Mechanism of the Postreceptor Defect in Insulin Action in Human Obesity

DECREASE IN GLUCOSE TRANSPORT SYSTEM ACTIVITY

THEODORE P. CIARALDI, ORVILLE G. KOLTERMAN, and JERROLD M. OLEFSKY,
Department of Medicine, University of Colorado Health Sciences Center, Division of Endocrinology/Metabolism, Denver, Colorado 80262

ABSTRACT We have studied insulin-stimulated 3-*O*-methyl glucose transport by isolated adipocytes prepared from 10 normal and 11 obese individuals. The results demonstrated that the insulin-glucose transport dose-response curves were shifted to the right in cells from the obese patients, and that the magnitude of this rightward shift was significantly correlated to the reduction in adipocyte insulin receptors in individual subjects ($r = -0.48, P < 0.01$). In three obese patients a rightward shift in the dose-response curve could be demonstrated and there was no decrease in maximal insulin effect. This corresponded to in vivo glucose clamp results showing only a rightward shift in the insulin dose-response curve for overall glucose disposal in these three subjects (1980. *J. Clin. Invest.* **65:** 1272–1284). In the remaining eight obese patients, the in vitro glucose transport studies showed not only a rightward shift in the dose-response curves but also a marked decrease in basal and maximally insulin-stimulated rates of transport, indicating a postreceptor defect in insulin action. Again, this was consistent with the in vivo glucose clamp studies demonstrating a marked postreceptor defect in these individuals. In conclusion, these results indicate that the mechanism of the postreceptor defect in insulin action, which exists in many obese patients, is related to a decrease in the activity of the glucose transport effector system.

INTRODUCTION

Insulin resistance is a well known characteristic feature of human obesity (1, 2), but the mechanisms underlying this insulin-resistant state are not well understood. Recently, we have used the multiple euglycemic glucose clamp technique to demonstrate that insulin resistance in obese man exists in the liver and in the periphery (3). Hepatic insulin resistance was found to be due to a pure decrease in insulin receptors (3). On the other hand, the defect in insulin's action to promote glucose disposal by peripheral tissues was heterogeneous. In those obese patients with mild insulin resistance and hyperinsulinemia, decreased insulin binding entirely accounted for the reduction in glucose disposal; as the hyperinsulinemia became more severe a postreceptor defect[1] in insulin action emerged, and in the most severely hyperinsulinemic patients the postreceptor defect was the predominant cause of the peripheral insulin resistance (3). Thus, a continuum of receptor and postreceptor defects exist in human obesity, but the cellular biochemical nature of the postreceptor abnormality has not been elucidated. Recently, we have reported studies of insulin-stimulated hexose transport in isolated human adipocytes and have described certain basic features of the normal system (4). We have now carried out similar studies in adipocytes from obese subjects; the results indicate that the previously demonstrated in vivo postreceptor defect can be localized to a decrease in glucose transport activity of peripheral tissues.

METHODS

Materials. Porcine monocomponent insulin was the kind gift of Dr. Ronald Chance of Eli Lilly & Company (Indianapolis, Ind.). 3-*O*-Methyl-[1-^{14}C]glucose, L-[^{14}C]glucose, and Na ^{125}I were purchased from New England Nuclear (Boston,

Address reprint requests to Dr. Olefsky.

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[1] In this context the term "postreceptor defect" refers to any abnormality in the insulin action sequence following the initial binding step. This could theoretically include an abnormality of the insulin receptor that does not affect insulin binding, but does affect insulin action.

Mass.), collagenase from Worthington Biochemical Corp. (Freehold, N. J.), bovine serum albumin (Fraction V) from Armour Pharmaceutical Co. (Phoenix, Ariz.), phloretin from Biochemical Laboratories Company (Redondo Beach, Calif.), and silicone oil from Union Carbide (New York).

Subjects. The study group consisted of 11 obese and 10 nonobese subjects whose clinical and metabolic features are outlined in Table I. All subjects had fasting glucose levels < 120 mg/100 ml and no subject had a disease (other than obesity) or was ingesting any agent known to affect carbohydrate or insulin metabolism. The relative weights of the obese patients ranged from 1.26 to 1.80 with a mean of 1.58, whereas the relative weights of the normal subjects ranged from 0.85 to 1.13 with a mean of 0.91. The mean (±SE) age for the obese group was 36±4 yr and 39±3 yr for the control group. After obtaining informed consent all patients were admitted to the University of Colorado Clinical Research Center but remained active to approximate their prehospitalization exercise level. 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, by calories. All subjects consumed the diet for at least 4 d before study.

