Observations on C-Peptide and Free Insulin in the Blood During Continuous Subcutaneous Insulin Infusion and Conventional Insulin Therapy

R. M. BERGENSTAL, J. DUPRE, P. M. LAWSON, R. A. RIZZA, AND A. H. RUBENSTEIN FOR THE KROC COLLABORATIVE STUDY GROUP

SUMMARY

As part of a multicenter trial, 70 individuals with insulin-dependent diabetes were randomized to either conventional insulin therapy (CIT) or continuous subcutaneous insulin infusion (CSII). In order to standardize patient selection in the six participating centers, one of the eligibility criteria was the demonstration that each patient had no residual endogenous insulin secretion as assessed by plasma C-peptide determinations. The patients were of average (±SEM) age of 33.0 ± 1.6 yr, had had diabetes for a mean (±SEM) duration of 17.4 ± 1.1 yr, and had both fasting and postglucagon stimulation C-peptide values of <0.1 pmol/ml, consistent with clinically insignificant endogenous insulin secretion. There was no change in C-peptide response at 4 or 8 mo compared with baseline values, whether or not the patient's glucose control remained unchanged (CIT group) or significantly improved to near-normoglycemia (CSII group). In a subgroup of 34 patients at three centers, the 24-h mean glucose concentration was significantly lower in the CSII group compared with the CIT group at 4 mo (126 ± 10 versus 176 ± 14 mg/dl) and at 8 mo (121 ± 5 versus 183 ± 15 mg/dl) ($P < 0.005$). Although the 24-h mean serum free immunoreactive insulin levels were shown to be no different at baseline (27.4 ± 3.8 versus 26.2 ± 3.1 μU/ml) or after 4 mo (22.5 ± 3.2 versus 25.6 ± 3.2) or 8 mo (26.5 ± 3.4 versus 28.8 ± 3.4) of CIT or CSII therapy, respectively, the mean increase of free insulin concentrations in relation to the main meals was greater in the CSII group ($P < 0.05$). The mean serum free insulin levels achieved with both CIT and CSII were significantly greater than the 24-h mean free insulin level obtained in 12 normal subjects (12.2 ± 1.4 μU/ml) ($P < 0.001$).

DIABETES 1985; 34 (Suppl. 3):31–36.

From the Departments of Medicine, University of Chicago, Chicago, Illinois (R.M.B. and A.H.R.); University of Western Ontario, London, Ontario (J.D.); Mayo Clinic, Rochester, Minnesota (R.A.R.); and Hammersmith Hospital, London, England (P.M.L.).

Address reprint requests to R. M. Bergenstal, M.D., International Diabetes Center, Park Nicollet Medical Center, 5000 West 39th Street, Minneapolis, Minnesota 55416.

The Kroc Collaborative Study Group was organized as a prospective randomized trial to determine if a group of investigators could establish and maintain significantly different levels of glucose control in patients with type I diabetes. Previous studies have described the effects of improved glucose control on various metabolic[1–6] or structural[7–10] features of the disease, yet none has been large enough or included the control group necessary to link the development of microvascular complications definitely to the level of blood glucose control.

The study group has attempted to design a trial that, while involving several centers, recruited (according to strict clinical eligibility criteria) a homogeneous group of patients with documented insulin deficiency. Since C-peptide is co-secreted with insulin in equimolar amounts from the beta cell[11,12] and is not significantly metabolized by the liver,[13,14] the peripheral plasma C-peptide concentration can be used as an indirect measure of endogenous insulin secretion. It has been suggested that the establishment of blood glucose control in the near-normal range soon after the time of diagnosis of type I diabetes may increase the likelihood of obtaining a clinical remission (honeymoon period)[15,16] and may even prolong the duration of this partial recovery of insulin secretion.[17] The study group, therefore, used C-peptide levels not only to select a homogeneous group of insulin-deficient patients, but also to determine if optimized glucose control had any influence on beta cell reserve in patients with a long duration of insulin-dependent diabetes mellitus (IDDM).

Few studies have compared the plasma insulin levels in groups of patients proven to have no endogenous insulin secretion randomized to conventional insulin therapy (CIT) or continuous subcutaneous insulin infusion (CSII).[18–20] In previous studies when CSII achieved better glucose control than CIT, this proved not to be due to more insulin being administered;[21,22] however, whether different levels of serum free insulin were attained in the two treatment groups was not evaluated. Concern has been expressed that hyperinsuli-

nemia may be implicated in the pathogenesis of complications of diabetes such as hypertriglyceridemia[23] and atherogenesis.[24] This study allowed us to determine whether the improved glucose control obtained with CSII is associated with the potential long-term risks that may accompany higher insulin levels in the blood, by comparison with those resulting from conventional insulin therapy. In addition, we compared the insulin levels obtained with CSII and CIT with those seen in normal subjects.

