Changes in Insulin Resistance With Long-Term Insulin Therapy

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Thirteen newly diagnosed diabetic subjects, 5 with insulin-dependent diabetes mellitus (IDDM) and 8 with non-insulin-dependent diabetes mellitus, mean age 37.1 yr (range 25–64 yr), underwent glucose-clamp studies at diagnosis of diabetes at plasma glucose 200 mg/dl. Each subject was then treated twice daily with insulin for 6 mo with improvement in glycemic control, and the glucose-clamp studies repeated. Changes in glucose uptake at an insulin infusion rate of 1.0 mU · kg^{-1} · min^{-1} varied greatly from diagnosis to 6 mo. There were significant negative correlations between change in glucose uptake and diabetes type ($r = -.78$, $P < .002$), C-peptide secretion ($r = -.66$, $P < .05$), and age ($r = -.62$, $P < .05$). At an insulin infusion rate of 10 mU · kg^{-1} · min^{-1} there was improvement in glucose uptake from diagnosis to 6 mo that did not reach statistical significance. During the steady-state periods of the glucose-clamp studies at diagnosis, growth hormone (GH) rose above basal, which reached statistical significance at the higher insulin infusion rate. This increase in GH was not apparent at the time of the glucose-clamp studies after insulin therapy. Our results indicate that in the clinical situation, only patients with IDDM can expect an improvement in their sensitivity to physiologic insulin levels with long-term insulin therapy. In all subjects, improvement in glycemic control leads to abolition of GH secretion in the presence of hyperglycemia. *Diabetes Care* 10:56–61, 1987

Insulin resistance is thought to play a part in the pathogenesis of both insulin-dependent (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) (1,2). Short-term insulin therapy has been shown to improve insulin resistance in both forms of diabetes (3–10), although there have been no reports of the effect of prolonged insulin therapy. We report on our observations of changes in insulin sensitivity with insulin treatment in a broad spectrum of IDDM and NIDDM subjects. This study differs from those above in that all subjects were newly diagnosed and had no prior treatment. Insulin treatment was continued for 6 mo on an outpatient basis.

PATIENTS AND METHODS

Thirteen newly diagnosed diabetic subjects were recruited for the study, and their clinical characteristics are given in Table 1. At diagnosis, no subject had more than moderate ketonuria or had experienced significant weight loss. None had proteinuria, as measured by Albustix (Ames, Elkhart, IN), and all had normal values for plasma urea and creatinine. Subjects 1 and 7 had mild background retinopathy; subjects 10, 11, and 12 had proliferative retinopathy. Based on clinical characteristics and the recommendations of the National Diabetes Data Group (11), 5 subjects were classified as IDDM and 8 as NIDDM.

Before commencing treatment, each subject underwent a glucose-clamp procedure to measure insulin sensitivity (Figs. 1 and 2). These studies were performed on an outpatient basis, with the patient attending the hospital on the morning of the study day after an overnight fast. The method used was modified from that of DeFronzo et al. (12). Blood samples were taken at −20 and −5 min for measurement of plasma glucose and insulin. A logarithmically decreasing infusion of short-acting insulin was then given for 10 min, followed by a continuous infusion at a rate of 1.0 mU · kg^{-1} · min^{-1}. A variable infusion of 50% dextrose was then given to maintain the plasma glucose of arterialized venous blood at 200 mg/dl. Insulin was infused in all subjects for 180 min, during which the plasma glucose was stabilized. The insulin infusion was maintained for 80 min thereafter, and this period was

