozen at −20° C until assay. After thawing, the urinary samples were diluted with a 0.04 mol/1 phosphate buffer containing 0.1% albumin and NaCl to make the buffer isotonic. Measurement of urinary C-peptide was performed in the concentration range 0.1–1.0 nmol/l. Immunoreactivity of C-peptide in the urine and plasma was determined by the method of Heding [5] using antibody M1230 [16]. The intra- and interassay coefficients of variation of the C-peptide assay is 3.2% and 9.2%, respectively. The detection limit of C-peptide in plasma is 0.06 nmol/l as determined from measurement of C-peptide in plasma from pancreatectomised patients [17]. Detection limit of C-peptide in urine is 0.1 nmol/l defined as the lowest concentration which with 95% confidence limits can be distinguished from zero. Twenty-four h urinary C-peptide values of 0.3 nmol/l, 0.06 nmol/mmol creatinine/24 h, or 0.6 nmol/24 h correspond to a plasma C-peptide value of 0.06 nmol/l, so-called "effective" detection limits [14]. The 24 h urinary C-peptide excretion was calculated as the mean value of the three samples and expressed as nmol/l, nmol/umol urinary creatinine/24 h, and nmol/24 h. Adjustment with urinary creatinine may correct for differences in body size [18] and glomerular filtration rate [19]. Peripheral plasma free insulin concentrations were measured according to Heding [20]. Fasting blood glucose was measured by a hexokinase method [21].

Informed consent was obtained in all patients. The study was approved by The Ethical Committee of Funen and Vejle Counties.

Statistical analysis

Kruskall-Wallis' non-parametric ranked-sum test was used in comparing median values in C-peptide groups. The level of significance was set at $2\alpha = 0.05$.

Figures for predictive values of positive and negative tests were calculated as follows:

Predictive value of positive test (%) = (number of patients classified as Type 2 diabetic subjects with a C-peptide value above the specified limit/total number of patients with a C-peptide value above the specified limit) × 100.

Predictive value of negative test (%) = (number of patients classified as Type 1 diabetic subjects with a C-peptide value below the specified limit/total number of patients with a C-peptide value below the specified limit) × 100.

Table 1. Predictive value of positive test in the detection of Type 2 (non-insulin-dependent) diabetes and of negative test in the detection of Type 1 was (insulin-dependent) diabetes at the plasma and urinary C-peptide levels separating class III and IV in Figures 1 and 2 (95% confidence limits). Values are given in patients with diabetes duration $> 1/2$ year and in patients with diabetes duration > 2 years

Diabetes duration	C-peptide limit	Predictive value of positive test	Predictive value of negative test	Predictive value of negative test when excluding patients of class I
$> 1/2$ year	F-CP 0.20 nmol/l	83% (70- 93%)	86% (76-92%)	81% (63-93%)
	S-CP 0.32 nmol/l	86% (73- 94%)	88% (79-94%)	88% (71-96%)
	UCP 3.1 nmol/l	76% (60- 88%)	79% (69-87%)	61% (41-79%)
	UCP 0.54 nmol/mmol/24 h	79% (63- 90%)	81% (71-89%)	69% (49-85%)
	UCP 5.4 nmol/24 h	78% (62- 89%)	80% (70-88%)	64% (44-81%)
	F-CP $\geq$ 0.20 nmol/l + S-CP $\geq$ 0.32 nmol/l	87% (74- 95%)	-	-
	F-CP $<$ 0.20 nmol/l + S-CP $<$ 0.32 nmol/l	-	88% (78-94%)	-
	UCP $\geq$ 0.54 nmol/mmol/24 h + S-CP $\geq$ 0.32 nmol/l	86% (71- 95%)	-	-
	UCP $<$ 0.54 nmol/mmol/24 h + S-CP $<$ 0.32 nmol/l	-	88% (78-94%)	-
> 2 years	F-CP 0.20 nmol/l	97% (86-100%)	86% (76-93%)	79% (58-93%)
	S-CP 0.32 nmol/l	95% (83- 99%)	88% (78-94%)	87% (66-97%)
	UCP 3.1 nmol/l	90% (74- 98%)	79% (68-88%)	57% (34-77%)
	UCP 0.54 nmol/mmol/24 h	97% (83-100%)	82% (71-90%)	68% (47-85%)
	UCP 5.4 nmol/24 h	94% (79- 99%)	82% (71-90%)	64% (41-83%)

F-CP = fasting plasma C-peptide, S-CP = glucagon stimulated plasma C-peptide, UCP = mean of three 24 h urinary C-peptide values