METHODS

Patients. The protocol for patient selection and randomization is detailed elsewhere (see articles by Champion et al., pages 5–12 and 13–16, this supplement). In general, all patients had type I diabetes clinically, were between ages 14 and 60 yr, with a diagnosis of diabetes before age 35 and for <30-yr duration. Each center randomly assigned successive pairs of patients meeting all the entry criteria and signing informed consent to either CIT or CSII therapy. C-peptide measurements performed in all patients from the six participating clinical centers as part of eligibility screening were taken as those prevailing at entry into the study at baseline (0 mo). C-peptide measurements were repeated on all patients at 4 and 8 mo during inpatient studies.

In addition, three clinical centers (Chicago, Western Ontario, and Mayo) collected samples from a total of 34 patients, 17 in the CSII group and 17 in the CIT group, for analysis of serum free insulin levels at baseline (0 mo), 4, and 8 mo, during the 24-h inpatient plasma glucose profiles. The clinical characteristics and parameters of glucose control in this subgroup of 34 patients (Table 1) did not differ from those of the whole group (see article by Champion et al., pages 13–16, this supplement).

TABLE 1
Characteristics of 34 subjects with diabetes on entry into the trial and after 4 and 8 mo of therapy (mean ± SEM)*

	Treatment group	
	CSII (N = 17)	CIT (N = 17)
Disease onset/duration		
Age at entry (yr)	30.2 ± 1.8	32.4 ± 2.2
Age at diagnosis (yr)	14.2 ± 2.1	14.3 ± 1.5
Duration of diabetes (yr)	15.9 ± 1.7	18.1 ± 1.9
Clinical characteristics		
Glycosylated hemoglobin (%)		
0 mo	10.4 ± 0.57	10.8 ± 0.49
4 mo†	8.1 ± 0.25‡	9.6 ± 0.38§
8 mo†	8.3 ± 0.38‡	10.5 ± 0.44
Total insulin dose (U/day)		
0 mo	52.7 ± 4.6	52.1 ± 4.5
4 mo	45.4 ± 2.5	54.0 ± 4.6
8 mo	46.2 ± 2.8	49.0 ± 3.3

Abbreviations: CSII, continuous subcutaneous insulin infusion; CIT, conventional insulin therapy.
*This represents the subgroup of patients on whom plasma free insulin levels were measured in three clinical centers.
†$P < 0.002$ between CSII and CIT glycosylated hemoglobin at 4 mo and 8 mo.
‡CSII glycosylated hemoglobin, $P < 0.001$ 4 and 8 mo versus 0 mo.
§CIT glycosylated hemoglobin, $P < 0.05$ 4 mo versus 0 mo.

TABLE 2
Mean plasma glucose and plasma free insulin concentrations (24 h) during CSII or CIT (mean ± SEM)

	Mo	CSII	CIT
Plasma glucose (mg/dl)	0	211 ± 14	183 ± 16
	4	126 ± 10*	176 ± 14
	8	121 ± 5*	183 ± 15
		Normal (90 ± 1)†	
Serum free insulin (μU/ml)	0	26.2 ± 3.1	27.4 ± 3.8
	4	25.6 ± 3.2	22.5 ± 3.2
	8	28.8 ± 3.4	26.5 ± 3.4
		Normal (12.3 ± 1.4)†	

Abbreviations as in Table 1.
*Differs from baseline (0 mo) and from corresponding CIT values ($P < 0.005$).
†Values from 12 normal subjects, which differed significantly from all CSII and CIT glucose and free insulin values. ($P < 0.01$ versus values marked with an asterisk and $P < 0.001$ versus all other values.)

PROTOCOL

C-peptide measurements. All patients who met the clinical criteria for entry into the study had a fasting venous blood sample obtained for C-peptide. They were then given an intravenous injection of 1.0 mg glucagon and 6 min later a repeat C-peptide sample was obtained. These tests were carried out before breakfast and before administration of insulin, although patients receiving CSII continued the basal infusion of insulin during the procedure. Samples (volume: 5 ml) were collected in chilled EDTA tubes containing 2000 KIU Traysolol in 0.1 ml of distilled water. The plasma was separated within 1 h and stored at −20°C. The frozen samples were shipped on dry ice to a central laboratory for C-peptide analysis (Chicago). One center (Hammersmith) transmitting C-peptide samples across the Atlantic to Chicago included a duplicate set of freeze-dried (lyophilized) samples for the baseline determinations, along with the frozen plasma samples. The lyophilized samples were reconstituted in assay buffer at the time of assay: The results of the assays on the frozen and lyophilized duplicate samples did not differ significantly.

