

# Improved In Vivo Insulin Effect During Continuous Subcutaneous Insulin Infusion in Patients with IDDM

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## SUMMARY

It has recently been shown that conventionally treated IDDMs are insulin resistant. Using the insulin clamp technique, we studied the influence of metabolic status on the in vivo insulin effect in these patients. Eleven IDDMs, treated conventionally with diet and insulin for  $10.7 \pm 5.6$  yr, were studied before and after continuous subcutaneous insulin infusion (CSII) treatment (with a portable pump) for 6 mo. We found that conventionally treated diabetic subjects were extremely insulin resistant with regard to peripheral glucose uptake. Glucose uptake, at an insulin concentration of about  $80 \mu\text{U/ml}$ , was  $4.3 \pm 2.0 \text{ mg/kg} \cdot \text{min}$  before treatment compared with  $11.5 \pm 4.0 \text{ mg/kg} \cdot \text{min}$  in normals ( $P < 0.01$ ). After pump treatment for 6 mo, metabolic control improved significantly ( $\text{HbA}_{1c}$  decreased from  $8.9 \pm 1.9\%$  to  $7.4 \pm 1.2\%$ ,  $P < 0.01$ ) and, parallel to that, glucose uptake increased about 80% to  $7.5 \pm 3.5 \text{ mg/kg} \cdot \text{min}$  ( $P < 0.01$ ). The mean daily plasma FFA level decreased from  $0.32 \pm 0.10 \text{ mmol/L}$  to  $0.21 \pm 0.07 \text{ mmol/L}$  ( $P < 0.01$ ); this variable was negatively correlated to the glucose clearance rate ( $r = -0.62$ ,  $P < 0.01$ ). There was no statistically significant change in mean daily plasma insulin and plasma growth hormone levels or in 24-h cortisol excretion in the urine ( $P > 0.1$ ). The insulin binding capacity of serum IgG was also unchanged, and there was no significant relationship between this quantity and glucose clearance rates ( $r = 0.18$ ,  $P > 0.1$ ). We conclude that conventionally treated IDDMs are insulin resistant with regard to peripheral glucose uptake. The insulin resistance may, at least in part, be secondary to the abnormal metabolic status of these patients. *DIABETES* 1984; 33:832-37.

In contrast to the findings in non-insulin-dependent diabetic subjects, insulin resistance has not been suggested to play any important role in insulin-dependent diabetes (IDDM).<sup>1</sup> Recently, however, it has been shown in both in vivo and in vitro studies that the cellular effect of insulin is reduced in IDDM.<sup>2-4</sup> DeFronzo et al. have shown

that the insulin resistance in these patients seems mainly located to the peripheral tissue.<sup>2</sup> They found reduced peripheral glucose uptake, as measured by the insulin clamp technique, and a normal suppression of the hepatic glucose release at an insulin concentration of about  $100 \mu\text{U/ml}$ . In accordance with these findings, Pedersen and Hjøllund showed that insulin-mediated glucose metabolism in isolated adipocytes was significantly reduced due to both insulin receptor and postreceptor defects.<sup>4</sup>

The etiology of insulin resistance in IDDM is still unknown. It has been hypothesized that it is secondary to the peripheral hyperinsulinism induced by subcutaneous (s.c.) insulin injection in these patients.<sup>4</sup> However, this hypothesis has not been investigated. Another possibility is that the insulin resistance is secondary to hormonal changes or to metabolic abnormalities. Many insulin-treated diabetic subjects produce insulin antibodies. The possible effect of these antibodies on the in vivo insulin effect are still incompletely elucidated.

In this study, we examined the in vivo insulin effect in IDDM using the insulin clamp technique, both before and after continuous subcutaneous insulin infusion (CSII) treatment for 6 mo. CSII treatment resulted in near-normalization of plasma glucose and FFA values. This study, therefore, gave us the opportunity to evaluate the influence of metabolic changes on insulin action in IDDM. Furthermore, we estimated the influence of plasma levels of insulin, insulin antibodies, growth hormone, and cortisol.

