

# Defect in Insulin Binding to Receptors in Obese Man

## AMELIORATION WITH CALORIE RESTRICTION

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**ABSTRACT** With insulin at 0.1 ng/ml, the binding of [<sup>125</sup>I]insulin in vitro to circulating lymphocytes from 11 obese patients was less than that observed with cells from 10 thin volunteers. Furthermore, with obese cells, unlabeled insulin was less effective in competing with labeled hormone for binding, both at low and high concentrations of unlabeled insulin. These differences were not accounted for by the high concentrations of insulin in the circulation of the obese patients at the time the blood was drawn, or by differences in degradation of hormone, or in the characteristics of the cell population. The decrease in binding appears to be due to a lowering of the receptor concentration, but some loss of affinity has not been excluded. Institution of a calorie restricted diet (nine patients) which ameliorated the hyperinsulinemia, produced an improvement in hormone binding. Since the insulin receptors of lymphocytes in metabolic disorders seem to reflect the state of insulin receptors in target cells such as liver and fat, the lymphocytes or other leukocytes appear to be ideal for studies of impaired cell responsiveness to hormones in man.

### INTRODUCTION

Obesity is the most common insulin resistant state in man. It is characterized by elevated concentrations of circulating insulin, both in the basal state and after various stimuli of insulin secretion, and by resistance

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to the effects of both endogenous and exogenous insulin (3, 4).

In rodents, both genetic and acquired forms of obesity are characterized by a decrement in the concentration of insulin receptors per cell (5-17). Insulin receptors in liver (5-7, 12-14), fat (6, 9, 15), and in thymic lymphocytes (10, 11) are similarly affected, and in the lymphocytes, a decrease in receptor concentration is associated with a loss in insulin-stimulated aminoisobutyric acid transport (10, 11, 18). Total fasting for 24 h or restriction of food intake to normal levels for a period of weeks elevated insulin binding to normal or near normal levels (13, 14).

In a previous study in humans, we reported that with circulating lymphocytes taken from an obese patient, the concentration of insulin required to decrease the specific binding of [<sup>125</sup>I]insulin was higher (i.e., the slopes of the competition curves were shallower) than with lymphocytes taken from normal subjects. Now, with more studies and using a lower concentration of [<sup>125</sup>I]insulin (0.1 ng/ml rather than 1.0 ng/ml), we have defined an additional difference between the normal and the obese. More importantly, a decrease in calorie intake by the obese patient, which produced improvements in weight, blood glucose, and plasma insulin, was associated with improvements in binding of [<sup>125</sup>I]insulin to lymphocytes.

### METHODS

*Patients.* 11 obese patients and 10 thin, normal, healthy volunteers (see Table I) were studied while inpatients in the Clinical Center, National Institutes of Health. We have used "normal" and "thin" interchangeably. In addition to their differences in body weight and composition, the group of normal subjects were more homogeneous; all were white, largely younger, predominantly male (9 of 10), and presumably more physically active than the obese who were on an average a decade older, racially mixed (6 white, 5 black), predominantly female (9 of 11), and presumably less physically active, despite encouragement to the con-

TABLE I  
Comparison of Body Weight, Fasting Blood Glucose, and Fasting Plasma Insulin  
between Thin and Obese Patients

	Patients		Total range (median value)				
	Total number	Sex		Age	Body weight	Fasting blood glucose	Fasting plasma insulin
		M	F	yr	% of ideal	mg/100 ml	$\mu\text{U/ml}$
Thin	10	9	1	19-25 (21)	93-111 (100)	80-92 (84)	10-25 (12)
Obese	11	2	9	23-51 (32)	162-352 (200)	87-200 (100)	19-42 (35)

trary. The thin subjects, who were maintaining a constant weight, ate a normal full diet. The obese subjects were on an ad lib. diet of approximately 3,500 cal, unless otherwise stated. Since we found no differences among obese subjects as a function of age, race, or sex, and because of our studies in animals, we have attributed differences between the thin and obese to effects of body weight, body composition, and diet. The contribution of other related variables has not been adequately studied. All studies were done after an overnight fast and after at least 48 h of hospitalization.