Results

Figure 1 shows the fasting, stimulated, and urinary C-peptide values in patients considered Type 1 and Type 2 diabetic subjects marked whether diabetes duration was above or below 2 years. A large overlap was found in C-peptide values between patients with Type 1 and Type 2 diabetes. Six patients were classified as Type 2 diabetic subjects but had plasma C-peptide levels below 0.06 nmol/l together with urinary C-peptide values below the corresponding "effective" detection limits. Figure 2 shows the prevalence of Type 1 and Type 2 diabetes in the 6 C-peptide classes. The majority of patients in class I–III were classified as Type 1 while the majority of patients in class IV–VI were classified as Type 2 diabetic subjects. In Fig. 3 are shown predictive values of positive and negative tests at the limits between the 6 C-peptide classes. The best discrimination between Type 1 and Type 2 diabetes was obtained at the limit between class III and IV. In Table 1 predictive values are shown with 95% confidence limits of negative and positive tests at the limits separating class III and IV. C-peptide after glucagon tended to give the best discrimination between the clinical types of diabetes while urinary C-peptide tended to give a less distinct discrimination. The prevalence of Type 1 diabetes was high in class I whether using C-peptide determination in plasma or in urine. Figure 3 also shows the predictive values of negative tests at the limits between class II–VI when patients belonging to class I are excluded. The predictive value of a negative test at the limit between class III and IV was now significantly higher ($p < 0.05$) using C-peptide determination post glucagon than using urinary C-peptide as islet B-cell estimate considering the 95% confidence limits of the predictive values (Table 1).

Table 1 also shows that the predictive values of stimulated plasma C-peptide concentrations above or below the limit between class III and IV would not increase if they were combined with fasting plasma C-peptide concentrations or with urinary C-peptide values above or below the corresponding limit between class III and IV.

Table 1 finally shows that the predictive values of positive tests using all estimates of islet B-cell function would increase to 90–97%, if only patients with a duration of diabetes > 2 years were included in the study. In this case, the predictive values of negative tests were, however, almost unchanged.

Patients belonging to class II + III and to class V + VI had preserved B-cell function and were each homogenous in the respect that the prevalence of Type 1 diabetes was very high in class II + III and the prevalence of Type 2 diabetes very high in class V + VI when using stimulated C-peptide as islet B-cell estimate. Table 2 shows the average number of Type 1 clinical features in the two types of diabetes in the stimulated C-peptide classes, when combining patients of class II + III and of class V + VI. There was no significant association between number of clinical features and C-peptide levels when analysing patients with Type 1 and Type 2 diabetes separately.

Table 3 shows different clinical characteristics of the patients also when combining the stimulated C-peptide class II + III and V + VI. Patients belonging to class IV and class V + VI were the oldest. Sex distribution was

Table 2. Mean number of features of Type 1 diabetes (ketonuria, early insulin treatment, age of diagnosis ≤ 40 years, and normal weight) in stimulated C-peptide classes in patients classified as Type 1 or Type 2 diabetic subjects

Type of diabetes	Class I	Class II+III	Class IV	Class V+VI
Type 1	3.3 $n=45$	3.0 $n=28$	3.0 $n=4$	2.3 $n=3$
Type 2	0.7 $n=6$	0.5 $n=4$	0.6 $n=12$	0.6 $n=30$

Class I: Glucagon stimulated plasma C-peptide < 0.06 nmol/l.
Class II+III: Glucagon stimulated plasma C-peptide 0.06- < 0.32 nmol/l.
Class IV: Glucagon stimulated plasma C-peptide 0.32- < 0.60 nmol/l.
Class V+VI: Glucagon stimulated plasma C-peptide ≥ 0.60 nmol/l.
n = number of patients studied

similar in the groups. Duration of diabetes was longest in patients belonging to class I. Body mass index was highest in patients belonging to class II+III. Insulin dose was inversely associated with C-peptide. Plasma peripheral insulin levels were highest in patients belonging to class V+VI. Fasting blood glucose was highest in patients without islet B-cell function.

The influence of duration of diabetes on median stimulated C-peptide in class II+III, class IV, and class V+VI is shown in Table 4. In patients of class II+III, an obvious decline in median C-peptide value was found when the duration of diabetes was ≥ 5 years as compared to < 5 years ($p < 0.01$). In the other classes, no significant association was found between stimulated C-peptide and the duration of diabetes.