TABLE 1
Patient characteristics

Subject	Sex (M/F)	Age (yr)	BMI at diagnosis	Weight (kg)		HbA_1 (%)		Fasting insulin (mU/L)	Insulin dose (U)	Diabetes type
				Before	After	Before	After			
1	M	50	29.7	82.9	88.0	13.1	10.1	17.1	24	NIDDM
2	M	29	23.8	71.4	75.3	9.6	5.9	6.4	30	IDDM
3	M	25	22.8	69.2	69.4	11.3	8.0	5.1	44	IDDM
4	M	30	19.4	54.0	67.9	13.9	10.1	5.1	47	IDDM
5	F	35	25.5	61.4	59.5	12.0	7.7	13.7	39	NIDDM
6	M	25	20.4	59.0	61.0	13.8	8.4	4.7	36	IDDM
7	M	53	23.0	65.8	71.2	13.4	10.0	15.9	28	NIDDM
8	M	32	29.1	83.2	84.0	16.4	8.7	10.6	24	NIDDM
9	M	30	23.2	67.0	73.0	14.4	8.5	6.6	30	IDDM
10	M	64	23.0	66.5	82.1	14.5	6.4	7.8	38	NIDDM
11	F	29	20.1	51.0	58.0	13.8	8.8	6.0	32	NIDDM
12	M	51	24.8	69.2	75.6	11.5	9.0	4.0	36	NIDDM
13	M	30	21.1	64.0	75.0	14.5	7.8	6.0	32	NIDDM
IDDM (mean ± SD)		27.8 ± 2.6*	21.9 ± 1.9	64.1 ± 7.3	69.3 ± 5.5	12.6 ± 2.1	8.2 ± 1.5	5.6 ± 0.9*	37.4 ± 7.9	
NIDDM (mean ± SD)		43.0 ± 13.1*	24.5 ± 3.5	68.0 ± 10.8	74.2 ± 10.9	13.6 ± 1.5	8.6 ± 1.2	10.1 ± 5.0*	31.6 ± 5.9	

Body mass index (BMI) = wt (kg)/ht^2 (m).
*$P < .05$, comparison of NIDDM and IDDM groups.

used for calculation. Another logarithmically decreasing infusion of insulin was then given to elevate the plasma insulin to ~1000 mU/L, followed by an infusion at a rate of 10 $mU \cdot kg^{-1} \cdot min^{-1}$. Thirty minutes was allowed for stabilizing the plasma glucose, and the next 80 min was used for calculation. The plasma glucose was measured at 5-min intervals and the plasma insulin at 10-min intervals throughout. Measurements of plasma growth hormone (GH), prolactin, cortisol, glucagon, and somatostatin were taken at −20 min and at 20-min intervals throughout both steady-state periods.

Insulin therapy was then begun twice daily for all subjects [subjects 7, 8, and 9 on Velosulin and Insulatard porcine insulins (Nordisk, Copenhagen); the remainder on Humulin S and Humulin I human insulins (Eli Lilly, Indianapolis, IN)]. All subjects were closely supervised to ensure good