9 of the 11 obese patients (7 female, 2 male; 5 white, 4 black) were studied further (Fig. 1). For 6 days they were fed a 3,500-cal diet of which approximately one-third was carbohydrate, during which time base-line studies were done for the weight reduction period that followed. On the 3rd day and on the 5th day, blood was drawn for electrolytes, glucose, insulin, and for a study of binding of [ $^{125}\text{I}$ ]-insulin to lymphocytes. On the 4th day, a standard glucose tolerance test (100 g by mouth) was performed, and on the 6th day of the base-line period an insulin tolerance test (0.1 U/kg body weight, i.v.) was performed (Fig. 1). The high cal diet was succeeded by periods of "starvation" (less than 50 cal/day) alternating with "severe calorie restriction" (600 cal/day). Allopurinol, 300 mg, was administered by mouth daily to prevent hyperuricemia and potassium chloride, 60 meq, and sodium bicarbonate, 1.8 g,

were given, to minimize electrolyte disturbances, as indicated in Fig. 1. On each occasion, when the patient had been returned to the 600-cal diet for a sufficiently long period to be free of ketonuria, blood was removed for measurements of electrolytes, glucose, insulin, and binding of [ $^{125}\text{I}$ ]-insulin to lymphocytes. Serum electrolytes remained normal throughout the study. For the final part of the study, the 6 days of high calorie-high carbohydrate diet and the measurements of electrolytes, blood glucose, plasma insulin, glucose tolerance, and insulin tolerance were repeated. A single study of hormone binding to lymphocytes was performed during this period. Thus, on each of the nine obese patients, the first two and the last of the studies of hormone binding were done while the patient was on the high calorie diet.

Serum electrolytes, blood glucose, and plasma insulin were determined as previously described (19). Ketones in urine were measured by sodium nitroprusside (Ketotest, Ames Co., Div. of Miles Lab, Inc., Elkhart, Ind.).

*Studies of insulin binding.* Whole blood was drawn into acid-citrate-dextrose and centrifuged. The red cells and plasma were returned to the patient while the buffy coat was centrifuged in a Ficoll-Hypaque gradient exactly as described by Boyum (20), except that 2 ml of water were added to the Ficoll-Hypaque (21) and the centrifugation was for 30 min. The cells in the final preparation were 90-

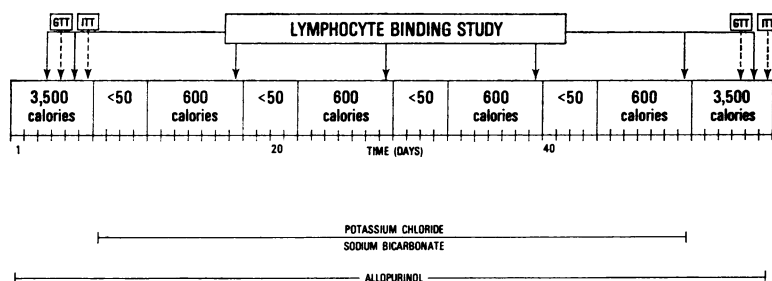


FIGURE 1 Study protocol for obese patients. Nine obese patients were studied during periods of high and low cal diets. A representative protocol is illustrated. Blood was drawn for studies of lymphocyte binding of [ $^{125}\text{I}$ ]-insulin on the days designated by the solid arrows. A 100-g oral glucose tolerance test and an i.v. 0.1 U/kg insulin tolerance test were done on the days designated by the broken arrows. All tests were performed in the morning, after an overnight fast. Allopurinol, 300 mg, sodium bicarbonate, 1.8 g, and potassium chloride, 60 meq, were administered orally each day during the periods marked by brackets. Note that the first two and also the last one of the lymphocyte studies were performed during periods of high calorie diets.

