Mechanisms of insulin resistance in human obesity: evidence for receptor and postreceptor defects.

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Research Article

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Mechanisms of Insulin Resistance in Human Obesity

EVIDENCE FOR RECEPTOR AND POSTRECEPTOR DEFECTS

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ABSTRACT To assess the mechanisms of the insulin resistance in human obesity, we have determined, using a modification of the euglycemic glucose clamp technique, the shape of the in vivo insulin-glucose disposal dose-response curves in 7 control and 13 obese human subjects. Each subject had at least three euglycemic studies performed at insulin infusion rates of 15, 40, 120, 240, or 1,200 mU/M²/min. The glucose disposal rate was decreased in all obese subjects compared with controls (101±16 vs. 186±16 mg/M²/min) during the 40 mU/M²/min insulin infusion. The mean dose-response curve for the obese subjects was displaced to the right, i.e., the half-maximally effective insulin concentration was 270±27 μU/ml for the obese compared with 130±10 μU/ml for controls. In nine of the obese subjects, the dose-response curves were shifted to the right, and maximal glucose disposal rates (at a maximally effective insulin concentration) were markedly decreased, indicating both a receptor and a postreceptor defect. On the other hand, four obese patients had right-shifted dose-response curves but reached normal maximal glucose disposal rates, consistent with decreased insulin receptors as the only abnormality. When the individual data were analyzed, it was found that the least hyperinsulinemic, least insulin-resistant patients displayed only the receptor defect, whereas those with the greatest hyperinsulinemia exhibited the largest postreceptor defect, suggesting a continuous spectrum of defects as one advances from mild to severe insulin resistance. When insulin's ability to suppress hepatic glucose output was assessed, hyperinsulinemia produced total suppression in all subjects. The dose-response curve for the obese subjects was shifted to the right, indicating a defect in insulin receptors. Insulin binding to isolated adipocytes obtained from the obese subjects was decreased, and a highly significant inverse linear relationship was demonstrated between insulin binding and the serum insulin concentration required for halfmaximal stimulation of glucose disposal. In conclusion: (*a*) decreased cellular insulin receptors contribute to the insulin resistance associated with human obesity in all subjects; (*b*) in the least hyperinsulinemic, insulin-resistant patients, decreased insulin receptors are the sole defect, whereas in the more hyperinsulinemic, insulin-resistant patients, the insulin resistance is the result of a combination of receptor and postreceptor abnormalities; (*c*) all obese patients were insensitive to insulin's suppressive effects on hepatic glucose output; this was entirely the result of decreased insulin receptors; no postreceptor defect in this insulin effect was demonstrated.

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INTRODUCTION

Most obese patients are insulin resistant (1–3), and various animal models of obesity have also been described in which insulin resistance is a prominent feature (4, 5). Decreased cellular insulin receptors have been observed in a variety of tissues from obese animals (6–10) and man (11–15), and the potential causal relationship between decreased insulin receptors and insulin resistance is obvious. However, the quantitative relationship between insulin receptors and insulin's biologic effects is not straightforward as a result of the presence of spare receptors on insulin target tissues (16–18). For example, the functional consequence of decreased insulin receptors is a rightward shift in the insulin:biologic function dose-response curve, resulting in a decreased response to submaximal insulin concentrations but no change in the maximal response to the hormone (9, 16).

Reports using isolated adipocytes from obese rats (9, 19–22) and soleus muscle from genetically obese

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mice (23) have shown that intracellular abnormalities of glucose metabolism are the major cause of the cellular insulin resistance, with the decrease in insulin receptors playing only a minor role. Thus, a modest rightward shift in the insulin dose-response curve was observed but, more importantly, these workers (9, 19–23) noted a marked reduction in insulin response even at maximally effective hormone concentrations. Extrapolating to the situation in obese man, one could infer that a postreceptor defect in the insulin action-glucose metabolism sequence is the predominant cause of insulin resistance, and that decreased cellular insulin receptors play a contributory but quantitatively less important role. However, it is not clear that one can readily extrapolate from in vitro studies in isolated tissues to the overall response of the intact organism; furthermore, it remains to be demonstrated that results in obese rodents are applicable to man. In an attempt to further explore this subject, we recently reported experiments in which the overall in vivo dose-response curve of normal and obese subjects was studied by measuring glucose disposal at various steady-state plasma concentrations of insulin, including maximally effective hormone levels (14). These studies demonstrated a rightward shift in the overall insulin dose-response curve, indicating that decreased insulin receptors contribute to the insulin-resistant state. However, the degree of insulin resistance demonstrated was quantitatively much greater than what would be predicted from the magnitude of the decrease in insulin receptors. Furthermore, the methodology employed in that study did not allow us to define the maximum responsiveness to insulin.

Consequently, in the present experiments, we have used the euglycemic glucose clamp technique to construct detailed in vivo insulin dose-response curves to accurately define both the functional form of the dose-response relationship and the maximum effect of insulin in promoting overall glucose uptake. An underlying premise in these studies is that if overall in vivo insulin resistance in human obesity is the result of decreased insulin receptors, one should see a rightward shift in the insulin dose-response curve which is quantitatively accounted for by decreased insulin receptors, with no impairment in the maximal response to the hormone. On the other hand, if postreceptor abnormalities are the predominant cause of in vivo insulin resistance in man, insulin's effects should be subnormal even at maximally effective plasma insulin concentrations. Finally, if biologically significant postreceptor and receptor defects coexist, one would predict a rightward shift in the dose-response curve combined with a subnormal response at maximally effective insulin concentrations. Therefore, we have determined overall glucose uptake at various steady-state plasma insulin concentrations in normal and obese patients.

METHODS

Materials. Porcine monocomponent insulin was generously supplied by Dr. Ronald Chance of Eli Lilly & Co. (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 antiinsulin antibody was kindly supplied by Dr. Edward Arquilla (Irvine, Calif.).

Subjects. The study group consisted of 13 obese and 7 nonobese subjects whose clinical and metabolic features are outlined in Table I. All subjects had fasting serum glucose levels <120 mg/100 ml, and no subject had a disease or was ingesting any agent known to affect carbohydrate or insulin metabolism. The relative weights of the obese patients ranged from 1.26 to 2.45 U, with a mean of 1.76, whereas the relative weights of the normal subjects ranged from 0.85 to 1.13, with a mean of 0.97 (24). The mean(±SE) age for the obese group was 38±4 yr and 32±3 yr for the control group. After obtaining informed consent, all subjects were admitted to the University of Colorado Clinical Research Center but remained active to approximate their prehospitalization exercise level. Subjects were chemically euthyroid and had no stimgata of renal, cardiac, or hepatic dysfunction.

Diet. All subjects were placed on an isocaloric liquid formula diet, with three divided feedings consisting of 1/5, 2/5, and 2/5 of total daily calories given at 0800, 1200, and 1700 h, respectively. The diet contained 45% carbohydrate, 40% fat, and 15% protein. All subjects consumed this diet for at least 48 h before study.

