Receptor and Postreceptor Defects Contribute to the Insulin Resistance in Noninsulin-dependent Diabetes Mellitus

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ABSTRACT We have assessed the mechanisms involved in the pathogenesis of the insulin resistance associated with impaired glucose tolerance and Type II diabetes mellitus by exploring, by means of the euglycemic glucose-clamp technique, the in vivo dose-response relationship between serum insulin and the overall rate of glucose disposal in 14 control subjects; 8 subjects with impaired glucose tolerance, and 23 subjects with Type II diabetes. Each subject had at least three studies performed on separate days at insulin infusion rates of 40, 120, 240, 1,200, or 1,800 mU/M^2 per min. In the subjects with impaired glucose tolerance, the dose-response curve was shifted to the right (half-maximally effective insulin level 240 vs. 135 μU/ml for controls), but the maximal rate of glucose disposal remained normal. In patients with Type II diabetes mellitus, the dose-response curve was also shifted to the right, but in addition, there was a marked decrease in the maximal rate of glucose disposal. This pattern was seen both in the 13 nonobese and the 10 obese diabetic subjects. Among these patients, an inverse linear relationship exists ($r = -0.72$) so that the higher the fasting glucose level, the lower the maximal glucose disposal rate.

Basal rates of hepatic glucose output were 74±4, 82±7, 139±24, and 125±16 mg/M^2 per min for the control subjects. subjects with impaired glucose tolerance, nonobese Type II diabetic subjects, and obese Type II diabetic subjects, respectively. Higher serum insulin levels were required to suppress hepatic glucose output in the subjects with impaired glucose tolerance and Type II diabetics, compared with controls, but hepatic glucose output could be totally suppressed in each study group.

We conclude that the mechanisms of insulin resistance in patients with impaired glucose tolerance and in patients with Type II noninsulin-dependent diabetes are complex, and result from heterogeneous causes. (*a*) In the patients with the mildest disorders of carbohydrate homeostasis (patients with impaired glucose tolerance) the insulin resistance can be accounted for solely on the basis of decreased insulin receptors. (*b*) In patients with fasting hyperglycemia, insulin resistance is due to both decreased insulin receptors and a postreceptor defect in the glucose disposal mechanisms. (*c*) As the hyperglycemia worsens, the postreceptor defect in peripheral glucose disposal emerges and progressively increases. And (*d*) no postreceptor defect was detected in any of the patient groups when insulin's ability to suppress hepatic glucose output was measured.

INTRODUCTION

Insulin resistance is a characteristic feature of patients with impaired glucose tolerance (1) and patients with Type II or noninsulin-dependent diabetes mellitus (NIDDM)[1] (2–11). Patients with impaired glucose tolerance have relatively mild insulin resistance, whereas patients with Type II NIDDM have more severe insulin resistance (1, 2). Furthermore, as the degree of carbohydrate intolerance worsens, the frequency of insulin resistance increases (1, 2). Thus, while not all patients with impaired glucose tolerance are insulin resistant, the majority of Type II diabetics with significant fasting hyperglycemia display this abnormality. These findings appear to be independent

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[1] *Abbreviation used in this paper*: NIDDM, noninsulin-dependent diabetes mellitus.

of the existence of obesity (1, 2, 4–11) and have been reported by a number of investigators using a variety of different techniques (1–11).

The available evidence indicates that this insulin-resistant state is due to a tissue defect in insulin action (4–6). Insulin action at the cellular level is the result of a complex sequence of events that is initiated by binding of the hormone to specific receptor sites on the cell membrane. Therefore, insulin resistance can be due to an abnormality at any step in the entire insulin action sequence, and, for convenience, these potential abnormalities can be divided into two categories: receptor defects and postreceptor defects in insulin action (4, 12). Knowledge of the insulin-biologic function dose-response curve allows a distinction to be made between these two general categories of defects (4, 12). Since cells possess spare receptors for insulin action, the functional consequence of a pure decrease in cellular insulin receptors is a decrease in insulin's effects at submaximal hormone concentrations with normal insulin action at maximally effective hormone concentrations. This produces a rightward shift in the insulin-biologic function dose-response curve and is termed a decrease in insulin sensitivity (4, 12,13). A postreceptor defect leads to a proportionate reduction in insulin action at all hormone levels, including maximally effective concentrations, and this is termed a decrease in insulin responsiveness (4, 12,13). If receptor and postreceptor defects coexist, a rightward shift in the dose-response curve as well as a decrease in maximal insulin action will result (i.e., decreased insulin sensitivity and responsiveness) (12, 13).

In the present study, we have used a modification of the euglycemic glucose-clamp technique originally devised by Andres and colleagues (14–16) to evaluate the mechanisms responsible for the insulin resistance in patients with varying degrees of decreased carbohydrate tolerance. To accomplish this, multiple glucose-clamp studies were performed in patients with impaired glucose tolerance and in patients with Type II NIDDM. Each study was performed at a different steady-state serum insulin level, which permits the determination of the dose-response relationship for insulin's ability to promote peripheral glucose disposal. Hepatic glucose output was monitored during each study to evaluate the impact of these insulin levels upon the liver in these subjects.

METHODS

Materials. Porcine monocomponent insulin was generously supplied by Dr. Ronald Chance of Eli Lilly & Company (Indianapolis, Ind.); ^{125}I-Na and [3-^{3}H]glucose were purchased from New England Nuclear (Boston, Mass.); bovine serum albumin (fraction V) was obtained from Armour Pharmaceutical Co. (Chicago, Ill.); collagenase was purchased from Worthington Biochemical Corp. (Freehold, N. J.); guinea pig anti-insulin antibody was kindly supplied by Dr. Edward Arquilla (Irvine, Calif.).

Subjects. The study group consisted of 14 nonobese control subjects, 8 subjects with impaired glucose tolerance, and 23 subjects with Type II NIDDM as defined by the criteria of the National Diabetes Data Group (3). The clinical and metabolic characteristics of the subjects are summarized in Table I.

The mean (±SE) age of the control group was 37±3 yr, compared with values of 44±5 yr for the subjects with impaired glucose tolerance, 55±2 yr for the nonobese Type II diabetics and 51±3 yr for the obese Type II diabetic subjects. The relative weights of the control subjects ranged from 0.85 to 1.13 with a mean value of 0.94 (17). For the subjects with impaired glucose tolerance, the corresponding values were 0.77–1.42 with a mean value of 1.02, whereas the nonobese Type II diabetics ranged from 0.74 to 1.09 with a mean value of 0.96 and the obese Type II diabetes ranged from 1.25 to 1.51 with a mean value of 1.31 (17).

