

Insulin Action and Insulin Secretion in Identical Twins With MODY

Evidence for Defects in Both Insulin Action and Secretion

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To evaluate the pathogenetic mechanisms responsible for development of diabetes in the genetically inherited disease maturity-onset diabetes of the young (MODY), we have investigated a pair of identical twins (19 yr old) from a MODY family. One twin had nondiabetic fasting plasma glucose values but impaired glucose tolerance (IGT), whereas the other suffered from frank diabetes (fasting plasma glucose 12.5 mM). Differences in insulin secretion pattern and/or insulin action between the twins is supposed to be responsible for development of hyperglycemia in MODY. On the other hand, identical defects in insulin secretion and action in the twins may point to the primary genetic defect in MODY. Therefore, our aim was to investigate insulin secretion and insulin action in the twins to find these differences and similarities. We found that fasting plasma insulin and C-peptide values were slightly increased in the twins, whereas the responses of insulin and C-peptide to oral glucose tolerance tests (OGTT) and meals were similar in the twins and within normal range. The insulin responses to OGTT were, however, lower than expected from the glucose values, indicating a β -cell defect. Despite elevated plasma insulin levels, basal hepatic glucose output (HGO) was normal in the IGT twin but increased by 75% in the diabetic twin. The maximally inhibitory effect of insulin on HGO, when estimated at euglycemia, was normal in the IGT twin but reduced by 60% in the diabetic twin. Furthermore, the maximal insulin-mediated glucose uptake in peripheral tissues was reduced by 40% in the diabetic twin. Estimated from isolated fat cells, we found that the cellular defect that is responsible for the insulin resistance appears to be a reduced cellular insulin binding and, in surplus, a postreceptor defect in glucose metabolism in the diabetic twin. These data indicate that a cellular defect in insulin action at

the postreceptor level may be responsible for the progression from a state of IGT to frank diabetes, whereas the genetic defect in MODY may be localized to the β -cells. A primary insulin-receptor defect, however, cannot be excluded. *Diabetes* 37:730-35, 1988

Non-insulin-dependent diabetes mellitus (NIDDM) in young people, often called maturity-onset diabetes of the young (MODY), is an autosomal-dominant inherited disease with a low prevalence (1). It is defined as a ketosis-resistant form of diabetes that develops in young subjects in certain families (2). MODY is a genetic disease, but the pathogenesis is poorly understood. Some patients are found to be insulin deficient without reaching the low insulin level of patients with insulin-dependent diabetes mellitus (IDDM). In other subjects with MODY a normal or even increased insulin production has been described (2,3). Gradually, however, insulin secretion seems to decline, and insulin treatment may be necessary in some patients after several years (2).

The in vivo insulin effect has never been investigated in Caucasian patients with MODY. Hence, the importance of insulin resistance for development of glucose intolerance is unknown. From studies in patients with NIDDM, we know that both insulin resistance and insulin deficiency may play a significant pathogenetic role.

We present a pair of HLA-identical twins from a MODY family. One of the twins was diabetic, whereas the other twin at the time of investigation had nondiabetic fasting plasma glucose values. Because both twins have the same genetic and environmental background, we had the opportunity to compare "the same subject" with and without diabetes. Therefore, we investigated glucose metabolism, insulin secretion, and insulin action in the twins to describe similarities and differences. Our aim was to address the following questions: What is the primary genetic defect in MODY? What is the cause of hyperglycemia that is present in one twin and not in the other?

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MATERIALS AND METHODS

SUBJECTS

A pair of twins (19 yr old) from a MODY family was investigated and compared with a group of normal subjects (Table 1). Informed consent was obtained according to the Helsinki Declaration II.

One of the twins (A) had impaired glucose tolerance (IGT) but nondiabetic fasting plasma glucose, whereas the other twin (B) had frank diabetes. Twin B's diabetes was ketosis-resistant, and she had been treated for 3 yr without need of insulin. In the family, NIDDM had been described in four generations. No islet cell antibodies were detectable in plasma of the twins. Thus, twin B fulfilled the criteria for the MODY group (2).