Oral glucose tolerance test. Oral glucose tolerance tests were performed by giving each subject 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 using the euglycemic "glucose clamp" technique as previously described (25, 26). 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 box (72°C) to facilitate venous sampling. After insertion of the catheters, the subject is allowed to stabilize for at least 30 min before beginning the study.

At the onset, a priming insulin dose is administered during the initial 10 min in a logarithmically decreasing manner to acutely raise the serum insulin to the desired concentration, which is then maintained by a continuous insulin infusion for the duration of the study. All studies were carried out for 120 mins. Serum glucose was maintained between 80 and 90 mg/100 ml throughout the study period by monitoring the glucose level at 5-min intervals and adjusting the infusion rate of a 20% glucose solution with a servo-control negative feedback principle (25, 26). Because serum potassium levels tend to fall during this procedure, KCl was administered during each study at a rate of 15–20 meq/h to maintain the serum potassium between 3.5 and 4.5 meq/liter. Thus, any potential deleterious effects of hypokalemia were avoided.

With this technique, steady-state euglycemia is maintained and all of the glucose infused is metabolized by peripheral tissues (25). Thus, the total amount of glucose metabolized serves as a measure of the subject's sensitivity to the prevailing serum insulin concentration (25, 26). The amount of glucose metabolized during each study was calculated for each 20-min interval after the initial 40 min of the study. The glucose disposal rates for the 20-min intervals were then averaged and the mean value used as the data point for

TABLE I
Clinical and Metabolic Features

Subject	Age	Sex	Relative* weight	Age at onset of obesity	Fasting serum glucose	2-h serum glucose (GTT)‡	Fasting serum insulin concentration	Mean adipocyte size	Percent [^{125}I]insulin bound§	Maximal glucose disposal rate
								pl/cell		*mg/H²/min*
Controls										
1	44	F	0.86	—	88	124	7	170	2.20	267
2	23	F	0.86	—	78	133	6	161	3.00	369
3	29	F	1.13	—	75	102	13	348	2.92	316
4	29	M	0.85	—	89	131	10	184	3.18	348
5	32	F	1.05	—	82	85	10	169	4.15	463
6	37	F	0.92	—	90	119	6	225	3.76	296
7	28	M	1.13	—	91	124	11	206	3.22	432
Mean±SE	32±3		0.97±0.05		85±2	117±7	9±1	209±25	3.20±0.24	356±27
Group I obese										
8	36	M	2.13	15	112	208	36	649	1.61	289
9	41	F	1.47	14	86	85	16	298	1.79	364
10	28	F	1.40	8	87	124	24	272	2.68	458
11	36	M	2.45	4	96	138	21	318	1.85	327
Mean±SE	35±3		1.86±0.26	10±3	95±6	139±26	24±4	384±89	1.98±0.24	360±36
Group II obese										
12	28	F	1.89	22	81	150	64	463	1.02	114
13	31	F	2.19	21	70	121	35	828	1.73	246
14	45	F	1.85	32	121	187	83	783	1.74	152
15	49	M	1.71	24	69	128	23	362	1.49	229
16	58	M	1.26	32	90	134	32	767	1.27	189
17	24	F	1.76	20	88	151	86	393	1.62	132
18	50	F	1.71	22	99	210	38	405	1.14	219
19	40	F	1.51	26	104	203	74	530	1.07	158
20	35	F	1.52	24	78	158	25	735	1.13	184
Mean±SE	40±4		1.71±0.09	25±1	89±6	160±11	51±8	585±64	1.36±0.10	180±15

* Source: Society of Actuaries. 1959. Build and Blood Pressure Study. **1:** 17.
‡ GTT, glucose tolerance test.
§ Percent ^{125}I-insulin bound per 2×10^5 cells at an insulin concentration of 0.2 ng/ml.

that individual study. Endogenous glucose production was measured in all cases, and this value was added to the amount of glucose infused to obtain the total amount of glucose metabolized (27). During each study, blood samples were obtained every 20 min for measurement of serum insulin and potassium concentrations.

Each subject was studied at an insulin infusion rate of 40 mU/M²/min to allow assessment of in vivo insulin sensitivity in a uniform manner. In addition, each subject had at least two additional euglycemic glucose clamp studies performed on separate days at different insulin infusion rates (15, 120, 240, or 1,200 mU/M²/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 one control subject, K.K., and three obese subjects; they received a continuous insulin infusion of 1,200 mU/M²/min, whereas the serum glucose level was acutely raised to 225 mg/100 ml and maintained within ±10% of that value throughout the study.

Hepatic glucose output. Hepatic glucose production was assessed during the basal state as well as during each of the glucose clamp studies by infusing [3-^{3}H]glucose in a primed continuous manner (27, 28). With this technique, 25 μCi of the tracer is injected as a bolus, followed by continuous infusion at the rate of 0.25 μCi/min. Blood samples are then obtained at 30-min intervals for the determination of the concentration and specific activity of serum glucose. Hepatic glucose output is then calculated using the Steele equations (29) in their modified derivative form (26, 29) because the tracer exhibits nonsteady-state kinetics under these conditions.

Insulin binding studies. Insulin binding to isolated adipocytes was studied with cells obtained from open adipose tissue biopsy of the lower abdominal wall. Details concerning the measurement and calculation of the amount of insulin bound to adipocytes have been published previously (13, 30).

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

Blood for the determination of serum insulin levels 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 with the technique of Desbuquois and Aurbach (31).

Data analysis. All calculations were performed using a programmable calculator (model 67, Hewlett-Packard Co., Palo Alto, Calif.).

RESULTS

Glucose tolerance tests. The fasting and 2-h serum glucose and fasting insulin levels are shown in Table I. All of the obese subjects had fasting glucose levels <120 mg/100 ml and, although five obese subjects had glucose levels at either the 1-h or 2-h points, which were abnormal according to the criteria of Fajans and Conn (32), only two subjects, 8 and 14, had curves that would be classified as chemical diabetes. The obese subjects exhibited marked hyperinsulinemia compared with the control group, and this is a well-recognized feature of human obesity.

Measurement of in vivo insulin sensitivity. A glucose clamp study was performed in each subject at an insulin infusion rate of 40 mU/M²/min. This resulted in comparable steady-state serum insulin levels in all subjects, 106±5 for the control group compared with 110±5 μU/ml for the obese subjects. On the other hand, the glucose disposal rates were 101±16 for the obese vs. 186±16 mg/M²/min for the control subjects. Thus, physiologic insulin concentrations were much less effective in promoting glucose removal in the obese subjects compared with controls, and this demonstrates the insulin resistance present in these patients.