After we obtained informed consent, all subjects were admitted to the University of Colorado Clinical Research Center but remained active to approximate their prehospital exercise level. All subjects were chemically euthyroid and had no stigmata of renal, hepatic, or cardiac dysfunction. None of the subjects had evidence of disease states other than diabetes, or were ingesting agents known to affect carbohydrate or insulin metabolism.

Diet. All subjects were placed on a weight-maintenance (32 kcal/kg) liquid formula diet, with three divided feedings containing ⅛, ⅜, and ⅜ of the total daily calories given at 0800, 1200, and 1700 h, respectively. The diet contained 45% carbohydrate, 40% fat, and 15% protein. All subjects equilibrated on this diet for at least 48 h before studies were performed.

Oral glucose tolerance test. Oral glucose tolerance tests were performed by giving subjects 40 g/M^2 glucose after an overnight fast. Serum was obtained at 0, 30, 60, 120, and 180 min for measurement of glucose and insulin levels.

Euglycemic glucose-clamp studies. In vivo insulin sensitivity was measured with a modification of the euglycemic glucose-clamp technique as previously described (14–16). With this technique, an antecubital vein is cannulated in an antegrade manner to administer the infusates. A dorsal hand vein is cannulated in a retrograde fashion and kept in a warming device (72°C) to facilitate venous sampling and provide arterialized venous blood. After insertion of the catheters, [3-^{3}H]glucose is infused for at least 30 min before initiating the insulin infusion. At the onset of the insulin infusion, a priming insulin dose is administered during the initial 10 min in a logarithmically decreasing manner to acutely raise the serum insulin level to the desired level, where it is then maintained for the duration of the study by a continuous insulin infusion. The serum glucose was maintained between 80 and 90 mg/100 ml throughout the study period with a coefficient of variation of 5% by monitoring the glucose level at 5-min intervals and adjusting the infusion rate of a 20%-glucose solution with a servocontrol negative feedback principle (14–16). In the subjects with fasting hyperglycemia, the insulin infusion was initiated as described and the serum glucose level allowed to fall to euglycemic levels before initiating the infusion of 20% glucose. The period required to achieve euglycemia ranged from 30 to 170 min and was directly proportional to the fasting glucose level. The glucose infusion was then adjusted as needed to maintain the serum glucose level between 80 and 90 mg/100 ml during the period the measurements were made. All studies were continued for at least 80 min (mean 140 min) after achieving euglycemia. Since serum potassium levels tend to fall during this procedure (13), KCl was ad-

ministered at a rate of 15–20 meq/h to maintain the serum potassium level between 3.5 and 4.5 meq/liter and thus avoid any potential deleterious effects of hypokalemia.

During these studies, steady-state euglycemia is maintained in order to avoid the endogenous secretion of either insulin or the various counterregulatory hormones. Under these conditions, all of the glucose infused is removed from the circulation and either metabolized by the peripheral tissues or taken up by the liver. While the exact fate of this glucose remains controversial (18–20), the total flux of glucose through the system serves as a measure of the steady-state glucose disposal rate at the prevailing serum insulin concentration (14–16). The overall glucose disposal rate was assesed isotopically (see below, *Hepatic glucose output*) for each 20-min interval after the initial 40 min of the study in subjects with fasting euglycemia and for each 20-min interval after the initial 20 min of euglycemia in the diabetic subjects with fasting hyperglycemia. The glucose disposal rates for the 20-min intervals were then averaged and the mean value used as the data point for the individual study. Urinary glucose loss is not a problem since these measurements were made under euglycemic conditions in all subjects.

Each subject was studied at an insulin infusion rate of 120 mU/M² per min to allow assessment of in vivo insulin sensitivity in an uniform manner. In addition, each subject had two to four additional euglycemic glucose-clamp studies performed on separate days at different insulin infusion rates (40, 240, 1,200, or 1,800 mU/M² per min) to define the shape of the in vivo insulin dose-response curve.

Hyperglycemic glucose-clamp studies. An additional glucose-clamp study was performed in two type II diabetic subjects under hyperglycemic conditions. These studies were conducted with an insulin infusion rate of 1,200 mU/M² per min. The serum glucose was acutely raised to 225 mg/100 ml by a bolus infusion of 20% glucose, if needed, and maintained within ±10% of that value for the duration of the study by a variable rate glucose infusion (13, 16).

Hepatic glucose output. R_a, the rate of glucose appearance, and R_d, the rate of overall glucose disappearance, were quantitated in the basal state and during each of the glucose clamp studies by infusing [3-³H]glucose in a primed continuous manner (13, 21, 22). With this technique, 25 μCi of the tracer is injected as a bolus, followed by a continuous infusion at the rate of 0.25 μCi/min. Blood samples are obtained at 20-min intervals for the determination of both the concentration and specific activity of serum glucose. R_a and R_d are then calculated with the Steele equations (23) in their modified derivative form (13, 21), since the tracer exhibits nonsteady-state kinetics under these conditions. The rate of hepatic glucose output can then be calculated since R_a represents the sum of hepatic glucose output and the rate of infusion of exogenous glucose. The values for R_d in the basal state were corrected for urinary glucose loss to reflect the actual rate of endogenous glucose disposal.

Insulin binding studies. Insulin binding to isolated adipocytes was studied with adipocytes obtained from open biopsy of the adipose tissue on the lower abdominal wall. This biopsy was performed on the day preceeding the first glucose-clamp study in all subjects. Details concerning the measurement and calculation of the amount of insulin bound to adipocytes have been published previously (24, 25).

Analytical methods. Blood for serum glucose determinations was drawn and serum immediately separated with a Beckman microfuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Serum glucose was measured by the glucose oxidase method using a Beckman Glucose Analyzer (Beckman Instruments, Inc., Clinical Instruments Div., Fullerton, Calif.).

Blood for the determination of serum insulin levels and serum glucose specific activity was collected in untreated tubes and allowed to clot. The specimens were then spun, and the serum removed and stored at −20°C until the determinations were made. Serum insulin levels were measured by a double antibody radioimmunoassay according to the method of Desbuquois and Aurbach (26).

Data analysis. All calculations were performed on a programmable calculator (model 67, Hewlett-Packard Co., Palo Alto, Calif.). Data presented represent the mean (±SE), unless otherwise stated. Statistical analysis was done with Student's *t* test for paired data and unpaired data as indicated. Correlation coefficients were calculated with the standard statistics package for the model 67 Hewlett-Packard calculator.