The twins were described as identical at birth, and clinically they are still identical. Furthermore, they have identical HLA-A and -B tissue type: HLA-A3, -B7, -DR2, -DQw1, -DRw52. Based on these criteria the twins are assumed to be identical. Both twins had normal body weight and had no signs of lipodystrophy or acanthosis nigricans. They did not receive any drugs.

PROTOCOL

All studies of twin B (the diabetic twin) were carried out at the diagnosis of the disease and before any treatment was started. Twin A was investigated during the same period. On admission to the hospital the twins ate a diet of 7000 kJ (20% protein, 30% fat, and 55% carbohydrate).

Insulin secretion. Insulin secretion was evaluated by measuring both the plasma insulin and plasma C-peptide responses to an oral glucose tolerance test (OGTT, 75 g glucose) and by following the pattern of these hormones from 0800 to 2200 h. During the day, the twins and normal subjects were fed a diabetic diet of 7000 kJ.

In vivo insulin action. The sequential euglycemic glucose-clamp technique in combination with [$3\text{-}^3\text{H}$]glucose infusion was used to estimate in vivo insulin action. All studies were started at 0800 h after an overnight fast. A catheter was inserted in the right cubital vein for infusion of all test substances. The left hand and forearm was heated to 50°C to obtain arterialized venous blood. A hand vein was cannulated for intermittent blood sampling, and a double-lumen catheter was placed in a separate hand vein for continuous measurement of blood glucose by an artificial pancreas (Biostator GCIIIS, Miles, Elkhart, IN). After collection of baseline blood samples, subjects were infused with [$3\text{-}^3\text{H}$]glucose (New England Nuclear, Boston, MA) in a primed (25 μCi) and continuous (0.25 $\mu\text{Ci}/\text{min}$) manner. In the diabetic twin the prime was increased in proportion to the increase in the fasting plasma glucose concentration. Two hours later, insulin infusion was started with increasing rates of 0.36, 0.8, and 3.2 $\text{mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in three periods of 2 h each.

All test solutions were mixed on the day of the study. Insulin (Velosulin, Nordisk, Gentofte, Denmark; 160 U in 500 ml 0.9% NaCl) tubes were flushed with 100 ml and prerun (1 ml/min) for 2 h before the insulin infusion was started.

During the insulin infusion, blood glucose was clamped at the fasting level (5 mM) with the Biostator mode 7:0 and the following constants: BD = 5.0 mM, RD = 1.5 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, QD = 20, and FD = 400.

Glucose 20% was infused via the glucose channel of the

TABLE 1
Clinical characteristics of twins and normal subjects

Characteristic	Twin A (IGT)	Twin B (NIDDM)	Normal subjects
Sex (M/F)	1F	1F	9F
Age (yr)	19	19	25 \pm 4*
Body weight (% of ideal)	98	95	101 \pm 5*
Islet cell antibodies	0	0	0
Fasting plasma glucose (mM)	7.2	12.5	5.4 \pm 0.4*
Fasting plasma insulin (nM)	0.08	0.09	0.04 \pm 0.01*
Urine ketone bodies (mM)	0	0	<0.3

IGT, impaired glucose tolerance.

*Means \pm SD.

Biostator, and a supplementary infusion of glucose 50% was supplied via a separate pump when necessary. In the diabetic twin, blood glucose was allowed to decline to 5 mM before clamping, and the 0.36- $\text{mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ insulin infusion was prolonged accordingly. Blood samples for measurement of plasma insulin, glucose, and [$3\text{-}^3\text{H}$]glucose activity were obtained at 30-min intervals and every 10 min during the last 30 min of each period. The amount of glucose infused to maintain euglycemia was calculated at 10-min intervals, but because $\geq 70\text{--}80$ min is required for the insulin effect to reach a stable plateau, only values from the last 30 min of each period were used in the calculations.

A steady-state plateau of glucose specific activity was obtained in all subjects before the insulin infusion was started. During the basal state, plasma glucose was constant, and therefore, in the absence of glucosuria, the glucose production rate must equal the glucose utilization rate. Glucose production in the basal state was calculated by dividing the rate of [$3\text{-}^3\text{H}$]glucose infusion [counts per minute (cpm)/min] by the steady-state level of glucose specific activity (cpm/mg). During the glucose and insulin infusion, a non-steady-state condition for glucose specific activity exists. The rates of total glucose appearance and glucose utilization were therefore calculated according to Steele's equations, with a value of 0.65 for the pool fraction. The rate of glucose production was then obtained by subtracting the exogenous glucose infusion rate from the total rate of glucose appearance as calculated by the isotope-tracer technique.

