Fluctuations in the Affinity and Concentration of Insulin Receptors on Circulating Monocytes of Obese Patients

EFFECTS OF STARVATION, REFEEDING, AND DIETING

ROBERT S. BAR, PHILLIP GORDEN, JESSE ROTH, C. RONALD KAHN, and PIERRE DE MEYTS

From the Diabetes Branch, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014

ABSTRACT The binding of ^{125}I-insulin to insulin receptors on circulating monocytes of obese patients and normal volunteers has been determined under various dietary states. In the basal, fed state the monocytes of obese patients with clinical insulin resistance (n = 6) bound less insulin than normals (n = 10) because of a decrease in insulin receptor concentration (obese = 6,000–13,000 sites per monocyte versus normals 15,000–28,000 sites per monocyte). The single obese patient without evidence of clinical insulin resistance demonstrated normal binding of insulin with 16,000 sites per monocyte. In all patients, the total receptor concentration was inversely related to the circulating levels of insulin measured at rest after an overnight fast.

For the obese patients with basally depressed insulin binding, a 48–72-h fast lowered circulating insulin and increased binding to normal levels but only at low hormone concentrations; this limited normalization of ^{125}I-insulin binding was associated with increased receptor affinity for insulin without change in receptor concentration. Refeeding after the acute fasting periods resulted in return to the elevated plasma insulin levels, the basal receptor affinity, and the depressed insulin binding observed in the basal, fed state. Chronic diet restored plasma insulin levels, insulin binding, and receptor concentration to normal without change in affinity.

When the data from this study are coupled with prevous in vivo and in vitro findings they suggest that the insulin receptor of human monocytes is more sensitive to regulation by ambient insulin than the receptors of obese mice and cultured human lymphocytes. The results further indicate that an insulin receptor undergoes in vivo modulation of its interaction with insulin by changing receptor concentration and by altering the affinity of existing receptors.

INTRODUCTION

Obesity is commonly associated with hyperinsulinemia and resistance to the action of endogenous and exogenous insulin (1, 2). In both genetic and acquired forms of obesity in rodents, the increased concentrations of circulating insulin are associated with decreased insulin binding to liver (3–6), muscle (7), fat (8–10), and thymus lymphocytes (11,12). The decrease in insulin binding is due entirely to a decreased concentration of insulin receptors; these residual receptors are normal receptors by multiple criteria (6). With chronic caloric restriction of obese mice, as well as with acute starvation of obese and thin mice, the plasma insulin concentrations fall and receptor concentrations rise (3). In both the fed and food-restricted states, the receptor concentration is inversely related to the level of circulating insulin.

The insulin receptors in obese humans are not as well characterized. With circulating mononuclear cells, we have previously shown that some obese patients have impaired binding of ^{125}I-insulin and that chronic caloric restriction improves this defect in hormone binding (13). The nature of the defect and the mechanism of this adaptation have not been clearly defined. These initial studies measured insulin binding to mixed mononuclear leukocyte preparations derived from Ficoll-Hypaque gradients (13, 14). We have re-

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TABLE I

Metabolic Characteristics of Obese Patients in Basal State and after Acute Fast, Refeeding, and Chronic Diet

Subject	Age, sex, and percent ideal body weight	Condition	Weight	Glucose	Insulin
			kg	*mg/dl*	*μU/ml*
A	19	Basal	114	85	28
	Female	72 h fast	111	54	8
	207%				
B	54	Basal	132	95	60
	Female	72 h fast	129	75	22
	224%	refeeding	132	93	50
		chronic diet	113	84	12
C	23	Basal	163	86	50
	Male	72 h fast	161	80	25
	199%	refeeding	162	91	55
		chronic diet	145	74	12
D	37	Basal	130	96	50
	Female	72 h fast	127	60	15
	215%	refeeding	130	85	35
		chronic diet	117	85	15
E	33	Basal	149	470	60
	Male	72 h fast	147	253	28
	215%	chronic diet	123	105	35
F	47	Basal	161	90	35
	Female				
	288%				
G	58	Basal	108	160	42
	Female				
	209%				

cently demonstrated that monocytes, which constitute about 20% of this mixed cell population, account for 85–90% of the insulin binding (15). Therefore, changes in monocyte number could result in major alterations in insulin binding. In an attempt to clarify the mechanisms of insulin resistance in human obesity, we have examined insulin binding to circulating monocytes of obese patients under various dietary conditions including the basal, fed state, after 24–72 h of starvation, after refeeding, and finally after chronic diet.

METHODS

Patients

A. *Obese patients.* Seven obese patients (A.–G.), selected to represent the range of carbohydrate metabolic defects seen in obesity, were studied while inpatients in the Clinical Center of the National Institutes of Health (Fig. 1, Table I). They were taking no medications and were free of disease except for patient E. who had congenital alkalosis and has previously been reported (16). In patient E. all metabolic and hormone binding studies were done after hypokalemia and alkalosis had been corrected.

Patients were initially maintained on a 3,500 calorie, weight-maintaining diet (≈45% carbohydrate, 20% protein, and 35% fat) for periods of 3–7 days. Oral glucose tolerance tests with 100 g of glucose and one to three insulin binding studies were done in this basal, fed state. Patient A. was further tested after a 72-h fast. The four patients demonstrating lowest basal insulin binding (B.–E.) were selected for detailed study. After basal studies were completed, these four patients were fasted (<50 calories per day = "acute fast") with insulin binding studies performed after 24, 48, and 72 h of fast (the designated time intervals are in addition to the initial 10 h overnight fasting period). Upon completion of the 72-h fast the 3,500 calorie diet was reinstituted ("refeeding") with repeat binding studies performed after 2–10 days. Patients were then begun on a 600 calorie diet (45% carbohydrate, 20% protein, 35% fat) for 6–12 wk ("chronic diet"). Plasma glucose and insulin levels were measured after overnight fasting on days that insulin binding studies were performed.