In vivo insulin dose response curves. To further define the mechanisms of this insulin-resistant state, dose-response studies were performed with additional euglycemic glucose clamp studies in each subject at insulin infusion rates of either 15, 120, 240, or 1,200 mU/M²/min. Each subject had at least three studies, but it was not feasible to study all subjects at all insulin infusion rates.

The individual dose-response curves for the control subjects are shown in Fig. 1A. The initial point on each curve was obtained by measuring hepatic glucose output in the basal state. Under these conditions, overall glucose disposal equals hepatic glucose production. The portal/peripheral insulin gradient that exists in this situation is not a factor and, because the liver does not take up glucose in the basal state (33), the measured basal glucose turnover rates accurately reflect the relationship between tissue glucose uptake and the basal insulin levels in the peripheral circulation. Based on previous reports, only 10–30% of basal glucose uptake proceeds by insulin-dependent processes (34–36). The data in Fig. 1A describe the overall in vivo insulin dose-response relationship in normal man. Although the expected biologic variability is evident, all of the individual curves fall within a reasonably narrow range.

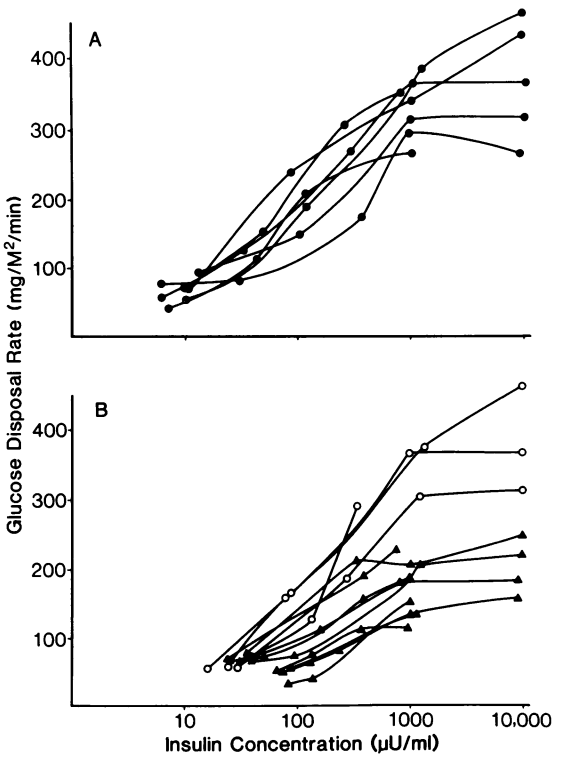

FIGURE 1 (A) Individual dose-response curves for control subjects. Results were obtained by performing additional euglycemic clamp studies in each subject with insulin infusion rates of 15, 120, 240, or 1,200 mU/M²/min. The initial point on each curve represents glucose disposal in the basal state as determined by a primed continuous infusion of [3-³H]glucose (see text for details). (B) Individual dose-response curves for the entire group of 13 obese subjects. Four subjects (○) had maximal rates of glucose disposal similar to those seen in the control subjects, whereas the remaining nine subjects (▲) had maximal rates that were markedly decreased.

Increasing the steady-state serum insulin level led to a dose-dependent, four- to sixfold increase in the glucose disposal rate, with an average half-maximal insulin level of 130 μU/ml. Thus, the physiologic range of peripheral insulin concentrations essentially falls entirely within the steep, or most responsive, segment of the dose-response relationship.

In vivo insulin dose-response curves for the 13 obese subjects are shown in Fig. 1B. When taken as a group, greater variability is evident in the response curves for the obese subjects than was observed in normals. On inspection of the individual data, it is apparent that all of the curves are shifted to the right. However, it is also clear that some patients achieve normal, maximum insulin-stimulated rates of glucose disposal (as indicated by the four upper curves with the open circles), whereas the remaining nine subjects (closed triangles) display markedly decreased maximum glucose disposal rates. Thus, the insulin resistance

associated with human obesity appears to be a heterogeneous disorder.

Because the obese subjects displayed two kinds of response patterns, they have been divided into two separate groups for the purpose of further analysis. The first group, hereafter referred to as group I obese, consists of the four obese subjects who achieved normal maximal rates of insulin-stimulated glucose disposal, whereas the second group (group II obese) is composed of the remaining nine obese subjects who displayed markedly decreased maximal glucose disposal rates. It should be emphasized at the outset that this division of the obese subjects is arbitrary and retrospective in nature and is done simply to demonstrate and contrast the different response patterns that can be seen in human obesity. We feel that the individual response curves of the 13 obese subjects comprise a spectrum, and we do not mean to imply that these arbitrarily defined groups represent distinctly different populations of obese patients. In the following figures, as well as in Table I, mean and standard error values have been calculated for various mesurements. This has been done to facilitate data comparison and to provide some measure of data variability. However, this is not meant to imply any statistically significant differences between these two obese groups because, given the nature of the division, this would obviously be a circular argument.

The mean dose-response curves for the control group and the two groups of obese subjects are plotted in Fig. 2A. Because all studies were not performed at the same steady-state insulin concentrations, when necessary, glucose disposal rates were estimated from each subject's individual dose-response curve at insulin levels of 100, 300, 1,000, and 10,000 μU/ml. With this approach, group I exhibits normal maximal glucose disposal with a rightward shift in the dose-response curve. In this group, increasing serum insulin levels lead to a normal sixfold increase in glucose disposal rate. The other nine obese subjects (group II) demonstrate both a rightward shift in their dose-response curve and strikingly decreased maximal glucose disposal rates. Thus, increasing insulin levels lead to only a threefold increase in glucose disposal in this group. With this analysis, the insulin resistance associated with the obese state in man appears to be heterogeneous; some patients display a dose-response relationship consistent with decreased insulin receptors as the sole abnormality, whereas the results in others are most consistent with a decrease in cellular insulin receptors and a postreceptor defect.

