C-peptide Response to Glucagon

A Test for the Residual β-cell Function in Diabetes Mellitus

O. K. Faber, M.D., and C. Binder, M.D., Klampenborg, Denmark

SUMMARY

Pancreatic β-cell secretory activity was measured in 17 patients with insulin-dependent diabetes mellitus of less than 19 months' duration and in 10 nondiabetic subjects by means of the peripheral plasma C-peptide response to 1 mg. of glucagon I.V. The C-peptide response to a meal was also measured in the diabetic patients.

Residual β-cell function was present in all the diabetic patients as indicated by significant amounts of C-peptide in plasma. Significant increases in C-peptide were observed in 16 after glucagon stimulation and in 15 after the meal. Both absolute and relative increase in C-peptide were reduced in the diabetic patients. The increase in C-peptide was correlated to the fasting C-peptide concentration both after glucagon ($r = 0.86, p < 0.001$) and after the meal ($r = 0.66, p < 0.01$).

The responses to the meal and to glucagon were correlated ($r = 0.77, p < 0.005$), indicating a high predictive value of the glucagon test as to how the β-cells will respond during normal daily life. DIABETES *26*:605-10, July, 1977.

Peripheral plasma C-peptide concentrations have provided a measure of pancreatic β-cell function applicable to subjects treated with insulin and to subjects in whom circulating insulin antibodies interfere with radioimmunologic measurements of insulin.[1-3]

By means of C-peptide determinations, insulin secretory activity has been demonstrated in some subjects with insulin-dependent diabetes mellitus.[4-8]

This reduced β-cell function exerts metabolic effects.[9] A classification of insulin-treated diabetics according to the individual functional β-cell secretory capacity might be of as much importance in clinical situations as in research. A short test of the functional secretory capacity of the β-cells is required.

This report describes the β-cell response to glucagon stimulation in insulin-dependent subjects, measured by means of peripheral plasma C-peptide concentrations. The predictive value of this test as to how the β-cell will respond during normal daily life was determined by a comparison of the response to glucagon with that to a meal.

Presented in part at the Thirty-sixth Annual Meeting of the American Diabetes Association (DIABETES *25*:329, 1976).

From Hvidøre Hospital, Klampenborg, Denmark.

Address reprint requests to O.K. Faber, Hvidøre Hospital, Emiliekildevej 1, DK-2930 Klampenborg, Denmark.

Accepted for publication December 10, 1976.

MATERIAL AND METHODS

Seventeen subjects with insulin-dependent diabetes mellitus were investigated on two consecutive days after one to 19 months (average seven months) of insulin treatment (table 1). The clinical diagnosis was established at the time of examination according to the following criteria: random blood glucose concentration higher than 12 mmol/L., significant ketonuria (Ketostix), body weight below 110 per cent of the ideal for sex and height,[10] and age less than 50 years. The material comprised all subjects with insulin-dependent diabetes mellitus referred to the Hvidøre hospital during the period June 1, 1974, to May 31, 1975. The patients were treated with intermediate-acting insulin preparations administered as one dose in the morning. At the time of study, one subject (OS) was treated only with diet and tolbutamide because of a partial remission of his disease. In the other subjects daily insulin requirements ranged from 6 to 44 I.U., mean 23 I.U.

The subjects were studied after an overnight fast. On the first day, free-flowing venous blood was ob-

tained 30, 10, and 0 minutes before and 30, 60, 90, and 120 minutes after the start of a morning meal composed of 40 per cent fat, 40 per cent carbohydrate, and 20 per cent protein, which amounted to 15 per cent of the total calories in the individual diets. On the second day, free-flowing venous blood samples were taken 10, 5, and 0 minutes before and 2, 4, 6, 8, 10, 15, and 20 minutes after an intravenous bolus injection of 1 mg. porcine glucagon (Novo).

The glucagon stimulation test was also carried out in 10 nondiabetic normal-weight subjects, 18 to 32 years of age (average 27 years).

Blood glucose concentration (BG) was measured by a glucose oxidase method. The circulating-insulin-antibody concentration was measured as the insulin binding to IgG.[11] Plasma insulin concentration was measured in the normal subjects by the method of Heding.[12]

Plasma C-peptide concentration was measured as described by Heding[13] with the antibody M 1230.[14] In this assay and with this antibody, proinsulin cross reacts no more than 13 per cent with C-peptide on a molar basis in the concentration range up to 1.0 pmol/ml. (unpublished observation). All the samples from any single study were analyzed in the same assay. The within-assay coefficient of variation in the low part of the working range of the C-peptide assay was 0.054.[14] As a consequence, increases in C-peptide concentration, after stimulation with the meal or glucagon, exceeding 16.2 per cent of the fasting level were considered significant. The effective detection limit of the C-peptide assay was 0.06 pmol/ml. This limit was defined as the upper range of values measured in six pancreatectomized patients.