RESULTS

Oral glucose tolerance tests. The fasting serum glucose and insulin levels for all subjects are shown in Table I. After the ingestion of oral glucose (40 g/M²), each of the 14 control subjects exhibited serum glucose levels within the normal range at all timepoints according to the criteria of the National Diabetes Data Group (3). The eight subjects with impaired glucose tolerance had fasting levels <115 mg/100 ml, but elevated levels at 1 (198±6) and 2 (171±11) h after ingestion of the glucose load according to the aforementioned criteria.

Insulin receptor studies. The competition curves for insulin binding by isolated adipocytes from the four subject groups are shown in Fig. 1. Adipocytes from the subjects with impaired glucose tolerance and type II diabetes bind less insulin at all insulin concentrations than do the cells from the control subjects. Scatchard analysis (27) and average affinity profile analysis (28) reveals that this decrease in insulin binding is due to a decrease in receptor number with no change in binding affinity (data not shown).

Measurement of in vivo insulin sensitivity. All subjects were studied at an insulin infusion rate of 120 mU/M² per min while their serum glucose was maintained at euglycemic levels to provide a uniform assessment of in vivo insulin resistance. Steady-state serum insulin levels of 324±17, 350±36, 359±21, and 383±55 μU/ml were achieved in the control subjects, subjects with impaired glucose tolerance, nonobese NIDDM, and obese NIDDM subjects, respectively. In the face of similar steady-state serum insulin levels, the overall glucose disposal rate was 324±31 mg/M² per min for the controls, compared with values of 260±32, 142±12, and 128±10 mg/M² per min for the subjects with impaired glucose tolerance, nonobese NIDDM subjects, and obese NIDDM subjects, respectively. Thus, the mean glucose disposal rate was reduced by 20% ($P < 0.05$) in the subjects with impaired glucose tolerance and by 56% ($P < 0.001$) and 60% ($P < 0.001$) in the nonobese and obese type II diabetic groups. (These results demonstrate the presence of insulin resistance in these subjects and also show that the insulin resistance is greatest in those patients with

TABLE I
Clinical and Metabolic Features

Subject	Age	Sex	Relative weight	Fasting serum glucose	Fasting serum insulin	^{125}I-Insulin bound	Maximal glucose disposal rate
	yr			*mg/100 ml*		%	*mg/M²/min*
Controls							
1	57	F	0.99	94	16	1.22	421
2	54	F	0.85	89	10	2.82	493
3	44	F	0.86	88	7	2.19	306
4	63	F	0.86	87	8	2.31	477
5	47	F	0.87	85	9	1.69	453
6	38	F	0.89	85	7	2.74	387
7	37	F	0.92	90	6	3.70	296
8	36	F	0.94	92	12	1.96	487
9	32	F	1.05	82	10	4.15	463
10	29	M	0.85	89	10	3.18	348
11	29	F	1.13	75	13	2.92	316
12	29	M	1.13	91	11	3.22	432
13	25	F	0.91	80	9	2.17	368
14	23	F	0.86	76	6	3.00	369
Mean±SE	37±3		0.94±0.03	80±6	9±1	2.66±0.21	399±21
Impaired glucose tolerance							
15	59	M	0.97	89	9	2.70	523
16	39	M	0.93	84	11	1.71	300
17	32	M	1.42	104	35	1.59	497
18	34	F	0.87	104	10	1.71	307
19	53	F	0.77	88	12	1.22	347
20	37	M	1.27	112	27	2.16	234
21	29	M	0.90	102	15	1.67	397
22	65	M	0.96	118	18	0.54	294
Mean±SE	44±5		1.01±0.08	100±4	17±3	1.66±0.22	366±35
Nonobese type II diabetics							
23	62	F	1.04	294	9	1.54	192
24	60	M	0.98	182	12	2.76	396
25	54	F	1.08	288	30	1.38	124
26	62	M	0.87	280	12	—	104
27	62	M	0.74	295	21	2.52	154
28	58	F	1.01	221	8	1.15	166
29	62	M	1.00	152	13	2.29	287
30	64	F	0.94	213	49	1.81	218
31	47	F	1.09	294	16	2.88	124
32	36	M	0.90	250	28	2.82	248
33	54	M	0.97	215	12	1.36	362
34	43	F	1.07	302	16	1.39	123
35	53	M	0.74	327	4	1.55	185
Mean±SE	55±2		0.96±0.03	255±15	18±3	1.95±0.19	206±26
Obese type II diabetics							
36	49	F	1.39	268	78	1.66	160
37	55	F	1.36	322	26	1.21	121
38	63	F	1.15	200	44	0.88	133

TABLE I—(*Continued*)

Subject	Age	Sex	Relative weight	Fasting serum glucose	Fasting serum insulin	^{125}I-Insulin bound	Maximal glucose disposal rate
	yr			*mg/100 ml*		%	*mg/M²/min*
Obese type II diabetics							
39	58	F	1.30	237	18	3.41	151
40	65	F	1.25	235	46	—	137
41	52	F	1.32	252	26	1.36	152
42	57	M	1.29	395	26	—	207
43	41	F	1.29	245	26	1.68	190
44	40	F	1.25	163	50	1.48	224
45	32	F	1.51	152	51	1.54	223
Mean±SE	51±2		1.31±0.03	254±23	39±6	1.64±0.24	158±20

more severe carbohydrate intolerance). An insulin infusion rate that produced steady-state insulin levels at the upper limits of the physiologic range was used for this comparative study since it was difficult to achieve euglycemia with lower insulin infusion rates in the most insulin-resistant diabetic subjects. Euglycemia is required to allow quantitative comparisons, since hyperglycemia itself enhances peripheral glucose disposal by mass action (29, 30).

In vivo insulin dose-response curves. To define the mechanisms responsible for this insulin resistance further, additional euglycemic glucose-clamp studies were performed in each subject at insulin infusion rates of 40, 240, 1,200, or 1,800 mU/M² per min. Each subject had at least three studies on different days, but it was not feasible to study every subject at all insulin concentrations. The sequence of insulin rates was chosen in a random manner.

