Insulin Therapy in Relation to Circulating C-peptide Levels

W. Beischer, M.D., W. Kerner, M.D., S. Raptis, M.D., L. Keller, Ph.D., B. Beischer, M.D., and E. F. Pfeiffer, M.D., Ulm, Germany

SUMMARY

Part 1. **Beta-cell secretion was stimulated maximally by an intravenous glibenclamide-glucose load in four groups of 10 adult-onset diabetics divided according to their therapy, which was determined by clinical criteria. Under the standardized conditions of this test, the 20 patients requiring insulin had a mean fasting blood glucose (BG) of 255 mg./100 ml. and showed no stimulation of immunoassayable C-peptide (IMCP) by glibenclamide-glucose. In contrast, the mean fasting BG was 160 mg./100 ml., but the IMCP rose in patients who were able to be managed with sulfonylurea tablets. These differences in IMCP proved to be of value for the clinician in predicting the efficacy of diabetes therapy with either insulin or sulfonylurea tablets.**

Part 2. **Twenty-four-hour profiles of BG and IMCP were performed in 28 insulin-requiring diabetics (age range: 14 to 74 years) who were 66 to 111 per cent of normal weight and had had diabetes for 0.2 to 31 years. These patients were divided into diabetics *with* IMCP (n=14) and *without* IMCP (n=14); the mean age and weight were similar in the two groups. The mean IMCP value and its standard deviation correlated inversely with the mean blood glucose (MBG) in all diabetics and healthy subjects. According to clinical criteria, diabetes control was better in the patients *with* IMCP. Analysis of variance showed that the mean BG concentrations for diabetics *with* and *without* IMCP were statistically different ($p<0.02$). Patients *with* IMCP were found to inject less insulin daily, and six of them were adequately treated with only one daily injection.** DIABETES *27* **(Suppl. 1):235-40, 1978.**

Oral and intravenous sulfonylurea-response tests for predicting the outcome of long-term treatment with sulfonylurea agents were suggested by Pfeiffer et al.[356,359,360] and others.[54,520] At that time methods were not available for assessing residual beta-cell function in insulin-treated patients who had developed circulating insulin antibodies. In the first part of this study we examined the response of immunoassayable C-peptide (IMCP) to an intravenous glibenclamide-glucose load in diabetic patients in order to predict which patients would be manageable with glibenclamide tablets and which would require insulin.

The work of Molnar et al.[309,310,380,418] has been important in assessing the degree of diabetic control and instability. Methods to quantify the quality of glucose control have also been devised by other authors.[176,199,203,204,409] In the second part of this study, IMCP was determined in insulin-requiring diabetics at standardized intervals and blood glucose (BG) levels were monitored continuously for 24 hours. These results were correlated with several criteria of diabetic control and to the patients' insulin dose.

From the Department of Endocrinology and Metabolism, Center of Internal Medicine, Pediatrics and Dermatology, University of Ulm, Germany.

MATERIAL AND METHODS

Material and Patients

Glibenclamide-glucose test. Forty adult diabetics admitted to our metabolic unit who had been treated with sulfonylureas or insulin were studied. Therapy with glibenclamide was discontinued if postprandial blood glucose values exceeded 200 mg./100 ml. and/or urine glucose 10 gm./24 hours. An intravenous glibenclamide-glucose test was performed in all patients under standardized conditions.

These patients were divided into four groups. Group 1: patients receiving insulin on admission and on discharge (insulin-insulin); group 2: patients re-

TABLE 1

Median values and range for clinical data of 4 groups of diabetic patients (I to IV) subjected to an i.v. glibenclamide-glucose load.

group of patients	sex	% of normal weight	age at examination (years)	duration of diabetes (years)	duration of insulin treatment (years)
I: insulin–insulin (n=10)	5 ♀ 5 ♂	102 77–112	52.5 36–74	10 1–33	–
II: tablets–insulin (n=10)	7 ♀ 3 ♂	94.5 71–133	67 42–78	11.5 1–15	–
III: insulin–tablets (n=10)	4 ♀ 6 ♂	113 84–186	63 50–76	11 2–16	3 0.3–10
IV: tablets–tablets (n=10)	6 ♀ 4 ♂	106 95–125	61.5 31–71	7.5 0–19	–

ceiving sulfonylureas on admission and insulin on discharge (tablets-insulin); group 3: patients receivin insulin on admission and sulfonylurea tablets on discharge (insulin-tablets); group 4: patients receiving sulfonylureas on admission and on discharge (tablets-tablets). The only criteria for selecting these 40 patients were a well-documented medical history, absence of other endocrine diseases, and a normal serum creatinine value.