Each center was notified whether or not its patient met the eligibility criteria of insulin deficiency defined as a fasting and/or postglucagon stimulation C-peptide value of <0.1 pmol/ml. Repeat fasting and glucagon-stimulated C-peptide measurements were made at 4 and 8 mo.

Serum free insulin. Patients satisfying the eligibility criteria were hospitalized for baseline studies (0 mo). They were maintained on their prestudy insulin regimens and meal plans. On the morning of the second hospital day, an indwelling venous catheter was put in place, and samples for plasma glucose were drawn before and 90 min after each meal, before the bedtime snack, at midnight, 0200, 0400, and 0600 h, for an 11-sample profile. Similar inpatient plasma glucose profiles were obtained at 4 and 8 mo of treatment. At three of the six clinical centers, samples at each of the 11 time points were also collected for determination of serum free insulin levels in a total 34 patients (17 CSII, 17 CIT).

ANALYTIC TECHNIQUES

Glucose. As described in the article by Home et al., pages 17–21, and the article by Tamborlane et al., pages 22–26,

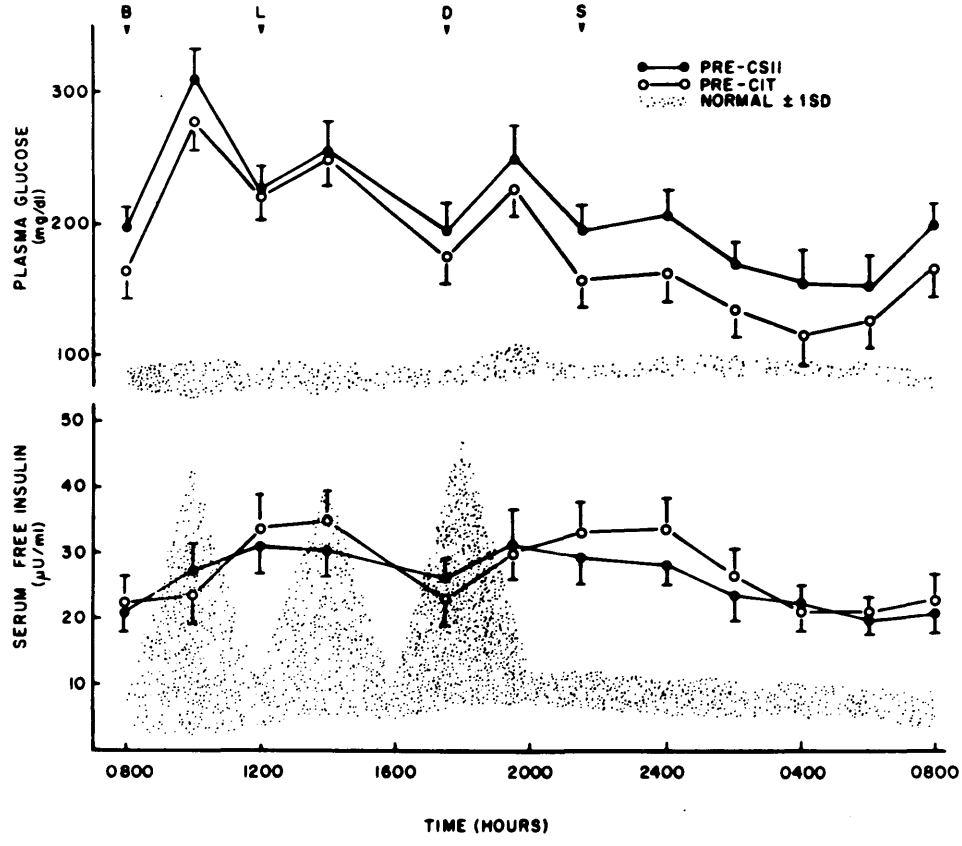

FIGURE 1. Mean plasma glucose and serum free insulin concentrations during 24-h inhospital monitoring before randomization to either continuous subcutaneous insulin infusion (CSII, ●——●) or conventional insulin therapy (CIT, ○——○). The shaded area represents one standard deviation above and below the mean of observations in 12 normal subjects. B denotes breakfast; L, lunch; D, dinner; S, snack.

this supplement, glucose samples were measured at each center and in a central laboratory by glucose-oxidase methods.

C-peptide. Plasma samples were measured in a central laboratory (Chicago) by a nonequilibrium ethanol precipitation radioimmunoassay[25] in which the tracer was ^{125}I-tyrosyl-biosynthetic human C-peptide. The sensitivity of the assay is 0.02 pmol/ml and the intra- and interassay coefficients of variation were 8.7% and 11.9%, respectively.

Serum free insulin. The three centers measuring serum free insulin used an insulin radioimmunoassay after the serum had been equilibrated at 37°C for 2 h, and the antibody bound insulin had been precipitated with polyethylene glycol.[25,26] The same free insulin assay technique was used to measure the insulin levels in the normal subjects reported in Table 2 and Figures 1 and 2, and gave similar results in samples from normal and diabetic subjects in the three laboratories.

