Effects of Continuous Subcutaneous Insulin Infusion Versus Multiple Injections on Insulin Receptors in Insulin-Dependent Diabetics

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We compared continuous subcutaneous insulin infusion (CSII) versus multiple injections (MI) in the treatment of insulin-dependent diabetes mellitus (IDDM) to assess the effect of glucose control on monocyte insulin receptors. Each IDDM patient (n = 8) was treated for 2 mo by MI (HS Ultralente and AC boluses of regular insulin) and for 2 mo by CSII in a randomized fashion. Prestudy preprandial/postprandial blood glucose levels were 199 ± 33/261 ± 28 mg/dl and improved to 124 ± 12/156 ± 13 mg/dl during MI and to 115 ± 11/151 ± 11 mg/dl during CSII. Glycosylated hemoglobin before the study was 10.1 ± 0.5% and decreased to 8.8 ± 0.4 and 8.3 ± 0.3% during MI and CSII, respectively. The specific ^{125}I-labeled insulin binding to circulating monocytes in a group of nonobese controls (n = 17) was 4.6 ± 0.2%. In our poorly controlled diabetics during conventional therapy, the ^{125}I-insulin binding was decreased to 3.7 ± 0.3 ($P < .025$). This was not significantly affected by MI despite good glucose control (4.0 ± 0.3%). With CSII, however, good glucose control was associated with normalization of ^{125}I-insulin binding to monocytes (4.7 ± 0.27%). The affinity of the insulin receptors was normal before the study and was not affected by either MI or CSII. In conclusion, these observations demonstrate that in IDDM, intensive therapy by MI and CSII resulted in similar good glucose control, but only CSII resulted in normalization of insulin receptors on circulating monocytes. *Diabetes Care* 10:300–305, 1987

Substantial evidence implicates hyperglycemia as a major factor involved in the development and progression of diabetic complications. The long-term study of Pirart (1) showed a positive correlation between poor glycemic control and the appearance of microangiopathy and neuropathy. Patients with diabetes secondary to pancreatectomy also develop the same degenerative lesions (2). Diabetic nephropathy is also observed in the kidney from a normal donor transplanted into a diabetic recipient (3). Finally, experimentally induced diabetes in animals is also associated with similar complications (4).

These observations have led to major efforts in the development of new approaches in the treatment of insulin-dependent diabetes mellitus (IDDM) in the hope of achieving better glycemic control. This was facilitated by the development of new techniques for self-monitoring of blood glucose (5–7) and the measurement of glycosylated hemoglobin, which is a reliable index of long-term glycemic control (8, 9). With this new technology, the treatment of IDDM was approached in a more physiological way, and new insulin regimens delivered by multiple injections (MI) or continuous subcutaneous insulin infusion (CSII) have been proposed (10–15).

With these new approaches, the achievement of long-term normoglycemia was made possible (16). Such normoglycemia has been shown to prevent the fetal complications observed in diabetic mothers (17). Diabetic neuropathy has been shown to regress with better glycemic control (18). More recently, we have shown that long-term normoglycemia achieved by either MI or CSII resulted in regression of peripheral neuropathy and proteinuria (19). In this study, we observed that, to achieve similar glycemic control, 20% more insulin was required with MI than with CSII. The question is therefore whether this is due to partial insulin degradation at the site of insulin injection or whether it reflects higher insulin levels required with MI. This is an important question because hyperinsulinemia has been suggested as a possible factor involved in the pathogenesis of atherosclerosis (20). Our study answered this question by looking at the effect of MI and CSII on monocyte insulin binding, which varies

TABLE 1
Clinical data

Patient no.	Age (yr)	Sex	Duration of diabetes (yr)	Complications		
				Retinopathy	Nephropathy	Neuropathy
1	22	M	11	+	−	−
2	24	M	2	−	−	−
3	40	F	10	−	−	−
4	36	M	10	+	−	−
5	28	M	3	−	−	−
6	30	M	2	−	−	−
7	39	F	26	+	−	+
8	27	F	13	−	−	−

Retinopathy defined by presence of microaneurysms; nephropathy defined by presence of >200 mg of protein in a 24-h urine collection; neuropathy defined by decrease in sural nerve conductivity of >2SD.

inversely with the insulin concentration. The results show that, although the insulin receptors normalized during CSII, they remained suppressed during MI despite similar metabolic control.