Statistical analysis of comparisons between groups was carried out by means of the Mann-Whitney rank-sum test. Significance of correlations were tested by means of Spearman's rank correlation test; 5 per cent was accepted as the level of statistical significance.

RESULTS

In the normal subjects C-peptide concentrations increased after glucagon from 0.36 pmol/ml. (range 0.26-0.63 pmol/ml.) to 1.28 pmol/ml. (range 0.91-1.88 pmol/ml.), with a mean relative increase of 274 per cent (range 130-377 per cent) (figure 1). Insulin concentration increased from 0.09 pmol/ml. (range 0.03-0.15 pmol/ml.) to 0.45 pmol/ml. (range 0.28-0.76 pmol/ml.), with a mean relative increase of 473 per cent (range 213-833 per cent). Individual insulin and C-peptide concentrations were significantly correlated in all, mean r = 0.82 (range 0.67-0.93).

TABLE 1

Individual clinical data, blood glucose concentration (BG), and C-peptide concentration (CP) before and after a meal and 1 mg. of glucagon I.V. "IgG" = insulin binding to IgG

		Age at onset,	Dura-tion,	Dose of insulin	IgG	Meal test				Glucagon test			
						BG mmol/L.		CP pmol/ml.		BG mmol/L.		CP pmol/ml.	
Subject	Sex	years	months	IU	mU./ml.	fasting	peak	fasting	peak	fasting	peak	fasting	peak
JL	M	13	11	16	0.277	6.0	18.8	0.06	0.13	5.9	10.0	0.06	0.10
MR	F	49	11½	24	0.204	10.7	19.4	0.16	0.32	10.6	13.3	0.14	0.25
MC	M	36	11	20	0.196	9.4	19.2	0.12	0.26	4.5	6.8	0.10	0.13
HR	M	36	19	36	2.625	12.8	16.4	0.34	0.39	9.3	11.4	0.25	0.41
OS	M	39	9¾	0	0.006	10.1	16.7	0.32	0.66	12.6	16.4	0.41	0.78
IN	F	15	9	32	0.844	11.7	20.8	0.15	0.20	15.4	15.5	0.14	0.26
CZ	M	25	10¼	44	0.124	12.6	20.2	0.12	0.13	13.6	15.8	0.12	0.12
GL	M	29	7½	28	0.762	11.7	19.4	0.09	0.12	16.1	17.5	0.11	0.13
HO	F	33	8½	20	0.000	21.1	29.0	0.17	0.20	16.9	21.8	0.15	0.21
TE	M	27	7	22	0.044	7.2	14.2	0.24	0.64	5.9	10.6	0.22	0.57
FH	M	48	2	36	0.005	14.8	20.1	0.13	0.14	6.5	11.0	0.06	0.08
PL	M	47	5¼	12	0.007	8.5	16.1	0.12	0.16	12.8	15.7	0.20	0.42
BA	M	25	1¼	18	0.013	10.0	12.9	0.30	0.47	8.5	13.8	0.24	0.56
PN	M	37	1¼	26	0.000	11.4	15.3	0.19	0.35	9.5	12.5	0.25	0.45
BP	M	44	2	16	0.002	6.6	11.6	0.40	0.94	6.1	8.6	0.35	0.61
ON	M	30	2¼	14	0.002	7.0	12.8	0.21	0.73	4.4	8.3	0.17	0.37
FK	M	13	2	6	0.153	7.3	14.2	0.19	0.31	6.3	9.4	0.18	0.38
Mean	—	32	7	23	0.307	10.5	17.5	0.19	0.36	9.7	12.8	0.18	0.34
Range	—	13-49	1¼-19	0-44	0.000-2.625	6.0-21.1	11.6-29.0	0.06-0.40	0.12-0.94	4.4-16.1	6.8-21.8	0.05-0.41	0.08-0.78