Statistical analysis. Twenty-four hour mean plasma glucose and serum free insulin values were calculated as the mean of the 11 determinations on each patient.[27] Statistical significance of the differences in glucose, free insulin, and C-peptide values were analyzed using Student's *t*-test.[27]

RESULTS

C-peptide. The mean ± SEM of the C-peptide values for patients in the six clinical centers are presented in Table 3. There were 70 patients (35 CSII, 35 CIT) at baseline (0 mo), and values were available from 68 patients at 4 mo and 66 patients at 8 mo. In order to be eligible for the study, all patients were required to have a fasting and glucagon-stimulated C-peptide at baseline of <0.10 pmol/ml. The mean C-peptide values obtained at baseline were considerably <0.10 pmol/ml with no difference between the CSII versus the CIT group (fasting 0.013 ± 0.003 versus 0.012 ± 0.003 pmol/ml, respectively, Table 3). Neither CIT nor CSII treatment for 4 or 8 mo resulted in any change in the mean fasting or stimulated C-peptide values (Table 3). Only 1 of 70 patients showed an elevation of C-peptide from baseline levels during treatment. This was a patient in the CIT group who had baseline fasting and stimulated C-peptide values of 0.02 and 0.03 pmol/ml, respectively. At 4 mo, these values were 0.15 and 0.22 pmol/ml, and at 8 mo they were 0.10 and 0.15 pmol/ml, respectively.

Glycemic control. The 24-h mean plasma glucose values shown in Table 2 represent the data from the 34 patients in whom free insulin measurements were made. The glucose control in the CSII and CIT groups did not differ significantly at baseline (211 ± 14 versus 183 ± 16 mg/dl), but the CSII group had significantly lower mean values at 4 mo (126 ± 10 versus 176 ± 14 mg/dl) and 8 mo (121 ± 5 versus 183 ± 15 mg/dl), $P < 0.005$. However, all mean glucose values for the diabetic subjects, including the CSII group, were significantly higher than the mean glucose values for the 12 normal subjects, which was 90 ± 1 mg/dl. The glycemic control of this subset of 34 patients (Table 1) did not differ significantly from

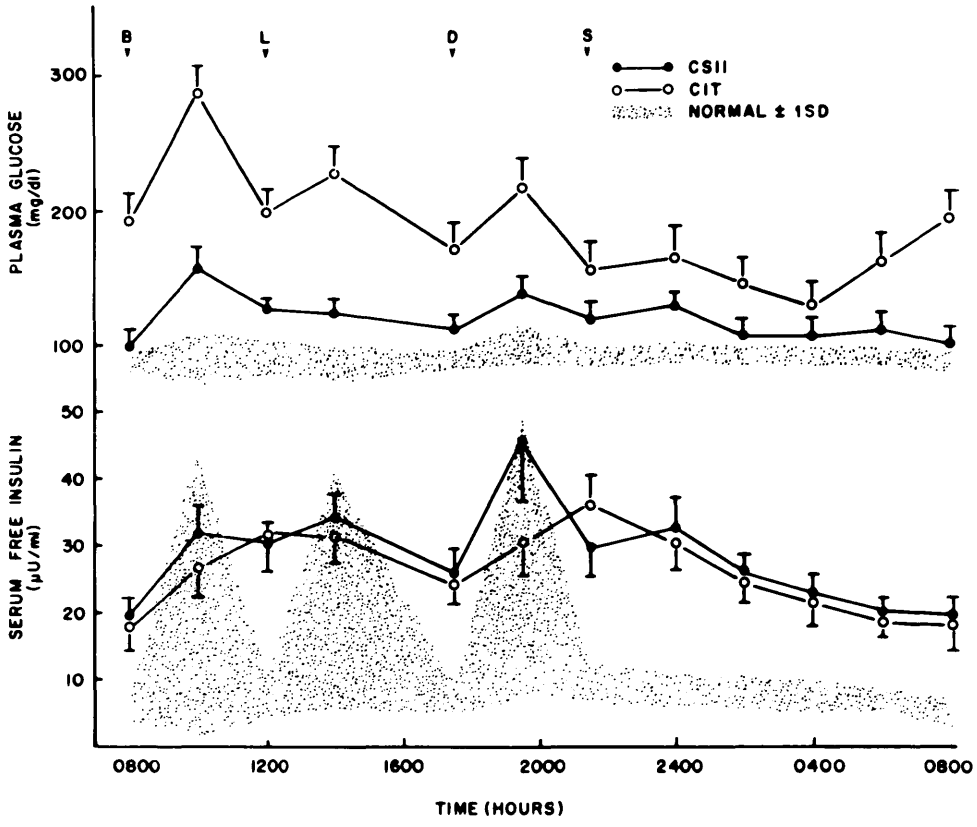

FIGURE 2. Mean plasma glucose and serum free insulin concentrations during 24-h inhospital monitoring 8 mo after randomization to CSII or CIT. Abbreviations as in Figure 1.

the complete group of 68 patients in the study[28] (see article by Tamborlane et al., pages 22–26, this supplement).