The clinical data are shown in table 1. For comparison, glibenclamide-glucose tests were also performed in six healthy volunteers, aged 22 to 27 years, within 85 to 99 per cent of normal weight.[25]

Twenty-four-hour profile. IMCP and BG were determined at standardized intervals over 24 hours during continuous monitoring. The criteria for selecting these patients were the same as for the previous group.

The clinical data of these diabetics are given in table 2. The patients were divided into diabetics *with* (n=14) and *without* (n=14) IMCP according to their mean IMCP concentration obtained during the 24-hour profile and its standard deviation. A mean IMCP level of 1 ng. per milliliter ($33\times1;^{-11}$ mol per liter) or a standard deviation of 0.25 ng./ml. (8.3×10^{-11} mol per liter) were arbitrarily chosen as the limits to divide the groups. The distribution of age and weight was similar within both groups. Insulin (table 2, columns 7 and 8) was given in an intermediate-acting form in one subcutaneous injection before breakfast (six patients *with* IMCP) or in two injections, one before breakfast and one before dinner. In most diabetics receiving two injections, regular insulin was also given in combination. Five to 10 determinations of the fasting BG, the BG one to two hours after breakfast, 24-hour glucose excretion, and urinary excretion of ketone bodies were used for classifying the quality of metabolic control. Control was considered *good* if 90 per cent of all values fell within the following limits: 100 to 150 mg./100 ml. for fasting glucose, 100 to 200 mg./100 ml. for postprandial glucose, 0 to 20 gm./24 hours for urinary glucose excretion, and if there was no ketonuria. Control was con-

TABLE 2

Median values and range for clinical data of insulin requiring diabetics subjected to 24 hour profiles of blood glucose (BG) and immunomeasurable C-peptide (IMCP).

group of patients	sex	% of normal weight	age at examination (years)	age at manifestation (years)	duration of diabetes (years)	duration of insulin treatment (years)	insulin therapy (U/24 h)	insulin therapy (U/kg/24h)	Metabolic Control	
"with" IMCP	8 ♀	90	32	23	5	1,8	27	0.55	good	: 4
(n=14)	6 ♂	68-108	14-74	13-61	0.2-30	0.1-30	15-72	0.22-1.07	tolerable	: 8
									bad	: 2
"without"	12 ♀	94	27	19	6.5	6	43	0.71	good	: 0
IMCP	2 ♂	72-111	16-68	2-64	1-31	1-31	18-84	0.3-1.53	tolerable	: 5
(n=14)									bad	: 9

sidered *tolerable* if at least 50 per cent and as *bad* if less than 50 per cent of all determinations were within these limits.

For comparison, 24-hour profiles were also done in four healthy volunteers (21 to 23 years; 76 to 92 per cent of normal weight).

Methods

Glibenclamide-glucose test. After an overnight fast and discontinuing insulin or sulfonylureas for 24 hours, 2 mg. glibenclamide and 0.33 gm. glucose per kilogram body weight were given intravenously within two minutes. The times of blood sampling are shown in figure 1.

Twenty-four-hour profile. Continuous BG monitoring was started between 7°° and 8°°. All subjects had lunch at 13°°; the other times of food intake as well as the times of insulin injection varied within the limits of one hour (figure 3). Periods of exercise were not included in the profiles.

IMCP was determined in 23 timed samples during the 24-hour profiles. From these data the mean value for each subject (MIMCP) and its standard deviation (SD-IMCP) was calculated (figure 2), as were the mean levels at similar time points for each group of subjects (figure 3). The BG concentration was read from the continuously monitored profiles at the 23 selected times, and the individual's mean concentration of blood glucose (MBG) as well as the mean levels for the groups of subjects were calculated.