To more accurately analyze the functional form of the dose-response curves, the absolute values for glucose disposal were converted to percentage terms (Fig. 2B). For each group, the maximal glucose disposal rate was taken as 100%, and the glucose disposal rate at

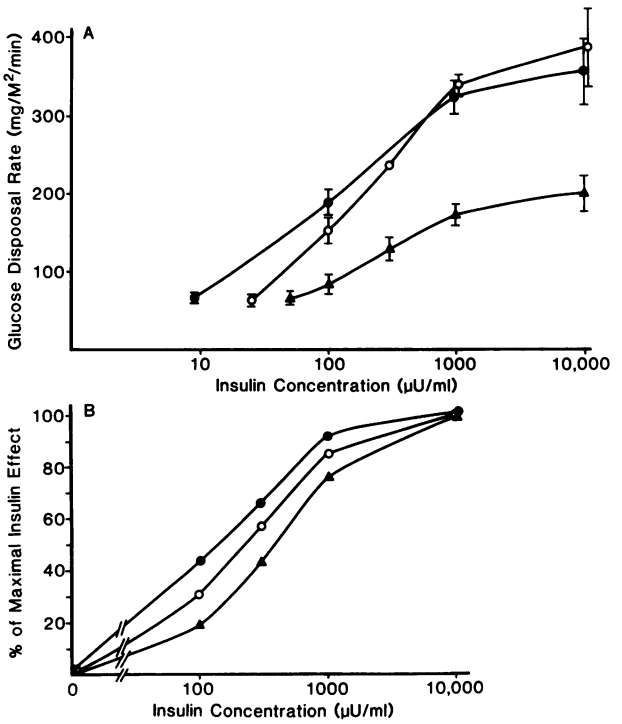

FIGURE 2 (A) Mean dose-response curves with the obese subjects separated into group I (○) and II (▲) obese groups (see text). ●, controls. (B) Dose-response curves plotted as percent of the maximal insulin effect.

each submaximal insulin level is plotted as a percent of this value. For purposes of this analysis, 70% of the absolute basal glucose disposal rate is initially subtracted from all values because this represents non-insulin-mediated glucose uptake (32–34). With this approach, potential influences of differences in post-receptor effector systems are eliminated because all maximal rates are taken as 100%, and the proportion of the total possible insulin effect elicited at any hormone level can be seen. Fig. 2B clearly shows that the dose-response curves are shifted to the right in obesity, and the magnitude of this effect is greatest in the patients with the most severe receptor reduction. Thus, the half-maximally effective insulin levels were 130, 210, and 370 μU/ml in the normal, group I and group II obese subjects, respectively.

From Table I and Fig. 1B, it is apparent that the patients with normal maximal rates of glucose disposal are less insulin resistant, less hyperinsulinemic, and have a smaller reduction in insulin receptors than the subjects with reduced maximal responsiveness. This is illustrated in Fig. 3, in which the magnitude of the postreceptor defect is plotted as a function of degree of insulin resistance (Fig. 3A) (as assessed by the rate of glucose disposal at an insulin level of 100 μU/ml) or the fasting insulin level (Fig. 3B). As can be

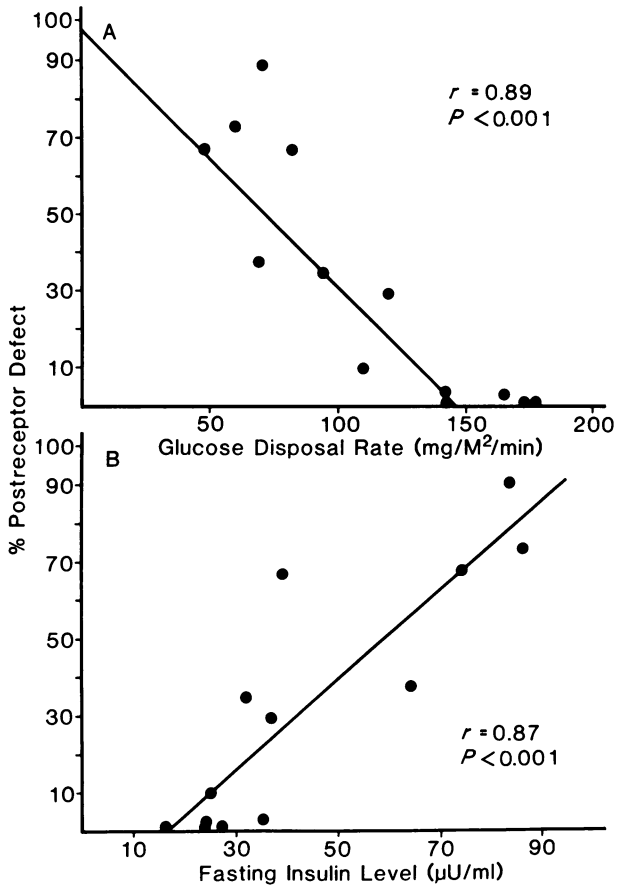

FIGURE 3 The magnitude of the postreceptor defect as a function of the degree of insulin resistance (A) or the fasting insulin level (B) in the 13 individual obese subjects. The degree of insulin resistance is represented by the glucose disposal rate (mg/M²/min) at an insulin concentration of 100 μU/ml (as in Fig. 1). The magnitude of the postreceptor defect at an insulin concentration of 100 μU/ml was calculated for each subject by determining the amount of insulin bound from the adipocyte insulin binding curve. The expected normal rate of glucose disposal, at this amount of insulin bound, was then calculated from the mean glucose disposal dose-response curve. The observed glucose disposal rate in the obese subject was then divided by the expected normal rate to compute the magnitude of the postreceptor defect, i.e., postreceptor defect = 1 − (observed glucose disposal rate ÷ expected glucose disposal rate × 100.

seen, both relationships are highly significant, and the greater the degree of hyperinsulinemia or insulin resistance, the greater the postreceptor defect. Furthermore, although all of the subjects were hyperinsulinemic and insulin resistant, no postreceptor defect exists in the least affected subjects. Consistent with previous observations (11, 15), the correlation between fasting insulin level and degree of insulin resistance is also highly significant ($r = 0.74$). These findings indicate a continuum of insulin resistance in human obesity so that, in the mildly hyperinsulinemic, insulin-resistant state, only a receptor defect exists; as the hyperinsulinemic, insulin-resistant state worsens, a postreceptor defect appears.

Hyperglycemic clamp studies. The receptor theory (14, 37) upon which the interpretation of the results in Figs. 1 and 2 is based presupposes 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). If this were not the case, then the glucose disposal rates at the highest insulin levels may not represent the maximum effect of the hormone, but could reflect the maximum capacity of tissues to metabolize glucose. To evaluate this, euglycemic and hyperglycemic glucose clamp studies were performed in a control, two group II obese, and one group I obese subject at the maximal insulin infusion rate (1,200 mU/M²/min). These results are seen in Table II. Increasing the substrate (serum glucose) concentration led to a marked increase in the overall glucose disposal rate in all subjects. Therefore, the maximal insulin-stimulated glucose disposal rates seen in Figs. 1 and 2 do not represent the maximal capacity of the tissues to metabolize glucose but, instead, reflect the maximal effect of insulin.

Hepatic glucose output. Hepatic glucose output was quantitated during each study by the administration of a primed continuous infusion of [3-³H]glucose. Hepatic glucose production was similar for the three groups during the basal state: 66±7, 67±6, and 63±3 mg/M²/min for the controls, group I, and group II obese, respectively. With the values obtained during each insulin infusion, the percent suppression of basal hepatic glucose output was calculated at each insulin concentration (Fig. 4). The mean values for each group are plotted as a function of serum insulin concentration, and this analysis provides dose-response curves for another in vivo insulin function. The curves for both obese groups are shifted to the right, and this effect is greater for the subjects with the greatest

TABLE II
*Glucose Disposal under Hyperglycemic Conditions**

	Euglycemia	Hyperglycemia
	mg/M²/min	
Normals (1)	404	698
Group I obese (1)	328	661
Group II obese (2)	156	302

* All studies were performed at a maximally effective insulin level (~10,000 μU/ml) and the numbers in parentheses indicate the number of patients studied in each group. During the euglycemic studies, the plasma glucose concentration was 89±2 (mean±SD) mg/100 ml, and was 225±4 mg/100 ml during the hyperglycemic studies.

