Correlation between Insulin Receptor Binding in Isolated Fat Cells and Insulin Sensitivity in Obese Human Subjects

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ABSTRACT This study examined the relationship between receptor binding of insulin in a metabolically significant target tissue in vitro and sensitivity to insulin in vivo in obese human subjects. Specific insulin binding was measured at 24°C in isolated enlarged fat cells obtained from 16 patients, by observing the effect of increasing concentrations of unlabeled insulin on the binding of [^{125}I]insulin. Scatchard plots of the binding data were curvilinear with an upward concavity, similarly shaped, and essentially parallel. Kinetic studies on the dissociation of [^{125}I]insulin from fat cells indicated that these curvilinear Scatchard plots could be explained by the presence of site:site interactions of the negative cooperative type. Differences in binding between individual patients were predominantly due to differences in the numbers of receptor sites whether expressed in relation to cell number, cell volume, or cell surface area. These findings were not accounted for by differences in [^{125}I]insulin degradation. Acute exposure of adipose tissue to insulin in vitro had no significant effect on [^{125}I]insulin binding to isolated cells. The number of receptor sites was directly correlated with insulin sensitivity in vivo, measured as the rate constant (K_{itt}) for the fall in blood glucose after intravenous insulin, and was inversely correlated with the level of fasting plasma insulin. These findings corroborate those from other studies using human mononuclear leukocytes and various tissues from the obese mouse, which indicate that decreased insulin binding is a characteristic feature of insulin resistance in obesity.

INTRODUCTION

Obesity is characterized by hyperinsulinemia both in the basal state and after various insulin secretagogues, and by tissue resistance to the action of both endogenous and exogenous insulin (1). The initial step in the action of insulin is its interaction with specific receptors on the surface membrane of the cell (2). Recently, a number of in vitro studies have shown that obesity in animals is associated with a decrease in the concentration of normal insulin receptors on cell membranes (3). This phenomenon has been demonstrated in the plasma membranes of fat cells (4), liver cells (5, 6), and cardiac muscle (7) as well as in thymic lymphocytes (8) from the obese hyperglycemic (ob/ob) mouse, and in isolated fat cells from rats with acquired obesity (9). A defect in insulin binding to circulating mononuclear leukocytes from obese humans (10) has recently been shown to be due to a decrease in receptor concentration (11). However the role of the insulin receptor in human obesity has not been extensively studied in metabolically significant target tissues. Olefsky et al. (12) first described the characteristics of insulin binding to human fat cells. Amatruda et al. (13) then reported that contrary to the results in monocytes, there was no difference in insulin binding per fat cell between obese and normal weight subjects. However, one brief symposium report by Marinetti et al. (14) had earlier suggested that insulin binding to fat cells from obese subjects was decreased.

The present study was designed to examine the relationships between insulin-receptor[1] binding in isolated fat cells from obese subjects and indices of insulin sensitivity in these subjects, and to further characterize the nature of insulin binding to human fat cells.

METHODS

Patient assessment. Of the 16 patients studied, 15 were obese patients admitted to The Royal Melbourne Hospital

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[1] Receptor is here defined as meaning specific binding site.