Discussion

Our study population consisted of a sample of insulin treated diabetic outpatients in whom insulin treatment was given without any rigid or reportable criteria. Many patients with Type 2 diabetes are treated with insulin in order to control hyperglycaemia [2], and we, therefore, expected a certain part of the patients to have Type 2 diabetes. However, no criteria exist that permit a mutually exclusive discrimination between Type 1 and Type 2 diabetes. Even for the same subject classification can change with the condition of the patient [22]. Any classification of patients into Type 1 and Type 2 diabetic subjects must be based on arbitrary criteria. In other studies, early insulin treatment and significant ketonuria have been used as criteria of Type 1 diabetes, while the lack of these criteria have been used to diagnose Type 2 diabetes [2]. In our study, we followed the clinical differences outlined by WHO [1]. Thus, the criteria for Type 1 diabetes were ketonuria at diagnosis or later, early insulin treatment, diagnosis before 40 years and normal weight. In order to reduce the number of "false positive" Type 1 diabetic subjects, at least two of the criteria should be present to allow the diagnosis Type 1 diabetes. Type 2 diabetes was considered in the abscence of any criterion, or if only one criterion was present. Measurement of islet cell autoantibodies or HLA-typing were not performed.

The predictive value of a stimulated plasma C-peptide concentration above and below 0.32 nmol/1 as indicator of Type 2 and Type 1 diabetes was as high as 86% and 88%, respectively. The predictive values of positive tests could be further increased at higher stimu-

Table 3. Median value and range of some clinical characteristics of 132 insulin treated diabetic subjects, when dividing the patients into different classes according to glucagon stimulated C-peptide levels

Variable	Class I $n=51$	Class II+III $n=32$	Class IV $n=16$	Class V+VI $n=33$
Sex (F/M)	35/16	16/16	11/5	20/13 NS
Age (years)	36 (19-83)	43 (18-76)	63 (21-77)	69 a (38-87)
Duration of diabetes mellitus (years)	17 (3-51)	6 (1-25)	9 (2-26)	7 a (1-43)
Body mass index (kg/m^2)	23.9 (18.1-34.0)	22.6 (17.9-29.8)	23.2 (17.7-33.1)	25.2 b (16.1-40.8)
Insulin dose (U/kg)	0.61 (0.42-1.27)	0.43 (0.24-1.87)	0.40 (0.22-0.63)	0.35 a (0.16-1.67)
Plasma free insulin (pmol/l)	54 (0-272)	34 (10-276)	50 (0-99)	76 b (17-580)
Blood glucose (mmol/l)	12.0 (3.2-21.0)	6.7 (2.0-18.2)	8.5 (3.9-14.6)	7.9 b (3.3-15.4)

Class I: Stimulated C-peptide < 0.06 nmol/l.
Class II+III: Stimulated C-peptide 0.06- < 0.32 nmol/l.
Class IV: Stimulated C-peptide 0.32- < 0.60 nmol/l.
Class V+VI: Stimulated C-peptide ≥ 0.60 nmol/l.
n = number of patients studied
[a] = $p < 0.01$, [b] = $p < 0.001$, NS = not significant

Table 4. Median and range of glucagon stimulated plasma C-peptide values in C-peptide classes, grouped by duration of diabetes

Duration of diabetes	Median plasma C-peptide (nmol/l) < 5 years	5- < 10 years	≥ 10 years
Class II+III	0.25 0.06-0.32 $n=11$	0.09 0.06-0.23 $n=12$	0.09 a 0.06-0.22 $n=9$
Class IV	0.41 0.37-0.57 $n=3$	0.35 0.32-0.53 $n=6$	0.52 NS 0.36-0.57 $n=7$
Class V+VI	1.02 0.73-2.80 $n=9$	0.90 0.61-1.50 $n=12$	0.81 NS 0.64-1.92 $n=12$

Class II+III: Glucagon stimulated plasma C-peptide 0.06- < 0.32 nmol/l.
Class IV: Glucagon stimulated plasma C-peptide 0.32- < 0.60 nmol/l.
Class V+VI: Glucagon stimulated plasma C-peptide ≥ 0.60 nmol/l.
n = number of patients studied
[a] = $p < 0.01$

lated plasma C-peptide levels. Thus, the predictive value of a stimulated C-peptide level above and below 0.60 nmol/l as indicator of Type 2 and Type 1 diabetes was 91% and 78%, respectively. A plasma C-peptide level of 0.60 nmol/l has previously been suggested to discriminate between Type 2 diabetic subjects with and without insulin requirement [8, 9]. In our study, approximately 75% of patients with a stimulated plasma C-peptide concentration from 0.32 nmol/l to 0.60 nmol/l were clinically Type 2 diabetic subjects. In another study, we discontinued insulin treatment in 25 patients with a stimulated value above 0.33 nmol/l [22]. After 3 months, only 4 patients had to resume insulin treatment due to hyperglycaemia. Ketonuria developed in none of the patients.

The predictive values of fasting C-peptide above or below 0.20 nmol/l were almost as high as those obtained using stimulated C-peptide above or below 0.32 nmol/l. Basal C-peptide and stimulated C-peptide, therefore, seem to give an almost similar information when used to distinguish between Type 1 and Type 2 diabetes. This agrees with our previous report of a very close correlation between fasting and glucagon stimulated plasma C-peptide in the same patients [23]. Welborn et al. also related basal C-peptide to clinical types of diabetes and found a basal C-peptide range from 0.16–0.32 nmol/l overlapping between Type 1 and Type 2 diabetes [24] corresponding to our value of 0.20 nmol/l.