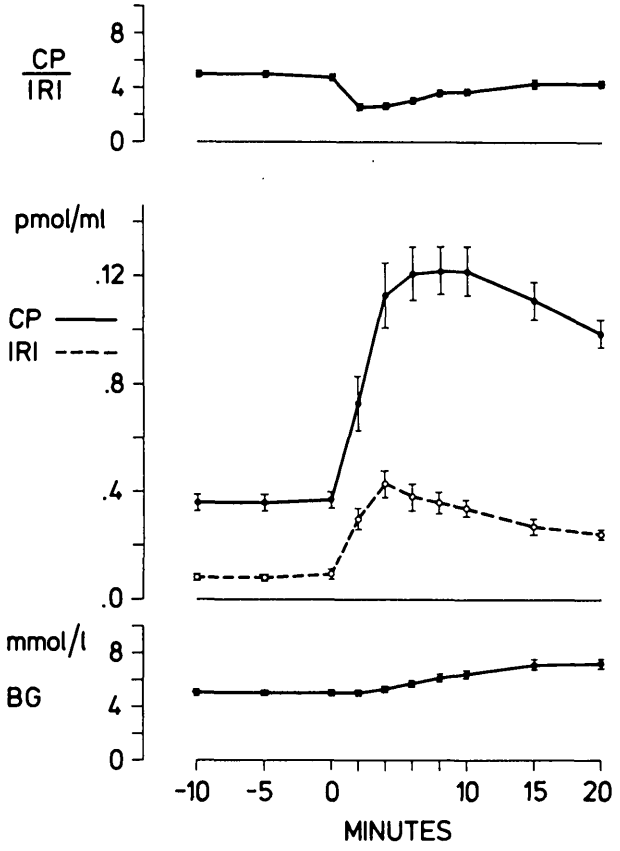

FIG. 1. Blood glucose (BG), insulin (IRI), and C-peptide (CP) concentrations (mean ± 1 × S.E.M.) before and after 1 mg. of glucagon I.V. (at time 0) in 10 normal subjects. The upper panel shows the molar ratio between C-peptide and insulin.

The molar ratio between C-peptide and insulin was 4.9 (range 2.9-10.1 dl.) in the fasting state. Two minutes after stimulation with glucagon a significant fall ($p < 0.01$) to 2.5 was observed. Thereafter the molar ratio increased steadily without reaching prestimulatory levels within the 20 minutes.

In the diabetic subjects (figure 2 and table 1) plasma C-peptide concentrations exceeded the effective detection limit of 0.06 pmol/ml. C-peptide concentration increased significantly after glucagon in all but one (CZ). The mean relative increase after glucagon was 76 per cent (range 0-157 per cent). Both fasting and maximum C-peptide concentrations and the relative increase after glucagon were significantly higher in the normal than in the diabetic subjects ($p < 0.01$). The average time to maximum C-peptide concentrations in the normal subjects (mean 8.7, range 4-15 minutes) was significantly ($p < 0.01$) longer than observed in the patients (mean 5.0, range 2-8 minutes).

After the meal, C-peptide concentration increased significantly in all patients but two (CZ and FH) (figure 2 and table 1). The mean relative increase was 92 per cent (range 8-248 per cent).

The C-peptide response to both the meal and glucagon, expressed as the maximum increase in C-peptide concentration after stimulation, was correlated to the fasting C-peptide concentration ($r = 0.66, p < 0.01$ after meal, $r = 0.86, p < 0.001$ after glucagon) (figure 3). The responses to the two tests were mutually correlated ($r = 0.77, p < 0.005$) (fi-

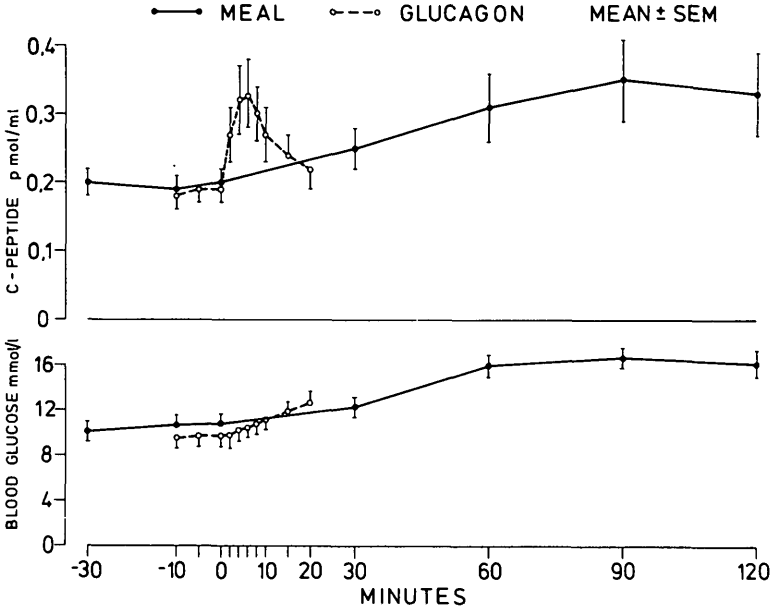

FIGURE 2

Blood glucose and C-peptide concentrations (mean ± 1 × S.E.M.) before and after meal and 1 mg. of glucagon I.V. in 17 insulin-dependent diabetic subjects.