The diet comprised the individual's basal caloric requirement plus 30 per cent. Breakfast, lunch, and dinner were the major meals, consisting of 20, 30 and 25 per cent of the total kcal's, respectively. The kcals were made up of 40 per cent carbohydrate, 40 per cent fat, and 20 per cent protein. The patients were given their usual insulin dose.

Determination of BG and IMCP. Single determinations of blood and urine glucose were carried out according to the GOD-Perid method.[412,499] Continuous BG monitoring was performed either by an Auto-Analyzer (Technicon, Bad Vibel, Germany) and the hexokinase/glucose-6-phosphatase-dehydrogenase method[413] or by the analyzer unit of the artificial beta-cell (Biostator, Miles, Elkhart, Ind.).[23,70] The methods for the determination of IMCP and of its proinsulin portion have been described previously.[24,262] The lower detection limit of IMCP was 0.5 ng. per milliliter (16.5×10^{-11} mol per liter).

Statistical analysis. Analysis of variance and covariance, including repeated measures, was carried out according to the Health Science Computing Facilities, University of California, Los Angeles.

RESULTS

Glibenclamide-glucose test. Following intravenous

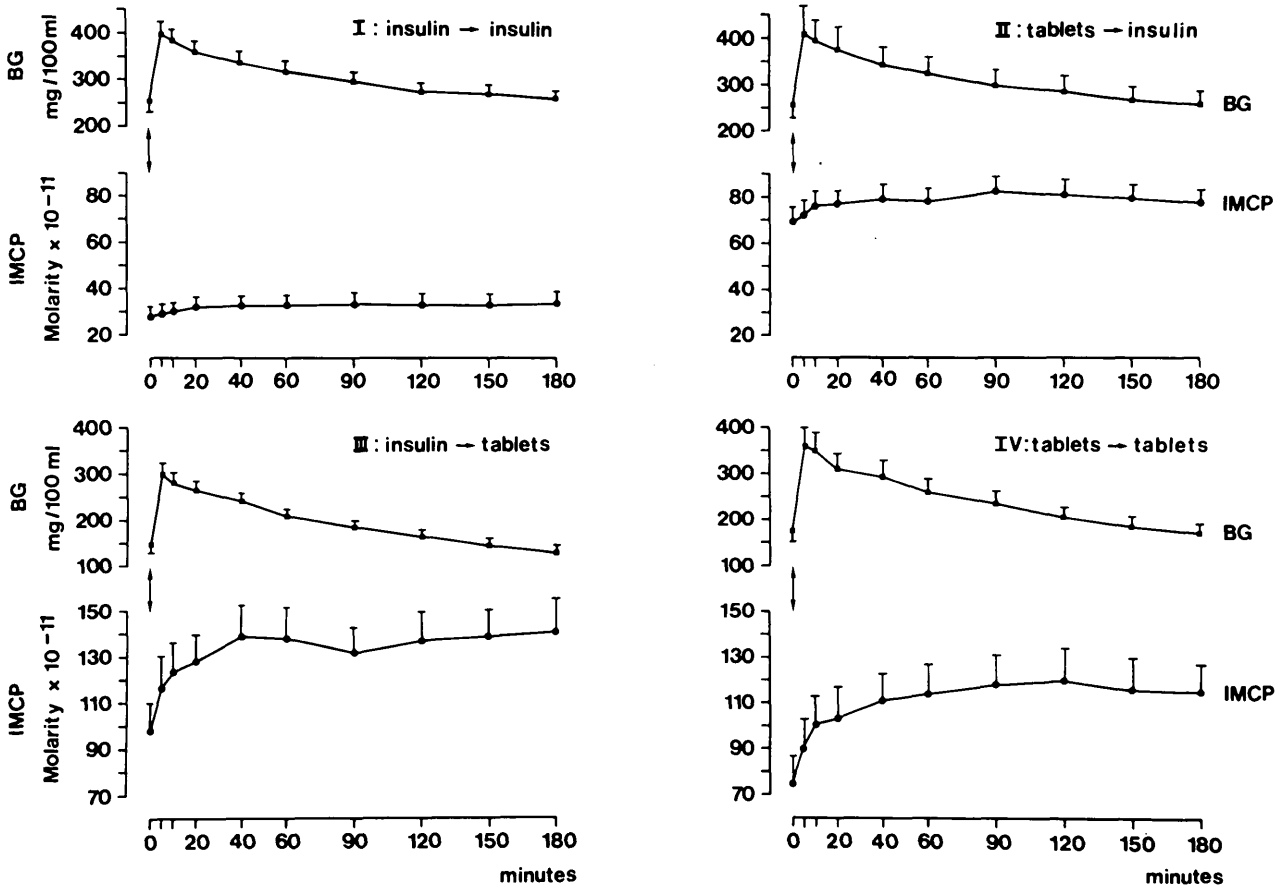

FIG. 1. Blood glucose (BG) and immunoassayable C-peptide (IMCP) concentrations after stimulation (↕) with glibenclamide-glucose intravenously (2 mg.-0.33 gm./kg.) in diabetics divided into four groups (n=10 each) according to therapy on admission and on discharge with either insulin or sulfonylurea tablets. Mean values + S.E.M.

glibenclamide-glucose the mean BG concentrations were much higher for the diabetics requiring insulin (groups 1 and 2) than for the patients requiring sulfonylureas only (groups 3 and 4) (figure 1). The BG pattern was similar in the four groups of diabetics, rising to a maximum at 5 minutes, followed by a steady fall to the fasting level at 180 minutes. The mean IMCP concentration was highest in group 3 followed by 4, 2, and finally 1 (figure 1). IMCP responded to glibenclamide-glucose in the patients who could be treated with sulfonylurea tablets (groups 3 and 4), and the mean IMCP levels were still maxi-