PATIENTS AND METHODS

Subjects. Eight IDDM patients [confirmed by the absence of C-peptide response to glucagon (21)] took part in the study. All were within 20% of their ideal body weight. The clinical data are listed in Table 1.

Protocol. The protocol was approved by the Ethics Committee, and all subjects signed informed consent. Before entering the study, all patients were treated by conventional insulin therapy, i.e., one insulin injection in the morning. All subjects were then treated by CSII for 2 mo with the programmable Autosyringe AS6MP insulin pump (Travenol, Flint Division, Deerfield, IL) and by MI with the Pen Pump Infuser (Markwell, Racine, WI) also for 2 mo. The choice of the first treatment was randomized. The Pen Pump is a 3-ml syringe with a screw-on piston. Each complete turn of the piston will infuse 4 U of U100 insulin. A catheter with a 27-gauge needle is hooked up to the syringe, and the needle is secured subcutaneously on the abdomen. The Pen Pump was filled with regular insulin (Iletin, Lilly, Indianapolis, IN) and used for the preprandial boluses only. The basal insulin was given as Ultralente insulin (Lilly) before bedtime with the conventional syringe. Half of the patients started with CSII and half with MI.

Patients were admitted to the Clinical Research Unit at the Hotel-Dieu of Montreal Hospital to initiate the treatment with either the Autosyringe or the Pen Pump. During their hospitalization they learned how to operate the insulin-delivering device, how to calculate the amount of carbohydrate in their diet, and how to modify their insulin according to specific algorithms described previously (19), with a slight modification. The basal rate was started at 50% of the current insulin dose and was readjusted to maintain the 0400 h, fasting, preprandial, and bedtime blood glucose between 70 and 120 mg/dl. The boluses were started at 1.5 U/10 g carbohydrate before breakfast and 1.0 U/10 g carbohydrate for other meals and snacks; these were readjusted to maintain 1-h postprandial blood glucose <160 mg/dl. A diabetic diary recording the insulin dose, preprandial (AC) and 1-h postprandial (PC) capillary blood glucose measured at home, and meal content (type of food and quantity) was obtained before the study and at the end of each 2-mo treatment during 3 representative days (2 during the week and 1 during the weekend but never on 2 consecutive days) and used for data analysis. Free plasma insulin and glycosylated hemoglobin were measured before the study and at the end of each study. Insulin binding to the monocytes was measured at the same time in a single-blind fashion, i.e., the one performing the assay was not aware of the type of treatment.

Analysis. Self-monitoring of blood glucose was performed with a Glucometer and Dextrostix (Miles, Elkhart, IN). The accuracy of the patients' determinations was checked during their hospitalization. Glycosylated hemoglobin was measured by column chromatography (Bio-Rad procedure kit, Rich-