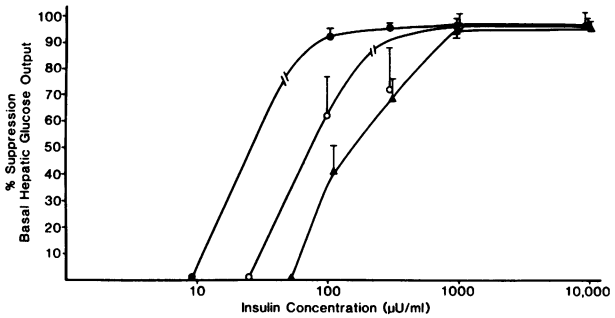

FIGURE 4 Mean dose-response curves for insulin-mediated suppression of basal hepatic glucose output for control (●), group I (○), and group II (▲) obese subjects.

reduction in insulin receptors. However, unlike the dose-response curves for glucose disposal, there is no difference in the maximal insulin effect on hepatic glucose output and glucose production is essentially completely suppressed in all subjects. These results suggest that the incomplete suppression at submaximal insulin levels in the obese subjects stems solely from the decrease in cellular insulin receptors.

Insulin receptor studies. The competition curves for insulin binding by isolated adipocytes are shown in Fig. 5. Adipocytes obtained from the obese patients bind less insulin than normals, and this decrease is greatest in the group II obese patients. Scatchard (38) and average affinity (39) analyses demonstrate that this decrease in insulin binding is the result of a reduced number of insulin receptors (not shown).

Decreased insulin receptors should lead to a rightward shift in the insulin-biologic function dose-response curve, and this was observed in all of the obese patients. This implies a relationship between decreased insulin binding to receptors and overall in vivo insulin action; to more quantitatively examine this relationship, insulin binding to adipocytes was plotted as a function of the serum insulin concentration required to achieve half-maximal stimulation of glucose disposal in each subject. The half-maximal insulin concentration should be primarily determined by the degree of insulin binding and, as can be seen in Fig. 6, this was the case. Thus, the lower the level of insulin binding, the higher the half-maximal insulin concentration; the relationship between these two variables was highly statistically significant.

The data in Fig. 6 demonstrate that the decrease in insulin binding in the obese groups quantitatively accounts for the rightward shift in the in vivo dose-response curves. However, to assess the absolute effectiveness of bound insulin, one must examine the relationship between the actual amount of insulin bound at a given insulin concentration and the in vivo biologic effect of insulin at that concentration. The amount of insulin bound was determined from the curves presented in Fig. 5 at the insulin concentrations indicated in Fig. 2. This analysis assumes that insulin binding to adipocytes accurately reflects insulin binding to other target tissues in vivo. In view of the heterogeneity demonstrated in obese subjects, one would expect to see different relationships for the two obese groups. As can be seen in Fig. 7A, this is the case. It is apparent that for any given amount of insulin bound, in vivo insulin effectiveness is quite comparable in the control and group I obese subjects; this, again, demonstrates

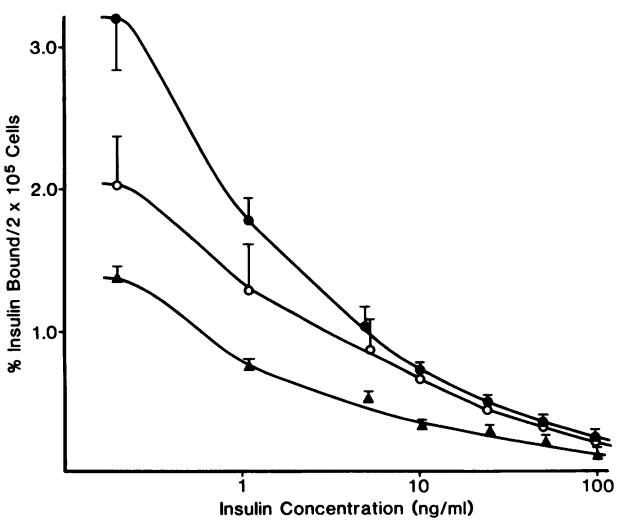

FIGURE 5 Insulin binding by isolated adipocytes from control (●), group I (○), and group II (▲) obese subjects. All data are corrected for nonspecific binding and represent the mean±SE percentage of ^{125}I-insulin specifically bound per 2×10^5 cells.

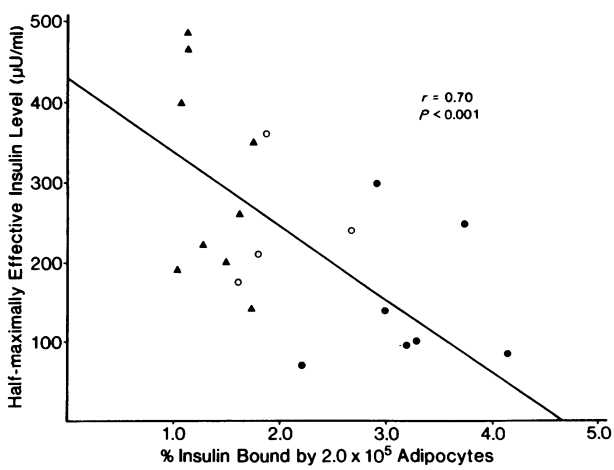

FIGURE 6 Relationship between the insulin concentration that produced half-maximal stimulation of glucose disposal (from the individual dose-response curves) and the percent ^{125}I-insulin bound (at 0.2 ng/ml) in individual subjects. ●, controls; ○, group I obese; ▲, group II obese.