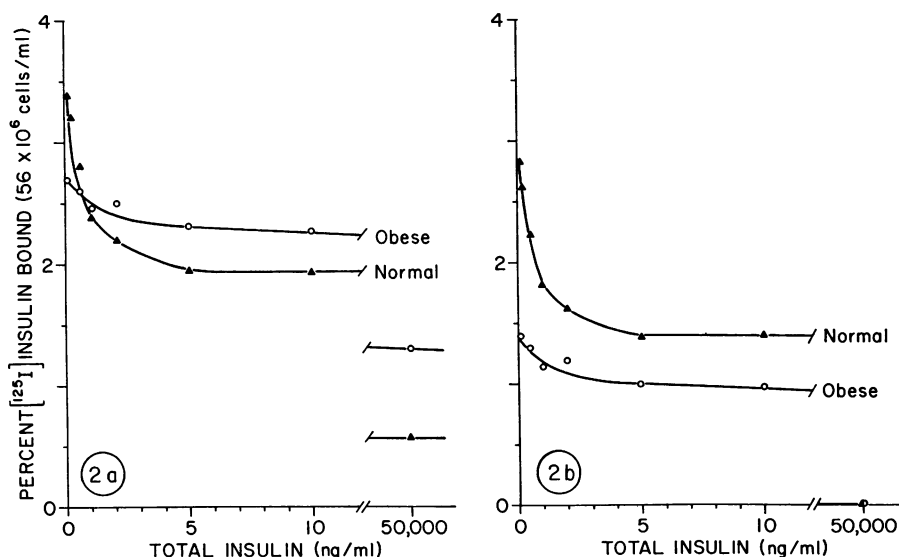


FIGURE 2 [<sup>125</sup>I]insulin binding to lymphocytes from one obese and one normal subject. One normal thin volunteer and one obese patient were studied simultaneously under the same conditions. Blood was drawn, and the lymphocytes were isolated by centrifugation in a Ficoll-Hypaque gradient. [<sup>125</sup>I]insulin (0.1 ng/ml) was incubated with lymphocytes ( $56 \times 10^6$ /ml) in the absence of unlabeled insulin and in the presence of a range of concentrations of unlabeled insulin up to 50,000 ng/ml. After 90 min at 15°C, duplicate aliquots of the incubation solutions were transferred to microfuge tubes and centrifuged. The supernates were aspirated and discarded; the cell pellet at the bottom of each tube was excised and the radioactivity counted. Each point is the mean of duplicates. In Fig. 2 a, total binding, expressed as the percentage of total radioactivity per milliliter that was bound to  $56 \times 10^6$  cells/ml, is plotted on the vertical axis as a function of the total insulin concentration. Nonspecific binding is the radioactivity bound to the cells in the presence of unlabeled insulin at 50,000 ng/ml. Specific binding is the difference between total binding and nonspecific binding. In Fig. 2 b, the same data are presented except that specific binding, rather than total binding, is plotted on the vertical axis. Although not shown in these graphs, points at insulin concentrations of 30, 100, and 1,000 ng/ml were also included in all experiments.

95% mononuclear leukocytes; the remainder were a mixture of erythrocytes, platelets, and other leukocytes.

Specially prepared [<sup>125</sup>I]insulin (22–24), 0.1 ng/ml, was incubated at 15°C with the lymphocytes,  $56 \times 10^6$  cells/ml, in the absence and presence of unlabeled insulin over a range of concentrations up to 50,000 ng/ml.<sup>1</sup> After 90 min, duplicate 0.2 ml aliquots of the incubation mixture were transferred to microfuge tubes and the cells were sedimented. The supernate was discarded, and the tip of the tube that contained the cell pellet was excised and the radioactivity counted.

*Technical comments.* Under these conditions, with  $56 \times 10^6$  cells/ml from normal subjects, about 3.5% of the [<sup>125</sup>I]-insulin radioactivity was bound, of which 85% was displaced in the presence of an excess of unlabeled insulin. Binding of insulin is proportional to cell concentration over a wide range (25); we chose the above concentration of cells so that a complete binding curve with duplicate aliquots of

cells could be performed with a single plasmapheresis. It should be noted that at this cell concentration, reproducibility and accuracy are reasonable and trapping of supernate in the cell pellet as well as degradation of labeled hormone are minimal, as are pH changes during the incubation.

## RESULTS

When complete competition curves (Fig. 2) for each of 11 obese patients and 10 thin subjects were obtained, "total binding", at very low insulin concentrations (0.1 ng/ml which is 10-fold less than in our previous study (21)), was significantly lower in the obese patients than in the thin subjects ( $P < 0.01$ ) (Fig. 3). Since nonspecific binding was generally increased with cells from the obese (21), specific binding showed an even greater difference ( $P < 0.005$ ) (Fig. 3).

At each insulin concentration within the physiologic range, the cells from the obese bound less insulin per cell. Stated in another way, obese cells need more insulin in the medium to bind as many molecules of insulin (Table II). Thus it can be seen in Table II that

<sup>1</sup>In our previous publication we stated that the final concentration of cells in the incubation mixture was  $70 \times 10^6$  cells/ml (21). Actually, the cell concentrations in the previous experiments were exactly as shown in this manuscript and should be corrected by multiplying the cell concentration stated in reference 21 by 0.8.

cells from obese patients require about twice the insulin concentration in the medium for equivalent binding to cells from thin subjects.