B. *Normal volunteers.* 10 normal volunteers, 7 males and 3 females, aged 18–32 yr were studied while inpatients. All were within 10% of their ideal body weight, had fasting plasma insulin concentrations of 5–18 μU/ml, and showed normal plasma glucose and insulin responses after ingesting

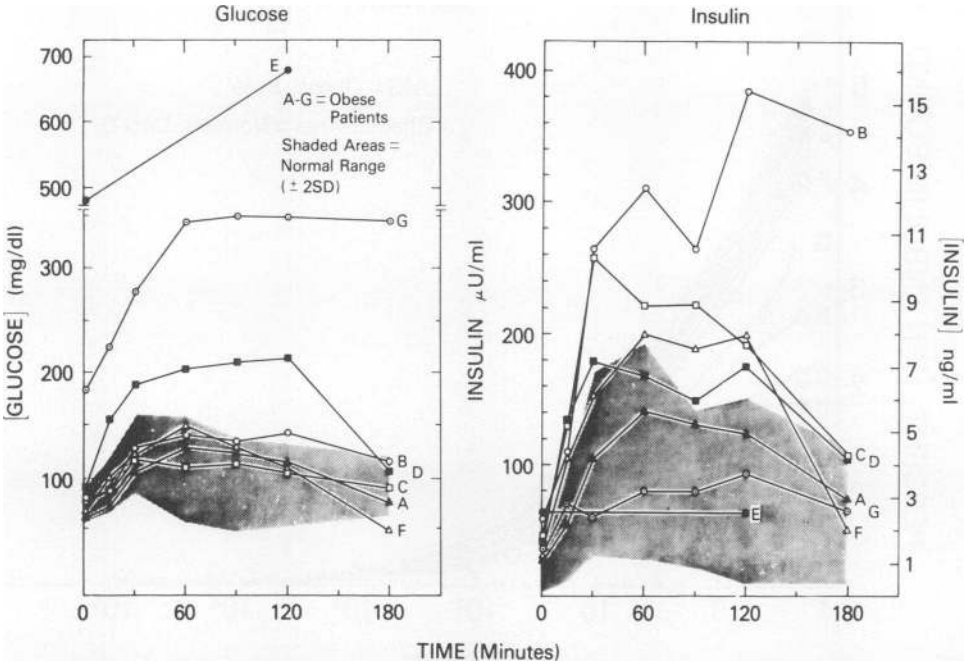

FIGURE 1 Basal glucose tolerance tests in obese patients A.–G. Shaded areas represent the normal range±2 SD of plasma glucose and insulin responses in 39 normal subjects.

100 g of glucose. Oral glucose tolerance tests, as well as insulin binding studies, were performed in the basal state in all normal volunteers. The normal volunteers were given a 3,500 calorie diet (≈45% carbohydrate, 20% protein, and 35% fat) for at least 4 days before all basal testing. Four normal volunteers also had insulin binding studies after periods of acute fast, three for 48 h and the other for 72 h.

^{125}I-insulin binding study. Whole blood was drawn into acid-dextrose-citrate solution (Fenwal plasmapheresis triple blood pack Fenwal Inc., Walter Kidde and Co., Inc., Ashland, Maine) and centrifuged. The erythrocytes and plasma were returned to the patient. The buffy coat was diluted 1:1 with phosphate buffered saline (pH 7.4), layered onto Ficoll-Hypaque gradients, and centrifuged according to the method of Böyum (17). The mononuclear cell layer was removed and diluted in 100 mM HEPES buffer (pH 8) to a final concentration of 50×10^6 mononuclear cells per ml. Viability, as assessed by trypan blue exclusion, was always greater than 98%. The percentage of monocytes in this final mononuclear preparation was determined by phagocytosis of latex beads (18) and verified by esterase staining of monocytes (19). Good agreement was found between these two methods, with esterase staining giving results ~10% higher than found with latex bead ingestion.[1] There was no systematic difference in monocyte content in mononuclear cell preparations of the obese or normal subjects (Table II).

^{125}I-insulin was prepared at a specific activity of 150–200 μCi/μg insulin (≈0.5 I per insulin) as previously described (20–22). ^{125}I-insulin, 0.2 ng/ml, was incubated with 40×10^6 mononuclear cells per ml in the absence or presence or unlabeled insulin over a range of insulin concentrations from 0.2 through 100,000 ng/ml with final incubation volume of 0.5 ml per assay tube. In this study the assay buffer was 100 mM

[1] The monocyte count actually used for calculation of binding data was determined by latex bead ingestion.

TABLE II

*Monocyte Percentages in Normal and Obese Patients**

	Basal‡	72 h fast	Refeeding	Chronic diet
I. Normal volunteers				
1	17			
2	29			
3	29,30,23	33		
4	26,36	19	29	
5	24,27			
6	21,30			
7	22,21			
8	26,29			
9	22			
10	23			
II. Obese				
A	25,28	37		
B	24,26	20	26	20
C	30,36	31	33	21
D	14,21	19	16	20
E	31,25	30		20
F	21			
G	21			

* Values represented are determined by latex particle ingestion.

‡ Patients were studied on one to three separate occasions.

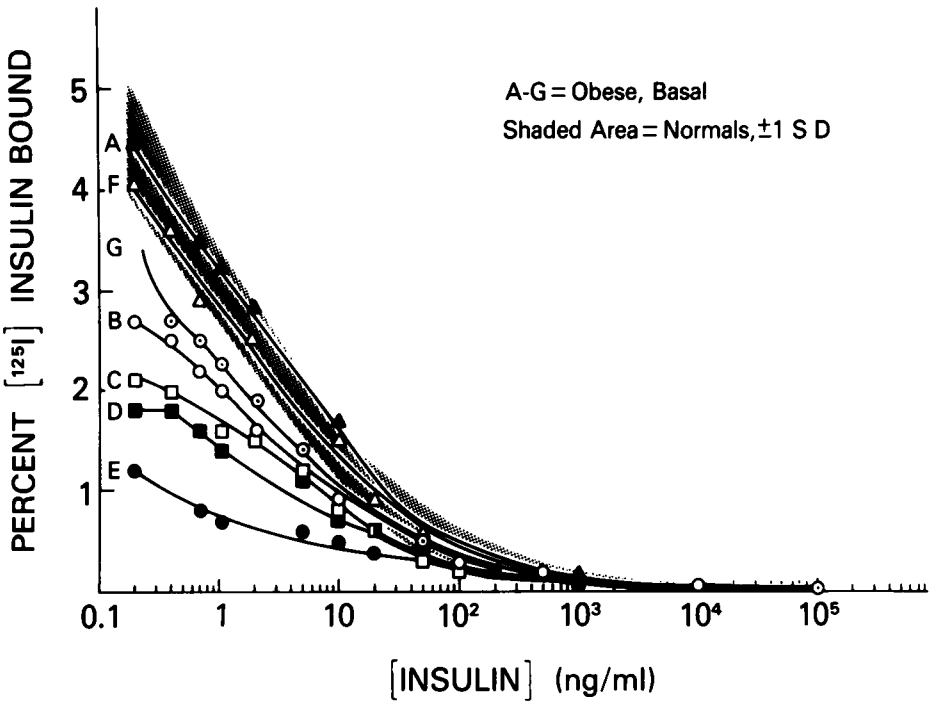

FIGURE 2 ^{125}I-insulin binding to monocytes of obese patients studied in the basal state. Monocytes were prepared by Ficoll-Hypaque gradient centrifugation of buffy coats. ^{125}I-insulin (0.2 ng/ml) was incubated with 40 × 10^6 mononuclear cells/ml in the absence or presence of unlabeled insulin over a range of insulin concentrations from 0.2 to 100,000 ng/ml. After 180 min at 22°C, replicate 0.2-ml-aliquots of the assay mixture were transferred to microfuge tubes, centrifuged, and the supernatant fluid was aspirated. The tips of the tubes containing the pellet were excised and counted. Nonspecific binding, defined as the radioactivity in the cell pellet in the presence of 10^5 ng/ml insulin, was subtracted from the total binding at each insulin concentration and normalized to a monocyte concentration of 10^7 cells/ml. Each point is the mean of replicate samples. The shaded area represents the mean ± 1 SD of 17 insulin binding studies performed in 10 normal volunteers.