In vitro insulin action. Isolated fat cells were used to estimate in vitro insulin action. The method for isolation of human fat cells has been described in detail (4).

Insulin binding to fat cells ($\sim 10^5$ cells/ml cell suspension) was measured in 10 mM HEPES buffer at 37°C after incubation for 60 min with ^{125}I -labeled A14-insulin with or without increasing concentrations of unlabeled insulin. Cell-associated radioactivity in the presence of 10 μM unlabeled insulin (nonspecific binding) averaged 2% of total binding. Specific insulin binding to adipocytes was expressed per 30 cm^2 of cell surface area per milliliter.

Glucose oxidation was measured by studies of the conversion of the D-[^{14}C]glucose to [^{14}C]CO₂. Isolated adipocytes were prepared in 10 mM HEPES buffer containing 0.5 mM glucose (volume fraction 0.05). The cells were preincubated for 45 min at 37°C with or without insulin in increasing concentrations. Then, 0.4 μCi D-[^{14}C]glucose was added to each tube (final glucose concentration 0.5 mM), and incubation was continued for 90 min. H₂SO₄ was added,

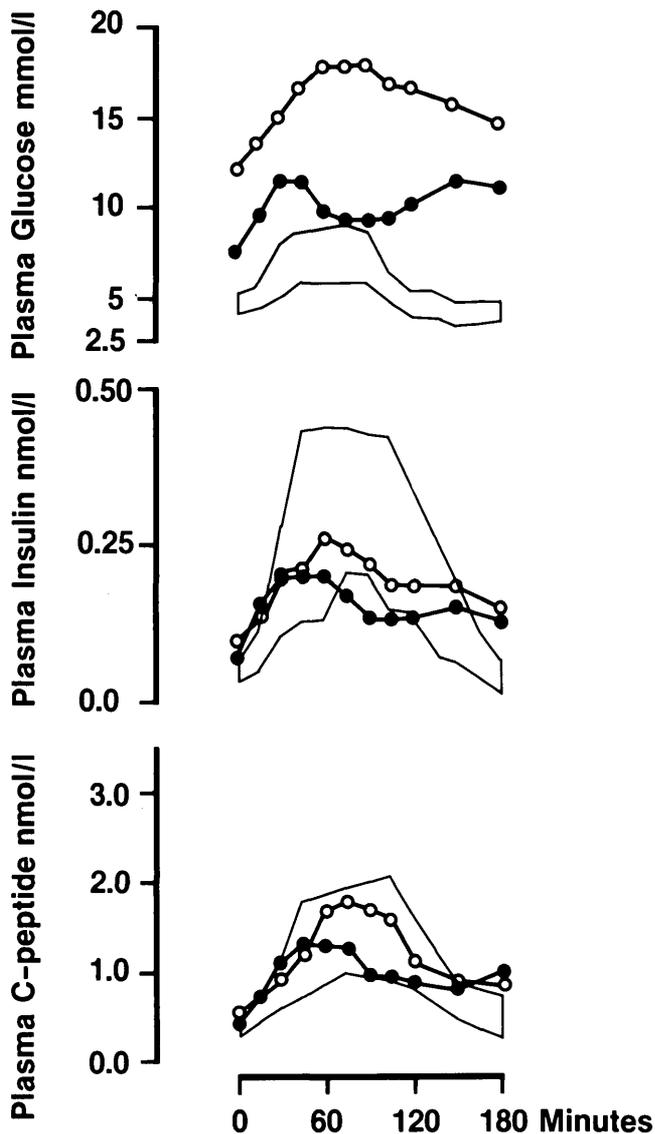


FIG. 1. Plasma glucose, insulin, and C-peptide responses during oral glucose tolerance test (75 g glucose) in twin A (impaired glucose tolerance, ●), twin B (diabetes, ○), and normal subjects (area enclosed by solid line; means ± SD).

and during the subsequent 60 min, $[^{14}\text{C}]\text{CO}_2$ was collected with phenethylamine as the trapping agent (5). When incubations were run in absence of fat cells (blank values), the apparent spontaneous decarboxylation rate averaged 17 pM per 90 min. All values for fat cell-produced CO_2 have been corrected for the individual blank values (4).