Preparation of isolated human adipocytes. Adipose tissue was obtained from the lower abdominal region as described (5). 1% xylocaine (lidocaine) was infiltrated in a square-field fashion and the biopsy obtained from the center. Tissue was obtained following a 14-h overnight fast. Isolated adipocytes were prepared by the method of Rodbell (6). Tissue was minced into polypropylene vials containing Krebs-Ringer bicarbonate buffer with collagenase (3 mg/ml) and albumin (40 mg/ml). The tissue was incubated for 70 min in a 37°C shaking water bath, and the cell suspension was filtered through a 250-μm nylon mesh. The cells were washed four times by centrifugation at 400 rpm for 3 min in a Krebs-Ringer bicarbonate buffer containing 118 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25.5 mM $NaHCO_3$, and 5% bovine serum albumin, pH 7.4. Adipocyte counts were performed according to a modification of method III of Hirsch and Gallian (7). All data were normalized to a cell number of 2×10^5 cells.

3-O-Methyl glucose transport. Isolated fat cells in a volume of 400 μl (~1×10^6 cells/ml) were preincubated for 60 min at 37°C in the absence or presence of the indicated concentrations of insulin. 3-*O*-Methyl glucose transport was assayed as described (4). The substrate was placed in a 20-μl vol in 17 × 100 mm polypropylene test tubes. The reaction was started by the rapid addition of 50 μl of the concentrated cell suspension (50–70,000 cells). Transport was terminated at 10 s by the addition of 11 ml of chilled Krebs-Ringer bicarbonate buffer containing 0.3 mM phloretin. Approximately 2 ml of silicone oil was layered over the phloretin mixture and the tubes were rapidly centrifuged (2,000 *g* for 15 s) in a Heraeus labofuge. The cells form a layer on the surface of the oil and can be collected by sweeping the oil with adsorptive material (such as pieces of cotton pipe cleaner). The pipe cleaners with collected cells were then added to a liquid scintillation cocktail and the radioactivity determined. The amount of cell-associated radioactivity due to diffusion and extracellular trapping of 3-*O*-methyl-[1-^{14}C]glucose was corrected for by performing parallel reactions where L-[^{14}C]glucose uptake was measured; the value for L-glucose uptake was subtracted from the total uptake of 3-*O*-methyl glucose to give the amount of specific transport. Transport studies were carried out for a reaction time of 10 s at a substrate concentration of 15.8 μM (0.4 μCi). These conditions insure measurement of initial rates of transport (4) since control studies demonstrated that the time-course of uptake was comparable in normal cells and cells from obese subjects.

Measurement of insulin binding. ^{125}I-insulin was prepared by the chloramine T procedure as modified by Freychet et al. (8). For binding studies isolated fat cells were suspended in a buffer containing 35 mM Tris, 120 mM NaCl, 1.2 mM $MgSO_4$, 2.5 mM KCl, 1.2 mM $CaCl_2$, 10 mM glucose, and 1% BSA, pH 7.6, and incubated with ^{125}I-insulin (0.2 ng/ml) and varying concentrations of unlabeled insulin in plastic vials for 90 min in a 24°C shaking water bath. Full details of both the measurement and calculation of the amount of insulin bound to fat cells have been published (5, 9).

It should be noted that in vitro conditions for measurement of glucose transport and insulin binding are not identical with respect to buffer and temperature conditions. However, these differences are necessary in order to study both systems under the most ideal and reproducible assay conditions.

Euglycemic glucose clamp studies were performed as described (3).

RESULTS

We have measured basal- and insulin-stimulated rates of 3-*O*-methyl glucose transport in adipocytes prepared from 11 obese subjects. Each of these patients has previously been studied using the multiple euglycemic clamp approach (3). In three of these subjects no postreceptor defect could be demonstrated with the in vivo approach, whereas a significant postreceptor defect was demonstrated in the remaining eight (3). For purposes of data presentation we have termed the three patients with no postreceptor defect as group I and the remaining eight patients as group II. The in vitro insulin dose-response curves for adipocyte 3-*O*-methyl glucose transport in normals, group I obese and group II obese are shown in Fig. 1. In group I, decreased glucose transport is seen at submaximal insulin concentrations, whereas maximal rates of glucose transport are normal. Thus, insulin sensitivity is decreased and insulin responsiveness is normal (10, 11), consistent with pure decrease in insulin receptors and no postreceptor defect (10, 11). In the group II obese patients, not only are the dose-response curves shifted to the right, but there is also a marked decrease in both basal- and maximal insulin-stimulated rates of glucose transport. Because of the large reduction in basal glucose transport values, the insulin-induced increase in glucose transport as a percent of basal is slightly, but not significantly, greater than in control subjects. Thus, both decreased insulin sensitivity and decreased insulin responsiveness are demonstrated in the group II obese patients, consistent with both a receptor and postreceptor defect. In this context, a decrease in insulin sensitivity is defined as a decrease in insulin effect at submaximal insulin levels with no decrease in the maximal absolute effect of the hormone. Decreased insulin responsiveness refers to a