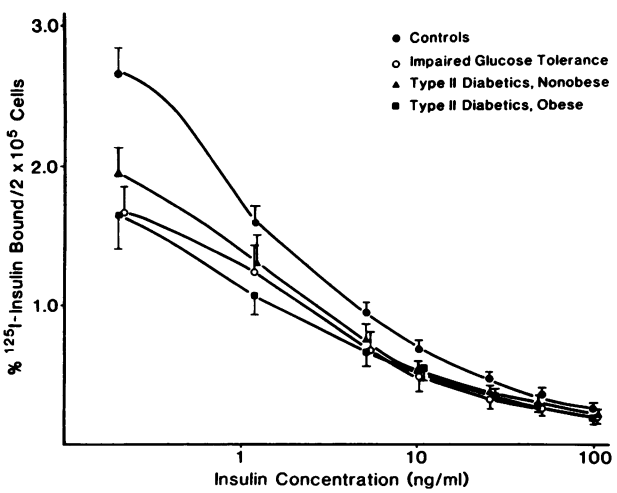

FIGURE 1 Insulin binding by isolated adipocytes from control subjects (●), subjects with impaired glucose tolerance (○), nonobese (▲), and obese (■) type II diabetics. All data are corrected for nonspecific binding and represent the mean ±SE of the percentage of ^{125}I-insulin specifically bound per 2×10^5 cells.

The individual dose-response curves for the 14 control subjects are shown in Figure 2A. These results are analogous to those previously reported from our laboratory for normal subjects (13) with increasing steady-state serum insulin levels leading to a four- to sixfold increase in the glucose disposal rate. Although the expected biologic variability is evident, the steepest portion of each curve resides within the range of physiologic insulin concentrations. The initial point on each curve represents basal hepatic glucose output; in the basal state, hepatic glucose output is equal to overall glucose disposal and the portal/peripheral insulin gradient is not a factor since hepatic glucose uptake is minimal in the basal state (18). Because of these factors, the basal hepatic glucose output accurately reflects the relationship between the basal insulin level in the peripheral circulation and overall glucose disposal. On the basis of several studies, only 10–30% of this glucose uptake in the basal state proceeds by insulin-mediated pathways (30–34).

The individual dose-response curves for the eight subjects with impaired glucose tolerance are displayed in Fig. 2B. Again, the initial point on each curve represents basal hepatic glucose output (equal to glucose disposal rate). Although somewhat greater variability in the dose-response pattern exists for these subjects than for the control subjects, a general pattern emerges, i.e., all the curves are shifted to the right, with the maximal response falling within the normal range for seven of the eight subjects. This decrease in insulin action at submaximal insulin levels (decreased insulin sensitivity) is reflected in the glucose tolerance

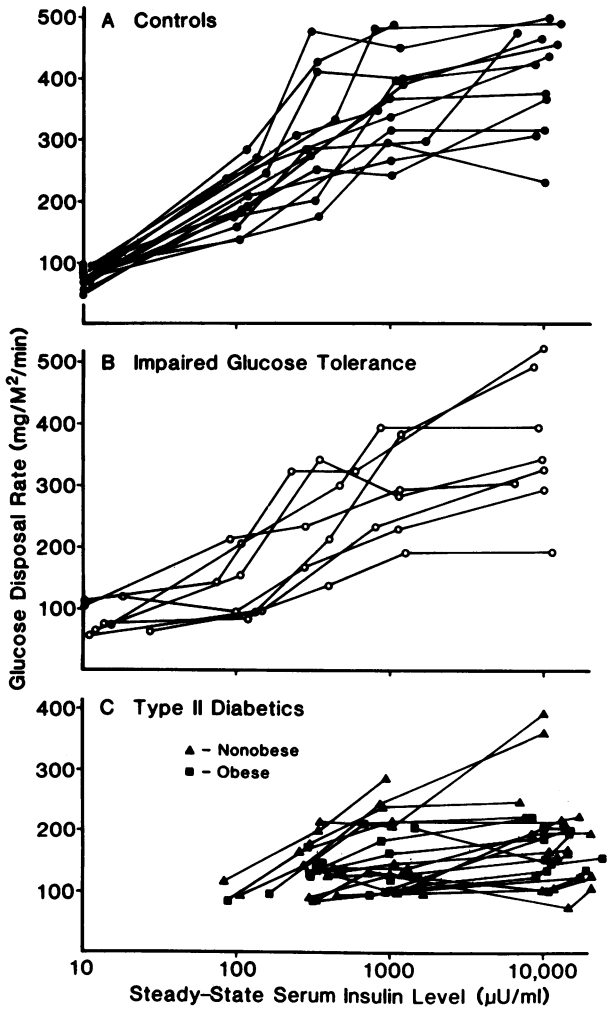

FIGURE 2 (A) Individual dose-response curves for control subjects. Results were obtained by performing additional euglycemic clamp studies in each subject with insulin infusion rates of 40, 240, 1,200, or 1,800 mU/M² per min. The initial point on each curve represents glucose disposal in the basal state, as determined by the primed continuous infusion of [³H]glucose (see text for details). (B) Individual dose-response curves for the eight subjects with impaired glucose tolerance. (C) Individual dose-response curves for the 13 nonobese (▲) and 10 obese (■) type II diabetic subjects.

tests in these subjects since the correlation between the 2-h postprandial glucose value and the glucose disposal rate at an insulin level of 100 μU/ml was highly significant ($r = -0.79$, $P < 0.01$).

Individual dose-response curves for the 23 subjects with type II diabetes are shown in Fig. 2C. Basal hepatic glucose output is measured at each patient's fasting glucose level. Thus, these values cannot be used as the initial point on the euglycemic dose-response curve, because these subjects exhibit fasting hyperglycemia, and elevated glucose levels accelerate peripheral glucose uptake independent of insulin (29, 30). Of the dose-response curves presented in Fig. 2C, only four diabetic subjects were studied at an insulin infusion rate <120 mU/M² per min, since most of these subjects were so insulin resistant that euglycemia could not be achieved in a reasonable period of time with lower insulin infusion rates. In the four subjects studied at the lower insulin level, a 63% decrease in glucose disposal rate was observed, compared with normals (94 vs. 201 mg/M² per min, respectively). The remainder of the results are also quite striking. Although the response pattern is somewhat heterogeneous, the curves are shifted to the right and the maximal glucose disposal rates are markedly decreased in the majority of subjects.