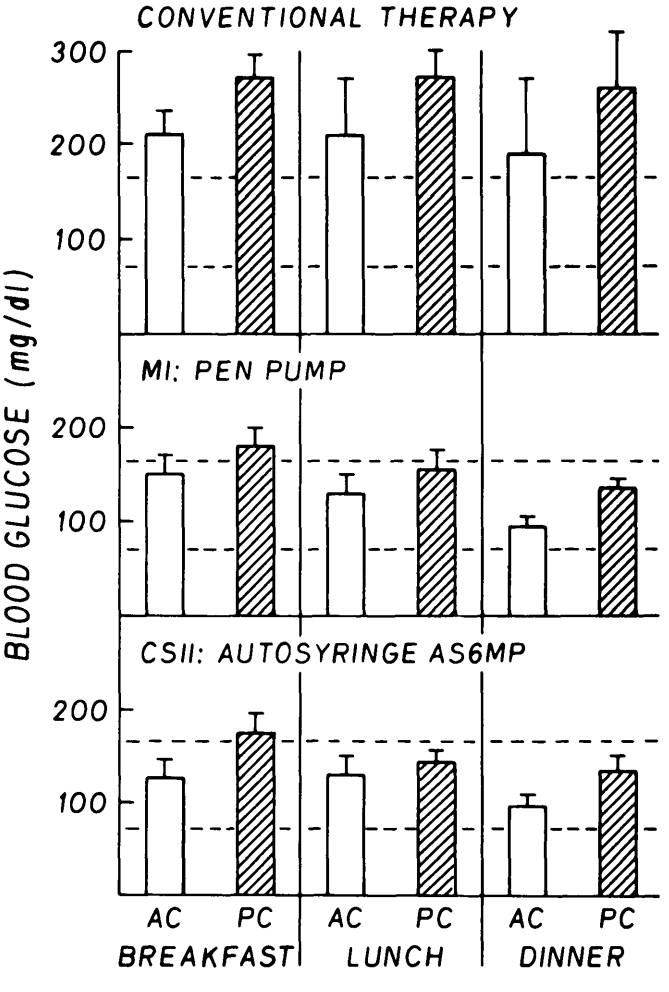

FIG. 1. Effects of conventional therapy, multiple insulin injections (MI), and continuous subcutaneous insulin infusion (CSII) on preprandial (*open bars*) and 1-h postprandial (*hatched bars*) capillary blood glucose for morning, midday, and evening meals. Values are means ± SE (*n* = 8).

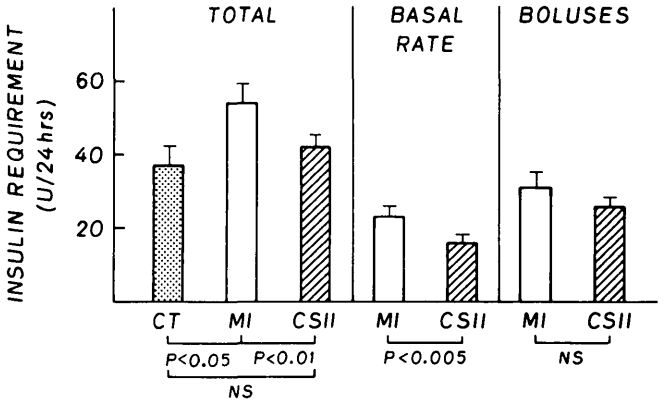

FIG. 2. Insulin requirements during conventional therapy (CT), multiple injections (MI), and continuous subcutaneous insulin infusion (CSII). Values are means ± SE (n = 8).

mond, CA). Free insulin was measured by Bio-RIA kit (Montreal, Canada) after precipitation by polyethylene glycol (22). Statistical analyses were done by paired t test, ANOVA (repeated measures), and χ^2-test wherever applicable.

Insulin-receptor measurement. One hundred milliliters of blood were centrifuged on Ficoll-Hypaque gradients (Pharmacia, Piscataway, NJ) according to the method of Boyum (23). Mononuclear cells were diluted in balanced salt solution (Pharmacia), centrifuged, and resuspended in the desired volume of HEPES buffer (100 mM, 1% bovine serum albumin, pH 8.0). The percentage of monocytes was determined with esterase staining (24). The viability was assessed by trypan blue exclusion and was always >97%. ^{125}I-labeled insulin was prepared at a specific activity of 200–250 mCi/mg by the method of Freychet et al. (25). Four hundred microliters of cell suspension (~5 × 10^6 monocytes/ml), 50 μl ^{125}I-insulin (0.2 ng/ml), and 50 μl unlabeled insulin (0–50 μl/ml) were incubated for 3 h at 20°C (26). After adding 500 μl of cold HEPES buffer, tubes were centrifuged in a Beckman microfuge for 5 min. Supernatants were removed for determination of tracer degradation (<10%), and the radioactivity in cell pellets was determined. The nonspecific binding (in the presence of 50 μg/ml of unlabeled insulin) was subtracted from the total binding (in the presence of ^{125}I-insulin only) to obtain the specific binding. The insulin concentration that displaced 50% of ^{125}I-insulin bound to the cells was used as an indicator of the insulin-receptor affinity.