Serum free insulin. At baseline (0 mo), there was no difference in the mean 24-h serum free immunoreactive insulin level of the 17 patients who were randomized to CSII (26.2 ± 3.1 μU/ml) compared with the 17 patients randomized to CIT (27.4 ± 3.8 μU/ml) (Table 2). After 4 and 8 mo of therapy, there was no change in the mean 24-h serum free insulin levels within each group, and no difference between values during the two treatments at 4 or 8 mo. At 8 mo, levels during treatment with CSII and CIT (28.8 ± 3.4 and 26.5 ± 3.4 μU/ml, respectively) were both significantly higher than the mean value obtained in 12 normal subjects (12.3 ± 1.4 μU/ml), $P < 0.001$.

The mean fasting serum free insulin levels (0800 h, prebreakfast) showed the same relationships to the two treatments, as did the mean of the values obtained during the 11-sample, 24-h profile. Thus, the mean fasting serum free insulin values in the CSII and CIT groups at baseline were 21.1 ± 4.2 and 21.7 ± 4.5 μU/ml, respectively. These were not significantly different from the values after 8 mo of either CSII or CIT (19.4 ± 2.7 versus 18.0 ± 3.2 μU/ml, respectively). Mean fasting free insulin levels in the diabetic subjects on both treatments were significantly greater at 0, 4, and 8 mo than the mean fasting insulin value in the 12 normal subjects (5.4 ± 2.2 μU/ml), $P < 0.001$.

Insulin requirements. At baseline the mean total insulin requirement (U/day) during CSII or CIT in the subgroup of 34 patients in whom serum free insulin levels were measured (Table 1) did not differ from the means reported in the entire study population (52.9 ± 3.2 U/day versus 53.1 ± 3.3 U/day, respectively).[28] It can be seen in Table 1 that the total insulin dose in the CSII and CIT subgroups before randomization did not differ significantly (means 52.7 ± 4.6 versus 52.1 ± 4.5 U/day, respectively). Although there tended to be a slightly lower insulin requirement on CSII (15% less than at baseline, and 6–19% less than required during CIT at corresponding times), the mean total insulin doses during CSII and CIT did not differ significantly from baseline or between treatment groups at 4 mo (45.4 ± 2.5 versus 54.0 ± 4.6 U/day) or 8 mo (46.2 ± 2.8 versus 49.0 ± 3.3 U/day), respectively.

DISCUSSION

One major goal of The Kroc Collaborative Study Group was to conduct a prospective, multicenter randomized trial to determine the feasibility of establishing and maintaining blood glucose control at different levels in IDDM treated with CIT or CSII.[28]

With this objective, and in order to address the issue of the relationship of blood glucose control to the development of microvascular complications in diabetes,[29,30] we established eligibility criteria that included not only clinical characteristics (see article by Champion et al., pages 5–12, this supplement), but also a reliable measure of beta cell function. Since C-peptide is secreted from the beta cell in equimolar concentration with insulin,[11,12] the plasma C-peptide level has

TABLE 3
C-peptide levels (pmol/ml) fasting and postglucagon stimulation during CSII and CIT (mean ± SEM)

	Treatment group			
	CSII		CIT	
Mo	Fasting†	Stimulated‡	Fasting	Stimulated
0*	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
4	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.02 ± 0.01
8	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.03 ± 0.01

Normal§	
Fasting	Stimulated
0.36	1.28
(0.26–0.63)	(0.91–1.88)

Abbreviations as in Table 1.
*Baseline (0 mo) determination made before randomization.
†Fasting sample.
‡Stimulated sample (6 min after 1.0 mg glucagon i.v.).
§Fasting and glucagon-stimulated values represent mean and range ( ) for 10 normal subjects using the same C-peptide antiserum.[15]

been shown to be an accurate marker of beta cell activity and can be employed in patients treated with exogenous insulin. Being certain all patients were insulin deficient at the start of the study allowed us to assume that the control they achieved depended mainly on the regimen of exogenous insulin treatment to which they were randomized. Fasting C-peptide is an acceptable indicator of beta cell reserve, but the beta cell secretory capacity can be assessed more effectively by means of a stimulation test dependent on ingestion of a standard meal or injection of glucagon intravenously.[31]

Since the glucagon test is easily standardized and rapid, it seemed most appropriate for a multicenter clinical trial. A C-peptide value of <0.1 pmol/ml in both the fasting and stimulated state is indicative of insulin deficiency.[31] However, values greater than this in type I diabetes are observed early in the course of the disease.[32] We wanted to avoid such subjects because their residual insulin secretion might influence the glucose control in a nonrandom way. It was therefore of interest that 15% of the patients, judged on clinical grounds to be insulin deficient and screened for C-peptide by the central lab, had values ≥0.1 pmol/ml and were not eligible for the study (see article by Champion et al., pages 5–12, this supplement).