## MATERIALS AND METHODS

**Subjects.** The study population consisted of 11 nonobese IDDM and 10 normal subjects. None of the diabetic subjects had signs of retinopathy or nephropathy. Age, sex, body weight, diabetes duration, daily insulin dose, insulin binding

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TABLE 1

Clinical and laboratory data of 10 normal and 11 insulin-dependent diabetic subjects, before and after continuous subcutaneous insulin infusion (insulin pump treatment) for 6 mo (mean  $\pm$  1 SD)

	Age (yr)	Sex	Obesity index	Duration of diabetes (yr)	Insulin dose (U)	HbA <sub>1c</sub> (%)	Fasting plasma glucose (mmol/L)	Fasting plasma FFA (mmol/L)	Fasting plasma ketone bodies (mmol/L)	Fasting plasma insulin ( $\mu$ U/ml)	Insulin binding capacity of IgG (mU/ml)
IDDs before pump treatment	28.8 $\pm$ 7.1	6 F 5 M	1.02 $\pm$ 0.05	10.7 $\pm$ 5.6	43.2 $\pm$ 5.6	8.9 $\pm$ 2.0	8.9 $\pm$ 4.5	0.66 $\pm$ 0.37	0.89 $\pm$ 0.76	15 $\pm$ 6	0.25 $\pm$ 0.47
IDDs after 6 mo of treatment	—	—	1.03 $\pm$ 0.06	—	37.6 $\pm$ 7.0	7.4 $\pm$ 1.2	5.6 $\pm$ 2.1	0.36 $\pm$ 0.25	0.29 $\pm$ 0.21	22 $\pm$ 10	0.21 $\pm$ 0.27
Normal subjects	26.0 $\pm$ 5.1	5 F 5 M	1.01 $\pm$ 0.04				5.4 $\pm$ 0.4	0.32 $\pm$ 0.12	0.34 $\pm$ 0.42	10 $\pm$ 4	

capacity of IgG, HbA<sub>1c</sub> values, and fasting plasma glucose, FFA, and ketone body levels are given in Table 1. None of the subjects were taking medicine other than insulin (diabetic subjects). The diabetic subjects were treated with a weight-maintaining diet consisting of 45% fat, 17% protein, and 38% carbohydrate. During conventional treatment, the last insulin dose was given at 5 p.m. the day before the clamp study. During CSII treatment, the pump was removed 1 h before the investigation. All studies were performed at 8 a.m. after a 12-h overnight fast. The insulin clamp was performed between 8 and 10 a.m. Informed consent was obtained from all subjects. The study protocol was reviewed by the hospital ethical committee.

**Protocol.** The IDDMs were studied before and after CSII treatment for 6 mo with a portable pump (Auto-Syringe Model AS 6C, Auto-Syringe, Inc., Hooksett, New Hampshire) using Velosulin, 40 IE/ml (Nordisk Insulin, Copenhagen, Denmark).

The pump injected insulin continuously, and 30 min before the meals a bolus was given by the patients when activating the pump. About 50% of the 24-h dose was given as boluses. Insulin was infused through a 25-gauge butterfly needle placed in the s.c. tissue of the abdominal wall. During pump treatment, diabetes control was evaluated by measuring fasting plasma glucose, 24-h glucose excretion, and HbA<sub>1c</sub> values. Furthermore, the following variables were measured both before and after 6 mo of treatment: diurnal pattern of plasma FFA, plasma free insulin, and serum growth hormone, as well as fasting plasma ketone bodies, insulin binding serum IgG, and 24-h cortisol excretion in urine.

**Insulin clamp studies.** Two catheters were inserted, one into an antecubital vein for injection and the other retrogradely into a forearm vein for blood sampling. The arm was heated to 50°C by a lamp. A primed-continuous (40 mU/m<sup>2</sup> · min) infusion of Velosulin was injected in all subjects to raise and maintain the plasma insulin concentrations at a level of about 100  $\mu$ U/ml. The glucose concentration was kept at the fasting level by measuring plasma glucose every 5 min and by adjustment of the infusion rate of a 20% glucose solution. Under steady-state conditions, glucose input equals glucose utilization. The glucose release from the liver is negligible in this situation (plasma insulin about 100  $\mu$ U/ml),<sup>2</sup> and, therefore, the glucose utilization is fairly identical to the glucose infusion minus the loss of glucose in the urine. The diabetic subjects were clamped at the same plasma glucose concentration after CSII treatment as before treat-

ment. Therefore, it was necessary to infuse glucose in some patients before the second clamp study began.