HEPES, pH 8.0, and the assay tubes were incubated for 180 min at 22°C. These assay conditions produce a steady-state binding with less than 10% degradation of labeled hormone, no receptor degradation, and pH change of less than 0.15 pH U during the 180-min incubation. ^{125}I-insulin binding with these modified conditions was also consistently greater than previously found.[2] After 180 min of incubation replicate 0.2-ml-aliquots of the incubation mixture were transferred to microfuge tubes and centrifuged. The supernate was aspirated and the cell pellet contained in the tip of the microfuge tube was excised and the radioactivity counted. Under these conditions nonspecific binding, defined as the amount of ^{125}I-insulin bound to the cell pellet in the presence of 10^5 ng/ml insulin, was 0.3–0.7% of the total radioactivity for both normal and obese patients and was subtracted from total binding to give specific binding. ^{125}I-insulin specifically bound was then expressed as a function of monocyte concentration.

The methodology of this study introduces modest changes in the preparation of the cells and several changes in the condition of the ^{125}I-insulin binding assay including pH, temperature, and buffer composition.[3] We have also normalized the data to correct for the variations in monocyte content (15). With the improved methodology, we now find that the total binding of ^{125}I-insulin to the cells of the normal subjects is in a narrower range and slightly higher than previously reported (13, 14). The nonspecific binding and the concentration of hormone that reduced the binding of ^{125}I-insulin by half, both of which had been higher with the cells of the obese (13, 14), were now the same for the obese and thin adults and lower than previously reported. More importantly, the improved precision of the data as well as a greater appreciation of the complexities of the interaction of insulin with its receptor, have permitted a more detailed analysis of the insulin binding.

Data analysis. The data on binding of ^{125}I-insulin to receptor is presented in three ways which emphasize different quantitative aspects of the binding interaction. (*1*) The percent of total radioactivity that is specifically bound to receptors is plotted as a function of log total insulin concentration; this most closely represents the actual experimental results. (2) Bound/free of ^{125}I-insulin (B/F)[4] is plotted as a function of bound (B) hormone (Scatchard plot) (23). For insulin binding to its receptors this plot is curvilinear.

[2] Manuscript in preparation.

[3] Previous reports from this laboratory performed binding studies with Tris buffer (25 mM, pH 7.6) and a 90-min incubation period at 15°C.

[4] *Abbreviations used in this paper*: B/F, bound/free; $\bar{K}$, affinity; $\bar{K}_e$, limiting high affinity state; $\bar{K}_f$, limiting low affinity state; R_0, total concentration of receptor sites per cell.

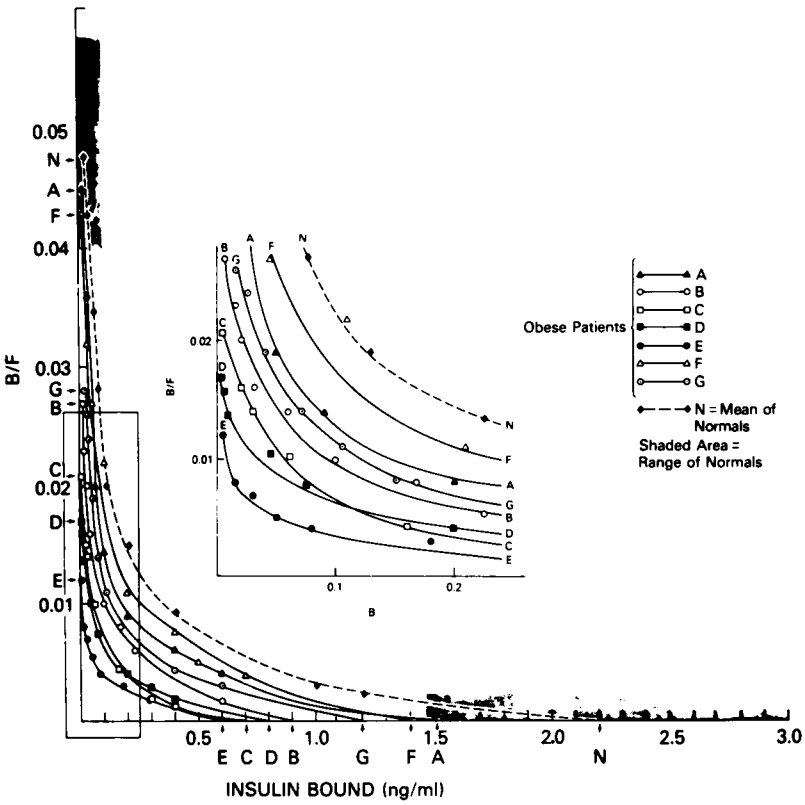

FIGURE 3 Scatchard analyses of ^{125}I-insulin binding to monocytes of obese patients A.–G. and normal volunteers. The ordinate, B/F, is the ratio of bound to free hormone and the abscissa represents the insulin bound (ng/ml), both expressed per 10^7 monocytes per ml. For each patient maximal B/F and the intercept at the abscissa are designated by arrows. The two shaded areas represent the range of normal for maximal B/F and abscissa-intercepts, respectively. The inset is an enlargement of the portion of the graph enclosed by the rectangle.

The total binding capacity or total concentration of receptor sites (R_0) is derived from the point where the linear extrapolation of the curve intersects the horizontal axis. (3) The third method of data analysis is the "average affinity profile". Because the insulin receptor sites are not independent of one another, traditional methods for deriving the affinities of the receptors from curvilinear Scatchard plots are not valid (24). Experimental data suggest that the insulin receptors are a single set of homogeneous binding sites that undergo negatively cooperative site-site interactions such that the affinity of the receptors for insulin is inversely related to the fractional occupancy (25, 26). The average affinity profile expresses the relationship between the average affinity for insulin ($\bar{K}$) and receptor occupancy ($\bar{Y}$) (27). At any point i on the Scatchard curve, the average affinity = $\bar{K}_i = \frac{(B/F)_i}{R_0 - B_i}$ where B_i = the concentration of bound hormone and $(B/F)_i$ = the bound/free hormone at that point (for details of this analysis see reference 27). When the affinity ($\bar{K}$) is graphed versus log of the fractional occupancy of the receptor ($\bar{Y}$ or B/R_0) the plot displays the average affinity of the receptor at all levels of receptor occupancy and is referred to as the "average affinity profile" (27). In this analysis, the limiting high affinity state, obtained at low levels of receptor occupancy, is designated $\bar{K}_e$; the limiting low affinity state, obtained at high levels of receptor occupancy, is $\bar{K}_f$. In this paper, we have designated $\bar{K}_e$ as the value of $\bar{K}$ when the ^{125}I-insulin concentration is 0.2 ng/ml.[5]

It should be noted that the validity of this analysis (and of the derived parameters) does not depend on assigning a particular model to the molecular mechanisms involved in the cooperativity (27).