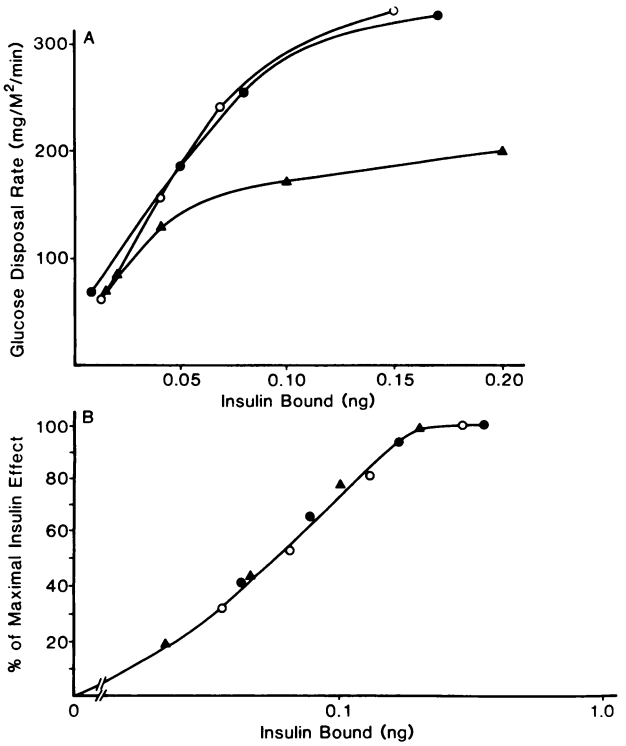

FIGURE 7 (A) Mean glucose disposal rates plotted as a function of the amount of insulin bound. The amount of insulin bound was calculated by multiplying the insulin concentrations plotted in Fig. 4 by the percent insulin bound at that concentration (as calculated from the competition curves in Fig. 7). ●, controls; ○, group I obese; ▲, group II obese. (B) Percent of the maximal insulin effect plotted as a function of the amount of insulin bound.

that the abnormality in the in vivo dose-response curves in these subjects was the result of a decrease in cellular insulin receptors. On the other hand, in vivo insulin action is less at any given amount of insulin bound in the group II obese group, and this suggests "uncoupling" between insulin-receptor complexes and the biologic function measured, i.e., overall glucose disposal. This is the predicted consequence of a postreceptor defect, and the uncoupling can be the result of any abnormality in the insulin action-glucose metabolism sequence after the initial binding event.

To further evaluate the site of this postreceptor defect, and to more accurately evaluate coupling between insulin-receptor complexes and glucose disposal, the relationship between percent of maximal insulin effect and the actual amount bound was also analyzed (Fig. 7B). As can be seen, the results in all three groups are quite comparable, indicating that when differences in postreceptor effector units are taken into account, insulin-receptor complexes are equally functional in all situations. Thus, actual coupling of insulin receptors to the biologic effect is intact in obesity, and the reduction in glucose disposal in the group II obese subjects is the result of a defect distal to this step.

Maximal insulin action occurs when only a minority of cellular insulin receptors are occupied; examination of Fig. 7 indicates the proportion of "spare receptors" for overall glucose disposal. In normals, fractional receptor occupancy is 11% at a half-maximally effective insulin level of 130 μU/ml (Table III). Insulin receptors are decreased in both obese groups, and the half-maximal insulin level is correspondingly increased. Thus, in group I, fractional receptor occupancy is 19% at a half-maximal insulin level of 210 μU/ml, and in group II, 24% at 370 μU/ml. On the other hand, the absolute number of insulin receptors that are occupied (26–30,000/cell) at the half-maximal insulin level is quite comparable for all subjects (Table III). The exact, maximally effective insulin level is difficult to quantitate because of the limited number of high insulin concentrations used and the smaller increments in insulin action at these levels. Nevertheless, if one assumes that 1,000 μU/ml is a fully effective concentration in normals, then fractional receptor occupancy is ~40% and is even higher in the obese groups. It should be noted, however, that the maximally effective insulin level is probably <1,000 μU/ml because in vitro studies (16–18, 23) indicate that the maximal insulin level is three to five times the half-maximal level. With this in mind, the true maximal insulin level may be closer to 400–700 μU/ml.

DISCUSSION

The potential causes of insulin resistance include abnormal beta-cell secretory products, circulating insulin antagonists, and tissue defects in insulin action (37); considerable evidence exists indicating that the insulin resistance of obesity resides at the level of the insulin target tissues (1–3, 37). In recent years, numerous studies have described decreased cellular insulin receptors in obese humans (11–15) and animals (6–10), and these observations have suggested that a causal relationship exists between the decrease in insulin-binding sites and the insulin-resistant state (6–15). However, insulin action consists of a complex sequence of events, and a defect at any step in this cascade could theoretically lead to insulin resistance.

TABLE III
Receptor Occupancy at Half-Maximal Insulin Concentration

	Normal	Group I obese	Group II obese
Fractional receptor occupancy, %	11	19	24
Number of receptors occupied	28,000	30,000	26,000

Indeed, in several animal models of obesity (9, 19–23), it has been shown that marked intracellular abnormalities in glucose metabolism exist. These intracellular defects lead to a marked decrease in the maximal capacity for glucose metabolism and indicate that decreased insulin receptors play a relatively minor role in the overall pathogenesis of the insulin resistance (9, 23).

From these observations, it is obvious that insulin resistance is a general term used to describe any defect in insulin action. This concept can be sharpened by dividing insulin resistance into abnormalities of insulin sensitivity or insulin responsiveness (40). Decreased insulin sensitivity indicates a decrease in the proportion of the total hormonal response, which occurs at any given submaximal concentration of insulin, with a normal response to a maximally effective concentration of insulin. This is usually the result of decreased insulin receptors and amounts to a rightward shift in the insulin-biologic function dose-response curve because a higher insulin level is required to achieve a given insulin effect. Decreased insulin responsiveness implies an impairment in the maximal effect of insulin on the particular process under study, and is the result of a postreceptor defect in insulin action.

The present study was performed to determine whether the overall insulin resistance associated with human obesity is the result of decreased insulin sensitivity, decreased insulin responsiveness, or both. The data presented show that the etiology of the insulin resistance associated with human obesity is complex. Some obese subjects were insulin resistant as the result of decreased insulin sensitivity secondary to a reduction in cellular insulin receptors, and exhibited normal insulin responsiveness at maximally effective insulin levels. The dose-response curves were displaced to the right, and the magnitude of this shift could be accounted for by the reduction in insulin binding to receptors. For example, the relationship between the amount of insulin bound and absolute glucose disposal rate, or the percentage of the maximal insulin effect, was normal. Likewise, at a physiologic insulin level (100 μU/ml), the decrease in insulin-stimulated glucose disposal rate (28%) was quite comparable with the decrease in insulin binding (26%). This demonstrates that the reduction in insulin receptors necessitated a higher insulin level to produce the same amount bound as in controls, but once this higher insulin level is achieved, insulin action is normal. On the other hand, the remaining nine obese patients were insulin resistant because of both decreased insulin sensitivity and 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, and the maximal response was also markedly reduced. The rightward shift in the dose-response curve was quantitatively accounted for by the decrease in insulin receptors, but this is only part of the reason for decreased absolute rates of insulin-mediated glucose disposal. For example, in the group II patients, if one sets the maximal insulin effect in these patients as normal and calculates the glucose disposal rate, which theoretically would have been achieved at an insulin level of 100 μU/ml, or if one divides the actual glucose disposal rate (100 μU/ml) by the rate predicted from an individual's binding defect, then it can be computed that the postreceptor defect accounts for 62% of the decrease in glucose disposal rate, whereas the receptor defect accounts for 38% of the decrease. At higher insulin levels, the contribution of the postreceptor defect to the overall deficit becomes greater because the amount of insulin bound becomes less rate determining for overall glucose disposal.