RESULTS

Effects of MI and CSII on metabolic control. The AC and PC capillary blood glucose levels for each meal during the three representative days while on conventional therapy (CT), MI, and CSII are shown in Fig. 1. Each value represents the mean ± SE from 8 subjects. Both MI and CSII resulted in similar good glucose control: AC/PC glucose values were 199 ± 33/261 ± 28 mg/dl with CT, 124 ± 12/156 ± 13 mg/dl with MI, and 115 ± 11/151 ± 11 mg/dl with CSII. During those days, the incidence of undesirable high blood glucose (>180 mg/dl) was 59% with CT and decreased to 18 and 13% with MI and CSII, respectively, whereas the incidence of low blood glucose (<70 mg/dl) was 1.2% with CT, 6% with MI, and 5.4% with CSII. Although the incidence of hypoglycemia tended to be slightly increased under intensive insulin therapy with either MI or CSII, these were less symptomatic than during CT. There was no episode of ketoacidosis or hypoglycemic coma during the study period.

Insulin requirement to achieve good metabolic control during MI and CSII. There was no difference between insulin requirement during CSII (42 ± 2.2 U/24 h) and during CT (37 ± 5 U/24 h) despite better metabolic control with the former. This is probably due to a better distribution of the insulin dose throughout the day with CSII. With MI, however, a significantly higher insulin dose was required (54 ± 4.2 U/24 h, $P < .05$) to achieve similar glucose control. When the boluses and basal rates are analyzed separately, it becomes obvious that the difference is in the basal rates (16 ± 1.9 U/24 h with CSII and 23 ± 3 U/24 h with MI, $P < .005$) but not in the boluses (26 ± 2.2 U/24 h with CSII and 31 ± 4.1 U/24 h with MI) (Fig. 2). The basal rate was changed during the day with the programmable pump to achieve better glycemic control (Fig. 3); this resulted in a peak insulin infusion rate at dawn (0400–0800 h: 1.0 ± 0.1 U/h) and a nadir by the early morning hours (midnight to 0400 h: 0.5 ± 0.1 U/h). The basal rate was changed according to AC glycemia during the day and tended to be lower during the active period of the day (0.5 ± 0.07 U/h) and higher during the evening (0.8 ± 0.1 U/h). The ratio between the basal rate and the total insulin dose was 0.38 ± 0.04 with CSII and 0.43 ± 0.05 with MI.

Glycosylated hemoglobin. Glycosylated hemoglobin was 10.1 ± 0.5% during CT. It decreased significantly to 8.8 ± 0.4% during MI ($P < .05$) and 8.2 ± 0.3% during CSII ($P < .005$). There was no significant difference between the MI and the CSII treatment periods.

Effect of MI and CSII on ^{125}I-insulin binding to monocytes. Compared with a group of 17 normal controls, the

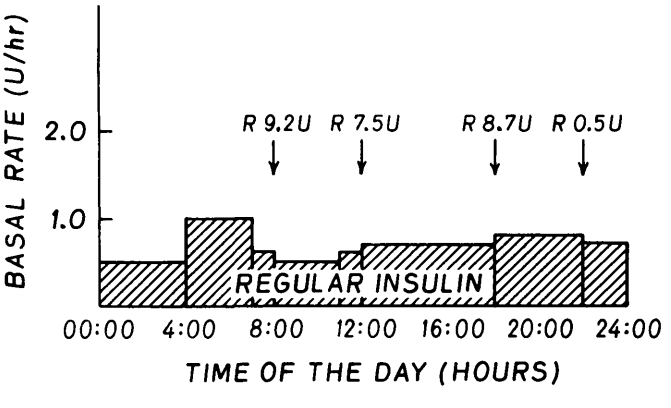

FIG. 3. Mean basal insulin infusion rate and boluses during the Autosyringe AS6MP pump treatment in IDDM patients (n = 8).