Lipogenesis was measured as conversion of D-[U- ^{14}C] glucose to $[^{14}\text{C}]$ total lipids. The experimental procedure was the same as that described for CO_2 production. After CO_2 collection had been terminated, a Dole extraction was performed, and a sample for liquid scintillation counting was taken from the upper phase (4). When incubations were performed in the absence of fat cells (blank values), the spontaneous "lipogenesis" (presumably because a proportion of the radioactivity present in the aqueous phase is extracted into the organic phase) rate averaged 234 pM/90 min. All values for fat cell-produced total lipids were corrected for the individual blank values (4).

Glucose transport rate was measured by a modification

of our previously described method (5). All studies were carried out at 37°C. Forty microliters of adipocyte suspension with a volume fraction of 0.4 ($\sim 6 \times 10^5$ cells/ml) were placed in polypropylene tubes and preincubated with or without insulin for 45 min. Twelve microliters (0.24 μCi) of tracer D-[U- ^{14}C]glucose (final glucose concentration 20 μM) was added at time zero and the uptake determined after 10 s by adding 3 ml phloretin (0.3 mM); 0.8 ml silicone oil (0.99 g/ml) was layered on top, and the tubes were spun within 2 min at 2500 g. The cells were collected from the top of the oil and placed in scintillation vials with 5 ml scintillation fluid. Extracellular trapped radioactivity was estimated by adding phloretin before tracer. All values were corrected for extracellular trapped radioactivity that averaged 25% of the tracer glucose uptake after 10 s in the absence of insulin. The initial uptake of tracer glucose (10 μM) is linear from 2.5 s to 5 min (5).

ANALYTICAL DETERMINATIONS

During the clamp, plasma was collected in fluoride-containing tubes for measurement of glucose content and $[3\text{-}^3\text{H}]\text{glucose}$ activity. Glucose in plasma was analyzed with a glucose dehydrogenase method (Merck enzymatic kit).

For measurement of $[3\text{-}^3\text{H}]\text{glucose}$ activity, 500 μl plasma was deproteinized with $\text{Ba}(\text{OH})_2$ and ZnSO_4 , and 500 μl of the supernatant was evaporated at 70°C under vacuum and resuspended in distilled water. After addition of 5 ml Aqua-

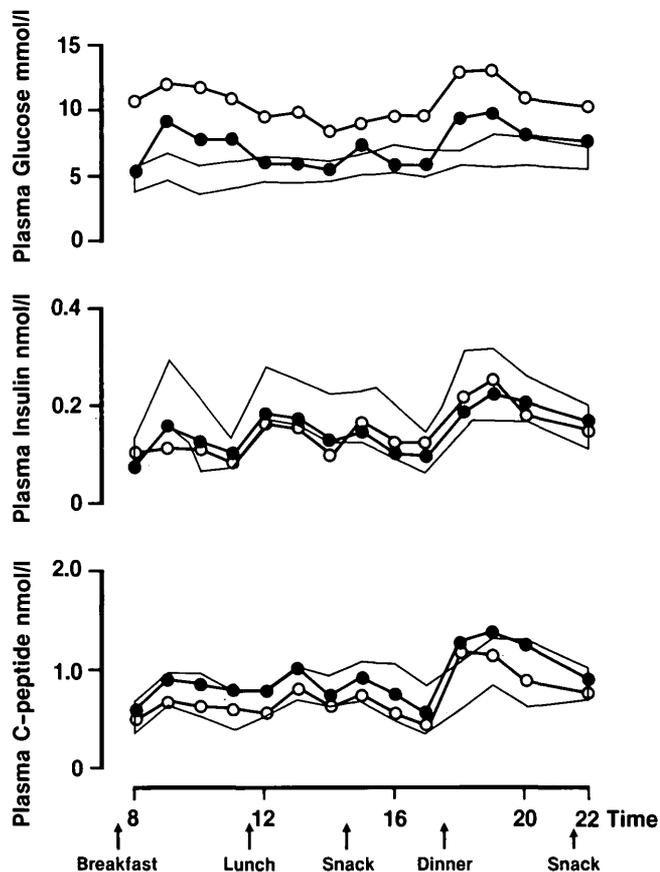


FIG. 2. Diurnal pattern of plasma glucose, insulin, and C-peptide in twin A (impaired glucose tolerance, ●), twin B (diabetes, ○), and normal subjects (area enclosed by solid line; means ± SD).