RESULTS

I. *Basal ^{125}I-insulin Binding in Normal and Obese*

A. *Normal volunteers.* 17 insulin studies were performed in 10 normal volunteers. At every insulin concentration insulin binding to monocytes fell within narrow limits (Fig. 2, shaded area). When a given individual was studied on separate occasions variability of data was also small.

[5] If ^{125}I-insulin binding to monocytes could be measured accurately under equilibrium conditions at insulin concentrations below 0.2 ng/ml, higher values of $\bar{K}_e$ might be expected.

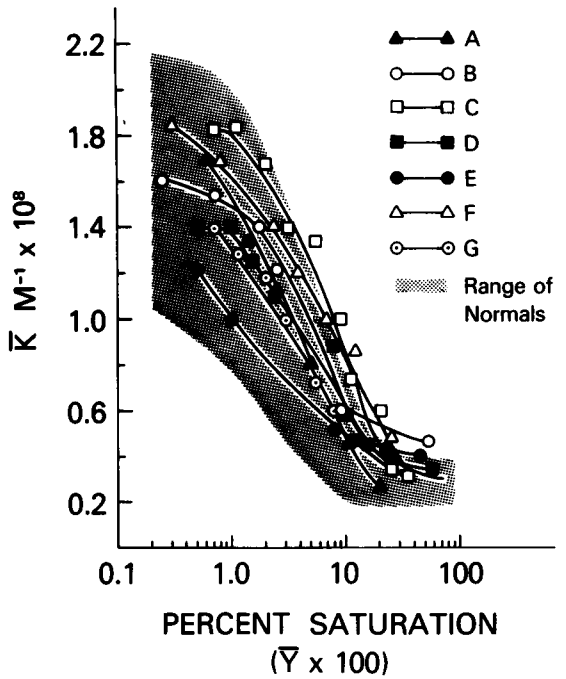

FIGURE 4 Average affinity profiles of ^{125}I-insulin binding to obese patients' monocytes. The average $\bar{K}$ is equal to $\frac{B/F}{R_0 - B}$. The percentage of R_0 that are occupied is given by $\bar{Y} \times 100$, where $\bar{Y} = B/R_0$. The shaded area is defined by the plots of the two normal volunteers with highest and lowest $\bar{K}_e$.

When these binding data were analyzed by the method of Scatchard, curvilinear plots were obtained. The insulin receptor concentrations on normal cells ranged from 15,000 to 28,000 sites per cell with a mean of 22,000 sites per cell. The relationship between changing receptor affinity for hormone and occupancy of receptor sites by the hormone is expressed by the average affinity profile. In monocytes from normal volunteers, the highest or "empty sites" affinity, $\bar{K}_e$, ranges from 0.10 to 0.23 nM^{-1} and begins to decrease when only 0.3% of total receptor sites are occupied. With increasing occupancy of receptors by hormone, apparent $\bar{K}$ progressively decreases until a $\bar{K}_f$, is reached. In normal volunteers $\bar{K}_f$ ranges between 0.02 and 0.04 nM^{-1} and is reached when only 10–20% of available receptor sites are occupied.

B. Obese. Studies of monocytes from the seven obese patients demonstrated a wide range of ^{125}I-insulin binding curves, consistent with the wide range of basal levels of circulating insulin seen in this obese group (Fig. 2). Three patients were within ±2 SD of normal while in the others binding was decreased by 40–70% as compared to normals. On Scatchard analysis the plots were all curvilinear and roughly parallel to one another and to the curves from the normal subjects (Fig. 3). This suggested that receptor affinities and negative cooperativity were largely unaltered and that the observed reduction in ^{125}I-insulin binding was due to a decrease in receptor concentration. The lack of change in receptor affinity was confirmed by the average affinity profiles (Fig. 4). These plots showed that both $\bar{K}_e$ and $\bar{K}_f$ as well as the entire affinity profile in the obese were within the range of the normal subjects. Thus the diminished basal binding of ^{125}I-insulin to monocyte receptors of obese patients was accounted for by changes in receptor concentration without any significant alteration in receptor affinity.

II. *Effect of Acute Fast and Chronic Diet in Obese Patients*

The four obese patients with the most impaired basal ^{125}I-insulin binding (B., C., D., and E.) were further studied during periods of acute fasting and after chronic diet. With acute fasting, elevated basal plasma insulin concentrations fell (Table I), body weight decreased by 2–3 kg, and urinary ketones became strongly positive in all patients. During the period of chronic diet restriction obese patients lost 13–19 kg of body weight and patients B., C., and D. lowered their plasma insulin to the normal range, while patient E. lowered his plasma insulin from 60 to 35 μU/ml. Both types of diet (acute fast and chronic diet) resulted in normalization of binding of tracer concentrations of ^{125}I-insulin in all four patients; however, the mechanisms whereby these changes were effected clearly differed. To illustrate these phenomena a single patient, E., will first be considered in detail.

A. *Obese patient E.* In the basal state, obese patient E. had the most profound depression of ^{125}I-insulin binding with monocytes binding ~30% as much insulin as normal (Fig. 5). This decreased ^{125}I-insulin binding was accompanied by a comparable decrease in R_0, i.e. ~6,000 sites per monocyte basally compared to a normal mean of 22,000 sites per monocyte. When acutely fasted, patient E. had no changes in insulin binding at 24 h but at 48 and 72 h of fast increases in ^{125}I-insulin binding became evident (Fig. 5). This increased hormone binding was present only at relatively low, physiologic concentrations of insulin. Thus, at an insulin concentration of 0.2 ng/ml (5 μU/ml) there was a fivefold increase in ^{125}I-insulin binding while at insulin concentrations greater than 1.0 ng/ml (25 μU/ml) no significant difference was seen between binding in the basal state and binding after a 72-h fast. The binding alterations with acute fasting were associated with little or no change in R_0 (Fig. 5) but with a marked increase in $\bar{K}_e$, the affinity of the "empty site" or high affinity state. Basally $\bar{K}_e$ was 1.2×10^8 (M^{-1}) and with acute fasting increased to 6.0×10^8 (M^{-1}), while $\bar{K}_f$ (0.04 nM^{-1}) and R_0 remained constant.