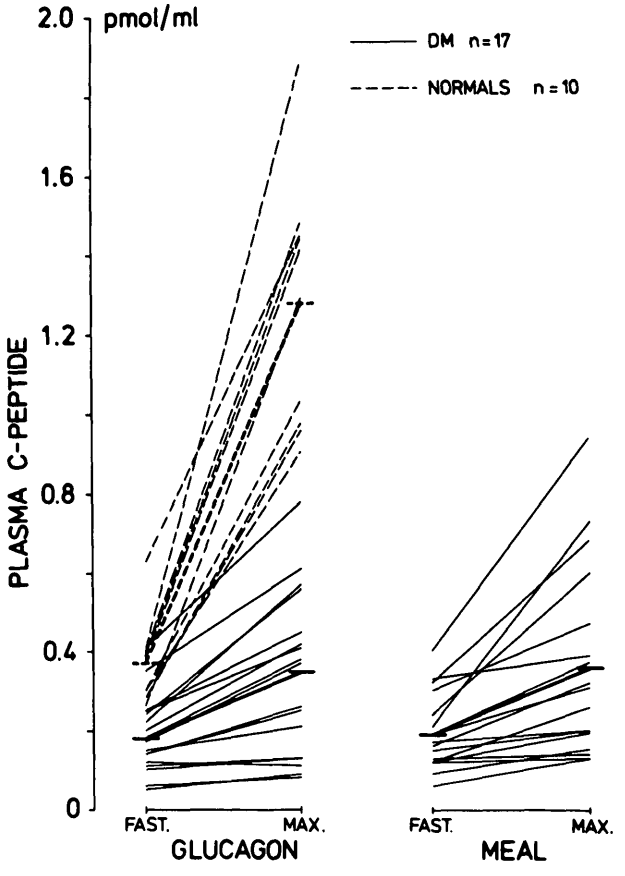

FIG. 3. Plasma C-peptide concentration before (Fast.) and maximum values after (Max.) 1 mg. of glucagon I.V. and a meal in 17 insulin-dependent patients and in 10 non-diabetic subjects. Heavy lines indicate mean values.

gure 4). The majority of the diabetics showed maximum concentrations of C-peptide 120 minutes after the meal and six minutes after glucagon. The same correlations were found when the C-peptide response to these fixed time points were calculated.

Average fasting blood glucose concentrations before the meal and glucagon test were not significantly different (table 1). Individual values of fasting blood glucose concentrations varied, however, with concomitant variations in fasting C-peptide ($r = 0.70, p < 0.01$). The increase in C-peptide concentration after the meal and glucagon were not correlated with the fasting blood glucose concentrations before the two tests.

DISCUSSION

Not only has a preserved β-cell function been demonstrated in insulin-dependent diabetes mellitus,[4-8] but it has also been shown that this, even minimal, function exerts metabolic effects.[9] It is therefore important, in future studies of intermediary metabolism in insulin-dependent diabetics, to have a quantitative as well as a qualitative measure of the functional secretory capacity of the pancreatic β-cells.

A simple test procedure for the insulin-secretory activity of the pancreatic β-cells applicable to subjects with insulin-treated diabetes mellitus should ideally be sensitive, specific, easy to perform, not time-consuming, physiologic, and without risk and discomfort to the patients. It should also have a high predictive value as to how the β-cells will respond during normal daily life.

The true physiologic stimulus to the β-cells is a meal composed like the meals the patients eat as a part of their prescribed diet. As, however, the diabetes diet varies both with time and between different diabetes clinics, a more reproducible stimulus to the β-cells