Degradation of insulin in this system is slight and does not appear to differ with cells from normal and obese patients (21); this is similar to results in thin and obese rodents with hepatocytes as well as isolated plasma membranes from liver and fat (6, 8). In a previous study, we showed that insulin added to the buffy coat, before isolation of the lymphocytes on the gradient, had no effect on the binding studies (21). To demonstrate further that the insulin in the medium bathing the cells at the time they were removed was without significant effect on the binding of [<sup>125</sup>I]-insulin, cells were obtained from normal subjects before and after elevation of the circulating insulin concentration. Cells obtained 90–120 min after oral glucose gave results indistinguishable from cells drawn after an overnight fast (Table III). Similar results were obtained in rats and mice, during studies of hepatocytes (6) and thymocytes, respectively (11). To establish that the differences in binding we obtained in obese subjects were not due to a difference in the population of lymphocytes in these subjects, the relative proportions of rosette-forming T cells and immunoglobulin-binding B cells were measured (26); the proportions varied widely in each group and did not appear to differ between the obese and the thin (Table IV).

*Effect of calorie restriction of obese patients on insulin binding to lymphocytes.* Since in obese patients calorie restriction even with only modest weight reduction is often associated with some amelioration of insulin resistance, glucose intolerance, and hyperinsulinemia, we examined insulin binding to lymphocytes from nine obese subjects during a period of high caloric intake and at intervals during 2 mo of calorie restriction (Fig. 1). All of the patients lost weight (mean

= 11.0%) and although the weight loss was modest in these short-term studies, most patients had a fall in the basal insulin and glucose concentration (Fig. 4). Six of the nine obese patients had improvement in glucose tolerance and five of the nine patients had some improvement in sensitivity to i.v. insulin after the period of calorie restriction. There was no con-

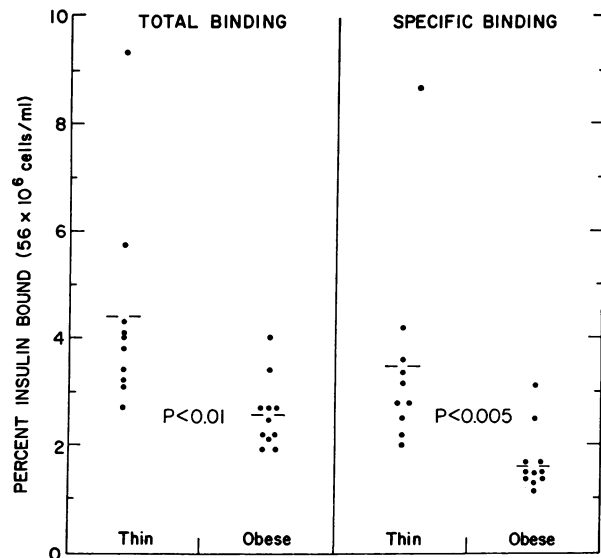


FIGURE 3 Insulin binding to lymphocytes from normal and obese subjects. All subjects had ingested a full carbohydrate-rich diet for 2 days, after which they were fasted overnight, and in the morning blood was drawn for studies of insulin binding. The freshly isolated lymphocytes were incubated with [<sup>125</sup>I]insulin (0.1 ng/ml) in the absence and presence of unlabeled insulin, and the binding of [<sup>125</sup>I]-insulin was measured as described in the legend to Fig. 2. Each point in this graph was derived from a complete binding curve such as that shown in Fig. 2. Total binding and specific binding are defined in the legend to Fig. 2.

TABLE II  
Insulin Binding to Lymphocytes as a Function of Insulin Concentration in Media\*

Patients	Insulin concentrations in media (ng/ml)					
	0.1	0.2	0.5	1.0	5.0	10.0
	<i>Insulin binding at pg/56 × 10<sup>6</sup> cells/ml</i>					
Thin	3.5 ± 0.6 <i>n</i> = 10	6.1 ± 1 <i>n</i> = 10	14.9 ± 4 <i>n</i> = 8	26.9 ± 5 <i>n</i> = 10	108.6 ± 20 <i>n</i> = 9	148.0 ± 13 <i>n</i> = 10
Obese	1.7 ± 0.1 <i>n</i> = 11	2.8 ± 0.1 <i>n</i> = 10	7.1 ± 0.7 <i>n</i> = 7	13.5 ± 1 <i>n</i> = 11	58.0 ± 7 <i>n</i> = 10	116.0 ± 13 <i>n</i> = 11
<i>P</i>	<0.01	<0.02	<0.10	<0.02	<0.05	<0.10

Values are means ± SEM; *n*, number of subjects studied at each concentration.