Clinical remission and partial recovery of insulin secretion in type I diabetic subjects within a few weeks or months of diagnosis[16] or after insulin therapy for an initial presentation of DKA[33] have been documented. Only a few studies have looked at the potential for beta cell recovery later in the course of type I diabetes,[34,35] and these were not randomized or controlled. The present study showed that there was no apparent improvement in insulin secretion, as indicated by plasma C-peptide levels, whether the glucose control was unchanged (CIT) or optimized (CSII) for 8 mo. While it must be noted that, during both CIT and CSII treatments, patients were subjected to supraphysiologic levels of free immunoreactive insulin in the blood that might have inhibited the secretion of insulin, it is clear from the data related to serum free insulin levels discussed below that such an effect would not have been greatly different in the two treatment groups.

Other studies have compared the total insulin requirements during CSII and conventional insulin regimens.[21,22] We found no significant difference in the total insulin requirements in our two groups, although there was a trend toward a slightly lower insulin dose in the CSII group. The total insulin dose may vary with the degree of control achieved and may not reflect the peripheral serum insulin levels achieved. There is evidence that hyperinsulinemia may be involved in some complications related to diabetes, including hypertriglyceridemia[23] and atherosclerotic vascular disease.[24] Only a few studies have compared the serum free insulin levels seen in type I diabetes treated with CSII and CIT.[18–20] Since endogenous insulin secretion (C-peptide levels) was being carefully monitored throughout the 8-mo study, our patients were considered to be an excellent group in which to evaluate the effect of different exogenous insulin regimens (CSII or CIT) on serum free insulin levels. We have shown that there were no significant differences in either the 24-h mean free insulin concentration as determined from the 11-point profiles described, or in the mean fasting free insulin concentrations after 4 or 8 mo of treatment with either the CSII or the CIT regimen, despite significantly improved glucose control in the CSII group. These data confirm findings in earlier shorter-term studies,[18–20] and fail to support suggestions that CSII-treated patients have improved glucose control because of relatively high free insulin levels overnight or in the fasting state.

An explanation for the similarity of mean serum insulin concentrations with concurrent difference in glucose control may be that the CSII treatment results in a more physiologic insulin profile than CIT. There is an impression from the data in the present study (Figure 2) that the CSII group has an insulin profile that more closely resembles the normal, with insulin peaks more clearly delineated at breakfast, lunch, and dinner. The rise of free insulin in the blood after delivery of supplementary doses by means of CSII is not slower, and is probably faster[36] than that following depot injection of mixed regular and modified insulins. After bolus delivery of supplements to CSII, the peak level of free insulin in the blood is reached between 60 and 90 min. Thus, although the sampling procedure employed in this study was inadequate to delineate the changes of free insulin in the blood closely, it is unlikely that the apparently greater rise of free insulin levels in the CSII group is artificial or exaggerated. The mean increase of free insulin levels was greater during CSII when the differences between all pre- and postmeal values were combined (mean ΔF-IRI, μU/ml) comparing the two treatment groups, CSII and CIT, respectively: at entry, 3.5 ± 1.4, 3.6 ± 1.5; at 4 mo, 10.3 ± 2.8, 4.8 ± 2.2; and at 8 mo, 12.2 ± 4.0, 5.4 ± 2.2. Differences were statistically significant at 4 and 8 mo ($P < 0.05$). Nevertheless, it is apparent that the change of insulin levels at the times of the meals cannot account in a simple fashion for the differences in glycemic control, since major differences in glucose control are found when comparing the mean overnight and pre-breakfast glucose levels.

It is also clear that both groups with diabetes had significantly higher fasting and mean 24-h serum free insulin values than those seen in normal subjects. This difference, though

its mechanism is not clear, may be related to effects of systemic delivery of insulin by comparison with delivery by the physiologic portal route.

That the major difference between the levels of blood glucose in the two groups is during the overnight interval, when the mean free insulin levels in the corresponding blood samples were not significantly different, raises the question of a possible difference in insulin sensitivity. The mechanisms of such a difference in insulin sensitivity are obscure. It has been shown that insulin receptor functions in erythrocytes of subjects receiving these two modes of treatment are not significantly different.[37] For these reasons, and on account of difficulties in interpretation of assays for free immunoreactive insulin in the blood of subjects with insulin antibodies, the data presented here emphasize the complexity of the relationship between insulin concentrations in blood and glycemic control, and point out the need for more sophisticated analyses of these relationships in terms of glucose turnover, and assessments of the distributed metabolic fate of glucose.