The above findings suggest that the cause of insulin resistance is heterogeneous in human obesity. A likely explanation for this apparent heterogeneity is that (*a*) the greater the hyperinsulinemia in obesity, the more severe the insulin resistance, and that (*b*) those patients with mild insulin resistance display only a defect in insulin receptors, whereas those obese patients with more severe hyperinsulinemia and insulin resistance also develop a postreceptor defect. Evidence for this hypothesis is seen in Fig. 3, in which the magnitude of the postreceptor defect in individual subjects has been calculated and plotted as a function of either the fasting insulin concentration or the degree of insulin resistance. As can be seen, a highly significant linear correlation exists, suggesting (*a*) that the hyperinsulinemia leads to reduced numbers of insulin receptors, and (*b*) that as this process advances, a postreceptor defect in insulin action develops. Furthermore, the magnitude of this post-receptor defect is directly related to the degree of hyperinsulinemia. This would indicate that high concentrations of circulating insulin (or some closely related factor) can desensitive the target tissues at both receptor and postreceptor levels. In vitro studies consistent with this concept have recently been obtained with isolated rat adipocytes (41).[1] However, to prove the sequential nature of this hypothesis, further studies will be necessary. For example, because the majority of the patients in group II were markedly obese (relative weight >1.70), it is quite possible that if additional subjects with moderate obesity were studied, a more defined continuum would be observed. Furthermore, if obese patients exhibiting both a receptor and postreceptor defect are induced to lose weight, they should first lose the postreceptor defect before the receptor number returns to normal.

[1] Marshall, S., and J. M. Olefsky. Unpublished results.

Additional points pertaining to these results deserve comment. First, it is interesting to note that the least insulin-resistant patients in this study became obese during childhood and have remained so ever since. In contrast, the most insulin-resistant patients all became obese during adulthood. No differences in degree of obesity, duration of obesity, age at the time of study, or oral glucose tolerance were noted between the two groups. Thus, for an equal degree of obesity, patients with childhood-onset obesity are less hyperinsulinemic and less insulin-resistant than adult-onset obese patients. This has been noted previously (13, 41, 42), and the reason for this remains unknown.

Second, the in vivo dose-response data obtained in this study demonstrate that the steepest section of the curve lies within the physiologic range of peripheral insulin concentrations, indicating that the changes in serum insulin concentration, which occur under normal conditions, will produce major increments in overall insulin-mediated glucose disposal. This is somewhat different from results obtained in vitro with animal tissues (16, 18), which have shown a much more sensitive dose-response relationship with maximal biologic effects occurring at low physiologic insulin levels.

Third, determination of the proper unit to which glucose disposal rates should be normalized represents a complicating factor in expressing the data from the various groups because the increase in body mass in obesity consists primarily of adipose tissue, which accounts for only a small proportion (4–5%) of total glucose consumption (41–44). If the results are expressed in terms of body weight, rates of glucose disposal will be reduced in obese subjects because a larger portion of their body weight consists of adipose tissue mass, which does not consume as much glucose as lean body mass. Expressing the data as amount of glucose consumed per total body per minute also introduces an artifact because changes in body mass are totally disregarded. For these reasons, we have elected to present glucose disposal rates as milligram per meter squared per minute. The rationale for this approach is that it more accurately represents the increase in major glucose consuming-tissues (lean body mass) as total body size increases. Most likely, it would be more accurate to express the results in terms of lean body mass; however, techniques for estimating lean body mass are cumbersome and relatively inaccurate. Consequently, although our approach is probably imperfect, we feel it represents the best available method at this time. It is worth noting that maximum rates of glucose disposal were 586±63, 843±100, and 386±39 mg/min when expressed on the basis of total body uptake, and were 9.6±0.9, 8.5±1.0, and 3.5±0.3 mg/kg/min when normalized to unit body weight in control, group I, and group II obese subjects, respectively. Thus, regardless of how the data are expressed, maximal rates of glucose disposal were not significantly decreased in the group I obese subjects and are always decreased in the group II obese subjects.

To properly interpret the dose-response curves depicted in Figs. 1 and 2, there should be a close relationship between increments in insulin binding and changes in insulin-mediated glucose disposal. 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. For example, if a postreceptor step were saturated, then the observed maximal glucose disposal rates would reflect the limiting capacity of this step rather than the maximum hormone effect. To be certain that this was not the case, hyperglycemic (225 mg/100 ml) glucose clamp studies were performed at maximal insulin levels in normal and obese 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. If some postreceptor step had been saturated during any of the euglycemic studies, one would predict that glucose disposal would not increase above the rates observed at euglycemia (and maximal insulin levels). However, the hyperglycemic infusion clearly led to marked increases in glucose disposal in the normal and obese subjects, demonstrating that postreceptor processes were not saturated. Even in the group II subjects, where a postreceptor defect exists, this step(s) is not saturated under euglycemic conditions, and the relationship between insulin binding and insulin action accurately reflects the interaction between formation of insulin-receptor complexes and the ability of insulin to augment glucose disposal. It should be noted that under both euglycemic and hyperglycemic conditions, De Fronzo et al. (23) have shown that the liver does not contribute to net glucose uptake; therefore, the measured glucose disposal rates reflect the events occurring at the site of peripheral insulin target tissues.

As shown in Fig. 5, the obese subjects exhibited decreased cellular insulin receptors and the dose-response data indicate that this is physiologically significant in both obese groups because all of the curves are displaced to the right. Thus, one should be able to demonstrate a significant relationship between insulin binding and an appropriate measure of biologic function. Such a relationship is shown in Fig. 6 where the half-maximally effective insulin concentration is plotted as a function of the percent insulin bound. The half-maximally effective insulin concentration was chosen as the index of biologic function because it provides an accurate representation of the shape of the dose-response curve and is primarily a function of in-

sulin receptor binding. A highly significant relationship exists between these two variables so that the lower the level of insulin binding, the greater the insulin concentration required for half-maximal effects. Similar correlations are observed when the glucose disposal rate at a given submaximal insulin concentration is plotted as a function of insulin binding.