\* These data represent the product of the specific binding of the labeled hormone and the total insulin concentration in the medium.

TABLE III  
*Effect of Acute Elevation of Endogenous Plasma Insulin in Thin Volunteers on [125I]Insulin Binding to Lymphocytes In vitro*

Volunteer	Patients		[125I]Insulin bound to lymphocytes		Plasma insulin			Blood glucose		
	Age	Sex	After overnight fast	After oral glucose	Hours after oral glucose			0	1	2
					0	1	2			
			% of total 125I radioactivity bound per 56 × 10 <sup>6</sup> cells		μU/ml			mg/100 ml		
D. L.	21	M	3.5	3.5	15	65	70	90	128	118
J. H.	20	M	3.3	3.6	12	—	22	77	120	75
J. M.	20	M	3.3	3.4	8	28	25	68	116	100

Blood was drawn from thin volunteers for studies of [125I]insulin binding to lymphocytes after an overnight fast and again 1½ (J. M.) or 2 h (D. L. and J. H.) after oral glucose (100 g). Complete curves of binding, similar to those in Fig. 2, were obtained. The studies before and after oral glucose were indistinguishable. Shown here is only the binding with [125I]insulin at 0.1 ng/ml.

sistent change, however, in the endogenous insulin response after the glucose load (Fig. 5). It should be noted that both the precalorie restriction test as well as the postcalorie restriction test were carried out while the patient was consuming a 3,500-cal diet (Fig. 1).

With each subject, total binding as well as specific binding of [125I]insulin to the cells was usually greater during the period of calorie restriction than in the studies carried out before the period of calorie restriction (Fig. 4). When the binding curves were analyzed further the initial portion of the curve (encompassing insulin concentrations of 0.1, 0.2, 0.5, and 1 ng/ml)

was significantly higher for the postcalorie restriction studies than for the precalorie restriction studies in six of the nine patients (Fig. 6). The remainder of the binding curve encompassing insulin concentrations beyond 1 ng/ml showed no significant differences. The three patients in whom the binding curves were not statistically improved by calorie restriction (patients G. S., L. P., and S. D.) were not distinguishable in their metabolic improvements from the other six, although two of the three lost the least amount of weight (Fig. 4, 5). Our failure to demonstrate statistically significant changes in these patients may result from

TABLE IV  
*Characterization of Lymphocytes in Thin and Obese Subjects*

	Patients			Percentage of total lymphocytes		[125I]Insulin bound to lymphocytes			Concentration of insulin that reduces specific binding by 50%
	Subject	Age	Sex	B	T	Total binding	Specific binding	Nonspecific binding	
									% of total 125I radioactivity bound per 56 × 10 <sup>6</sup> cells
Thin	R. D.	19	M	13	10	3.2	2.6	0.6	11
	J. M.	20	M	29	0	4.1	3.5	0.6	10
	J. K.	20	M	31	41	4.0	2.9	1.1	7
	B. A.	31	M	31	26	—	—	—	—
Obese	R. A.	32	M	31	17	2.4	1.40	1.0	30
	L. D.	45	F	41	42	3.4	2.7	0.7	15

Blood was drawn for studies of [125I]insulin binding to lymphocytes (cf. Fig. 2). In addition an aliquot of the lymphocytes that were used in the binding study were reacted with sheep red cells to detect rosette formation (T) and with fluorescent antibody to detect surface immunoglobulins (B) as in reference 26 except that in our study lymphocytes surrounded by at least four sheep red blood cells were required for designation as a T cell. This probably accounts for the relatively low percentage of T cells in both groups.