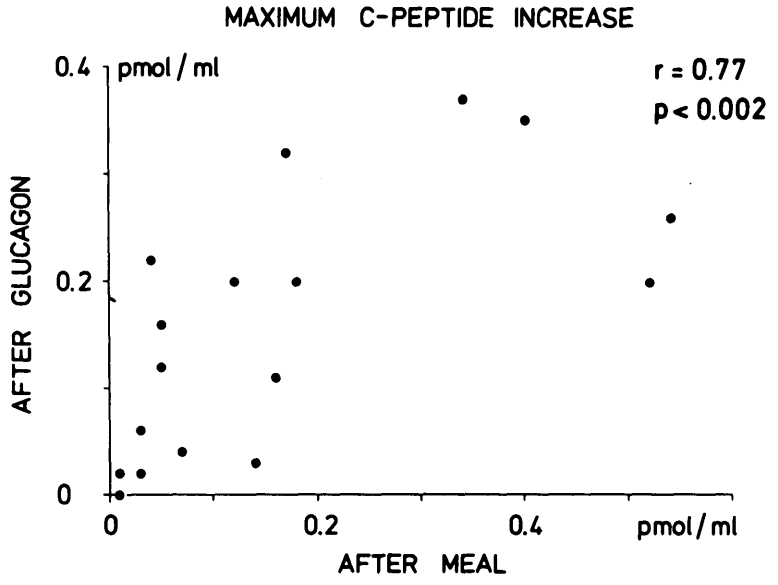

FIGURE 4

Correlation between maximum increase in plasma C-peptide concentration after a meal and after 1 mg. of glucagon I.V. in 17 insulin-dependent patients.

should be considered. Glucagon, a potent stimulator of insulin secretion,[15] fulfills the criteria mentioned above except that, in the concentrations used, it is not physiologic.

The sensitivity and specificity of a β-cell function test mostly depend on how the β-cell response to stimulation is measured. Quantitative estimation of endogenous insulin is hampered by circulating insulin antibodies, which interfere in the radioimmunoassay of insulin, and by the exogenously administered insulin. Radioimmunoassay of human C-peptide has provided a specific measure of the β-cell function even in insulin-treated diabetics with insulin antibodies.[1-3] In such patients codetermination of human proinsulin in the C-peptide assay may, however, be a problem.

In plasma devoid of insulin antibodies, human proinsulin is not codetermined in the C-peptide assay used in this study, because plasma, before being assayed, is preincubated with excess of insulin antibodies bound to Sepharose, whereby free proinsulin is removed.[13]

Inappropriately elevated concentrations of human proinsulin may be found in diabetic patients with intact β-cell function and circulating-insulin antibodies induced by exogenous insulin. In such patients proinsulin is bound to the insulin antibodies. This leads to an accumulation of proinsulin in plasma due to the prolonged disappearance time of the proinsulin-antibody complexes.[16] It also reduces the effectivity of the solid-phase-antibody removal of proinsulin. If significant amounts of human proinsulin had been codetermined in these 17 patients an inverse correlation between the insulin-antibody level, as determined by the insulin binding to IgG, and relative C-peptide increase would have been expected. This was, however, not found, suggesting that the contribution of endogenous human proinsulin to the plasma C-peptide concentration was negligible. The low cross reactivity of human proinsulin in this C-peptide assay further supports this concept.

Plasma from all 17 patients contained significant amounts of C-peptide. C-peptide concentration increased after the meal in all but two, indicating that these insulin-dependent diabetics secrete C-peptide, and thus presumably insulin, during normal daily life.

Fasting and maximum mean values after glucagon were nearly identical with those before and after the meal, suggesting that the easier and less time-consuming glucagon test is of equal value in the assessment of pancreatic β-cell secretory activity. This concept is also supported by the significant correlation between fasting C-peptide and increase in C-peptide during the two tests (figure 3) and is further strengthened by the significant correlation between the increase in C-peptide during the two tests (figure 4).

If an even simpler test is desired, we would suggest glucagon as the stimulator, but only with measurement of C-peptide concentration just before the injection and six minutes after, as these values gave nearly identical results with the 30-minute test. The simplest way to obtain an impression of the β-cell function would be to measure the fasting C-peptide concentration, since it is correlated to the increase after stimulation (figure 3). Thus, in this material of 17 adolescent and adult insulin-dependent subjects, all patients with fasting C-peptide concentration higher than 0.13 pmol/ml. showed a significant increase in C-peptide concentration after breakfast. When the fasting C-peptide concentration alone is taken into account, the concomitant fasting blood glucose concentration should be considered, because the individual fasting C-peptide concentration varied with that of blood glucose.

It has been shown that the insulin response to glucagon is directly related to the prevailing plasma glucose concentration in nondiabetic subjects.[17] Such a relationship was not found in these insulin-dependent diabetics, presumably because other factors, such as the duration of the disease, determine the functional capacity of the β-cells. The possible effect of the metabolic control on the β-cell function in insulin-dependent diabetic subjects remains, however, to be elucidated.

ACKNOWLEDGMENTS

We are indebted to Miss B. Mathiassen and Miss J. Falk for their technical assistance.

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