The mean dose-response curves for the normals, subjects with impaired glucose tolerance, and patients with type II NIDDM are seen in Fig. 3A. Since it was not feasible to study all of the subjects at each steady state insulin concentration, when necessary, glucose

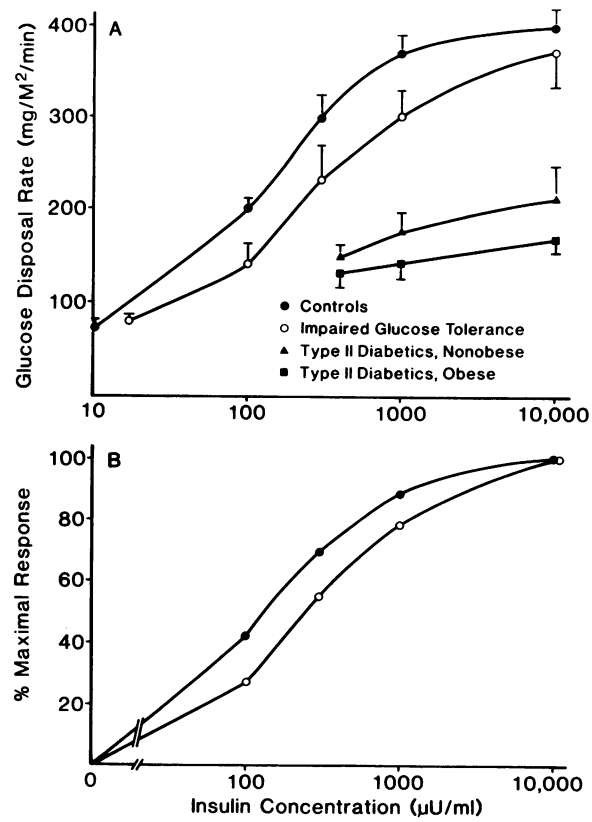

FIGURE 3 (A) Mean dose-response curves for the control subjects (●), subjects with impaired glucose tolerance (○), and nonobese (▲) and obese (■) type II diabetics. Results are plotted as mean±SE (B) Mean dose-response curves for the control subjects (●) and subjects with impaired glucose tolerance (○), plotted as the percentage of maximal response (see text for details).

disposal rates were estimated from the individual dose-response curves at steady-state serum insulin concentrations of 100, 300, 1,000, and 10,000 μU/ml for control subjects and subjects with impaired glucose tolerance, and at 400, 1,000, and 10,000 μU/ml for the type II diabetic subjects.

Inspection of the results in Fig. 3A, reveals that the curves for the subjects with impaired glucose tolerance and the type II diabetic patients lie to the right of the curve for the control subjects. Thus, the mean glucose disposal rates at insulin levels of 100, 300, and 1,000 μU/ml are significantly less ($P < 0.01$) when compared with controls. But the subjects with impaired glucose tolerance achieve a maximal rate of glucose disposal that is not significantly different from that of the control subjects. The type II diabetic subjects exhibit both a rightward shift in their dose-response curve and a marked decrease in the maximal rate of glucose disposal. There is a tendency for these changes to be more pronounced in the obese diabetic subjects, with the difference between the two groups being greatest at the highest insulin concentration. The differences in the glucose disposal rates for the two groups of type II diabetic subjects are not significant at the two lower insulin levels, but do reach statistical significance ($P < 0.05$) at the highest insulin level.

The assumptions upon which the interpretation of the results in Figs. 2 and 3 are based presuppose that glucose uptake is relatively rate determining for overall glucose disposal and that intracellular processes of glucose metabolism are not saturated (especially at maximal insulin levels) (13). If this were not the case, the glucose disposal rates at the highest insulin levels may not represent the maximal effect of the hormone, but could reflect the maximal capacity of tissues to metabolize glucose. To evaluate this, hyperglycemic glucose-clamp studies at a glucose level of 225 mg/100 ml and euglycemic studies (85 mg/100 mg) were performed in two type II NIDDM patients at the maximal insulin infusion rate (1,200 mU/M^2 per min). In the studies at euglycemia, the mean glucose disposal rate was 150 mg/M^2 per min and increased to 325 mg/M^2 per min during the hyperglycemic clamp studies. Thus, increasing the substrate (serum glucose) concentration 2.6-fold led to a 2.7-fold increase in the overall glucose disposal rate. Therefore, the maximal insulin-stimulated glucose disposal rates in Figs. 3 and 4 do not represent the maximal capacity of the tissues of the diabetic patients to metabolize glucose, but reflect the maximal effect of insulin.

Since the maximal glucose disposal rates are not the same among the different study groups, the functional form of the dose-response curves can be better appreciated by plotting the data as a percentage of the maximal insulin effect (13). This method of analysis eliminates the potential influence of defects in postreceptor effector units, since the maximal response is taken as 100% and the remaining values expressed as a percentage of that response. For the purpose of this analysis, it is necessary to examine only insulin-stimulated glucose disposal for the conclusions to be valid. We have previously suggested that this can be done by subtracting 70% of the basal glucose disposal rate from all points on the curve, since this is a reasonable approximation of noninsulin-mediated glucose uptake (13). Recent studies have addressed this issue directly and indicate that the actual value for non-insulin-mediated glucose disposal is 1.1 mg/kg per min (30, 34). This represents 65 and 69% of the basal glucose disposal rate in the normals and subjects with impaired glucose tolerance, respectively, which indicates that our initial estimate of 70% was reasonably accurate. In the present study, the value of 1.1 mg/kg per min has been used to correct for noninsulin-mediated glucose disposal and the results are shown in Fig. 3B for the control subjects and subjects with impaired glucose tolerance. This analysis could not be done accurately for the type II diabetic subjects because their dose-response curves were too flat. The results show that the half-maximally effective insulin level is 135 μU/ml for the control subjects, compared with 240 μU/ml for the subjects with impaired glucose tolerance (note the log scale on the abscissa). Therefore, this form of analysis quantitates the rightward shift in the dose-response curve for the subjects with impaired glucose tolerance.

Decreased cellular insulin receptors should lead to a shift to the right of the insulin dose-response curve, and this was observed in the subjects with impaired glucose tolerance. Implicit in this observation is a relationship between cellular insulin binding and in vivo insulin action. Since the half-maximally effective insulin level is largely determined by the degree of insulin binding, this value was plotted as a function of insulin binding for individual control subjects and subjects with impaired glucose tolerance (Fig. 4). Since the half-maximally effective insulin concentration cannot be accurately assessed in the type II diabetic subjects (because of the flat curves), they were not included in this analysis. As can be seen, a significant inverse relationship exists ($r = -0.53$, $P < 0.02$), indicating that subjects with higher levels of insulin binding require lower insulin concentrations to elicit a half-maximal response.