TABLE I
*Clinical and Metabolic Features of the Study Group**

	n	Age	Relative weight	Fasting glucose (mg/100 ml)	Fasting insulin level (μU/ml)
Control subjects	10	39±3	0.91±0.02	88±2	8±0.7
Group I obese	3	36±8	1.48±0.12	90±3	22±5
Group II obese	8	36±6	1.62±0.24	90±5	46±8.9

* Data represent means±SE.

decrease in the maximal absolute rate of 3-*O*-methyl glucose uptake in the presence of a maximally effective insulin concentration (10, 11). These findings in Fig. 1 are comparable to what was observed during the in vivo multiple glucose clamp studies in these same patients (3).

Because the maximal rates of transport differ among the three groups, the functional form of the dose-response curves can be better appreciated by plotting the data as a percentage of the maximal effect (Fig. 2). With this analysis the rightward shift in the curves from the obese groups can be clearly seen and the one-half maximally effective insulin concentrations were 0.46±0.1, 0.95±0.25, and 0.85±0.14 ng/ml in normal subjects, group I obese, and group II obese, respectively.

From the data in Figs. 1 and 2 it is apparent that the in vitro adipocyte glucose transport data are quite consistent with the in vivo gluose disposal rates as measured by the glucose clamp technique (3). The specifics of this relationship can be better appreciated by comparing the in vivo and in vitro results in individual patients as presented in Table II. This table lists the maximal insulin-stimulated overall in vivo glucose disposal rates and maximal insulin-stimulated in vitro adipocyte 3-*O*-methyl glucose transport rates in individual subjects. As can be seen, the maximal absolute rates of in vivo glucose disposal and in vitro glucose transport are quite comparable in the normal and group I obese subjects, whereas these values are markedly reduced in the group II obese patients. The correlation between these two variables is presented in Fig. 3, and a highly significant positive relationship ($r = 0.68$, $P < 0.01$) exists between these two variables. This indicates that measurement of the adipocyte glucose transport rate provides an accurate estimate of the overall capacity of target tissues to dispose of glucose in vivo.

We (9, 12) and others (13) have shown that insulin receptors are decreased in adipocytes from hyperinsulinemic, insulin-resistant patients with adult onset obesity. Adipocyte insulin binding was also decreased in the obese patients in this current study, and the functional significance of this decrease in insulin receptors can be appreciated by comparing the magnitude of the decrease in insulin binding with the rightward shift in the glucose transport dose-response curve (as assessed by the one-half maximally effective insulin level) in individual patients. Fig. 4 demonstrates a significant inverse relationship ($r = 0.48$, $P < 0.01$) between these two variables such that the greater the

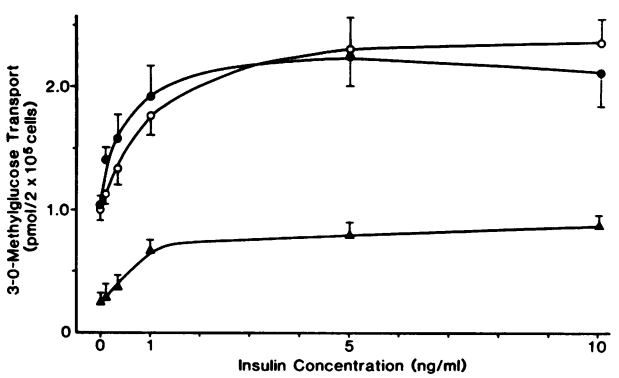

FIGURE 1 Dose-response curves for insulin's ability to simulate 3-*O*-methyl glucose transport in isolated adipocytes from normal (●), group I (○), and group II (▲) obese subjects. Isolated fat cells were incubated for 60 min at 37°C with the indicated concentrations of insulin and initial rates of 3-*O*-methyl glucose transport measured. All values are corrected for the L-glucose space.