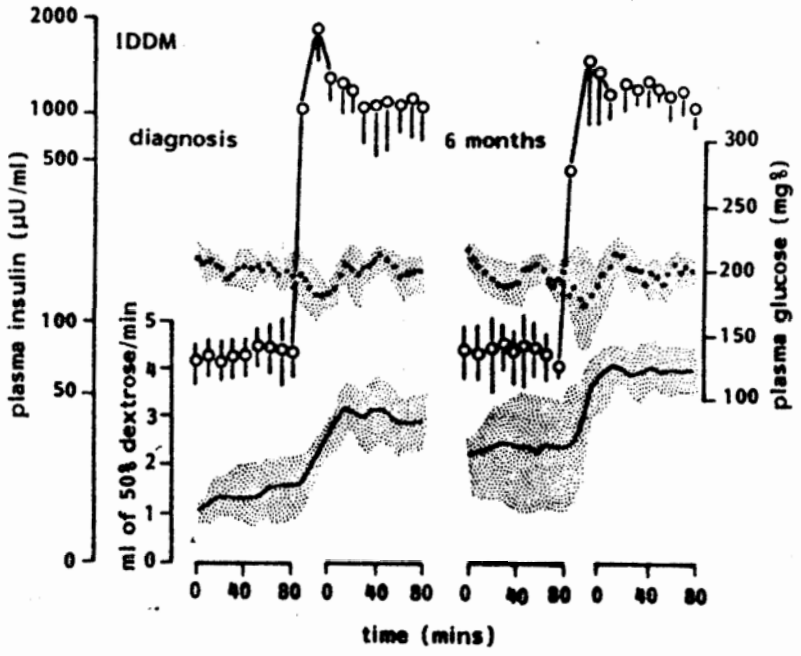

FIG. 1. Mean plasma glucose (*dotted line*), plasma insulin (O), and glucose infusion rate (*solid line*) during final 190 min of glucose-clamp procedure at diagnosis and after 6 mo of insulin therapy in patients with IDDM. *Shaded areas and error bars indicate means ± SD.*

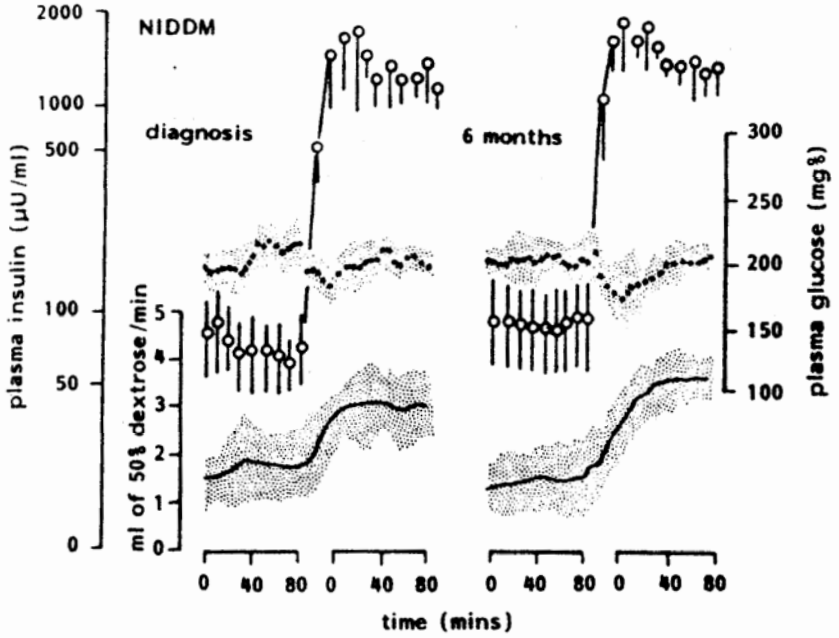

FIG. 2. Mean plasma glucose (*dotted line*), plasma free insulin (○), and glucose infusion rate (*solid line*) during final 190 min of glucose-clamp procedure at diagnosis and after 6 mo of insulin therapy in patients with NIDDM. *Shaded areas* and *error bars* indicate means ± SD.

glycemic control. After 6 mo, the glucose clamp was repeated as described above (Figs. 1 and 2). On this occasion, subjects missed their long-acting insulin the previous night and took no insulin on the study day.

C-peptide measurements were taken at diagnosis and at 3 and 6 mo. On each occasion, fasting, 1-, and 2-h postprandial blood samples were taken, and the maximum C-peptide value was used for calculation. Glycosylated hemoglobin (HbA_1) was measured at each clinic visit. Plasma insulin was measured with double-antibody radioimmunoassay (13), and at the time of the second clamp studies, plasma free insulin was separated with the method of Kuzuya et al. (14). Plasma GH and prolactin were measured by double-antibody radioimmunoassay (15). Plasma C-peptide, somatostatin, and glucagon were measured with radioimmunoassay kits (Novo, Copenhagen, and RIA UK, Tyne and Wear, UK). Plasma cortisol was measured with solid-phase radioimmunoassay with Guildhay antiserum HP/S/631g–1g (Guildhay, Surrey, UK). Plasma glucose was measured with the glucose oxidase method (Beckman glucose analyzer). The HbA_1 was measured by ion-exchange chromatography at 22°C (Bio-Rad kit) (normal range 5–7.5%).