luma Plus (Lumac, Schaesberg, The Netherlands), the samples were counted in a Beckman liquid scintillation counter. Ten microliters of the infusate were added to 500 μl of the subject's baseline plasma, and the mixture was run through the same procedure together with the plasma samples. Both plasma and infusate determinations were run in duplicate. Plasma insulin and C-peptide were measured with radioimmunoassay (6,7). Antibodies to pancreatic islets were measured according to Marner et al. (8). HLA typing for the HLA-A and -B antigens was performed as described previously (9). Ketone bodies in the urine (acetoacetate) was measured according to Wildenhoff (10). Mean values \pm SD are used to characterize the normal subjects.

RESULTS

Glucose tolerance. In twin A, we found a nondiabetic fasting plasma glucose value but IGT. However, the curve was not diabetic. On the other hand, twin B had a fasting plasma glucose value of 12.5 mM, and the OGTT was severely abnormal (Fig. 1).

Insulin secretion. Fasting plasma insulin and C-peptide values were similar in the twins but slightly elevated compared with control subjects (Table 1). The insulin response to OGTT was within the normal range in the diabetic twin, whereas the insulin curve for the IGT twin was below normal in the interval 60–120 min. The C-peptide responses to glucose were within the normal range in both twins but higher in twin B than in twin A (Fig. 1). Also, the insulin secretion pattern during the day, when investigated on the same diet, was

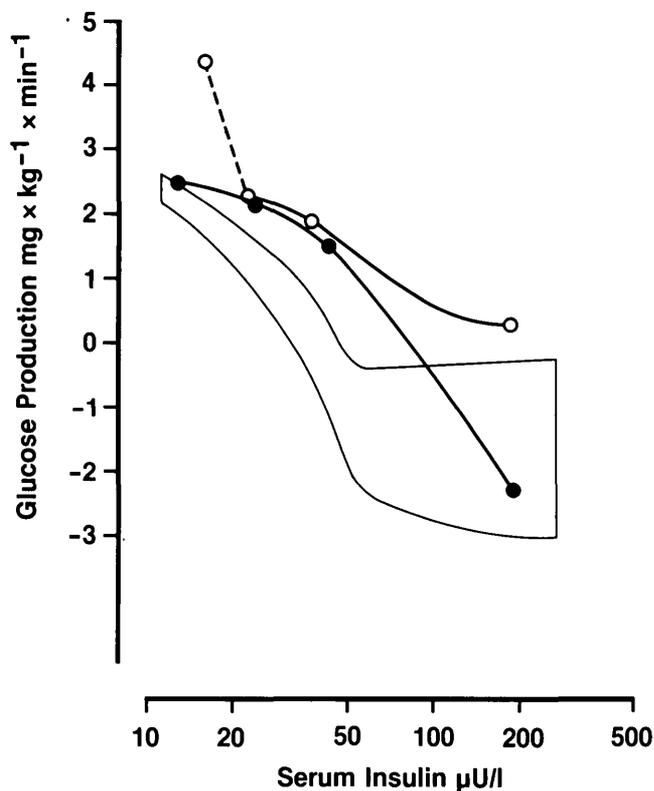


FIG. 3. Basal hepatic glucose output (HGO) measured at fasting plasma glucose values and inhibitory effect of insulin on HGO measured at euglycemia in twin A (impaired glucose tolerance, ●), twin B (diabetes, ○), and normal subjects (area enclosed by solid line; means \pm SD).