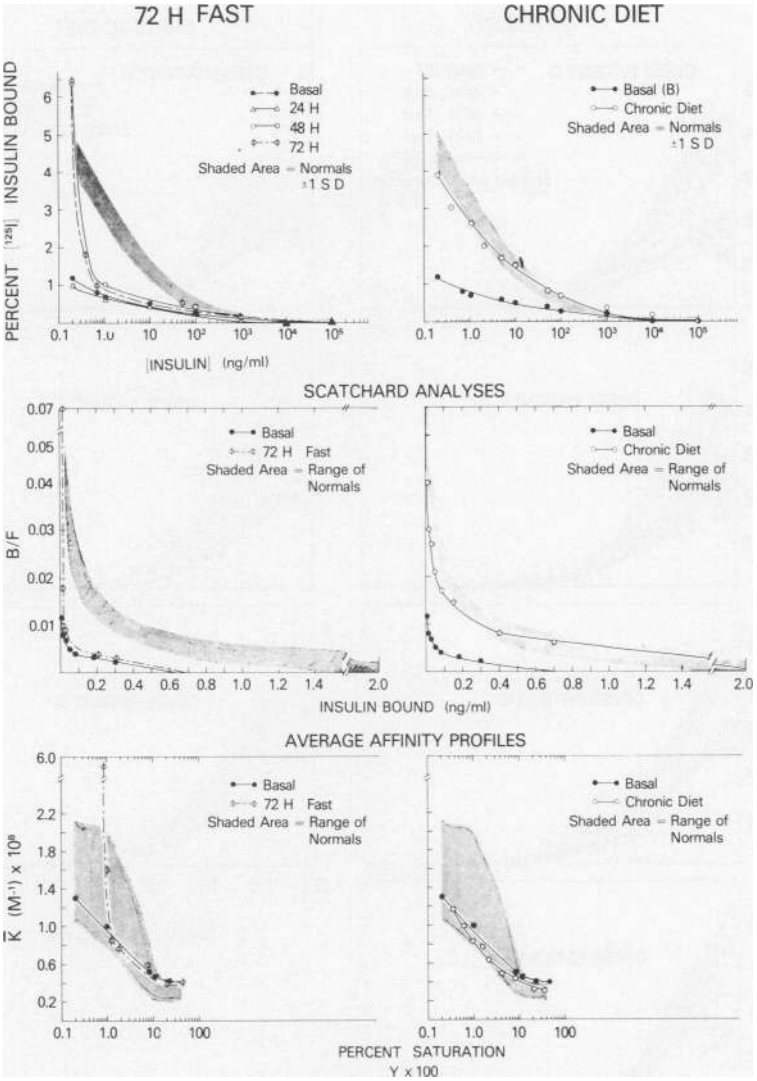

FIGURE 5 ^{125}I-insulin binding during a 72-h fast and after chronic diet in obese patient E. Binding-competition curves are determined by the methods described in Fig. 2. Scatchard analyses and average affinity profiles are calculated as described in Analysis of Data. For Scatchard analyses, the shaded area is defined by the two normal volunteers with highest and lowest R_0; for the average affinity profiles the shaded area is defined by plots of the two normal volunteers with highest and lowest $\bar{K}_e$.

Chronic diet also produced increases in ^{125}I-insulin binding (Fig. 5). In this case, however, the binding increase was apparent at all hormone concentrations and Scatchard plots demonstrated that this was due to an increase in R_0 from 6,000 sites per cell basally to 22,000 sites per cell after chronic diet. This increase in R_0 was not accompanied by any change in affinity (Fig. 5). Therefore, the restoration of insulin binding to normal by the chronic diet was solely caused by an increase in receptor concentration.

B. Obese Patients B., C., and D. During acute fasting, patients B., C., and D. showed binding changes similar to those of patient E. (Fig. 6). In these patients after 72 h of fasting ^{125}I-insulin binding was increased to normal levels but the increase was only found at insulin concentrations less than 5 ng/ml (100 μU/ml). Scatchard plots and average affinity profiles of these data revealed that the increased binding was associated with little change in R_0 but with markedly increased $\bar{K}_e$. At higher concentrations of hormone, the affinity differences between acute fast and basal study became less pronounced, so that when more than 7–10% of total receptor sites were filled, the affinity curve with acute fasting became indistinguishable from the affinity curve derived from basal data. After 2–10 days of refeeding in patients B.–D., body weight, plasma

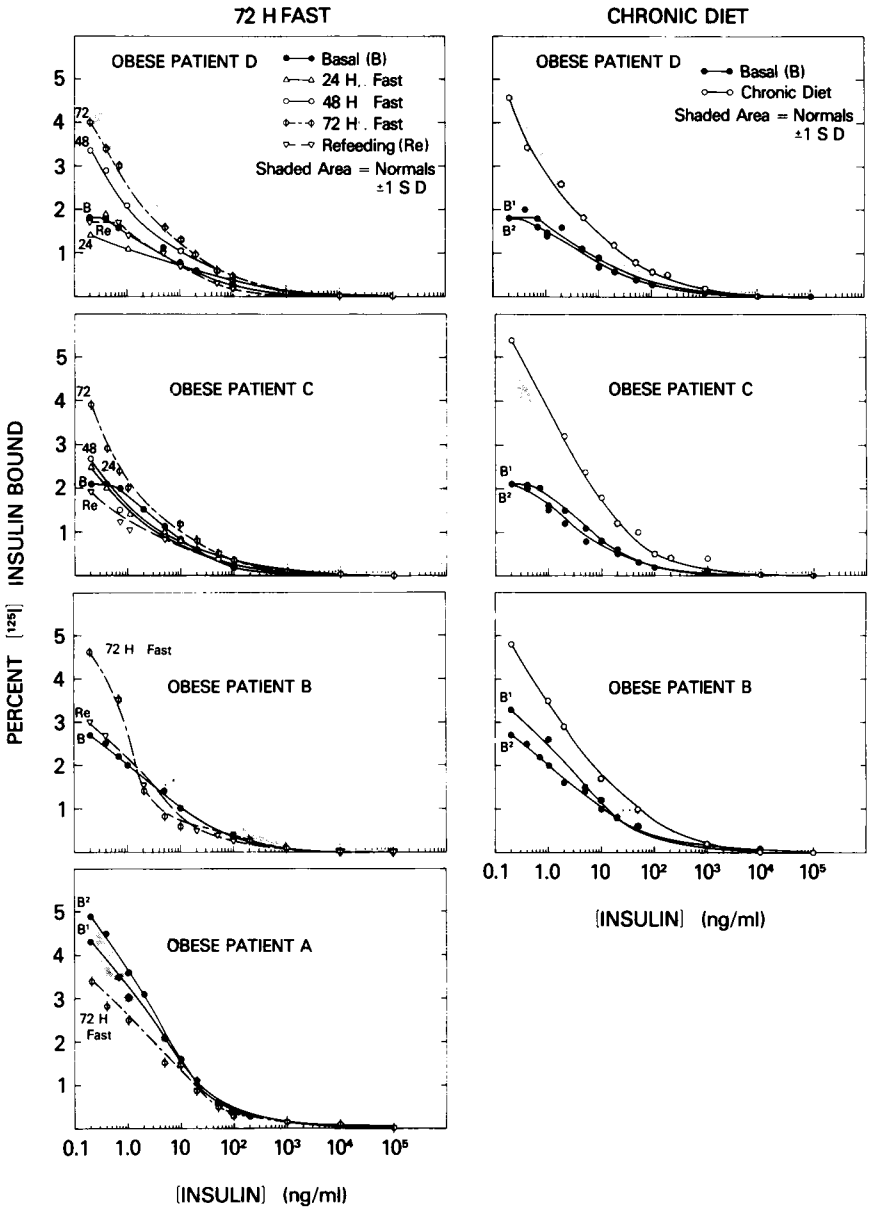

FIGURE 6 ^{125}I-insulin binding during a 72-h fast, with refeeding and after chronic diet in obese patients A.–D. The binding competition curves shown were determined by the method described in Fig. 2. The shaded area represents the mean±1 SD of 17 ^{125}I-insulin binding curves in 10 normal volunteers.