To provide further evidence that the alterations in in vivo insulin action seen in the subjects with impaired glucose tolerance were due to decreased cellular insulin binding, the glucose disposal rate was plotted as a function of the amount of cellular bound insulin (Fig. 5). The amount of insulin bound at each of the insulin concentrations shown in Fig. 3 was determined from the adipocyte binding data plotted

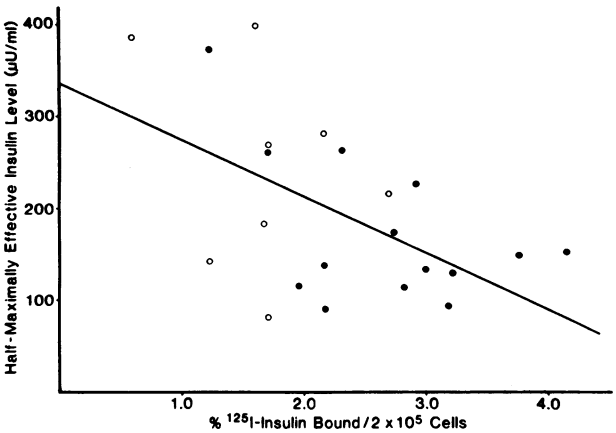

FIGURE 4 Relationship between the insulin concentration that produced half-maximal stimulation of glucose disposal (from the individual dose-response curves) and the percentage of ^{125}I-insulin bound (at 0.2 ng/ml) in individual control subjects (●) and subjects with impaired glucose tolerance (○) r = −0.53, $P < 0.02$.

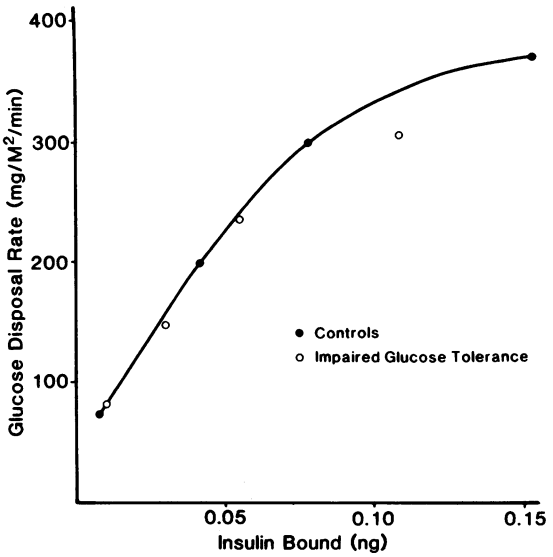

FIGURE 5 Mean glucose disposal rates for the control (●) and impaired glucose tolerance subjects (○), plotted as a function of the amount of insulin bound. The amount of insulin bound was calculated by multiplying the insulin concentrations plotted in Fig. 4 by the percentage of insulin bound at that concentration (as calculated from the competition curves in Fig. 1).

in Fig. 1. This assumes that insulin binding to adipocytes accurately reflects insulin binding to other target tissues in vivo. As can be seen in Fig. 5, when the biologic effect is examined as a function of insulin binding, the same biologic effect is elicited in both groups of subjects by a given amount of bound insulin. This indicates that when one accounts for the decreased ability of tissues from patients with impaired glucose tolerance to bind insulin, no defect in the steps of insulin action distal to the binding event can be detected.

From Table I and Fig. 2C, it is apparent that the diabetic subjects with the lower fasting glucose levels are less insulin resistant and have the smallest reductions in maximal glucose disposal rates. This is shown directly in Fig. 6, where the fasting serum glucose level is plotted as a function of the maximal glucose disposal rate in the subjects with impaired glucose tolerance and Type II diabetes (the decrease in maximal glucose disposal is a measure of the magnitude of the postreceptor defect). When this group of subjects is examined, a highly significant inverse linear relationship is found ($r = -0.72$, $P < 0.001$), indicating that as the maximal glucose disposal rate falls, the fasting glucose level rises. This relationship is also found when the patients with type II diabetes are considered alone ($r = -0.48$, $P < 0.05$), which suggests that a continuum of defects exists. In the subjects with mild impairment of glucose tolerance, i.e., normal fasting glucose levels, maximal insulin-stimulated glucose disposal rates are normal.

Hepatic glucose output. Hepatic glucose output was quantitated during each study by the administration of a primed continuous infusion of [3-^{3}H]glucose. Basal rates of hepatic glucose output were 74±4, 87±8, 139±24, and 125±16 mg/M² per min for the control subjects, subjects with impaired glucose tolerance, nonobese NIDDM, and obese NIDDM subjects, respectively. Using the values obtained for residual hepatic glucose output during each insulin infusion, we calculated the percentage of suppression of basal hepatic glucose output for each insulin concentration. The mean values (±SD) for the various groups are plotted as a function of the serum insulin

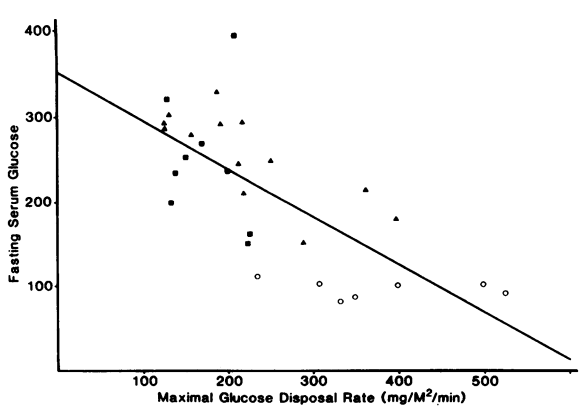

FIGURE 6 Relationship between the fasting serum glucose level and the maximal glucose disposal rate in individual subjects with impaired glucose tolerance (○) and type II diabetes nonobese (▲) and obese (■), r = −0.72, $P < 0.001$.

concentration (Fig. 7), which provides an assessment of another important in vivo insulin action.

It should be pointed out that the lowest insulin concentrations used during these studies achieved >70% suppression of hepatic glucose output. Therefore, the portion of the curves connecting the initial data points with the base line are hypothetical (indicated by the broken lines), which prevents accurate assessment of the half-maximally effective insulin concentration for this insulin effect. Two findings are clear, however. First, the initial data point on the curves for the subjects with impaired glucose tolerance and type II diabetes shows less suppression of hepatic glucose output for that insulin concentration ($P < 0.01$ in both cases). This demonstrates that the dose-response curves for these two groups are right-shifted, compared with controls, but the precise magnitude of this shift cannot be calculated. Second, there is no difference in the maximal response for this hepatic insulin action in any of the study groups, since glucose output is totally suppressed in all groups at maximally effective insulin concentrations.

Since basal rates of hepatic glucose output are greater in the type II diabetic subjects, it is of interest to compare suppression of this function in absolute terms. Thus, at a steady-state serum insulin concentration of 400 μU/ml, the type II diabetic subjects exhibit 75% suppression of their basal hepatic glucose output, compared with 99% suppression for the controls. When expressed in absolute terms, these values correspond to 100 and 73 mg/M² per min, respectively. The physiological significance of this observation remains unclear.