Approximately 10% ($n=6$) of our patients with plasma C-peptide < 0.06 nmol/l were classified clinically as Type 2 diabetic subjects. These 6 patients had as few Type 1 clinical features as Type 2 diabetic subjects with higher C-peptide values (Table 3). In other studies, it has been suggested that undetectable C-peptide in plasma can be used as indicator of Type 1 diabetes [2]. Our findings reveal that classification based on clinical characteristics makes it difficult to differentiate between patients with Type 2 and slowly progressing Type 1 diabetes. The prevalence of patients diagnosed as Type 2 diabetic subjects in the post glucagon C-peptide range 0.06– < 0.32 nmol/l was also approximately 10%. It seems possible that these patients also were Type 1 diabetic subjects. Our data, therefore, suggest that a plasma C-peptide value post glucagon below 0.32 nmol/l might be used as a criterion of Type 1 diabetes in future studies. The finding that patients with a stimulated plasma C-peptide range from 0.06 to < 0.32 nmol/l showed a progressive decline in stimulated C-peptide values along with duration of diabetes while this was not the case in patients with higher C-peptide values seems to support such a classification of patients.

Plasma C-peptide post glucagon showed a significantly better distinction between Type 1 and Type 2 diabetes than urinary C-peptide if patients without B-cell function were excluded from the study. Similarly, Koskinen et al. reported that urinary C-peptide was less sensitive than post glucagon C-peptide in the discrimination between Type 2 diabetic subjects with and without insulin requirement [9]. The most important reason probably is a large variability of urinary C-peptide excretion [14]. This large variability is probably mainly due to the fact that only approximately 4% of secreted C-peptide from the pancreas is excreted in the urine [25]. Thus, a small change in the renal handling of C-peptide may result in a large change of the amount of C-peptide excreted in the urine [26]. Other factors of importance could be different blood glucose and plasma insulin levels during the urinary sampling with subsequent different amounts of stimulation [11, 27, 28] and inhibition of the islet B-cells [29, 30]. Similarly, standardisation of the pre-stimulatory blood glucose concentration might have increased the predictive values of C-peptide in plasma as indicators of Type 1 and Type 2 diabetes.

Including more C-peptide data in each patient did not influence the discrimination of patients. Thus, the distinction between Type 1 and Type 2 diabetes was unchanged if stimulated plasma C-peptide was combined with fasting plasma C-peptide or urinary C-peptide.

Agner et al. reported in a prospective study of basal plasma C-peptide during the first 3 years of Type 1 diabetes that total remission would last maximally 18 months after diagnosis [31]. After 2 years, median basal C-peptide levels were a little lower than at diagnosis. In our study, we therefore re-analysed data excluding patients with a duration of diabetes below 2 years since these patients might be in the so-called honeymoon phase. This procedure resulted in a better distinction between Type 1 and Type 2 diabetes since the predictive values of positive tests increased while the predictive values of negative tests were unchanged. It would be of interest to evaluate our predictive values in a prospective study of Type 1 and Type 2 diabetic subjects. In the study by Agner et al., less than 25% of newly diagnosed Type 1 diabetic subjects would reach fasting C-peptide levels above 0.30 nmol/1 during the honeymoon phase [31].

The urinary C-peptide excretion rate of 5.4 nmol/24 h gave the best discrimination between Type 1 and Type 2 diabetes. This value corresponds to an insulin secretion rate of 19 U/24 h (Fig. 2) using the information that approximately 4% of C-peptide secreted from the pancreas appears in the urine [25]. The excretion rate of 19 U/24 h is just below the range from 25–125 U/24 h reported in normal subjects [25]. Similarly, the fasting plasma C-peptide value of 0.20 nmol/l is in the lowest range from 0.15–1.43 nmol/l reported in 928 non-diabetic subjects [28].

In most diabetic subjects, the diagnosis of Type 1 or Type 2 diabetes is not difficult from clinical data of the patients [32]. However, we believe that measurement of basal C-peptide or C-peptide post glucagon may be of value in the choice of treatment for example in patients in whom insulin withdrawal is considered due to a de-

creasing need of exogenous insulin. Furthermore, measurement of C-peptide might be of value in dysregulated perhaps obese subjects on maximal doses of oral antidiabetic agents in whom insulin may be the correct choice of therapy.

We conclude that plasma C-peptide after glucagon stimulation and basal C-peptide give a good discrimination between clinical Type 1 and Type 2 diabetes, while 24 h urinary C-peptide seems to be less sensitive in this discrimination.

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