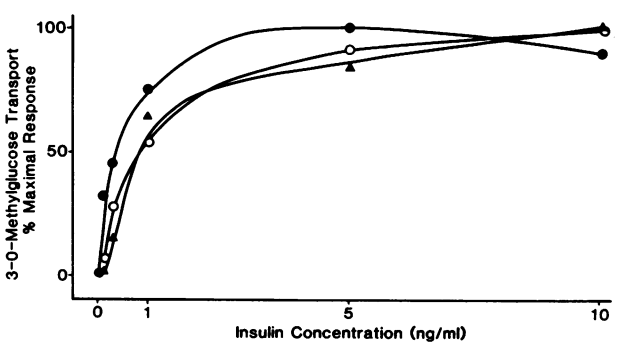

FIGURE 2 Insulin simulation of 3-*O*-methyl glucose transport in adipocytes isolated from normal (●), group I (○), and group II (▲) obese subjects. The data in Fig. 2 are expressed as a percentage of the maximal insulin effect.

TABLE II
Comparison of In Vivo and In Vitro Data

	Maximal in vivo glucose disposal rate	Maximal in vitro glucose transport	½ Maximally effective insulin concentration
	mg/M²/min	*pmol/2 × 10⁵ cells/10 s*	*ng/ml*
Normals (Mean±SEM)	395±35	2.45±0.30	0.46±0.10
Group I obese			
1	364	3.34	1.43
2	458	1.59	0.82
3	351	2.62	0.61
Mean (±SEM)	386±28	2.52±0.79	0.95±0.25
Group II obese			
4	184	0.99	1.32
5	219	1.04	0.82
6	158	0.89	0.83
7	132	0.71	0.47
8	189	1.06	1.49
9	246	0.40	0.60
10	301	1.45	0.31
11	235	1.15	0.98
Mean (±SEM)	208±19	1.15±0.17	0.85±0.14

decrease in insulin receptors, the greater the one-half maximally effective insulin level. If one compares Figs. 2 and 4, it can be seen that the decrease in insulin binding is greater in the group II obese patients as compared with group I obese patients. This is true despite the fact that the one-half maximally effective insulin concentrations (Fig. 2) are about the same in both groups. However, it should be pointed out that group I consists of only three patients and as Fig. 4 shows, one of these patients (patient 1, Table II) had a very high one-half maximally effective insulin concentration of 1.3 ng/ml, and this accounts for the discrepancy. We have no unique explanation for this discordant value in this patient.

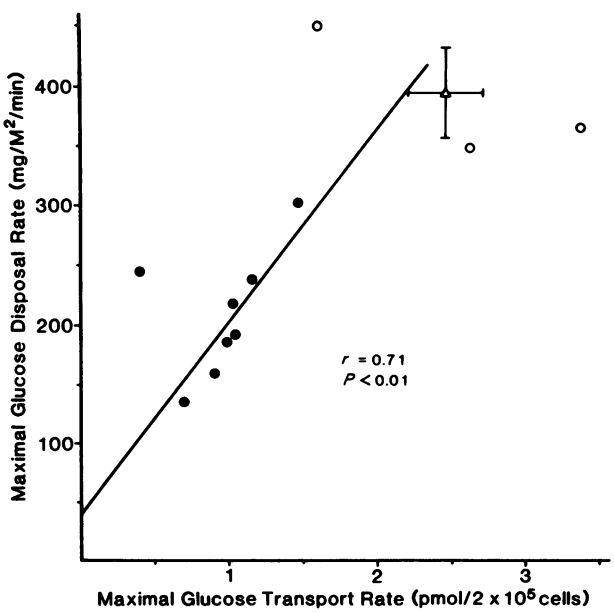

FIGURE 3 Relationship between the maximal insulin-stimulated overall in vivo glucose disposal rate (mg/M² per min), as measured by the euglycemic glucose clamp technique (3), and the maximally stimulated rate of adipocyte 3-*O*-methyl transport (pmol/2 × 10^5 cells per 10 s) in individual subjects. Individual data for group I obese subjects (○), group II obese patients (▲) are given. The open triangle gives the mean (±SE) value for normal subjects for comparative purposes, but this value has not been used in calculating the correlation coefficient between the two variables.