In conclusion, these results suggest that individuals with insulin-dependent diabetes mellitus who have had marked insulin deficiency for prolonged periods of time are unlikely to manifest detectable improvement of insulin secretion after a period of improved glucose control. The study also showed that the levels of free insulin in the blood of insulin-dependent diabetic subjects are similar in the postabsorptive state during CSII and CIT. The same is true of the mean integrated levels of plasma insulin determined by means of the 11-point profiles described. It is also clear that these levels are significantly higher by comparison with those of normal subjects determined under the same conditions. Thus, improvement of glycemic control by means of CSII is not associated with a significant increase in postabsorptive or mean integrated levels of insulin in the blood, by comparison with CIT. How the serum free insulin levels relate to glucose control and to the potential complications of diabetes remains uncertain, and clearly deserves further study.

REFERENCES

[1] Skyler, J. S.: Complications of diabetes mellitus: relationship to metabolic dysfunction. Diabetes Care 1979; 2:499–509.

[2] Pickup, J. C., Keen, H., Parson, J. A., Alberti, K. G. M. M., and Rowe, A. S.: Continuous subcutaneous insulin infusion: improved blood glucose and intermediary-metabolite control in diabetics. Lancet 1979; 1:1255–58.

[3] Tamborlane, W. V., Sherwin, R. S., Genel, M., and Felig, P.: Restoration of normal lipid and amino acid metabolism in diabetic patients treated with a portable insulin infusion pump. Lancet 1979; 1:1258–61.

[4] Tamborlane, W. V., Sherwin, R. S., Koivisto, V., Hendler, R., Genel, M., and Felig, P.: Normalization of the growth hormone and catecholamine response to exercise in juvenile onset diabetic subjects treated with portable insulin infusion pump. Diabetes 1979; 28:785–88.

[5] Pietri, A., Dunn, F. L., and Raskin, P.: The effect of improved diabetic control on plasma lipid and lipoprotein levels: a comparison of conventional therapy and continuous subcutaneous insulin infusion. Diabetes 1980; 29:1001–1005.

[6] Raskin, P., Pietri, A., and Unger, R. H.: Changes in glucagon levels after four to five weeks of glucoregulation by portable insulin infusion pumps. Diabetes 1979; 28:1033–35.

[7] Tchobroutsky, G.: Relation of diabetic control to development of microvascular complications. Diabetologia 1978; 15:143–52.

[8] Pietri, A., Ehle, A. L., and Raskin, P.: Changes in nerve conduction velocity after six weeks of glucoregulation with portable insulin infusion pumps. Diabetes 1980; 29:668–71.

[9] White, N. H., Waltman, S. R., Krupin, T., and Santiago, J.: Reversal of abnormalities in ocular fluorophotometry in insulin dependent diabetes after five to nine months of improved metabolic control. Diabetes 1982; 31:80–85.

[10] Irsigler, K., Kritz, HI., and Najemnik, C.: Reversal of florid diabetic retinopathy. Lancet 1979; 2:1068.

[11] Steiner, D. F., and Oyer, P. E.: The biosynthesis of insulin and a probable precursor of insulin by a human islet cell adenoma. Proc. Natl. Acad. Sci. USA 1967; 57:473–80.

[12] Rubenstein, A. H., Clark, J. L., Melani, F., and Steiner, D. F.: Secretion of proinsulin C-peptide by pancreatic beta cells and its circulation in blood. Nature 1969; 224:697–99.

[13] Horwitz, D. L., Starr, J. I., Mako, M. E., Blackard, W. G., and Rubenstein, A. H.: Proinsulin, insulin and C-peptide concentrations in human portal and peripheral blood. J. Clin. Invest. 1975; 55:1278–83.

[14] Polonsky, K. S., and Rubenstein, A. H.: C-peptide as a measure of the secretion and hepatic extraction of insulin: pitfalls and limitations. Diabetes 1984; 33:486–94.

[15] Faber, O. K., and Binder, C.: B-cell function and blood glucose control in insulin dependent diabetics within the first month of insulin treatment. Diabetologia 1977; 13:263–68.

[16] Grajwer, L. A., Pildes, R. S., Horwitz, D. L., and Rubenstein, A. H.: Control of juvenile diabetes mellitus and its relationship to endogenous insulin secretion as measured by C-peptide immunoreactivity. J. Pediatr. 1977; 90:42–48.

[17] Mirouze, J., Selam, J. L., Pham, T. C., Mendoza, E., and Orsetti, A.: Sustained insulin induced remissions of juvenile diabetes by means of an external artificial pancreas. Diabetologia 1978; 14:223–27.