Correlations were performed by the Spearman rank-sum test. Statistical comparisons were performed with a paired Student's *t* test. Glucose uptake during the clamps was calculated from the quantities of glucose infused, corrected for variations of the plasma glucose from the target value and urinary losses as described by DeFronzo et al. (12).

RESULTS

The HbA_1 fell in all subjects with insulin treatment (13.2 ± 0.5 vs. 8.4 ± 0.4, $P < .001$; Table 1). The fasting plasma glucose also fell significantly (237.6 ± 19.8 vs. 160.2 ± 14.8 mg/dl, $P < .03$). There was no correlation between the fasting plasma glucose or HbA_1 at the time of the glucose clamp and the subsequent measurement of glucose uptake. Insulin infusion rates of 1.0 and 10 mU · kg^{-1} · min^{-1} resulted in plasma free-insulin levels of 86.8 ± 5.1 and 1129 ± 54 mU/L, respectively. Coefficients of variation (C.V.) for insulin levels were 33% at the lower insulin infusion rate and 32% at the higher infusion rate. There was no significant difference between insulin levels of the first and second studies. During the glucose clamps, at the lower insulin infusion rate, the mean plasma glucose was 199.1 mg/dl, C.V. 6.3%, and at the higher infusion rate 201.3 mg/dl, C.V. 5.5% (Figs. 1 and 2).

Subjects varied greatly in their changes in glucose uptake at insulin infusion 1.0 mU · kg^{-1} · min^{-1} from diagnosis to 6 mo (Fig. 3). There was a negative correlation between the change in glucose uptake and the fasting insulin at diagnosis ($r = -.50$) that did not reach statistical significance. The correlation between change in glucose uptake and the maximum C-peptide secreted by each individual during insulin treatment was stronger ($r = -.66$, $P < .05$; Fig. 4). There was also a significant correlation between change in glucose uptake at an insulin infusion rate 1.0 mU · kg^{-1} · min^{-1} and age ($r = -.62$, $P < .05$) but no correlation with body mass index (BMI), change in BMI during the study, or change in HbA_1.

Separate analysis of the glucose-clamp data for subjects with IDDM and NIDDM demonstrate that the two groups behaved differently regarding change in glucose uptake with insulin therapy. The IDDM group showed a significant increase in glucose uptake at both the lower and higher insulin infusion rates (11.0 ± 1.4 vs. 15.9 ± 1.8 mg · kg^{-1} · min^{-1},