DISCUSSION

Insulin resistance and decreased cellular insulin receptors are important pathophysiologic features of human obesity (1, 2). However, due to the presence of spare (or excess) insulin receptors, the relationship between decreased insulin receptors and insulin resistance is not straightforward (11, 14–17). A pure decrease in insulin receptors will lead to a rightward shift in the insulin-biologic function dose-response curve with no decrease in maximal insulin action, and this is termed a decrease in insulin sensitivity (1, 3, 10). A pure postreceptor defect should lead to a reduction in insulin action at all insulin concentrations, including maximally effective ones, and this is termed a decrease in insulin responsiveness (1, 3, 10). Because the precise mechanisms of insulin's cellular actions are not well understood it is possible that certain kinds of postreceptor defects could also result in a rightward shift in the dose-response curve. A combination of receptor and postreceptor defects will lead to decreased insulin

sensitivity and responsiveness manifested by both a rightward shift in the dose-response curve and a decrease in maximal insulin action.

In previous in vivo studies we have used the multiple euglycemic glucose clamp technique to measure peripheral glucose disposal at a variety of steady-state plasma insulin levels and thus to construct the overall in vivo dose-response curves in normal and obese subjects (3). The results showed that in the mildly hyperinsulinemic insulin-resistant obese patients the dose-response curve was shifted to the right consistent with a pure decrease in insulin receptors (3). As the degree of hyperinsulinemia and insulin resistance increased, cellular insulin receptors declined further, the dose-response curves became more right shifted, and a marked postreceptor defect in insulin action emerged. In those patients with the most severe hyperinsulinemia the postreceptor defect was the predominant abnormality. These results indicated that a postreceptor defect in insulin action played a significant role in the insulin resistance of many obese patients (3).

However, the mechanisms of this postreceptor defect have not been elucidated, and in the current studies we have examined the possibility that this abnormality could be located at the level of the glucose transport system. To this end we have measured basal and insulin-stimulated glucose transport in adipocytes freshly isolated from normal and obese subjects according to previously published techniques (4). The results demonstrated that the in vitro dose-response curves were shifted to the right in obese subjects and that the degree of this shift was well correlated with the magnitude of the decrease in insulin binding. More importantly, in those patients in whom in vivo studies showed decreased insulin sensitivity with only a rightward shift in the dose-response curve (3), in vitro studies also demonstrated only a rightward shift in the glucose transport dose-response curve with normal maximal responsiveness. However, in those patients who demonstrated a postreceptor defect as well as decreased insulin sensitivity in vivo (3), similar findings were observed in vitro. Thus, in these patients the adipocyte glucose transport dose-response curves were shifted to the right (reflecting their decrease in insulin receptors), but in addition, a marked decrease in basal and maximally insulin-stimulated rates of glucose transport were observed, indicative of a postreceptor defect in insulin action localized to the glucose transport system.

These results demonstrate a strong correlation between the in vivo demonstration of a postreceptor defect in human obesity and the in vitro demonstration of reduced activity of the insulin-stimulated glucose transport system in individual obese subjects. Because adipocytes are a known peripheral target tissue for insulin, and because physiologic and pathophysiologic changes in adipocyte glucose transport are reflected by

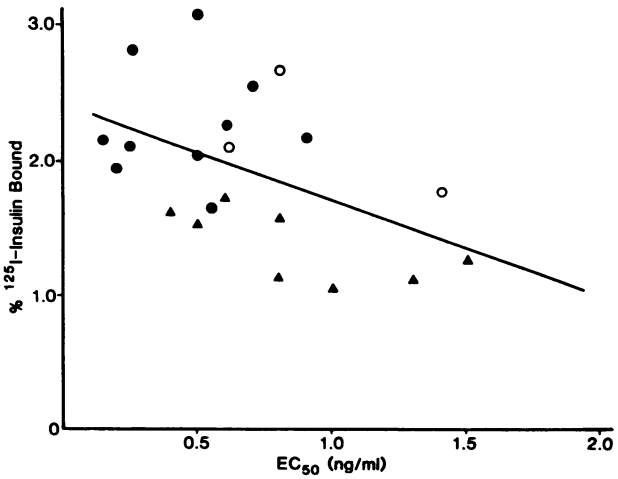

FIGURE 4 Relationship between the half-maximally effective insulin concentration (EC_{50}) and the percentage of ^{125}I-insulin bound (at 0.2 ng/ml) to isolated adipocytes from individual normal (●), group I (○), and group II obese (▲) subjects.