**Calculations.** The steady state for glucose utilization was not obtained until after 80 min of infusion. Therefore, the glucose infusion rate was estimated from 80 to 120 min. The glucose utilization rate is equal to the glucose infusion rate minus loss of glucose in the urine. The glucose clearance was calculated by dividing the total amount of glucose metabolized during 80–120 min by the mean plasma glucose concentration during the same period.

**Analytic procedures.** Plasma glucose was analyzed by a glucose-dehydrogenase method (Merck enzymatic kit) and plasma 3-hydroxybutyrate and acetoacetate were measured separately by an enzymatic method.<sup>5</sup> In the RESULTS, the sum of the two concentrations is given. Plasma free fatty acids (FFA, aliphatic carboxylate C<sub>8</sub> to C<sub>18</sub>, nonesterified) were analyzed according to Itaya and Uj.<sup>6</sup> In controls, plasma insulin was measured by an RIA technique.<sup>7</sup> Free insulin in plasma of diabetic subjects was measured by the same assay after the plasma had been incubated at 37°C for 150 min and subsequently precipitated with polyethylene glycol.<sup>8</sup> The insulin-binding capacity of IgG was determined by means of immunoelectrophoresis.<sup>9</sup> The cortisol concentration in the urine was determined according to Murphy.<sup>10</sup> Stable HbA<sub>1c</sub> (percent of total hemoglobin) was measured ad modum Svendsen et al.<sup>11</sup> Human growth hormone in serum was estimated ad modum Ørskov et al.<sup>12</sup>

**Statistical methods.** Wilcoxon's test for paired differences and Spearman's correlation were used. In the text and in Table 1, the results are expressed as mean  $\pm$  1 SD, whereas the mean value  $\pm$  1 SEM is used in the figures.

## RESULTS

**Effect of CSII treatment on metabolic status.** During CSII treatment for 6 mo, we found that fasting plasma glucose values were reduced from 8.9  $\pm$  4.5 mmol/L to 5.6  $\pm$  2.1 mmol/L ( $P < 0.01$ ) and 24-h glucose excretion was reduced from 273  $\pm$  164 mmol to 18  $\pm$  16 mmol ( $P < 0.01$ ). HbA<sub>1c</sub> fell from 8.9  $\pm$  1.9% to 7.4  $\pm$  1.2% ( $P < 0.01$ , Table 1).

During the study period, the daily insulin dose was reduced from 43.5  $\pm$  10.1 IE/day to 37.6  $\pm$  8.4 IE/day ( $P < 0.05$ ). The diurnal variations in plasma free insulin concentrations are given in Figure 1. At 7:30 a.m., the plasma insulin values were significantly higher during pump treatment compared with conventional treatment ( $P < 0.05$ ). However, at

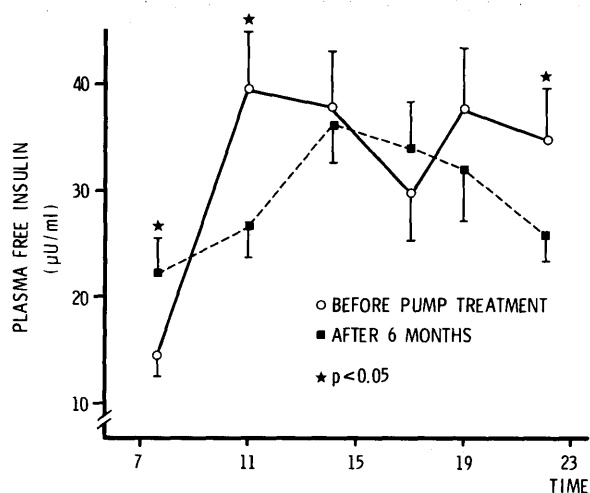


FIGURE 1. The diurnal variation in plasma free insulin concentrations before and after CSII treatment with a portable pump for 6 mo.

11 a.m. and 10 p.m. the insulin concentrations were lower during pump treatment ( $P < 0.05$ ), whereas the insulin values were similar at all other time points tested ( $P > 0.1$ ). The mean diurnal plasma free insulin concentrations (from 7:30 a.m. to 10 p.m.) were not statistically significantly different in the two situations,  $32 \pm 12 \mu\text{U/ml}$  before and  $29 \pm 8 \mu\text{U/ml}$  after pump treatment for 6 mo ( $P > 0.1$ ).