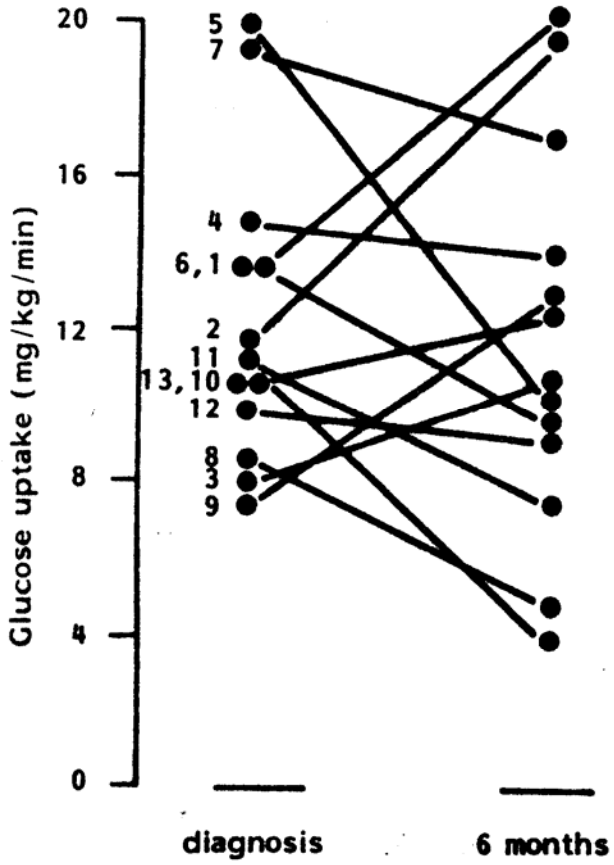

FIG. 3. Change in glucose uptake at insulin infusion rate of 1.0 mU · kg^{-1} · min^{-1} from diagnosis to 6 mo in all subjects. Numbers in *left-hand column* refer to subject number in Table 1.

$P = 0.04$, and 22.5 ± 0.9 vs. 26.4 ± 1.7 mg · kg^{-1} · min^{-1}, $P = 0.025$, respectively). In contrast, the NIDDM group demonstrated a decrease in glucose uptake at the lower insulin infusion rate (13.0 ± 1.6 vs. 9.3 ± 1.5 mg · kg^{-1} · min^{-1}, $P = .02$) but no change at the higher infusion rate (22.8 ± 1.3 vs. 22.1 ± 1.5 mg · kg^{-1} · min^{-1}, NS).

Hormonal measurements during the steady-state periods of the glucose-clamp experiments revealed no change from basal in glucagon and somatostatin levels and in levels lower than basal in cortisol and prolactin. Furthermore, there was no difference between levels at the first and second glucose-clamp studies in the above hormones. In contrast, at diagnosis there was a modest rise in GH above basal during the steady-state period at the insulin infusion rate of 1.0 mU · kg^{-1} · min^{-1} (1.9 ± 0.9 vs. 3.22 ± 1.1 ng/ml, NS) and a further rise at insulin infusion rate of 10 mU · kg^{-1} · min^{-1} that was significantly greater than basal (8.4 ± 2.4 ng/ml, $P < .002$). During the second glucose-clamp study, this increase in GH was no longer pronounced (1.65 ± 0.75 vs. 0.69 ± 0.2 vs. 2.65 ± 0.95 ng/ml, NS; Fig. 5). The increase in GH during the studies at diagnosis was present in patients with IDDM and NIDDM, and there was no significant difference between GH levels in the two groups at the time of either study. There was no significant correlation between change in glucose uptake during the glucose-clamp studies and change in GH levels ($r = .3$, NS).

On analysis of all the above factors that might contribute to changes in glucose uptake from diagnosis to 6 mo (diabetes type, age, BMI, change in BMI, HbA_1, change in HbA_1, GH levels, change in GH levels, and C-peptide secretion) by stepwise multiple regression analysis with change in glucose uptake as the dependent variable, only diabetes type enters the equation at a significant level ($r = -.79$, $F = 18.1$, $P = .0014$).

DISCUSSION

These studies were performed on an outpatient basis, which has the advantage of enabling study of the patient with minimal interference with diet or other routine, but consequently we decided to perform the glucose-clamp studies at a plasma glucose of 200 mg/dl. This figure is close to the patients' fasting plasma glucose at diagnosis and after having missed their insulin dosage during the 6-mo study. Rapid changes in plasma glucose that might have affected results were thus avoided. However, when considering the results of this study, certain factors should be kept in mind. At the lower insulin infusion