glucose and insulin concentrations, and insulin binding returned to basal, fed state levels (Table I, Fig. 6). The binding changes induced by refeeding were due entirely to a reduction of $\bar{K}_e$ to the level observed in the basal state.

Chronic diet, which was associated with a fall in plasma insulin concentrations to normal levels, also resulted in normalization of ^{125}I-insulin binding in patients B.–D. As with patient E., chronic diet was associated with an increase in R_0 to normal while all parameters of affinity were unchanged and well within the normal range.

C. Patient A. Patient A. was an obese patient with normal basal ^{125}I-insulin binding to her circulating monocytes (Fig. 6). Although equally obese (207% of ideal body weight), her basal plasma insulin concentration was the lowest of the obese group (Table I). Further, this patient had normal plasma glucose and insulin responses to oral glucose challenge (Fig. 1).

After 72 h of fasting her insulin binding did not sig-

nificantly change (Fig. 6) and no change in R_0 or $\bar{K}_e$ was detected during the acute fast. Similar results were obtained when four normal volunteers were fasted for 48 or 72 h (data not shown).

III. *Summary and relationship of R_0 to circulating plasma insulin concentrations*

The effects of acute fast, refeeding, and chronic diet on insulin binding parameters in patients B.–E. are summarized in Fig. 7. In the basal, fed state patients B.–E. have decreased ^{125}I-insulin binding, reduced R_0, but normal $\bar{K}_e$ suggesting the presence of decreased numbers of normal receptors accounting for the diminished insulin binding by these monocytes. With acute fasting insulin binding increased to normal (B., C., and D.) or supranormal (E.) levels with little change in R_0 and 1.5 to 5-fold increase in $\bar{K}_e$. Refeeding of a high calorie diet resulted in return of all binding and metabolic parameters to the basal state. With chronic diet, insulin binding, receptor number, and receptor affinity for hormone became indistinguishable from normal (Fig. 7).

In the basal state, when plasma insulin concentration is plotted against receptor concentration (or initial B/F), an inverse relationship is found (Fig. 8A), i.e., the higher the patient's plasma insulin concentration the lower the R_0. This inverse relationship between R_0 and circulating insulin concentration was also found during the refeeding stage and with chronic diet (Fig. 8B); however, after acute fasting R_0 was essentially unchanged despite significant falls in plasma insulin concentrations (Fig. 8B).

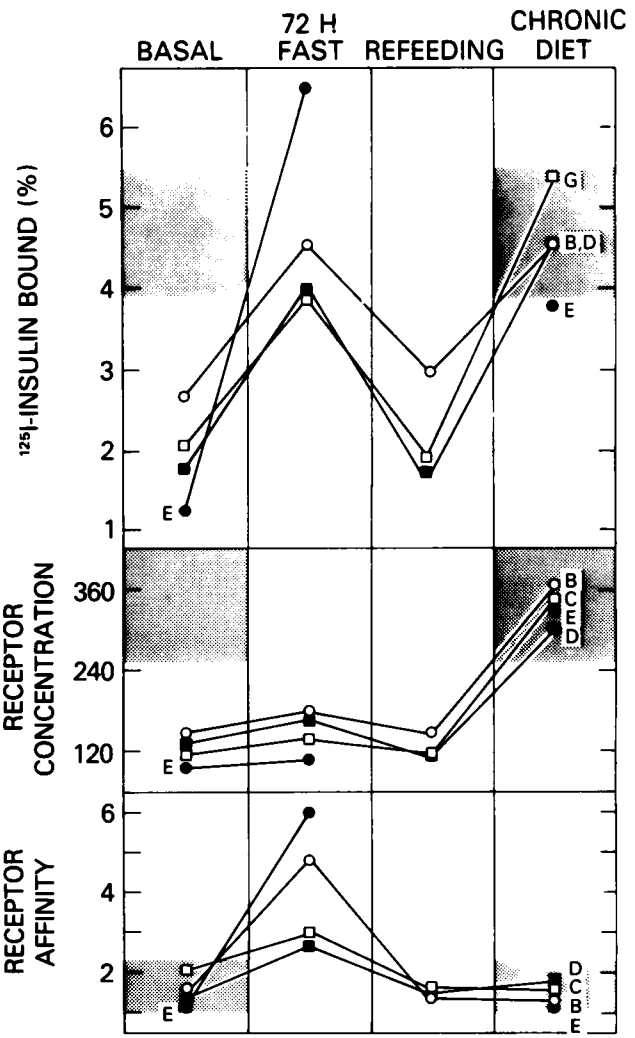

FIGURE 7 Summary of acute fast, refeeding, and chronic diet on ^{125}I-insulin binding to monocytes of obese patients. The upper panel is the percent of tracer insulin (0.2 ng/ml) specifically bound to 10^7 monocytes/ml, the middle panel is the total R_0 expressed as femtomoles per monocyte $\times 10^{-7}$; the lower panel is the receptor affinity at tracer concentration of insulin expressed as nM^{-1}.

DISCUSSION

Clinical states of hormone resistance can be due to an impairment in the delivery of the hormone to the target cell (for example, antihormone antibodies) or to defects at the target cell itself. At the target cell, the defect can be in the first step, the binding of the hormone to its receptor, or at any one of the multitude of steps between binding to receptor and the final effects of the hormone. Of the many conditions characterized by hormone resistance, the insulin resistance of obesity is the most common and best studied. In obese mice, insulin resistance is associated with a decreased concentration of insulin receptors (3–6). This decrease in insulin receptor concentration is reversible since receptor concentration can be restored toward normal by both acute fasting and chronic dieting (3).