Plasma FFA values were reduced by pump treatment. The diurnal pattern is given in Figure 2. It can be seen that the FFA values were lower during pump treatment at 7:30 a.m. ( $P < 0.01$ ) and at 2, 5, and 7 p.m. ( $P < 0.05$ ). The mean diurnal FFA level was reduced from  $0.32 \pm 0.10 \text{ mmol/L}$  to  $0.21 \pm 0.07 \text{ mmol/L}$  ( $P < 0.01$ ). In parallel, the fasting plasma ketone body level was also reduced, as indicated in Table 1.

The mean diurnal growth hormone level was unchanged during pump treatment,  $2.5 \pm 0.51 \text{ ng/ml}$  versus  $2.3 \pm 0.3 \text{ ng/ml}$  ( $P > 0.1$ , not shown). However, the fasting plasma growth hormone level decreased from  $3.2 \pm 0.9 \text{ ng/ml}$  to  $1.5 \pm 0.3 \text{ ng/ml}$  ( $P < 0.05$ , not shown). The 24-h cortisol excretion in the urine was unchanged,  $256 \pm 99 \text{ nmol}$  versus  $234 \pm 86 \text{ nmol}$  ( $P > 0.05$ ).

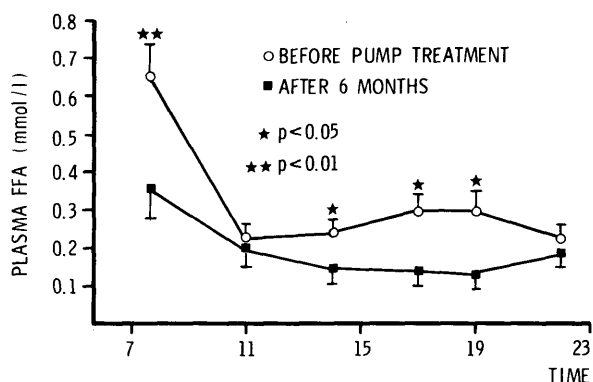


FIGURE 2. The diurnal variation in plasma FFA concentrations before and after CSII treatment with a portable pump for 6 mo.

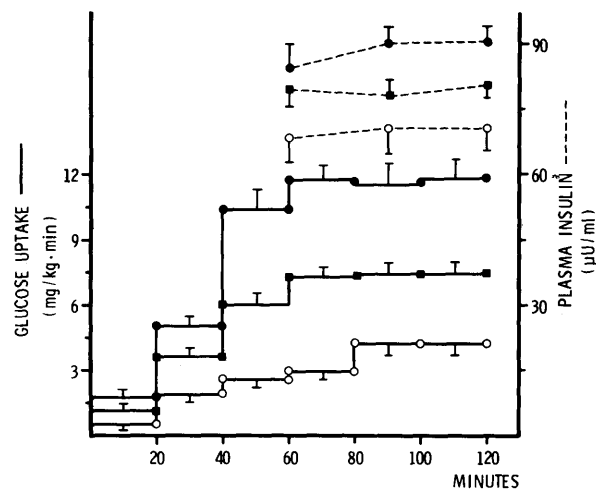


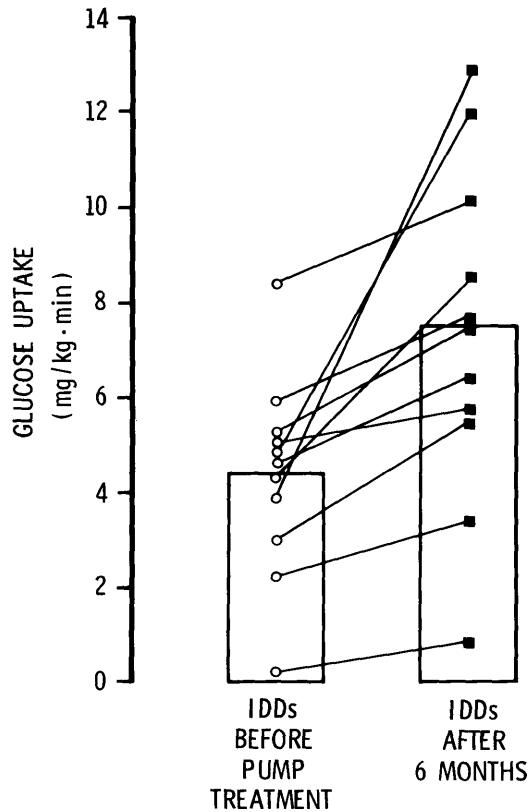
FIGURE 3. Glucose uptake during insulin clamp in normals (●—●) and in IDDMs before (○—○) and after (■—■) CSII treatment for 6 mo. Steady-state plasma insulin (in diabetic subjects, plasma free insulin) concentrations are given at the top of the figure.