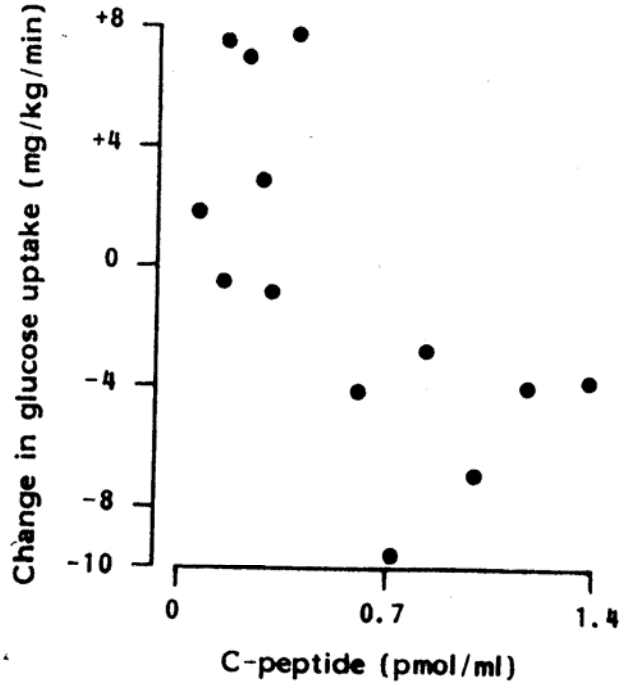

FIG. 4. Maximum C-peptide secreted by each subject during study plotted against change in glucose uptake from diagnosis to 6 mo at insulin infusion rate of 1.0 mU · kg^{-1} · min^{-1}.

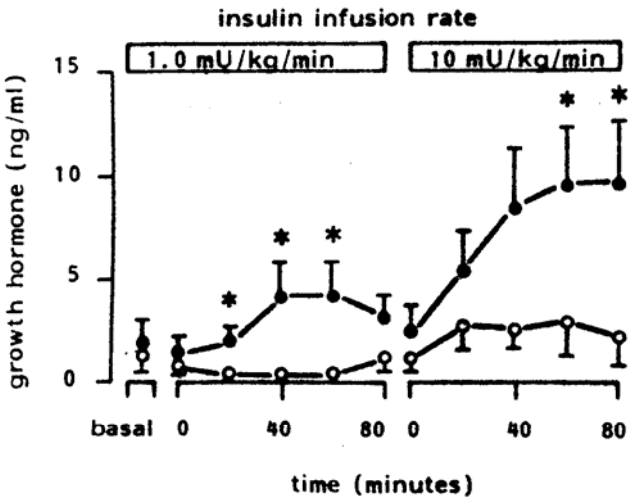

FIG. 5. Plasma GH levels in all subjects during steady-state periods of glucose-clamp studies at diagnosis (●) and after 6 mo of insulin therapy (○). *P < .05.

rate, a complex combination of factors contributing to glucose uptake is measured. The receptor and postreceptor components of insulin action are primary factors, but the higher insulin infusion rate, with saturation of insulin binding sites, provides information principally at the postreceptor level. Hepatic glucose output could interfere with measurements of glucose uptake, especially at the lower insulin infusion rate, because complete suppression of glucose output from the liver by the insulin infusion cannot be assumed, particularly in subjects with NIDDM. However, the glucose-clamp experiments were performed at hyperglycemia, which, when combined with the high insulin levels, is more likely to lead to net hepatic glucose uptake (16,17). Glucose output by the liver is therefore probably not significant. The fact that the studies were performed at hyperglycemia also contributes to the glucose-uptake measurements, because the high glucose levels would lead to an increase in non-insulin-mediated glucose uptake (18). However, this factor should not change with insulin therapy, and because both parts of the study were performed at the same plasma glucose level, comparison of results will denote changes in insulin-mediated glucose disposal.