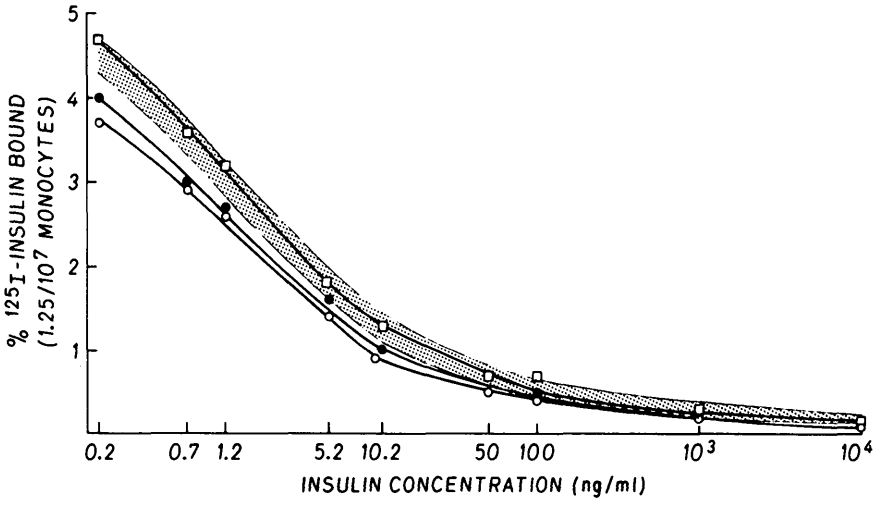

FIG. 4. Monocyte ^{125}I-labeled insulin binding in a group of normal subjects (n = 17; means ± SE, *stippled area*) and IDDM patients (n = 8) during conventional treatment (○), multiple injections (●), and CSII (□).

specific ^{125}I-insulin binding to monocytes in the diabetic patients was significantly lower during conventional therapy (P = .049; Fig. 4). The binding remained suppressed during MI despite good glycemic control. Interestingly, during CSII the insulin binding to monocytes normalized (Fig. 4) and became significantly different from the insulin binding during CT (P = .009) and MI (P = .018). The mean specific ^{125}I-insulin binding at 0.2 ng/ml insulin was 3.7 ± 0.3% with CT and increased to 4.0 ± 0.3% (NS) during MI and 4.8 ± 0.27% (P < .02) during CSII (Fig. 5). These are compared with 17 normal subjects who had a specific ^{125}I-insulin binding to monocytes of 4.6 ± 0.2%. The affinity for the receptors was normal before the start of the study and was not affected by either treatment. The concentration of insulin required to displace 50% of the bound ^{125}I-insulin was 2.8 ± 0.3 ng/ml during CT, 3.6 ± 0.8 ng/ml during MI, and 2.9 ± 0.4 ng/ml during CSII.

Plasma free insulin. The fasting plasma free insulin was determined at 0800 h before the study and at the end of each study. No difference was observed during the different treatments (11.2 ± 2.2 μU/ml during CT, 11.2 ± 1.1 μU/ml during MI, and 11.8 ± 2.2 μU/ml during CSII). However, serial diurnal insulin measurements were not made to evaluate plasma insulin around the clock.

DISCUSSION

As we (19) and others (11–14) have previously shown, good glucose control can be achieved by either MI or CSII. Both types of intensive insulin therapy resulted in a decrease in the excursion of the blood glucose. It was also noted that the patients on intensive insulin therapy tolerated lower blood glucose (45–50 mg/dl) without any hypoglycemic symptom, probably because of the very slow and progressive drop in blood glucose. No episode of hypoglycemic coma was observed during either intensive insulin treatment, and there was no episode of ketoacidosis due to malfunction of the pump or obstruction of the catheter as observed in other studies (27–28).

The insulin required to achieve normal blood glucose during CSII was similar to that used during CT. This may be due to a better distribution of the insulin throughout the day, which is similar to our previous observation (19). Also similar to our previous observation was the higher insulin requirement (20–30%) during MI compared with CSII. This higher insulin requirement was due mainly to the differences in the basal insulin requirement. Note that with the Auto-