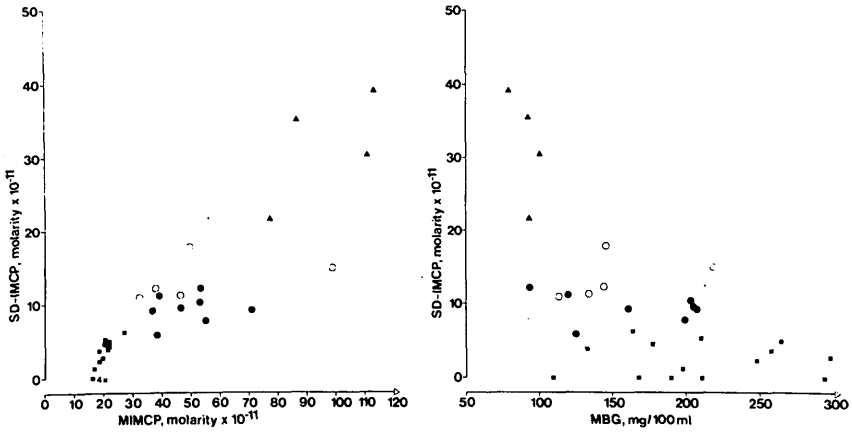

FIGURE 2

Correlation of the individual mean immunoassayable C-peptide (MIMCP, left side) and blood glucose (MBG, right side) with the standard deviation of MIMCP (SD-IMCP). The values were calculated from 24-hour profiles in healthy subjects (▲) and in three groups of diabetics: o diabetics *with* IMCP, one insulin injection per day; ● diabetics *with* IMCP, two insulin injections per day; and ■ diabetics *without* IMCP.

mally raised at 180 minutes in these diabetics. However, an absent or very low response to glibenclamide-glucose was found in the insulin-requiring diabetics (groups 1 and 2).

In healthy subjects the mean BG rose from 77 mg./100 ml. at zero minutes to a maximum of 206 mg./100 ml. at 5 minutes; thereafter the mean value declined to 52 mg./100 ml. at 90 minutes and then returned to 75 mg./100 ml. at 180 minutes. IMCP rose from 45×10^{-11} mol per liter at zero minutes to peak at 158×10^{-11} mol per liter at 40 minutes and returned to 40×10^{-11} mol per liter at 180 minutes.

Twenty-four-hour profile. Patients *with* and *without* IMCP had a similar duration of diabetes, whereas the duration of insulin therapy and the amount of insulin required differed more between the groups (table 2). The four patients with *good* and the majority of patients (8 of 13) with *tolerable* metabolic control belonged to the group *with* IMCP. The majority of patients (9 of 11) in *bad* metabolic control were in the group *without* IMCP (table 2). A subgroup of six patients *with* IMCP injected insulin only once daily; three of these patients were in *good* and three in *tolerable* metabolic control.