DISCUSSION

Previous studies have shown that insulin resistance exists in patients with either impaired glucose tolerance or type II NIDDM, and that this is due to decreased insulin action at the level of the target tissues (1–11). Decreased cellular insulin receptors have also been widely described in patients with impaired glucose tolerance (1, 4–6) and in patients with type II NIDDM (1, 4–6, 10, 11). But the relationship between decreased insulin binding and decreased insulin action is complex, and the observation that insulin receptors are decreased does not entirely explain the insulin resistance of type II diabetic patients. For example, it has been shown that although a strong correlation exists between the decrease in insulin binding and the degree of insulin resistance in patients with impaired glucose tolerance (1), no such relationship exists in type II diabetic patients with fasting hyperglycemia (1, 11). Furthermore, patients with fasting hyperglycemia are more insulin resistant than patients with impaired glucose tolerance despite a comparable decrease in insulin receptors (1, and Figs. 1 and 2). From these observations, it is apparent that insulin resistance is a general term used to describe any defect in insulin action. The concept can be sharpened by dividing insulin resistance into abnormalities of insulin sensitivity or insulin responsiveness (13). Decreased insulin sensitivity implies a reduction in insulin action at submaximal insulin concentrations with normal responses to maximally effective hormone levels. This results in a rightward shift of the dose-response curve and is usually due to a decrease in cellular insulin receptors (4, 12). It should be noted, however, that since the cellular mechanisms of insulin action are incompletely understood, it is theoretically possible that certain kinds of postreceptor defects could lead to rightward shifted curves.

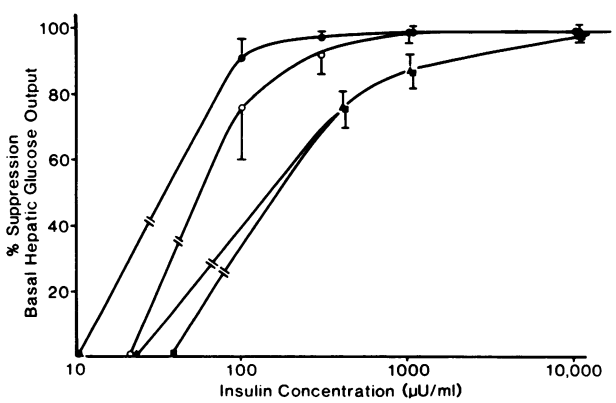

FIGURE 7 Mean dose-response curves for insulin-mediated suppression of hepatic glucose output for the control subjects (●), subjects with impaired glucose tolerance (○), nonobese type II diabetics (▲), and obese type II diabetics (■). Results are plotted as mean±SD.

In the present study we have defined the overall in vivo insulin dose-response curve to delineate the mechanisms of insulin resistance in patients with impaired glucose tolerance and type II NIDDM. The results indicate that the mechanisms of insulin resistance in these groups of patients are complex and result from heterogeneous causes. In the patients with the mildest disorders of carbohydrate homeostasis (patients with impaired glucose tolerance) the dose-response curves were shifted to the right, but maximal insulin action was normal. In other words, more insulin was necessary to achieve a given biologic effect, but when enough insulin was used, the absolute magnitude of the biologic effect was normal. Furthermore, an excellent relationship was observed between the magnitude of the decrease in insulin binding and the rightward shift in the dose-response curve (Fig. 4), and for any given amount of bound insulin the biologic response was comparable in normals and patients with impaired glucose tolerance (Fig. 5). Thus, the insulin resistance in these patients is most likely solely due to decreased insulin receptors and no postreceptor

defect could be detected. On the other hand, the patients with fasting hyperglycemia were insulin-resistant because of both decreased insulin sensitivity and decreased insulin responsiveness resulting from a combination of decreased insulin receptors and a postreceptor defect. In these patients, the dose-response curve was shifted to the right, but the predominant defect was a marked decrease in the maximal response. Thus, it is probable that in those patients with mild abnormalities of glucose tolerance, the insulin resistance is entirely due to decreased insulin receptors. As the magnitude of the diabetes worsens (as assessed by the degree of fasting hyperglycemia) a postreceptor defect emerges that is greatest in the most severely diabetic patients. Evidence for this latter formulation is seen in Fig. 6, which shows that the magnitude of the postreceptor defect increases in parallel with the degree of fasting hyperglycemia, and in those patients with the higher fasting glucose levels (>200 mg/100 ml), the postreceptor defect is most likely the predominant abnormality causing the insulin resistance.

To interpret properly the dose-response curves depicted in Figs. 2 and 3, there should be a close relationship between increments in insulin binding and changes in insulin-mediated glucose disposal (13). For such a relationship to exist, glucose uptake must be relatively rate determining for overall glucose disposal, and the intracellular processes of glucose metabolism should not be saturated (13). For example, if a postreceptor step was saturated, the observed maximal glucose disposal rates would reflect the limiting capacity of this step rather than the maximal hormone effect. To be certain that this was not the case, hyperglycemic (225 mg/100 ml) glucose-clamp studies were performed at maximally effective insulin levels in two type II NIDDM subjects. Under these conditions, the increase in extracellular substrate (glucose) concentration will lead to an increase in net glucose influx into cells by mass action, independent of any insulin-mediated mechanism (13). If some postreceptor step had been saturated during the euglycemic studies, glucose disposal would not increase above the rates observed at euglycemia (and maximal insulin levels). However, the hyperglycemic infusions clearly led to marked increases in glucose disposal in the diabetic subjects, which demonstrates that postreceptor processes were not saturated. When compared with normals studied under similar hyperglycemic, hyperinsulinemic conditions (13), the glucose disposal rates are still markedly reduced in the diabetic patients.

The ability of insulin to suppress hepatic glucose output was also defined. At submaximal insulin concentrations, hepatic glucose output was suppressed less in the groups with impaired glucose tolerance and type II diabetes, which indicates that the dose-response curves are shifted to the right. However, the number of data points obtained at submaximal insulin levels were insufficient to define with precision the degree of this rightward shift, or to calculate half-maximally effective insulin levels. On the other hand, complete suppression of hepatic glucose output was achieved in all groups, which indicates that no postreceptor defect exists for this important hepatic insulin action in any of the study groups. However, the insulin levels required to suppress hepatic glucose output in the patients with type II diabetes are sufficiently high to make it unlikely that these are ever achieved in the in vivo setting. Since basal rates of hepatic glucose production are also elevated in the diabetic patients (see below), it would appear that unrestrained glucose production by the liver contributes to both the fasting and postprandial hyperglycemia observed in these patients.