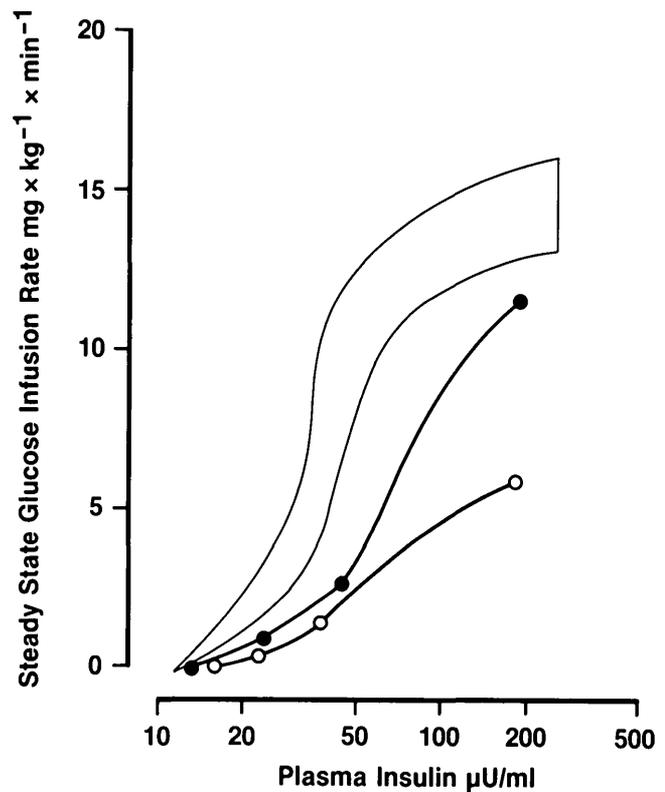


FIG. 4. Steady-state glucose infusion during euglycemic clamp in twin A (impaired glucose tolerance, ●), twin B (diabetes, ○), and normal subjects (area enclosed by solid line; means \pm SD).

similar in the twins and within normal range. However, the responses to breakfast were slightly lower or delayed compared with control subjects (Fig. 2). Thus, both the amount of insulin secreted as estimated by the C-peptide values and the insulin concentrations reaching the target tissues for insulin appear to be similar in the twins.

In vivo insulin action. In twin B, basal hepatic glucose output (HGO) was nearly two times higher than in normal subjects (4.4 vs. 2.4 ± 0.2 mg \cdot kg⁻¹ \cdot min⁻¹), despite the high normal plasma insulin concentration and the hyperglycemia in this patient (Fig. 3). In twin A, the basal HGO was normal (2.5 mg \cdot kg⁻¹ \cdot min⁻¹). In the diabetic twin (B), HGO at euglycemia was still elevated at all insulin concentrations tested compared with normal subjects (Fig. 3). The reduced insulin effect was due to both a rightward shift of the dose-response curve and a reduced maximal response. However, in twin A, the maximal response was normal, whereas the curve was shifted to the right, indicating a reduced insulin sensitivity in the liver. These data suggest that the liver of twin B is much more insulin resistant than the liver of twin A.

The insulin-mediated glucose uptake in peripheral tissues (mainly muscles and fat) as estimated by steady-state glucose-infusion (SSGI) values during the clamp are given in Fig. 4. The most impressive finding was the reduced maximal response in twin B, but a rightward shift of the curve was also observed. In twin A the dose-response curve was rightward shifted, whereas the maximal response was only slightly reduced. K_m values were similar in the twins (A, 80 $\mu\text{U}/\text{ml}$; B, 65 $\mu\text{U}/\text{ml}$).

In vitro insulin action. Insulin binding to isolated fat cells was reduced to approximately the same extent in the twins