similar changes in the glucose transport system of skeletal muscle in animals (18), it seems reasonable to postulate that the current findings in human adipocytes are representative of peripheral insulin target tissues in general. This conclusion is supported by the strong correlation observed (Fig. 3) between maximal insulin-stimulated in vivo overall glucose disposal and in vitro adipocyte glucose transport rates. From these results we conclude that the postreceptor defect demonstrated in many obese patients is due to a defect in the activity of the plasma membrane glucose transport carrier. Whether this is due to a decrease in the number of glucose carriers, a decrease in the affinity of the carrier for glucose, or a decrease in the accessability of the carriers to the cell surface (19) cannot be determined by the present data, but these possibilities are currently being explored. In addition it should be noted that although these results indicate that the rate-limiting step of the postreceptor defect in human obesity is localized to the glucose transport system, this does not exclude further, more distal, postreceptor defects in insulin action located intracellularly. Indeed, it seems quite possible that such intracellular defects may exist that could contribute to the overall insulin resistance of human obesity under certain conditions.

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REFERENCES

1. Olefsky, J. M., and O. G. Kolterman. 1981. Mechanisms of insulin resistance in obesity and non-insulin dependent (type II) diabetes. *Am. J. Med.* **70:** 151–168.
2. Rabinowitz, D. 1970. Some endocrine and metabolic aspects of obesity. *Ann. Rev. Med.* **21:** 241–258.
3. Kolterman, O. G., J. Insel, M. Saekow, and J. M. Olefsky. 1980. Mechanisms of insulin resistance in human obesity. Evidence for receptor and postreceptor defects. *J. Clin. Invest.* **65:** 1272–1284.
4. Ciaraldi, T. P., O. G. Kolterman, J. A. Siegel, and J. M. Olefsky. 1979. Insulin-stimulated glucose transport in human adipocytes. *Am. J. Physiol.* **236:** E621–E625.
5. Olefsky, J. M., P. Jen, and G. M. Reaven. 1974. Insulin binding to isolated human adipocytes. *Diabetes.* **23:** 565–571.
6. Rodbell, M. 1964. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* **239:** 375–380.
7. Hirsch, J., and E. Gallian. 1968. Methods for the determination of adipose cell size in man and animals. *J. Lipid Res.* **9:** 110–119.
8. Freychet, P., J. Roth, and D. M. Neville, Jr. 1971. Monoiodoinsulin: demonstration of its biological activity and binding to fat cells and liver membranes. *Biochem. Biophys. Res. Commun.* **43:** 400–408.
9. Olefsky, J. M. 1976. Decreased insulin binding to adipocytes and circulating monocytes from obese subjects. *J. Clin. Invest.* **57:** 1165–1172.
10. Kahn, C. R. 1978. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. *Metab. Clin. Exp.* **27**(Suppl. A): 1893–1902.
11. Olefsky, J. M. 1981. Insulin resistance and insulin action: an in vitro and in vivo perspective. *Diabetes.* **30:** 148–162.
12. Kolterman, O. G., G. M. Reaven, and J. M. Olefsky. 1976. Relationship between in vivo insulin resistance and decreased insulin receptors in obese man. *J. Clin. Endocrinol. Metab.* **48:** 487–494.
13. Harrison, L. C., F. I. R. Martin, and R. A. Melick. 1976. Correlation between insulin receptor binding in isolated fat cells and insulin sensitivity in obese human subjects. *J. Clin. Invest.* **58:** 1435–1441.
14. Gammeltoft, S., and J. Gliemann. 1973. Binding and degradation of ^{125}I-insulin by isolated rat fat cells. *Biochem. Biophys. Acta.* **320:** 16–32.
15. Kono, T., and F. W. Barham. 1971. The relationship between the insulin-binding capacity of fat cells and the cellular response to insulin: studies with intact and trypsin-treated fat cells. *J. Biol. Chem.* **246:** 6210–6216.
16. Olefsky, J. M. 1976. The effects of spontaneous obesity on insulin binding, glucose transport, and glucose oxidation of isolated rat adiopocytes. *J. Clin. Invest.* **57:** 842–851.
17. Olfesky, J. M. 1976. The insulin receptor: its role in insulin resistance of obesity and diabetes. *Diabetes.* **25:** 1154–1165.
18. LeMarchand-Brustel, Y., B. Jeanrenaud, and P. Freychet. 1978. Insulin binding and effects in isolated soleus muscle of lean and obese mice. *Am. J. Physiol.* **234:** E348–E358.
19. Cushman, S. W., and L. J. Wardzala. 1980. Potential mechanisms of insulin action on glucose transport in the isolated rat adipose cell-apparent translocation of intracellular transport system to the plasma membrane. *J. Biol. Chem.* **255:** 4758–4762.