The basal rates of hepatic glucose production were comparable for the normal subjects and the subjects with impaired glucose tolerance (74±4 vs. 87±8 mg/M^2 per min, respectively) but are considerably higher (139±24 and 125±15 mg/M^2 per min) in the nonobese and obese type II diabetic patients with fasting hyperglycemia. Elevated rates of glucose production in type II diabetic patients have been previously described (9, 10, 35–37), and exist despite the presence of hyperglycemia, a factor which inhibits hepatic glucose production in normal man (38–40). Because basal insulin levels are normal or elevated in these diabetic patients, it is possible that some additional neural or humoral factor is responsible for the elevated rates of hepatic glucose output. In this event, one can speculate that because glucose uptake by insulin-sensitive tissues is subnormal at euglycemia in type II diabetic patients, an increased rate of hepatic glucose production is necessary to produce fasting hyperglycemia, which, by mass action, will reestablish a normal absolute rate of glucose uptake in insulin-sensitive tissues. With this formulation, the diabetic may pay the price with an obligatory increase in glucose uptake by noninsulin-dependent tissues to satisfy the glucose demands of the insulin-sensitive tissues. It is possible that this phenomenon may bear some relationship to the complications of uncontrolled diabetes in those tissues which are not insulin sensitive.

Since the majority of type II diabetic patients are obese and insulin resistance is a well-known feature of obesity, one must consider the impact of obesity on our results. We have been able to address this issue directly in the present study, since both obese and nonobese type II diabetic patients were studied. The results indicate that while there is a trend toward lower glucose disposal rates in the obese NIDDM subjects at all insulin concentrations, these differences are relatively small and reach statistical sig-

nificance only at the highest insulin level. Furthermore, marked differences from normal are seen when only the nonobese subjects are considered (Fig. 3A). When the relationship between relative weight and maximal glucose disposal rate was examined, a trend was observed, but this did not reach statistical significance ($r = 0.28$, $P > 0.10$). Finally, if the data are subjected to multiple linear regression analysis, with the fasting glucose level held constant, and the relationship between relative weight and maximal glucose disposal rate examined, no significant relationship is found. Therefore, it seems likely that the postreceptor defect and insulin resistance observed in type II diabetic patients with moderate to severe fasting hyperglycemia are predominantly related to the diabetic state, and are so severe that additive effects of obesity are hard to appreciate. On the other hand, in the obese patients with less severe fasting hyperglycemia, the postreceptor defect attributable to the diabetes is less severe (Fig. 6) and, in these patients, obesity can contribute significantly to the insulin-resistant state.

The potential impact of aging upon the current results also deserves comment. This is particularly relevant since, as a group, our control subjects are younger than either group of diabetic subjects, and insulin resistance has been reported to occur with age (41). In this regard, a subset of our control groups, i.e., subjects 1–8, have a mean age of 47±3 yr, which is comparable to the mean age of the group of obese diabetics and approaches that of the nonobese diabetics. The mean maximal glucose disposal rate for this subgroup of older control subjects is 428±29 mg/M^2 per min, which is greater than the value for the entire control group or the value for the remaining six younger controls (mean age 28±2 yr) of 383±34 mg/M^2 per min. Thus, when this group of older control subjects is used for comparison, the differences between them and the subjects with abnormalities of carbohydrate metabolism are slightly enhanced. Furthermore, when the relationship between age and the maximal glucose disposal rate in the entire group of control subjects is examined, a positive relationship, $r = 0.42$, which does not reach statistical significance ($0.10 < P < 0.20$) is found, suggesting that, if anything, insulin responsiveness improves with age. Moreover, age had no impact on the maximal glucose disposal rate within the diabetic group. For these reasons, the results of the present study cannot be accounted for on the basis of age. Although we recognize that the number of control patients studied and the age ranges covered (particularly in the older decades) are insufficient to make firm conclusions about aging and insulin resistance in the general population, they do suggest that even if such a relationship existed, its quantitative magnitude would be small.

The causal sequence of events leading to the abnormalities observed are not completely elucidated by the current studies. In the patients with impaired glucose tolerance, decreased insulin sensitivity with normal insulin responsiveness was observed, and it seems likely that decreased insulin receptors are the cause leading to the insulin resistance and glucose intolerance. Of course, the cause of the initial decrease in insulin receptors remains to be defined. In type II diabetic patients with fasting hyperglycemia, the sequence is less clear since a postreceptor defect exists in combination with a receptor defect. In these patients, a number of metabolic abnormalities exist that could play a role in the pathogenesis of the defects in insulin action. For example, since a strong correlation exists between the fasting glucose level and the magnitude of the postreceptor defect, it is conceivable that sustained hyperglycemia induces this abnormality. These patients also have elevated free fatty acid levels and Randle et al. (42) have postulated that this leads to shifts in intracellular metabolic pathways that are responsible for the carbohydrate intolerance. Finally, although these patients may have normal or elevated basal insulin levels, they exhibit hypoinsulinemia after glucose or meal ingestion, and we have previously proposed that insulin deficiency is the primary lesion in these patients leading to the insulin resistance (1, 5, 6). Clearly, insulin deficiency could result in decreased tissue glucose uptake, elevated rates of hepatic glucose production, and fasting hyperglycemia. Additionally, it is possible that the postreceptor defect, which is a major cause of the insulin resistance in these patients, is also secondary to insulin deficiency and we have previously proposed such a hypothesis (43, 44). In support of this, we have recently obtained preliminary evidence that when type II diabetic patients are treated with frequent insulin injections so that they become adequately insulinized with normalization of serum glucose levels, the postreceptor defect can be reversed (45).

In conclusion, these studies have shown that the mechanisms of insulin resistance in patients with varying degrees of carbohydrate intolerance are heterogeneous and that a spectrum of defects exist. In the subjects with impaired glucose tolerance who have mild insulin resistance, the defect in insulin action is due to decreased numbers of cellular insulin receptors, leading to decreased insulin sensitivity. In type II diabetic patients with the greatest degree of fasting hyperglycemia and most severe insulin resistance, decreased insulin receptors and a postreceptor defect in insulin action coexist, but the postreceptor defect appears to be the major abnormality. Between these extremes, the relative roles of receptor and postreceptor defects vary, but the general trend is that as the insulin resistance and fasting hyper-

glycemia become more severe, the postreceptor defect becomes more prominent. Further studies will be necessary to elucidate the biochemical basis for the postreceptor defect in the type II diabetic patient. Although we can estimate the relative roles of receptor vs. postreceptor defects in causing the insulin resistance in the type II diabetic subjects with fasting hyperglycemia, the current results do not allow us to assess the relative contribution of insulin resistance vs. insulin deficiency in causing the hyperglycemic diabetic state itself.

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