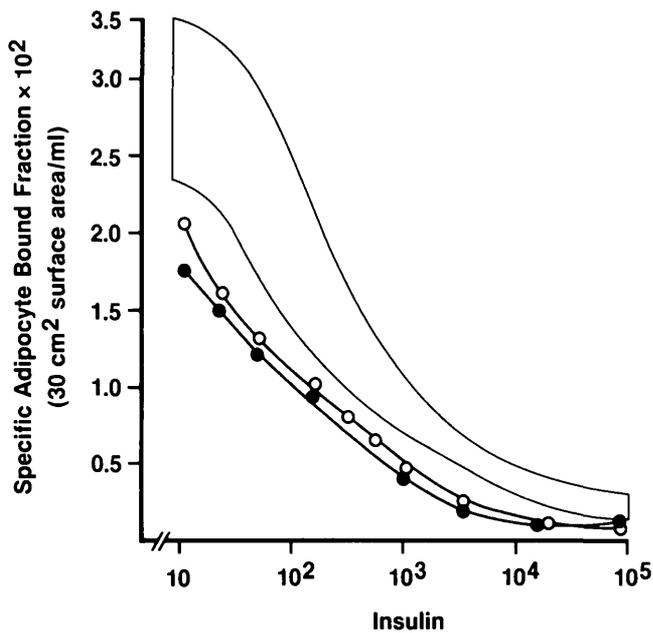


FIG. 5. ^{125}I -labeled insulin binding to adipocytes in twin A (impaired glucose tolerance, ●), twin B (diabetes, ○), and normal subjects (area enclosed by solid line; means \pm SD).

(~60%; Fig. 5). Also, the glucose transport was similar in the twins, and both the basal and insulin-stimulated values were normal (Fig. 6). However, the dose-response curves were shifted to the right. K_m was 240 and 205 pM for twins A and B, respectively. In normal subjects, K_m was 179 ± 90 pM. In contrast, the adipocyte glucose metabolism was different (Fig. 7). The lipogenesis rate in twin B was much lower than in twin A, mainly due to a reduction in the non-insulin-mediated lipogenesis, but also the maximal response to insulin was lower in twin B than in twin A. In both twins the K_m values (28 pM in A, and 15 pM in B) were higher than in normal subjects (11 ± 8 pM). Because of the wide normal range, both twins were within this scatter (Fig. 7). The results from studies of glucose oxidation were comparable with

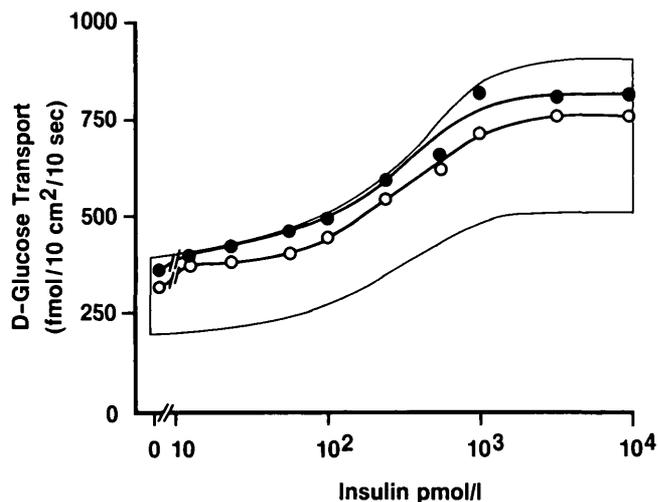


FIG. 6. Glucose transport into isolated adipocytes in twin A (impaired glucose tolerance, ●), twin B (diabetes, ○), and normal subjects (area enclosed by solid line; means \pm SD).

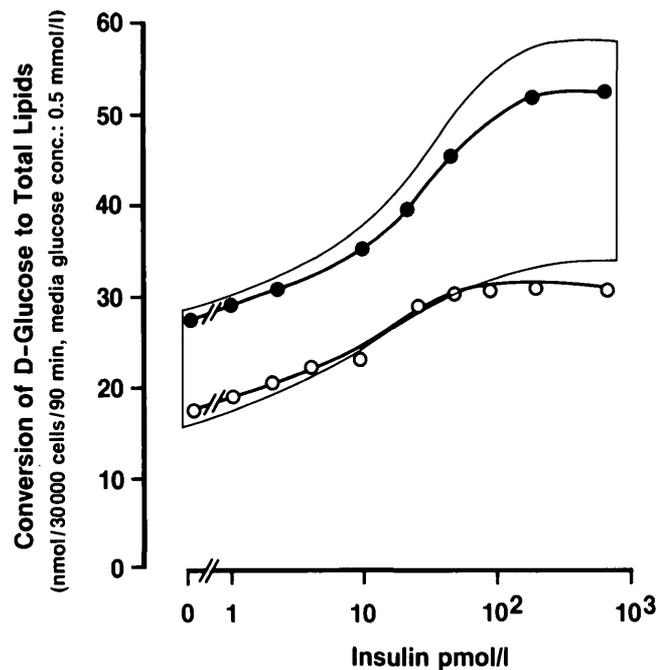


FIG. 7. Lipogenesis from glucose in isolated adipocytes from twin A (impaired glucose tolerance, ●), twin B (diabetes, ○), and normal subjects (area enclosed by solid line; means \pm SD).