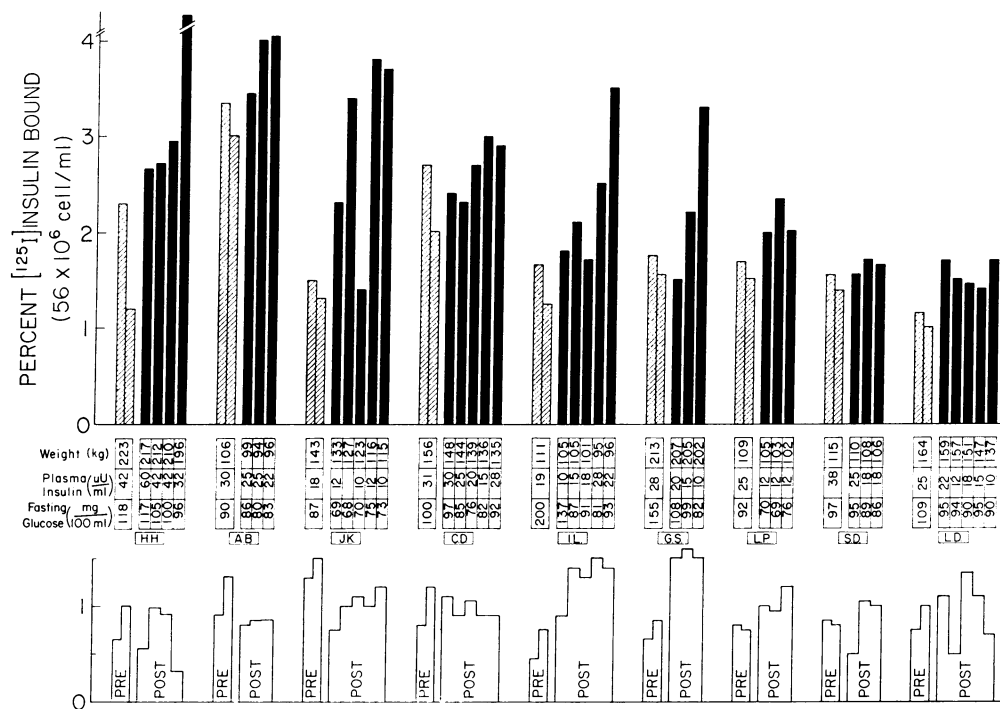


FIGURE 4 Effect of calorie restriction on insulin binding to lymphocytes from obese subjects. Nine obese subjects were studied at multiple intervals during a 5-8-wk period of dietary manipulations (see Fig. 1). On each occasion, after an overnight fast, blood was drawn for measurements of plasma insulin, blood glucose, and for a full standard curve for the binding of [<sup>125</sup>I]insulin to isolated lymphocytes such as that shown in Fig. 2. For each test the specific binding of [<sup>125</sup>I]insulin at 0.1 ng/ml (in the absence of unlabeled insulin) is plotted as a bar graph at the top and nonspecific binding as a bar graph at the bottom; body weight plasma insulin and blood glucose are in the chart in the middle. The first two tests on each patient (hatched bars) were performed during the initial period of high carbohydrate, high calorie feeding; the weights, insulins, and glucoses for this set are mean values, and these two binding studies are not necessarily in chronological order. The subsequent tests (solid bars) were performed in chronological order during the period of weight reduction. The last test on each patient was again during a period of high carbohydrate, high calorie feeding (see Fig. 1) which accounts for the occasional reversal in the trend of the plasma insulin, blood glucose, and [<sup>125</sup>I]insulin binding. The intervening tests were done during the period that the patient was ingesting 600 cal/day (see Fig. 1). All patients had normal serum electrolytes and were free of ketonuria for at least 2 days before any test.