An inverse correlation between the mean blood glucose (MBG) and the standard deviation of the mean IMCP (SD-IMCP) in the healthy subjects and all diabetics is obvious from the right side of figure 2. A tendency for lower MBG is seen in the group of patients *with* than the patients *without* IMCP. A correlation between the individual mean IMCP (MIMCP) and its standard deviation (SD-IMCP) in the healthy subjects and all diabetics can be seen from the left side of figure 2.

The mean BG concentration was highest for the diabetics *without* IMCP, followed by the diabetics *with* IMCP and two daily insulin injections (except for the period between 9°° and 11°°), the diabetics *with* IMCP and one daily insulin injection and finally the healthy subjects (figure 3, upper half). The opposite result was found for the mean IMCP concentration (figure 3, lower half). The changes in BG following meals were of similar magnitude in all the diabetic groups and were greater than in healthy volunteers. The swings of IMCP following meals were absent in the patients *without* IMCP, were small but delayed in the patients *with* IMCP and two daily insulin injections, and were still subnormal and delayed in the diabetics *with* IMCP and one daily insulin injection.

The mean BG concentrations during the period between 13°° to 17°° in the diabetics *with* and *without* IMCP were subjected to an analysis of variance. The probability of the error of the difference found was below 2 per cent. No influence on the BG concentration was detected when the total amount of insulin injected was introduced as a covariate. Interactions between times and groups were not significant.

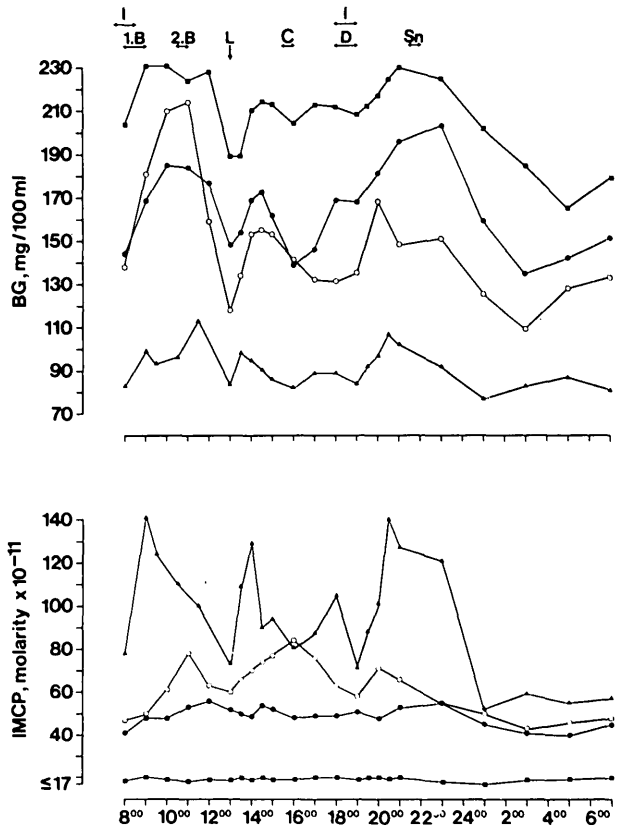

FIG. 3. Mean concentrations of blood glucose (BG, upper half) and immunoassayable C-peptide (IMCP, lower half) during 24-hour profiles in healthy subjects and three groups of diabetics. ▲ healthy subjects, mean standard deviation of IMCP (MSD-IMCP)=25.0, mean standard deviation of BG (MSD-BG)=13.8, n=4; o diabetics *with* IMCP, one insulin injection per day, MSD-IMCP=42.1, MSD-BG=50.9, n=6; • diabetics *with* IMCP, two insulin injections per day, MSD-IMCP=14.6, MSD-BG=64.9, n=8; ■ diabetics *without* IMCP, MSD-IMCP=4.7, MSD-BG=76.1, n=14. Arrows indicate: I=insulin injections, 1.B=first breakfast, 2.B=second breakfast, L=lunch, C=coffee, D=dinner, Sn=snack.