TABLE I

Clinical and Experimental Findings in Individual Subjects

Patient	Sex	Age	Relative weight	Mean fat cell volume	Fasting blood glucose	Fasting plasma insulin	K_{itt}*	B_{max}‡	R_0§
		yr		*ml × 10⁷*	*mg/100 ml*	*μU/ml*	*%/min*	*nmol/10⁹ cells*	
T. B.	M	24	2.39	9.29	99	54	3.00	0.185	0.430
P. S.	M	34	2.33	9.58	104	56	—	0.130	0.268
L.Y.	F	36	2.70	8.17	123	45	0.59	0.145	0.205
L. O.	F	30	2.12	8.54	54	13	2.39	0.220	0.415
R. W.	M	32	1.76	10.17	75	30	1.73	0.265	0.425
J. H.	F	39	1.91	6.39	54	14	4.33	0.260	0.525
M. W.	F	31	1.80	9.92	93	34	1.05	0.105	0.175
W. T.	F	49	2.40	6.02	73	15	4.84	0.495	0.905
M. M.	F	34	2.05	10.44	75	37	0.93	0.160	0.200
G. S.	F	52	2.36	9.27	108	25	1.31	0.140	0.215
C. P.	F	25	2.03	9.84	81	10	4.62	0.340	0.885
M. R.	F	41	2.20	10.23	72	20	1.73	0.170	0.320
L. S.	F	33	1.73	7.88	79	18	1.56	0.225	0.405
N. N.	F	21	2.68	10.37	63	28	2.72	0.180	0.445
N. W.	F	34	2.40	10.58	75	56	0.74	0.025	0.045
J. A.	F	30	1.00	9.93	90	45	1.58	0.165	0.280

* K_{itt} is the rate constant for the fall in blood glucose after 0.05 U/kg intravenous insulin.

‡ B_{max} is the insulin bound at an insulin concentration of 16.7 nM.

§ R_0, the maximal binding capacity, was estimated by extrapolation of the Scatchard plot (Fig. 2) to the abscissa.

Professorial Medical Unit primarily for jejunoileal bypass operation. The other subject (J. A.) had Werner's syndrome (short stature and early aging), and although of normal body weight, was studied because of hyperinsulinemia and known fat cell enlargement. Informed consent to the study was obtained from all the patients. They were fed a 2,500–3,000-cal diet containing 37% carbohydrate, 33% fat, and 30% protein. On the 4th day, after a 12-h overnight fast, an oral glucose tolerance test was performed with a glucose load of 40 g/m² body surface area, to a maximum of 100 g glucose. Glucose and insulin areas were calculated as the total integrated areas above the base line and as the integrated areas above fasting values. The insulinogenic index was calculated as the ratio of insulin to glucose areas. On the 5th or 6th day, after a 12-h overnight fast, an insulin tolerance test was performed with a dose of 0.05 U/kg neutral regular porcine insulin (Novo Research Institute, Copenhagen, Denmark). K_{itt}, the rate constant for the fall in blood glucose, was calculated with the value for the half-time of glucose disappearance (15).[2] The lower limit of the range for K_{itt} in normal weight, nondiabetic adults in our laboratory is 3.0 %/min.

Blood glucose was measured on the AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N. Y.) by the method of Hoffman (16) (normal fasting range 60–100 mg/100 ml). Plasma immunoreactive insulin was measured in duplicate with a charcoal separation technique (17) (normal fasting range 3–20 μU/ml).

Insulin binding studies. Abdominal subcutaneous adipose tissue samples were obtained at the beginning of operation in all subjects (including J. A.) on the 8th day, after a 12–14-h overnight fast. General anesthesia was induced with a barbiturate derivative and was maintained with a N_2O-halothane-O_2 mixture. Tissue was collected and washed in warm (37°C) Krebs-Ringer-bicarbonate buffer, pH 7.4, containing 54 mg/100 ml glucose and 1% insulin-free human albumin (Commonwealth Serum Laboratories, Melbourne, Australia). This buffer was used for all procedures, including binding studies. Isolated fat cells were prepared according to the method of Rodbell (18) and washed three times by flotation in buffer at 24°C before use. [^{125}I]Insulin was prepared from human monocomponent insulin (Novo Research Institute), by a modification of the chloramine-T method (19) and separated from free I^- on a cellulose column (20). This preparation was further purified by Sephadex G-50 chromatography (South Seas, Pty. Ltd., North Ryde, N. S. W., Australia) to remove a high molecular weight component. The specific activity of the purified [^{125}I]insulin, determined by radioimmunoassay, was 120–150 μCi/μg (average of 0.4–0.5 iodine atoms per insulin molecule). Its biological activity (conversion of [1-^{14}C]glucose to $^{14}CO_2$ by isolated rat fat cells) was identical with that of unlabeled human insulin.