To study the insulin receptors of obese humans under dietary conditions similar to those in the obese rodents, we should ideally study the insulin receptors of the liver, since the function of that organ is the overwhelming determinant of glucose homeostasis in vivo (1). Because the insulin receptors of human liver are inaccessible to direct study we have employed the insulin receptor of the circulating monocyte. Although the human monocyte has no defined role in glucose homeostasis, we have recently found that macrophages from the mouse spleen and in cell culture, which both have properties similar to human monocytes, have immunologic functions that are sensitive to insulin at physiological concentrations (10^{-10}–10^{-9} M) (28). These cultured macrophages have insulin receptors which are indistinguishable from those of the human monocyte and also very similar to insulin receptors of liver and other tissues by numerous

sensitive and precise physico-chemical criteria (28). Furthermore, in patients with the syndromes of insulin resistance associated with acanthosis nigricans, the degree of impairment of binding of ^{125}I-insulin to the circulating monocytes of these patients correlated very closely with the severity of the insulin resistance observed clinically (29, 30). Thus, we consider that the specific insulin binding sites of human monocytes are representative of the state of insulin receptors in liver and other tissues throughout the body.

In the present study, we have confirmed the finding that hyperinsulinemic obese patients have decreased insulin binding (13, 14) and we now find that the decrease in insulin binding is due entirely to a decrease in the concentration of insulin receptors. In these hyperinsulinemic obese patients chronic diet lowered circulating insulin levels and returned insulin binding and receptor concentrations to normal. In the basal (fed) and chronically dieted states, the total receptor concentration on the cells of the obese patients was inversely related to the resting levels of circulating insulin. Thus, obese people and obese mice have quite similar defects in insulin binding in the fed state and respond identically to chronic dieting.

In addition to the previous reports from our laboratory (13, 14), three other groups have evaluated the status of insulin receptors in human obesity. Marinetti et al., in a preliminary study with fat cell membranes from obese people, was the first to suggest that insulin binding was decreased in obesity (10). In a detailed study of obese patients, Amatruda and his co-workers reported that, when expressed on a per cell basis, fat cells from obese patients bound insulin as well as did cells from normal subjects (31). However, if these data are expressed per adipocyte surface area rather than per whole cell, the obese patients in Amatruda's study then show a receptor concentration decrease comparable to that of our patients.[6] In a recent study (32), Olefsky found that the concentration of insulin receptors on fat cells of obese patients is decreased whether expressed per cell or per unit of cell surface although clearly the defect is more marked when expressed per unit of cell surface. He also found

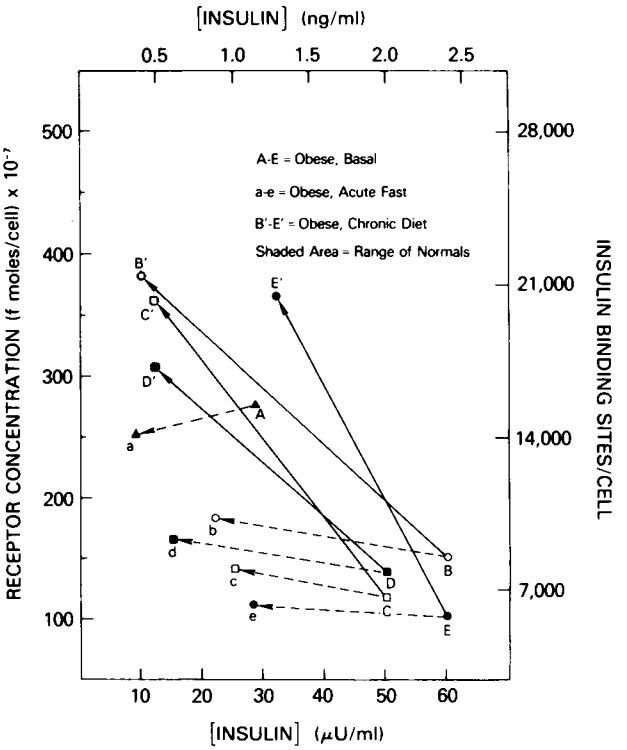

FIGURE 8B Effect of acute fast and chronic diet on plasma insulin concentration and insulin receptor concentration in obese patients. Basal values are designated by capital letters without superscripts, those after acute fast by small letters, while values after chronic diet are designated by prime (') superscripts.

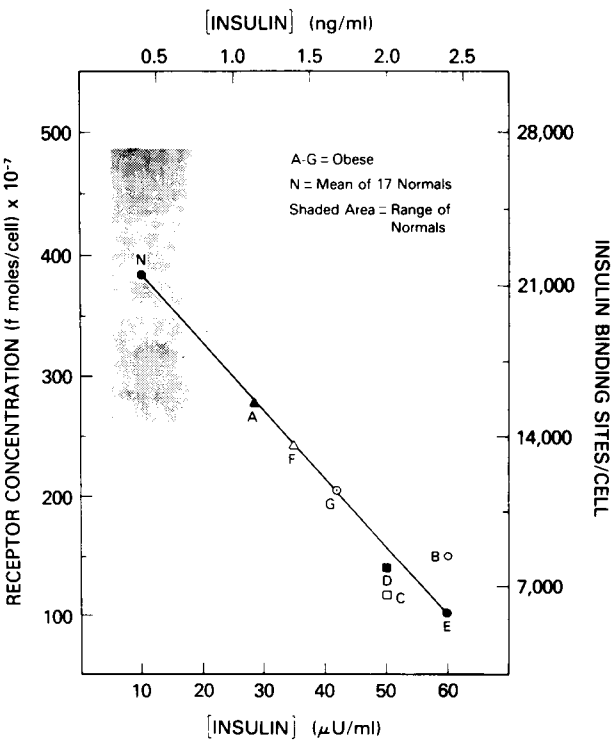

FIGURE 8A Basal insulin receptor concentration as a function of plasma insulin concentration in obese patients. The total insulin R_0 is expressed both as femtomoles (fmoles) per monocyte and as binding sites per monocyte.

[6] Fat cell size is known to increase in the obese state, consistent with the data from Amatruda's study in which the diameter and surface area of the fat cells from the obese patients were 1.6 and 2.5 times greater, respectively, than those in the normal subjects.

excellent correlation in his patients between insulin binding to monocytes and insulin binding to fat cells.

The present studies add support to the idea that one of the major influences on the receptor concentration is the chronic level of insulin to which the cells are being exposed (3, 33). We show that in obese patients under basal conditions, the concentration of insulin receptors is inversely related to the resting level of circulating insulin, and in obese patients who are not hyperinsulinemic or insulin resistant, receptor concentrations are normal. Chronic dieting of these hyperinsulinemic obese patients is accompanied by a fall in the ambient insulin and a commensurate rise in the receptor concentration. In thin hyperinsulinemic diabetics and in hyperinsulinemic obese patients, Olefsky and Olefsky and Reaven demonstrated that the concentration of insulin receptors on circulating monocytes is decreased (32, 34). Correlations between the ambient insulin concentration and the receptor concentration are even more extensive in mice. In both obese and thin mice studied in the fed, fasted and dieted states, there is an excellent correlation between circulating levels of insulin and the receptor concentration. When obese mice are fasted for 24 h, plasma insulin levels fall and receptor concentrations increase (3). Treatment of the mice during the fast with repeated doses of insulin prevents the restoration of the receptor concentration. Streptozotocin treatment of obese mice also corrects the hyperinsulinemia, and elevates receptor concentrations (35). Additionally, diabetic Chinese hamsters, who have abnormally low levels of insulin in both plasma and pancreas, have elevated concentrations of insulin receptors in plasma membranes of liver (36).