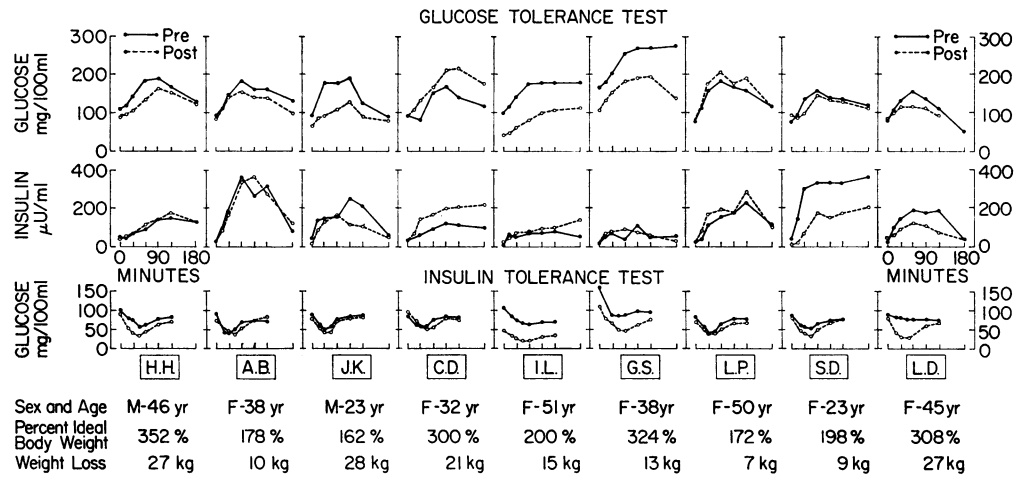
the fact that these patients were studied on only three occasions during postcalorie restriction, whereas all but one of the other patients were studied on more than three occasions (Fig. 4).

### DISCUSSION

This study shows that circulating lymphocytes from obese subjects bind less insulin than do cells from thin subjects. In the present study, [<sup>125</sup>I]insulin was at 0.1 ng/ml; in our previous studies (21), we reported data with [<sup>125</sup>I]insulin at 1.0 ng/ml. It can be seen in the present study (Fig. 2) that with cells from the normal sample, a reduction in insulin concentration from 1.0 ng/ml to 0.1 ng/ml produced a marked increase in binding of [<sup>125</sup>I]insulin, and which in turn caused a

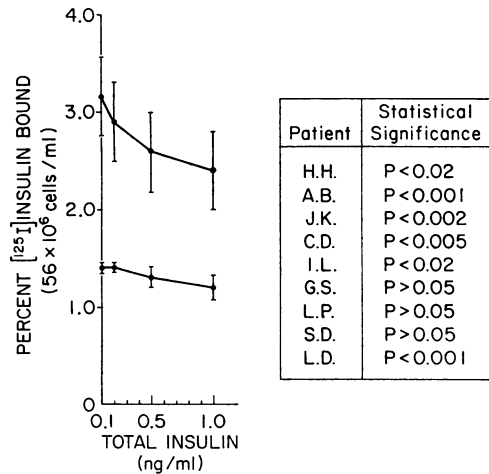
substantial increase in both total binding and specific binding at the initial points on the curves. With cells from the obese, on the other hand, the reduction in tracer [<sup>125</sup>I]insulin from 1.0 to 0.1 ng/ml produced only a slight increase or no increase in binding of the labeled hormone (Fig. 2). The net effect is that the differences between normal and obese are more marked here than in the previous study (cf. Fig. 3 in this paper with Fig. 3 in reference 21).

The differences observed here between cells of obese and thin subjects seem not to be due to other differences between the groups, nor to any systematic trivial differences in characteristics of the cells studied. A large number of studies with several cell types from rodents have also shown clearly the decrease in insulin



**FIGURE 5** Glucose tolerance and insulin tolerance in obese subjects before and after weight loss. Nine obese subjects were subjected to several weeks of calorie restriction; they were fed a high calorie, high carbohydrate diet for 6 days before and for 6 days after this period of calorie restriction. Standard glucose tolerance tests (100 g, by mouth) and insulin tolerance tests (0.1 U/kg i.v.) were performed on the 4th day and 6th day, respectively, of the pre- (●---●) and post- (○---○) diet period (see Fig. 1).

binding to cells from obese animals (5-17). We are also convinced that this difference is not due to the high insulin levels bathing the cells at the time of



**FIGURE 6** Representative example of the initial portion of the binding curve as described in Fig. 4. The lower points represent the mean  $\pm$  SEM of the two precalorie restriction studies and the upper points represent the mean  $\pm$  SEM from the three to five studies performed after the institution of calorie restriction (see Fig. 4). The statistical significance of the differences in the precalorie restriction and postcalorie restriction studies for each patient (shown at the right) was computed by the Student's *t* test. The mean for all points encompassing insulin concentrations between 0.1 ng/ml through 1.0 ng/ml for the precalorie restriction studies was compared to the mean of the same points from the calorie restriction period. Note that these differences are similar to the differences shown over the same insulin concentration between normal and obese subjects (Fig. 2).