Binding studies were performed in 5-ml polyethylene tubes; 0.3 ml of fat cell suspension (1.3–3.3 × 10^5 cells/ml) was added to 0.2 ml of buffer containing [^{125}I]insulin (final concentration 0.011–0.125 nM) and unlabeled human insulin in a final concentration range up to 0.67 μM. Incubations were performed in triplicate at each concentration of unlabeled insulin. The tubes were gently shaken for 30 min at 24°C and then rapidly cooled in iced water. Fat cells were immediately separated from the incubation medium by centrifuging 0.3-ml aliquots of each incubate through dinonyl phthalate in Beckman microfuge tubes (Beckman Instruments, Inc., Fullerton, Calif.), according to the method of Gliemann (21). Bound radioactivity in the excised cells and free radioactivity in an aliquot of the infranatant was measured in a gamma counter. Insulin degradation was estimated by measuring the ability of

[2] *Abbreviations used in this paper*: B_{max}, insulin specifically bound at an insulin concentration of 16.7 nM; K_{itt}, rate constant for the fall in blood glucose; R_0, maximal binding capacity.

[^{125}I]insulin in an aliquot of the infranatant to precipitate with 10% TCA or to bind to excess antiinsulin antibody, compared with that of [^{125}I]insulin not incubated with cells.

The maximum radioactivity bound to the cells ranged from 0.3 to 7.9% of the tracer [^{125}I]insulin (mean 2.1%), of which 10–20% was nonspecifically bound, i.e., nondisplaceable by the highest concentration of unlabeled insulin used (0.67 μM). Blank binding (in the absence of cells) was always insignificant. Specific, receptor-bound radioactivity was calculated as the difference between total and nonspecific-bound radioactivity. Binding was normalized to a standard cell number (10^9) on the basis that specific binding of tracer concentrations of [^{125}I]insulin was a linear function of cell number up to 3.1×10^5 cells/ml, the maximum concentration tested (data not shown). It should be noted that the insulin concentration of the extracellular phase (including the contribution of [^{125}I]insulin mass) was determined by correcting the incubation suspension for fat cell volume, before calculating binding results for each subject.

Fat cell size and viability and lipid mass were estimated as previously described (22).

RESULTS

Table I summarizes the findings in individual subjects. Two subjects (L. Y. and G. S.) were diagnosed by the oral glucose tolerance test as having adult-type diabetes with hyperinsulinemia.

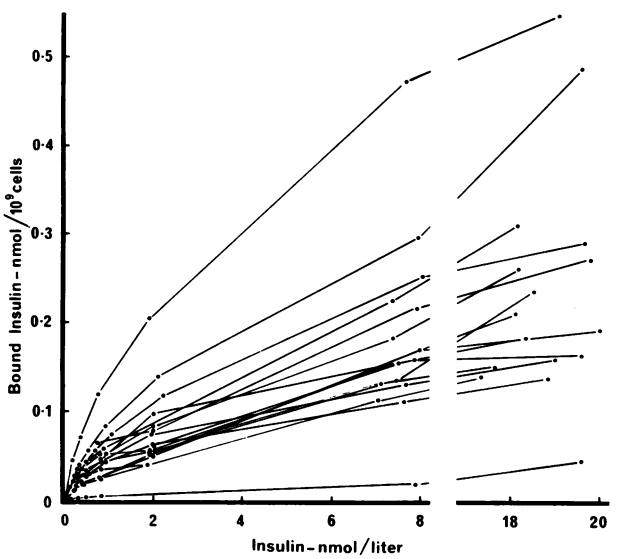

FIGURE 1 Insulin binding to isolated fat cells in 16 obese subjects. Fat cells were incubated for 30 min at 24°C with [^{125}I]insulin (0.011–0.125 nM) and unlabeled insulin in increasing concentrations up to 0.67 μM. Incubations were performed in triplicate at each concentration of unlabeled insulin. Cells were separated from the medium by centrifugation through the oil dinonyl phthalate at 4°C. Radioactivity bound to the cells in the presence of the highest concentration of unlabeled insulin (0.67 μM) was called nonspecific binding; this was subtracted from the total radioactivity bound at each insulin concentration to obtain specific, or receptor-bound, radioactivity. After correcting for the fat cell volume in the incubate suspension and taking into account the contribution of [^{125}I]insulin mass, specific bound [^{125}I]insulin radioactivity was used to calculate the total amount of insulin specifically bound at each concentration of unlabeled insulin.