DISCUSSION

Insulin-requiring diabetics had a higher fasting BG level than patients treated successfully with oral sulfonylureas and showed no response of IMCP during a standardized glibenclamide-glucose test. The lack of response of IMCP might be explained by maximal stimulation of beta cells by the high fasting BG concentrations. In clinical practice the response of IMCP to the glibenclamide-glucose stimulus proved to be valuable for predicting the response to therapy with

either insulin or sulfonylureas. Further details concerning the results of the glibenclamide-glucose test have been described previously.[25]

The 24-hour profiles were designed to determine the relationship between the quality of diabetic control and the capacity of residual beta-cell secretion in insulin-requiring diabetics of various ages and duration of disease.

The blood glucose level, urine glucose excretion, and ketonuria were chosen for the clinical classification of diabetic control, which was graded as *good*, *tolerable*, or *bad*.[100,286] The mean blood glucose (MBG) was also included as an additional measure of the adequacy of control. We did not have sufficient data for calculating the other values derived from blood glucose patterns by way of continuous monitoring, as suggested by Molnar and co-workers.[310,418] The MBG depends, to some extent, on the intensity of therapy with insulin and may fall because of hypoglycemic episodes.[418] All our patients were taking intermediate-acting insulin once or twice daily, the majority in combination with regular insulin. BG levels of below 50 mg./100 ml. during the 24-hour profiles were found in four patients *with* as well as in four diabetics *without* IMCP. Hence the influence of exogenous insulin on MBG was similar in both groups of patients.

To characterize the residual beta-cell function, the mean immunoassayable C-peptide (MIMCP) was calculated similarly to the MBG. As a measure of the variation of IMCP and thus of the capacity of the beta cell to be stimulated, the standard deviation of MIMCP (SD-IMCP) was introduced. An inverse correlation was demonstrated between MBG and SD-IMCP and between MBG and MIMCP, although to a lesser degree. The following consideration may explain this observation: in each patient MBG will correlate positively with MIMCP provided that residual beta-cell function is not exhausted; however, the correlation of MBG with SD-IMCP will depend on the absolute MBG level and may well be inverse. Hence MBG, MIMCP, and SD-IMCP are all interrelated in a complex fashion.

The influence of residual beta-cell function on diabetes control was also evaluated in the groups *with* and *without* IMCP. The limits of MIMCP (1 ng./ml.) and SD-IMCP (0.25 ng./ml.) for the classification *with* and *without* were arbitrarily chosen. Of 28 diabetics examined, only four showed a total lack of residual beta-cell function.

Studies done in our laboratory[262] and by Ludvigsson and Heding[201] showed a higher incidence of detectable IMCP concentrations in juvenile-onset diabetes of shorter duration. The duration of diabetes was similar in the two groups (5 vs. 6.5 years), but the duration of insulin therapy differed markedly between insulin-requiring diabetic patients of all age groups *with* and *without* IMCP. This implies that patients who could be treated with diet and sulfonylureas before requiring insulin have a slower deterioration of beta-cell function than those requiring insulin from the time of diagnosis.

With the use of insulin bioassays a decreased insulinogenic reserve was noted in adult-onset diabetics.[357,358,416] The delayed response of the low mean IMCP concentration to meals in the insulin-requiring diabetics as compared with the healthy subjects confirms this finding.

Our goal in the diabetics studied with 24-hour profiles was to achieve optimal control. The six patients who received only one daily insulin injection were found to belong to the group *with* IMCP and, as a subgroup, showed a higher mean IMCP level than the diabetics *with* IMCP who required two daily injections. Metabolic control was *good* in three of them and *tolerable* in the other three. MBG was below 150 mg./100 ml. in five, the mean BG level of this subgroup being closest to that of the healthy subjects. These data imply that patients who can be adequately treated with one daily injection of intermediate-acting insulin (alone or in combination with regular insulin) have especially well-preserved beta-cell function.

The daily insulin dosage was lower in diabetics *with* than in those *without* IMCP. This observation confirms the report of Faber and Binder,[100] who found an inverse correlation of IMCP with the insulin requirement and MAGEM in insulin-dependent diabetics within the first month of insulin treatment.

We conclude that the response to therapy with either sulfonylureas or insulin in one or two daily injections as well as the quality of diabetic control is essentially determined by the extent of residual beta-cell secretory function.

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