**Insulin clamp studies.** In Figure 3, the glucose uptake during the clamp study is shown for both normal and diabetic subjects before and after pump treatment for 6 mo. The steady state of glucose uptake was reached after 60 min in normals and in CSII-treated diabetic subjects, whereas a steady state was first obtained after 80 min in conventionally treated diabetic subjects. The steady-state plasma free insulin values are also given in Figure 3. In normals, the level was  $88 \pm 11 \mu\text{U/ml}$  and in conventionally treated diabetic subjects,  $71 \pm 16 \mu\text{U/ml}$  ( $P < 0.05$ ). After pump treatment for 6 mo, the insulin level was unchanged,  $80 \pm 16 \mu\text{U/ml}$  ( $P > 0.1$ ). The steady-state plasma glucose concentration during the clamp was, in normals,  $4.9 \pm 0.6 \text{ mmol/L}$  and, in diabetic subjects,  $11.0 \pm 4.1$  and  $11.1 \pm 4.2 \text{ mmol/L}$ , respectively, before and after pump treatment.

Estimated from 80 to 120 min, we found a glucose uptake of  $11.5 \pm 4.0 \text{ mg/kg} \cdot \text{min}$  (Figure 3) in normals. In conventionally treated diabetic subjects, the glucose uptake was significantly lower,  $4.3 \pm 2.0 \text{ mg/kg} \cdot \text{min}$ , indicating an extreme insulin resistance in these patients ( $P < 0.01$ , Figure 3). However, during CSII treatment for 6 mo, the glucose uptake was significantly increased to  $7.5 \pm 3.5 \text{ mg/kg} \cdot \text{min}$  ( $P < 0.01$ , Figure 4).

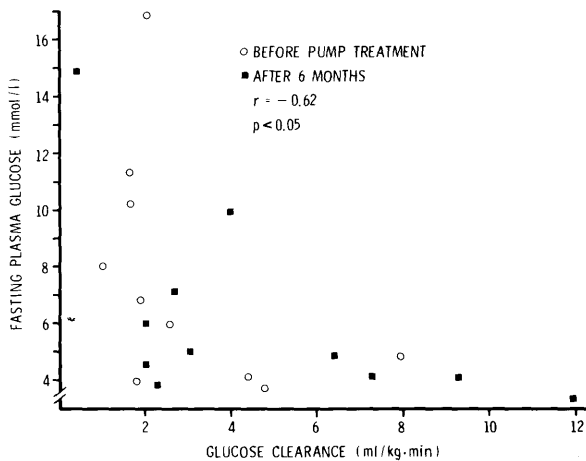
We found that the glucose clearance rate in diabetic subjects (before and after pump treatment) was negatively correlated to the fasting plasma glucose values ( $r = -0.62$ ,  $P < 0.05$ , Figure 5). Furthermore, the glucose clearance rate was negatively correlated to both the fasting plasma FFA levels ( $r = -0.53$ ,  $P < 0.05$ , not shown) and the mean diurnal FFA level ( $r = -0.62$ ,  $P < 0.01$ , Figure 6), whereas no statistically significant correlation was found between the glucose clearance rate and the mean diurnal growth hormone levels or the 24-h cortisol excretion ( $P > 0.1$ , not shown). However, the glucose clearance rate was negatively correlated to the fasting plasma free insulin levels ( $r = -0.60$ ,  $P < 0.05$ , not shown), but not to the mean daily plasma insulin level ( $r = -0.24$ ,  $P > 0.1$ , not shown).