their removal, i.e., that the insulin is not causing a reduction in binding by occupying sites. (A possible regulatory effect of hyperinsulinemia on the receptor concentration has been considered elsewhere (27).) Several lines of evidence against a role for occupancy are as follows: (a) cells from acromegalics, whose plasma insulin, and blood glucose concentrations are indistinguishable from those of the obese, bind [ $^{125}$ I]insulin as they do in thin subjects (21); (b) acute elevation (up to 1-2 h) of the ambient insulin concentration in vitro or in vivo with these cells, or in vitro with cultured lymphocytes which are very similar to the circulating cells, produces little or no effect on [ $^{125}$ I]insulin binding (28); (c) numerous studies with rodents confirm this lack of effect of acute hyperinsulinemia on [ $^{125}$ I]insulin binding (6, 11, 13).

The insulin receptors on lymphocytes are extraordinarily similar to those found in liver, fat, and other tissues (25, 28). In mice, the insulin receptors on thymic lymphocytes (10-11) from obese animals had the same defect as that observed with the liver (5, 6) and fat cells (8, 9) from these animals, which suggested that irrespective of whether the lymphocyte receptor for insulin has biological importance, the lymphocyte is an accurate mirror of events in liver and fat. Interestingly, lymphocytes do respond to insulin and with respect to aminoisobutyric acid transport, at least, there is a reasonable quantitative correlation between hormone binding and aminoisobutyric acid transport (18). Furthermore, lymphocytes from obese mice are resistant to this stimulatory effect of insulin (10, 11).

In obese animals, total fasting for 1 day produces an increase in hormone binding. In contrast to the effect of total fasting, diet restriction in the obese mice produces modest improvement only after weeks of dieting (14). These changes are similar to the modest effects of dieting on insulin binding that we observed in the present study. In the animal studies, chronic hyperinsulinemia seems to be an important feature of the deficit in insulin binding. For example, obese animals who are fasted and given depot insulin fail to restore their receptors (in liver) while those given insulin acutely, before sacrifice, have restored receptors (13, 14).

In previous studies of insulin as well as studies of many other hormones, we have found that binding data under steady-state conditions was consistent with two orders of binding sites that differed in affinity and numbers (16, 25, 29). For such an interpretation to be precisely correct, several conditions need to have been met, including the assumption that the binding sites act independently of one another. De Meyts has recently presented direct experimental evidence, in both intact cultured lymphocytes and with purified liver plasma membranes, that the insulin receptor sites do interact with one another, and this interaction conforms to the type described under the heading of "negative cooperativity" (29). He finds that the affinity of the receptors for insulin can be affected by a variety of alterations in the milieu, including the state of occupancy of receptors. Previously we would have interpreted our present data to indicate that the cells from the obese had a marked decrease in the concentration of high affinity receptors. Also, if one had assumed that the "nonspecific" binding, as we have defined it, was truly nonspecific, then low affinity receptors were also reduced in number (if, however, one had rejected the notion of nonspecific binding, one would have concluded that the low-affinity receptors were in fact increased). At present, with De Meyts's findings in mind, we conclude that cells from obese people bind less insulin in the physiological range of insulin concentrations. Although it is not possible to apply standard analytical techniques to the data, the data does make it seem that the reduced binding, both here and in the animal models, is due to a reduction of receptor concentrations per cell (6). We have, however, not excluded, in addition, some alterations in affinity. The distinction between changes in receptor concentration and changes in receptor affinity may be very difficult to make since De Meyts's data suggest that affinity of receptors for hormone may depend on the concentration of receptors.

*Note added in proof.* Since this manuscript was submitted for publication, further studies have indicated that, although the lymphocyte is the predominant cell in the in-

cubation medium, the monocyte accounts for most of the insulin binding (30). While the monocyte has not been specifically isolated in all these studies the monocyte content of each mononuclear cell preparation has been determined, and these cells exhibit the same properties of "negative cooperativity" as have been shown for cultured human lymphocytes (29). In preliminary studies we have not detected any systematic alteration in monocyte content among mononuclear cell populations obtained from normal volunteers, obese subjects, or females with extreme insulin resistance (1). When data from females with extreme insulin resistance are expressed per mononuclear cell or per monocyte and compared to normal subjects, the results are qualitatively the same. A small number of studies in obese subjects reveals similar results. Thus our general conclusion that a circulating cell (albeit monocyte rather than lymphocyte) mirrors the status of insulin receptors in other tissues remains unchanged.

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