With these considerations in mind, this study suggests that in a broad spectrum of diabetic subjects, some patients become more insulin resistant and some more insulin sensitive within the physiologic insulin range with 6 mo of insulin therapy and improved glycemic control. The strength of the correlation between change in glucose uptake and classification of diabetes, whether NIDDM or IDDM, suggests that this is the most important factor in predicting changes in glucose uptake with insulin therapy. Accordingly, it is not surprising that there are also significant correlations between change in glucose uptake, age, and endogenous insulin secretion, because patients with NIDDM are older and more likely to have residual insulin secretion at diagnosis. Whether these factors are simply markers of diabetes type or are contributory to changes in glucose uptake is not clear; e.g., insulin administered to patients with residual insulin secretion could lead to downregulation of insulin receptors (19). If this were true, reduced glucose uptake during the second clamp studies would only be seen at the lower and not at the higher insulin infusion rate, where insulin receptors are saturated, and we found this to be the case. However, from these data, we can only speculate on the mechanisms involved and observe that younger IDDM subjects can expect improvement in their insulin sensitivity with long-term insulin therapy, whereas the reverse is true of older NIDDM subjects. A complicating factor in these considerations, and a potential problem in any similar study, is the changes in body weight that often occur with time and improved glycemic control. However, we were unable to demonstrate a correlation between change in BMI during the study and change in glucose uptake. A further consideration is that five of our patients had some degree of retinopathy at diagnosis. These patients obviously fall into the NIDDM group, and all showed decreased glucose uptake at the lower insulin infusion rate after insulin therapy. However, whereas patients with retinopathy are thought to be more insulin resistant than patients without (20), there is no reason to believe that this factor would make these patients any less amenable to changes in insulin sensitivity with insulin therapy.

Several studies have examined the effect of short-term insulin therapy on insulin sensitivity. Investigators that have examined IDDM subjects agree that insulin therapy will improve insulin sensitivity in this group (3–6). The results of our study indicate that this improvement can be achieved in the clinical situation, without the use of intensive insulin therapy, and is maintained in the long term.

Early studies demonstrated that short-term insulin therapy in NIDDM subjects leads to variable improvement in insulin resistance (7) but not in obese NIDDM subjects (8). Short-term intensive therapy has since been shown to improve postbinding insulin action in NIDDM (9,10), but neither study showed improvement in insulin binding. In this study, we found a decrease in insulin sensitivity in subjects with NIDDM, measured at the lower insulin infusion rate, and no change in postbinding insulin action. Several explanations could account for these findings. First, improvement in postbinding insulin action in NIDDM may require rigorous glycemic control and would not be found in a clinical study where glycemic control was improved but not normalized. Second, whether improvement in insulin resistance in NIDDM is maintained with insulin therapy is unknown. The fact that the patients in this study were followed for a longer period than subjects in the above studies with no improvement in insulin sensitivity may indicate that such improvement is temporary. Finally, as discussed above, weight gain may nullify any improvement in insulin resistance obtained with insulin therapy.

The rise of GH during the steady-state period of the glucose-clamp studies at diagnosis, in the absence of any change in the other measured hormones, is surprising. Growth hor-

mone secretion is suppressed in normal subjects under conditions of hyperglycemia (21); GH secretion can persist in diabetic subjects in the presence of hyperglycemia (22). However, the fact that GH levels are increasing in the presence of a constant plasma glucose and hyperglycemia is clearly abnormal. The origin of the increase in GH levels in the current study is not clear, but the simplest explanation would be that even the slight fluctuations in plasma glucose during the glucose-clamp study and during transition to the higher insulin infusion rate are capable of stimulating GH release in newly diagnosed diabetes. Our studies indicate that, although GH is capable of antagonizing insulin action (23), the GH changes we noted during the clamp studies at diagnosis do not affect measurements of glucose uptake within the time scale of the experiments, and changes in GH levels with improved glycemic control do not appear to contribute to changes in glucose uptake. Furthermore, the abnormal GH secretion at diagnosis can be improved with insulin therapy and improved glycemic control.

We conclude that in a group of diabetic subjects treated with insulin for 6 mo in a routine diabetic clinic, younger subjects with IDDM and little residual insulin secretion will improve and maintain their sensitivity to physiologic insulin levels. In older subjects with NIDDM and significant residual insulin secretion, insulin resistance is increased with long-term insulin therapy. Regardless of changes in insulin sensitivity, evidence for improvement in GH regulation with improved glycemic control is presented.

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