The effect of insulin on the concentration of its own receptors is direct and can be reproduced in vitro by incubating cultured human lymphocytes (IM-9 line) in the presence of insulin (33, 37). When exposed to insulin these cells show a decrease in the concentration of insulin receptors; the speed and magnitude of the fall in the insulin receptor concentration is dependent on the concentration of insulin in the medium (33, 37, Fig. 9). Recent studies employing insulin analogues and inhibitors of protein synthesis further

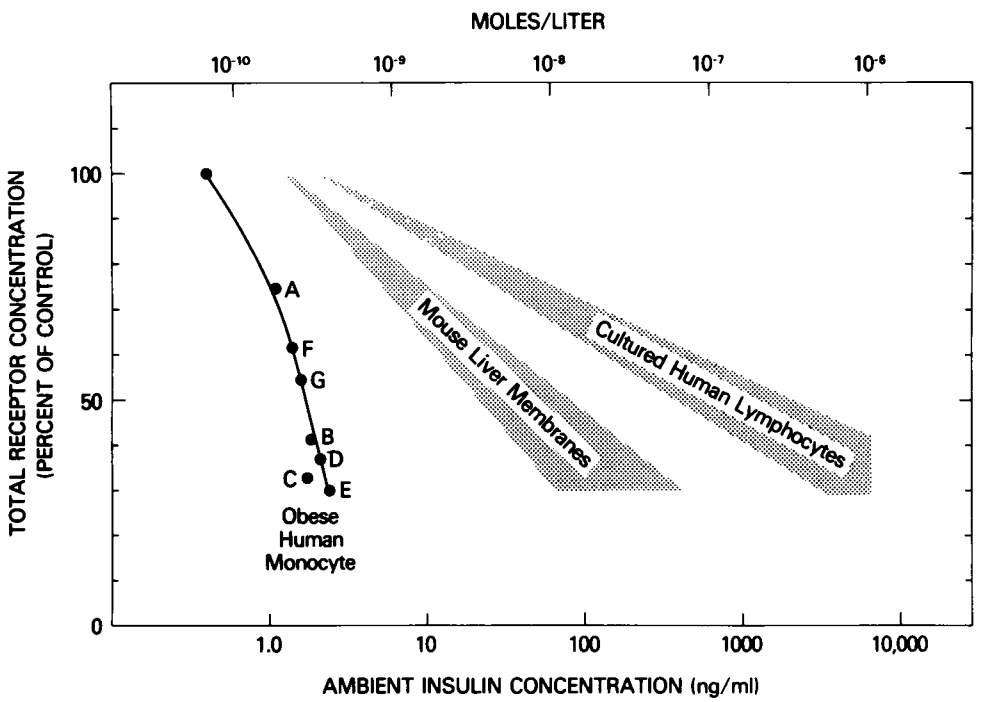

FIGURE 9 Effect of ambient insulin concentration on insulin R_0 in human monocytes, mouse liver cell plasma membranes, and in cultured human lymphocytes. For the human monocytes and mouse liver membranes, the insulin concentration is the basal level of plasma insulin in vivo measured by radioimmunoassay with porcine insulin and mouse insulin standards, respectively; the receptor concentration is expressed as percent of normal volunteers (mean) for human studies and percent of thin littermates for the obese mice. The cultured human lymphocytes were preincubated with the designated insulin concentrations for 18 h, then washed extensively before binding studies were performed. For the cultured lymphocytes, 100% receptor concentration represents the R_0 when cells were preincubated with culture medium alone. Data for the obese mice and cultured lymphocytes are found in references 3, 33, and 37.

indicate that the effect of insulin on the concentration of its own receptors is a direct regulatory process effected at the target cell via the insulin receptor itself (37).

The insulin receptors on human monocytes appear more sensitive to the effects of chronic elevations in ambient insulin than either mice in vivo or cultured human lymphocytes in vitro (Fig. 9). For humans a 50% decrease in receptor concentration was found with circulating levels of ≃2 ng/ml (50 μU/ml), while in mice, a comparable 50% decrease in receptor concentration required ambient insulin concentrations of 30 ng/ml (Fig. 9). This is consistent with the observations that, in general, rodents in vivo are less sensitive to insulin than are humans. The IM-9 lymphocytes are even more resistant; 400 ng/ml of insulin is needed to reduce receptor concentrations by 50%. Further studies will be required to determine if this reduced sensitivity is a general characteristic of lymphocytes in culture or varies from cell line to cell line.

The receptor affinity changes associated with acute fasting of hyperinsulinemic obese patients represent a unique finding of our study. When obese mice were acutely fasted, receptor concentrations increased while changes in receptor affinity were not observed (3). The affinity changes seen in the hyperinsulinemic obese humans were rather specific since they were not observed when thin normals and obese patients with minimal hyperinsulinemia were similarly fasted. This change in affinity would have the effect in vivo of normalizing insulin binding at low resting levels of circulating insulin while maintaining the relatively impaired binding at stimulated levels of hormone. The molecular mediators of these affinity changes are unknown. It is unlikely that the ambient insulin level directly effected these affinity changes since the plasma insulin levels were similar with acute fast and chronic diet yet no change in receptor affinity was seen with chronic diet. It should be pointed out that there are other examples where the affinity of the receptor for insulin can be regulated widely under biologically relevant conditions. Occupancy of only a small minority of the receptor binding sites by insulin produces a marked fall in the affinity of all of the receptors because of negatively cooperative site–site interactions among the receptors (25, 26). Fluctuations in pH within the range observed in vivo also produce major changes in receptor affinity. Conceivably, one or more small molecules, possibly metabolic intermediates, may act in vivo to regulate the affinity and cooperativity of the insulin receptor, analogous to the role of 2,3 diphosphoglycerate in regulating the binding and dissociation of oxygen and hemoglobin. It is important to emphasize that receptor concentration, as well as receptor affinity, are probably subject to numerous other potential influences, including the effects of other hormones, dietary composition, age, metabolic alterations such as acidosis or ketosis, as well as stages of growth, development, and differentiation. In addition, observations here and elsewhere of significant changes in plasma insulin levels without concomitant changes in receptor concentrations (as well as the converse) should serve to emphasize that the ambient insulin concentration is but one of the many influences on the receptor (38–40).

In summary, we have demonstrated that an insulin receptor undergoes changes in both its affinity and concentration in vivo. By coordinating changes in receptor affinity and receptor concentration with other regulatory events within the cell, the target cell is thereby capable of a broad range of responses to hormonal stimuli.

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