**Insulin antibodies.** In 7 diabetic subjects, a significant insulin-binding capacity of serum IgG ( $> 0.05 \text{ mU/ml}$ ) was

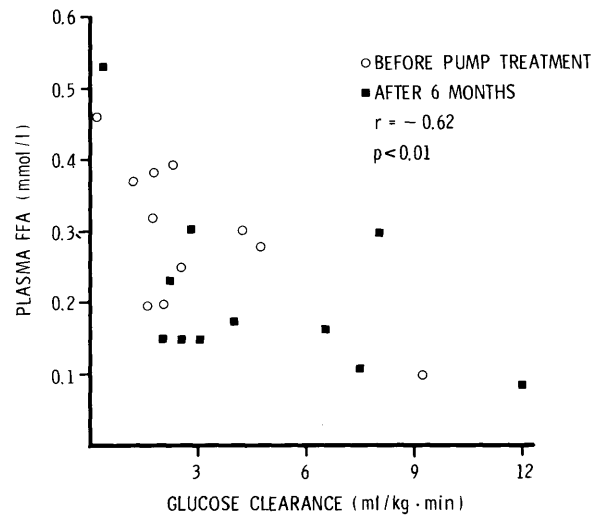


**FIGURE 4.** Glucose uptake in IDDMs before and after 6 mo of CSII treatment.

demonstrated. Before pump treatment, the fasting antibody level was  $0.25 \pm 0.47$  mU/ml and after treatment,  $0.21 \pm 0.27$  mU/ml ( $P > 0.1$ , Table 1). Insulin antibodies did not seem to influence the results of the clamp study, since the glucose clearance rate did not correlate with the antibody level ( $r = 0.18$ ,  $P > 0.1$ , Figure 7). Furthermore, no difference was found between glucose clearance rates in diabetic subjects with and without antibodies ( $P > 0.1$ , Figure 7). The reduced steady-state plasma insulin levels in diabetic subjects compared with normals may, at least in part, be caused



**FIGURE 5.** Correlation between fasting plasma glucose concentrations and glucose clearance rates in IDDMs.

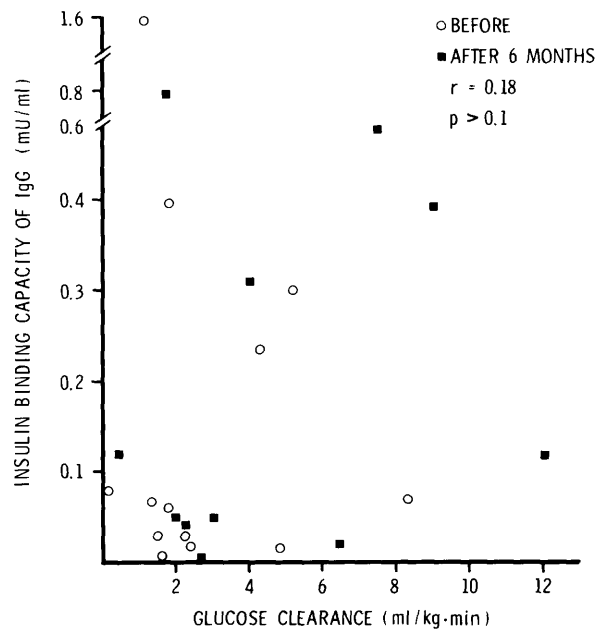


**FIGURE 6.** Correlation between the mean diurnal plasma FFA values and glucose clearance rates in IDDMs.

by insulin-binding antibodies. The antibodies seem not to be responsible alone, as no significant correlation was found between the antibody levels and the steady-state plasma free insulin levels ( $r = 0.31$ ,  $P > 0.1$ , not shown).

**DISCUSSION**

In this study, we found that IDDMs treated for about 10 yr with diet plus insulin are severely insulin resistant. The peripheral glucose uptake was reduced to a level of about 40% of that found in normals. CSII treatment for 6 mo resulted in an 80% increase in the peripheral glucose uptake. However, normal and diabetic subjects were not clamped at the same glucose level (4.9 versus 11.1 mmol/L). If the normals had been clamped at a glucose level of 11.1 mmol/L, the glucose uptake measured would have been even higher, resulting in



**FIGURE 7.** Correlation between the insulin-binding capacity of serum IgG (insulin antibodies) and glucose clearance rates in IDDMs.