A postreceptor abnormality should lead to a decrease in overall insulin action for any given amount of insulin bound. The results in Fig. 7A illustrate this concept. For each amount of insulin bound, the absolute rates of glucose disposal are less in these patients, and the difference between the curves represents the magnitude of the postreceptor defect. When the amount of insulin bound is plotted as a function of the percent of maximal insulin effect, the contribution of differences in postreceptor effector systems is eliminated, and with this analysis (Fig. 7B), the effectiveness of any given number of insulin-receptor complexes is the same in all subjects. Thus, even in group II obese subjects, a given amount of bound insulin stimulates the appropriate proportion of the total effector system, despite the fact that the effector system itself is impaired. This further demonstrates that the insulin-receptor complexes are fully functional in obese patients and that the decreased absolute rates of glucose disposal (Fig. 7A) are a result of an abnormality distal to the insulin binding and coupling steps. In a sense, then, the overall biologic effect (glucose disposal) is dissociated from the insulin receptor. In the group I subjects, the biologic effect of bound insulin is normal when the data are plotted in either absolute or percentage terms. This indicates that the insulin receptors are fully functional, that the steps distal to the insulin binding step are intact, and that no uncoupling exists with the overall biologic effect.

It is well recognized that maximal insulin effects are achieved when only a portion of the available receptors are occupied, and that cells possess spare receptors for insulin action (16–18). However, the proportion of receptors necessary for maximal action differs among cell types and is dependent upon which insulin function is measured. The current results provide an estimate of the proportion of spare receptors for overall in vivo insulin action. If one assumes that insulin binding to one target tissue (adipocytes) reflects the status of insulin receptors on other target tissues, one can then calculate that in normal subjects the half-maximally effective insulin level for glucose disposal (130 μU/ml) leads to 11% receptor occupancy. Although the half-maximal insulin level can be accurately assessed, the insulin concentration that produces a maximal effect is more difficult to quantitate, but maximal insulin action is clearly achieved at an insulin level of 1,000 μU/ml, which corresponds to a fractional receptor occupancy of ~40%. However, it is likely that maximal insulin stimulation of glucose disposal occurs at insulin concentrations <1,000 μU/ml because in vitro studies (16–18, 23) show that maximal insulin effects are achieved at insulin concentrations that are three to five times those that elicit a half-maximal response. Furthermore, fractional receptor occupancy at the maximally effective insulin level is ~2.5 times the fractional occupancy at the half-maximal insulin concentration (16–18, 23). Combining these observations with the data generated in the present study, one would predict that maximal insulin stimulation of glucose disposal should be achieved at an insulin concentration between 400 and 700 μU/ml. Thus, a maximal response would be achieved when 20–30% of the receptors are occupied.

An underlying premise in these studies is that target tissues possess spare receptors for insulin action and that a reduction in the number of cellular insulin receptors will lead to a decrease in insulin sensitivity as manifested by a rightward shift in the dose-response curve with no change in the maximal response to insulin. However, to generate a normal maximal response, the degree of receptor loss must not exceed the number of spare receptors. If the decrease in insulin receptors is great enough so that there are not enough receptors left to generate a maximal biologic effect, a decrease in insulin responsiveness would be observed. For a variety of reasons, we believe that our obese patients had more than enough receptors to generate a maximal response, and that the decrease in maximal insulin effect was therefore the result of a postreceptor abnormality. First, as stated above, the maximal insulin effect occurs at an insulin concentration of 1,000 μU/ml, which corresponds to a fractional receptor occupancy of ~40%, but, as discussed previously, this is most likely an overestimate, and the maximal response most likely occurs at a fractional receptor occupancy between 20 and 30%. If one examines the individual binding curves, none of the patients demonstrated more than a 60% reduction in insulin binding over the insulin concentration range used in the in vivo studies. Second, if receptor loss exceeds the number of spare receptors, the insulin-glucose disposal dose-response curve will be a continuous function of the insulin concentration. However, as can be seen in Fig. 2, plateau responses occurred in all of the obese patients studied at the higher insulin concentrations. Clearly, this is inconsistent with a pure receptor defect. Finally, if one assumes a system where 11% receptor occupancy leads to a half-maximal response and 40% occupancy leads to a maximal response, then a full maximal response can still occur provided the reduction in receptors does not exceed 60%. If the reduction in receptors is 70%, then decreased maximal responsiveness will occur, but this reduction in maximal response will be minimal. For

example, if one needs to occupy 40% (about 100,000 receptors) of the normal number of receptors to generate a maximal response and only 75,000 receptors are present, then there are still enough receptors to generate 90–95% of the maximal response. Stated another way, if one doubles the fractional receptor occupancy from 11 to 22%, this will raise the biologic response from 50 to 90% of maximal, based on in vitro data (16–18). The average decrease in maximal glucose uptake observed in the group II obese patients was ~50%. To have a 50% reduction in maximal insulin action as the result of a pure loss of receptors, one would need to lose 89% of the total receptor complement (because 11% occupancy leads to a half-maximal response. Because no patient displayed a degree of receptor loss that approached this value, we believe that all the patients had the necessary complement of receptors to generate a maximal biologic response in terms of glucose uptake and that a postreceptor defect in insulin action is the best explanation for the decreased maximal insulin responsiveness observed in the group II patients.

Hepatic glucose output was measured during all studies, and several conclusions can be made from the results obtained. First, basal hepatic glucose output is comparable for all groups when expressed as milligram per meter squared per minute, i.e., 66±5, 63±4, and 67±5 mg/M²/min for control, group I and II obese, respectively, but was significantly higher for the normal subjects when expressed on a per kilogram basis, i.e., 2.0±0.2 vs. 1.4±0.1 and 1.3±0.1 mg/kg/min for the group I and II obese. Second, hepatic glucose production can be totally suppressed in all subjects, demonstrating that, in contrast to peripheral glucose disposal, livers of group II obese subjects do not exhibit a postreceptor defect. Third, the dose-response curves for hepatic glucose output are shifted to the right in the obese groups and the half-maximally effective insulin levels were 33, 75, and 130 μU/ml in normal, group I, and group II obese subjects, respectively. This parallels the results for peripheral glucose disposal and reflects the magnitude of the decrease in insulin receptors in the two obese groups. Finally, it is apparent that lower insulin levels are required to suppress hepatic glucose output than are required to stimulate glucose disposal. During the glucose clamp studies, portal and peripheral insulin levels are the same, and the half maximal insulin levels are ~3.5–4.0-fold greater for glucose disposal. This finding, plus the fact that under physiologic conditions portal insulin levels are two- to threefold greater than peripheral levels (45), demonstrates the sensitivity of hepatic glucose production to insulin and indicates that this process should be suppressed under physiologic conditions where stimulated insulin levels exist.

In conclusion, we have shown that the mechanisms underlying the insulin resistance of human obesity are complex and may differ from subject to subject. Decreased insulin receptors play a role in the insulin resistance of all the patients studied by reducing the sensitivity to any prevailing submaximal insulin level. In some patients, this appeared to be the only defect and these patients displayed the least degree of hyperinsulinemia and insulin resistance. In contrast, the more hyperinsulinemic-insulin resistant patients demonstrated a combined defect with decreased insulin sensitivity as a result of decreased insulin receptors and decreased insulin responsiveness as a result of a postreceptor defect.

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