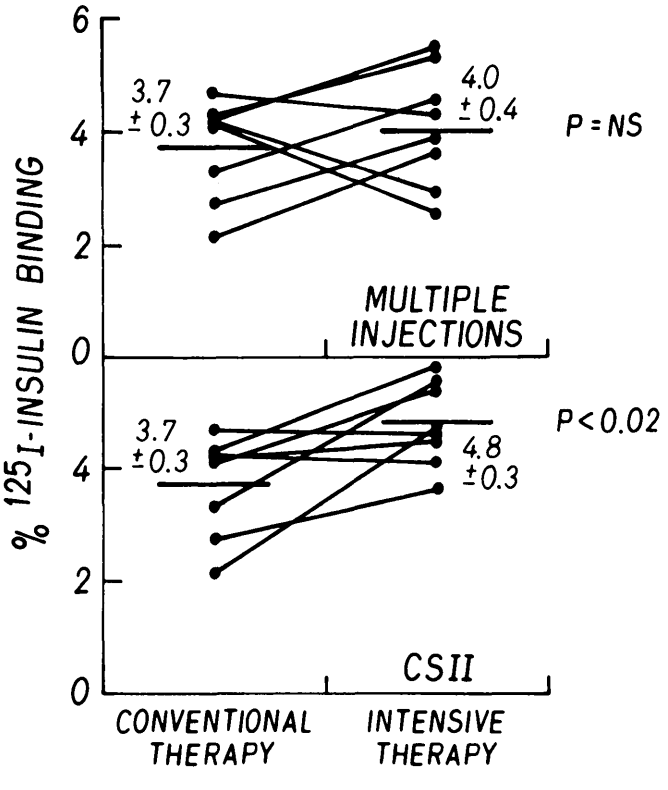

FIG. 5. Monocyte ^{125}I-labeled insulin binding at low insulin concentration (0.2 ng/ml) in IDDM patients (n = 8) during multiple injections and continuous subcutaneous insulin infusion (CSII) compared with conventional therapy.

syringe programmable pump, the basal rate varied throughout the day from 0.5 to 1.0 U/h. Note also that all patients under CSII had a relatively low insulin requirement during the early morning hours (midnight to 0400 h) with a significant increase in the requirement (2–3 times) to avoid morning hyperglycemia. This is compatible with the suggested dawn phenomenon (29). Note that the insulin requirement was also lower between 0800 and 1700 h than in the evening. This could be explained at least partly because the subjects were more active physically in the daytime. Also, the lower basal insulin rate between 0800 and 1200 h can be explained in part by the bigger bolus required before breakfast to prevent postprandial hyperglycemia; the basal rate had to be decreased to prevent hypoglycemia before the subsequent meal.

This higher insulin requirement during MI compared with CSII raised the question as to whether this was due to increased degradation at the site of injection or whether higher insulin levels were required to achieve similar glucose control. We could not show any significant difference in the fasting plasma free-insulin concentrations during the different treatment periods. However, because of the presence of circulating insulin antibodies, these had to be precipitated with polyethylene glycol, which introduces variations in the assay that could have overshadowed small yet important differences. More important, however, the plasma free-insulin concentration was measured at only one time (0800 h). Because that time coincides with the highest basal insulin rate during the CSII treatment, differences might have been found if we had measured the free-insulin levels throughout the day. Because this was not practical, we chose to look at the effect of each treatment regimen on the ^{125}I-insulin binding to monocytes. It has been shown that the insulin concentration is inversely proportional to the number of insulin receptors on lymphocytes (30), hepatocytes (31), fibroblasts (32), and adipocytes (33). The half-life of the insulin receptors is 30–40 h in vivo and therefore gives us a good appreciation of the insulin levels over the 24–48 h preceding the blood sampling (34).

This study showed that the insulin monocyte receptors were suppressed during CT when compared with a control group. The insulin receptors normalized during CSII but were not significantly affected by MI despite comparable glucose control. Under many conditions, in vivo and in vitro, the concentration of insulin receptors decreases in the presence of high insulin levels (30,34). Thus, the decreased number of insulin receptors observed during CT suggests higher overall insulin concentrations compared with controls. Normalization of insulin receptors during CSII therapy suggests that more physiologic insulin levels were obtained than during either CT or MI therapy. Pederson and Hjøllund (35) have also shown a decrease in the number of adipocyte insulin receptors in IDDM treated by CT compared with normal subjects; however, they did not observe any modification in the number of monocyte and erythrocyte insulin receptors. Tki-Jarvinen and Koivisto (36) did not find a change in the erythrocyte insulin receptors during pump treatment. This difference, however, may reflect the lower sensitivity of the erythrocyte receptors to changes in insulin concentrations (37). Our study is therefore the first to show a different effect between MI and CSII on insulin receptors. This observation could suggest that MI is associated with higher insulin levels than CSII for a similar good glucose control; such a conclusion, however, should be confirmed by a 24-h insulinemia profile. This could have important clinical implications if the epidemiological studies showing a correlation between hyperinsulinemia and cardiovascular disease are confirmed. (20,38)

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