a greater difference between normal and diabetic subjects.<sup>13,14</sup>

Until recently, insulin resistance was not supposed to play any role in IDDM. However, several years ago, insulin resistance was demonstrated in IDDM, but these studies have not been followed up.<sup>15,16</sup> Recently, Harrano et al., DeFronzo et al., and Pedersen and Hjøllund have shown that insulin resistance is a common feature of IDDM.<sup>2-4</sup> DeFronzo et al. found, with the insulin clamp technique, that the peripheral glucose uptake in IDDM was reduced to a level of 35% of that in normals. The basal hepatic glucose release was increased in IDDM, whereas the suppression of the hepatic glucose release at an insulin concentration of 100  $\mu\text{U}/\text{ml}$  was similar in IDDMs and in normals. These data concur with the results of Bearn et al. and Sacca et al., who used the hepatic venous catheter technique and the radioisotope technique, respectively.<sup>17,18</sup> Therefore, these authors concluded that the insulin resistance in IDDM is located in the peripheral tissues. At the cellular level, using the adipocyte model, Pedersen and Hjøllund found that the maximal insulin-stimulated glucose oxidation and lipogenesis in IDDMs were reduced to a level of about 30%.<sup>4</sup> Furthermore, the number of insulin receptors per surface area was reduced and accompanied by a rightward shift of the insulin dose-response curve for glucose transport. Our data are consistent with these findings, and, therefore, it may be concluded that IDDMs are insulin resistant with regard to glucose uptake in the peripheral tissues. The resistance may be due to both receptor and postreceptor defects; however, other mechanisms may also be operative.

It could be argued that the insulin resistance may, in part, be due to the presence of insulin antibodies. In fact, steady-state plasma insulin levels were about 20% lower in diabetic than in normal subjects, which may be due to the antibodies. However, the antibody level was neither correlated to the steady-state plasma insulin levels nor to the glucose clearance rates. Furthermore, the glucose clearance rate increased 80% during CSII treatment, whereas the antibody level was unchanged. Thus, the antibodies seem not to have any significant impact on the calculated glucose uptake in IDDM.

The 20% lower steady-state plasma free insulin concentration in diabetic subjects only results in a slight reduction of the glucose uptake (about 10%) if one extrapolates the results on an insulin dose-response curve obtained with the insulin clamp technique at different insulin concentrations.<sup>19</sup> Thus, the reduced insulin effect in the IDDMs is not explained by the lower steady-state insulin level during the clamp.

We have recently found that the insulin effect on glucose metabolism in adipocytes is further reduced by CSII treatment. This finding emphasizes the importance of the muscles for the improvement of insulin resistance seen during pump treatment.

After CSII treatment for 6 mo, the plasma glucose and FFA values were nearly normalized. Both glucose and FFA levels were negatively correlated with the glucose clearance rates. Thus, patients with poor metabolic control are more insulin resistant than are patients in good control. Therefore, we may conclude that the insulin resistance found in IDDM, at least in part, is secondary to metabolic derangements.

It has been shown that an increase in the growth hormone

level in a few hours can induce insulin resistance.<sup>20</sup> In this study, we found a lower morning growth hormone level in the patients treated with CSII for 6 mo. This reduction may, in part, add to the improved insulin effect measured. Accordingly, it is important to emphasize that an increase in growth hormone concentrations results in an increase in FFA values.

It has been claimed that the insulin resistance in IDDM is due to peripheral hyperinsulinism.<sup>3</sup> In fact, there was a negative correlation between insulin-mediated glucose clearance and fasting plasma free insulin levels. However, the diurnal plasma free insulin level was not reduced during pump treatment. The improved insulin effect, therefore, seems not to be due to a reduction in plasma insulin concentrations. Likewise, changes in the cortisol level do not seem to be of any significance.

In this study, the diabetic subjects were clamped at their fasting plasma glucose level the first time and at the same glucose level the second time. As the glucose uptake varies with the glucose concentration, it is impossible to compare the glucose uptake values when the patients are studied at different glucose concentrations.<sup>13,14</sup> Therefore, in correlation studies, we used glucose clearance rates. However, these values are not completely independent of glucose concentrations.<sup>13,14</sup> On the other hand, at a high insulin level of about 100  $\mu\text{U}/\text{ml}$ , the glucose clearance rates in normals seem to be fairly independent of the glucose values.<sup>14</sup> The relationship between the two variables in diabetic subjects is still unknown.

In this study, the *in vivo* insulin action was estimated at a single insulin concentration. At this concentration, the glucose release from the liver seems to be completely suppressed. It was not possible in this study to evaluate if the insulin resistance seen in IDDM is due to an alteration of insulin sensitivity or maximal insulin responsiveness in the peripheral tissues. Finally, it is unknown whether CSII may affect the insulin sensitivity of hepatic glucose release.

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