those of lipogenesis (not shown). Thus, the only difference between the twins concerning in vitro studies of fat cells was the reduced lipogenesis and glucose oxidation in twin B.

DISCUSSION

We have described a pair of twins with several relatives, at least in four generations, suffering from NIDDM. The twins had the same tissue type that did not contain the DR3 and DR4 antigens coupled to IDDM but contained the DR2 antigen, which seems to protect against this disease. Furthermore, islet cell antibodies were not detectable. The diabetic twin has now been treated for 3 yr without insulin. Therefore, we conclude that they belong to the MODY type. Because this disease appears to be autosomal-dominantly inherited (1), both twins must have the gene(s), but only one of them has diabetes. This situation has given us the opportunity to investigate both the pathogenesis of hyperglycemia and the primary genetic defect in MODY.

Insulin resistance and cellular defects. The insulin resistance in MODY subjects seems to be localized to both periphery and liver. The basal HGO was very high in the diabetic twin despite slightly elevated fasting plasma insulin levels. This finding together with the reduced inhibitory effect of insulin on HGO during the clamp indicates that the liver is extremely resistant to insulin. The muscles and fat cells also appear to be insulin resistant. Thus, the insulin resistance seems to be generalized. The cellular defects found in the isolated fat cells may therefore be representative of other cells. In twin A the only cellular defect was the reduced insulin binding, which may, if it is a general phenomenon, be responsible for the rightward shift in the dose-response curves in the liver and peripheral tissues. This finding is in accord with previous studies in patients with IGT (11). In the

diabetic twin the same defects were evaluated, but the major cellular defect was the reduced intracellular glucose metabolism at the posttransport level. The postreceptor defect was due to changes in both the non-insulin-mediated and the insulin-mediated glucose metabolism. These findings are in accord with the findings in newly diagnosed patients with NIDDM (12). Because the postreceptor defect was only found in the diabetic twin, it cannot be the primary genetic defect. On the other hand, the receptor defect responsible for the rightward shift in all dose-response curves may be the primary genetic defect because it was found in both twins. However, studies of cultured fibroblasts from MODY subjects seem to have normal insulin responsiveness (13). The degree of insulin resistance in the diabetic twin was higher than in the IGT twin, indicating that this difference may play a major role in the development of hyperglycemia in twin B.

Insulin secretion. When estimated from the plasma C-peptide response during OGTT, the insulin secretion was normal in the diabetic twin and higher than in the IGT twin. However, the glucose values obtained in the diabetic twin were much higher than in the IGT twin, indicating that the β -cell response was reduced in the diabetic twin. The insulin response to OGTT in the IGT twin was low normal or reduced. Our finding contrasts with that generally found in subjects with IGT, in whom the insulin responses are higher than in control subjects (11). Thus, the β -cell response was reduced in both twins. It may, of course, be a primary event, but it has also been shown that the β -cell sensitivity to glucose decreases in hyperglycemic situations (14). However, the relatively low response in the IGT twin indicates that this may be a primary defect in MODY, e.g., due to a lower β -cell volume.

The daily profiles of plasma insulin were identical in the twins. If we assume that the portal and intercellular insulin values are almost identical (there is no evidence to the contrary), we may conclude that the amount of insulin presented throughout the day to the target cells for insulin action is almost identical in the twins. Therefore, the reduced glucose metabolism in the diabetic twin must be due to changes in variables other than insulin.

We conclude that MODY subjects are severely insulin resistant in both liver and periphery. The insulin resistance is due to both receptor and postreceptor defects and seems to be the major cause of progression from IGT to frank diabetes. On the other hand, a β -cell defect may be the genetic

defect in MODY, but a primary insulin-receptor defect cannot be excluded.

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