[18] Champion, M. D., Shepherd, G. A. A., Rodger, N. W., and Dupre, J.: Continuous subcutaneous infusion of insulin in the management of diabetes mellitus. Diabetes 1980; 29:206–12.

[19] Rizza, R. A., Gerich, J. E., Haymond, M. W., Westland, R. E., Hall, L. D., Clemens, A. H., and Service, F. J.: Control of blood sugar in insulin dependent diabetes: comparisons of an artificial endocrine pancreas continuous subcutaneous insulin infusion and intensified conventional insulin therapy. N. Engl. J. Med. 1980; 303:1313–18.

[20] Buysschaert, M., Marchand, E., Ketelslegers, J. M., and Lambert, A. E.: Comparison of plasma glucose and plasma free insulin during CSII and intensified conventional insulin therapy. Diabetes Care 1983; 6:1–5.

[21] Nathan, D. M., Lou, P., and Avruch, J.: Intensive conventional and insulin pump therapies in adult type I diabetes. Ann. Intern. Med. 1982; 97:31–36.

[22] Reeves, M. L., Seigler, D. E., Ryan, E. A., and Skyler, J. S.: Glycemic control in insulin dependent diabetes mellitus: comparison of outpatient intensified conventional therapy with continuous subcutaneous insulin infusion. Am. J. Med. 1982; 72:673–80.

[23] Olefsky, J. M., Farquhar, J. W., and Reaven, G. M.: Reappraisal of the role of insulin in hypertriglyceridemia. Am. J. Med. 1974; 57:551–60.

[24] Stout, R. W.: Diabetes and atherosclerosis—the role of insulin. Diabetologia 1979; 16:141.

[25] Kuzuya, J., Blix, P. M., Horwitz, D. L., Steiner, D. F., and Rubenstein, A. H.: Determination of free and total insulin and C-peptide in insulin-treated diabetics. Diabetes 1977; 26:22–29.

[26] Nakagawa, S., Nakayama, H., Sasaki, T., Yoshino, K., Yu, Y. Y., Shinozaki, K., Aoki, S., and Mashimo, K.: A simple method for the determination of serum free insulin levels in insulin-treated patients. Diabetes 1973; 22:590–600.

[27] Snedecor, G. W., and Cochran, W. G.: Statistical Methods, 6th edit. Ames, Iowa State University Press, 1967.

[28] The Kroc Collaborative Study Group: Blood glucose control and the evolution of diabetic retinopathy and albuminuria: a preliminary multicenter trial. N. Engl. J. Med. 1984; 311:365–72.

[29] Cahill, G. F., Etzwiler, D. D., and Freinkel, N.: "Control" and diabetes. N. Engl. J. Med. 1976; 294:1004–1005.

[30] Siperstein, M. D., Foster, D. W., Knowles, H. C., Jr., Levine, R., Madison, L. L., and Roth, J.: Control of blood glucose and diabetic vascular disease. N. Engl. J. Med. 1977; 296:1060–62.

[31] Faber, O. K., and Binder, C.: C-peptide response to glucagon: a test for the residual β-cell function in diabetes mellitus. Diabetes 1977; 26:605–10.

[32] Madsbad, S., Faber, O. K., Binder, C., McNair, P., Christiansen, C., and Transbøl, I.: Prevalence of residual beta-cell function in insulin dependent diabetics in relation to age at onset and duration of diabetes. Diabetes 1978; 27 (Supp. 1):262–64.

[33] Block, M. B., Mako, M. E., Steiner, D. F., and Rubenstein, A. H.: Diabetic ketoacidosis: evidence for C-peptide and proinsulin secretion following recovery. J. Clin. Endocrinol. Metab. 1972; 35:402–406.

[34] Pietri, A., Alford, M., and Raskin, P.: The effect of continuous subcutaneous insulin infusion on endogenous insulin secretion in type I diabetes. Diabetes Care 1982; 5:330–33.

[35] Seigler, D. E., Reeves, M. L., and Skyler, J. S.: Lack of effect of improved glycemic control on C-peptide secretion in patients without residual β-cell function. Diabetes Care 1982; 5:334–36.

[36] Dupre, J., Champion, M. C., and Rodger, N. W.: Free immunoreactive insulin (FIRI) in the blood in insulin-dependent diabetes mellitus (IDDM) treated by subcutaneous injection (SCD) or continuous subcutaneous insulin infusion (CSII). Abstract. J. Clin. Invest. Med. 1982; 5:11B.

[37] Schade, D. S., Eaton, R. P., Friedman, N. M., and Spencer, W. J.: Normalization of plasma insulin profiles with intraperitoneal insulin infusion in diabetic man. Diabetologia 1980; 19:35–39.