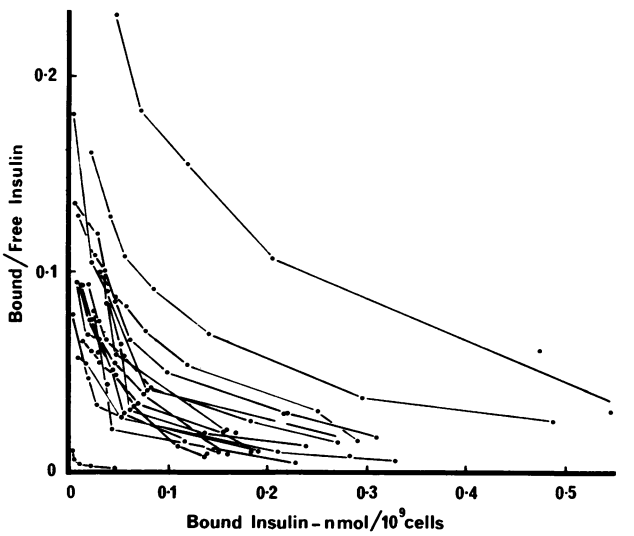

FIGURE 2 Scatchard analysis of the insulin binding data. Bound/free insulin was expressed as a function of bound insulin and a series of curves, all concave upwards and approximately parallel, were obtained. This suggests that the apparent overall affinity of the fat cell insulin receptors was similar in different subjects, but decreased as the fractional saturation of the receptor population increased. Differences in binding capacity are indicated by differences in the position of individual curves. Maximal binding capacity, R_0, was obtained by extrapolation of each curve to the abscissa.

Insulin binding in individual subjects. Specific bound [^{125}I]insulin radioactivity was used to calculate the total amount of insulin specifically bound at each concentration of unlabeled insulin used and a series of binding curves obtained (Fig. 1). The most obvious difference between subjects is in the amount of insulin bound for a given insulin concentration; this suggests that individual subjects differed in the number of receptors per cell able to bind insulin. This conclusion is supported by Scatchard analysis (23) of the binding data (Fig. 2). When the bound/free insulin is expressed as a function of the bound insulin, a series of similarly shaped, essentially parallel curves are obtained. These types of Scatchard plots, concave upwards, indicate a decreasing affinity of the insulin receptor population as fractional saturation increases. They may result from the presence of two or more discrete populations of receptors with differing affinities (24), from site:site interactions of the negative cooperative type (25), or from both. De Meyts and Roth have demonstrated that dissociation of [^{125}I]insulin caused by infinite dilution is enhanced by the addition of unlabeled insulin, a finding consistent with a negative cooperative model in which a homogenous population of insulin receptors switches from a high to a low affinity state as receptor occu-

pancy increases. The dissociation rate of [^{125}I]insulin from human fat cells was significantly greater with dilution plus unlabeled insulin (83 nM) than with dilution alone (Fig. 3), indicating that the curvilinear Scatchard plots (Fig. 2) could be explained, at least in part, by the presence of negative cooperativity. Under such circumstances, the estimation of binding affinities becomes difficult (26). It can be seen, however, (Fig. 2) that for a given fractional saturation the slopes of the curves are approximately the same; this parallelism reflects similar apparent binding affinities between subjects.

In the presence of cooperativity, the Hill plot (27) provides a method for assessing the average affinity and for estimating cooperative interactions. Data for the majority of subjects (11/16) were superimposable (Fig. 4). All lines had a slope (Hill coefficient) of 0.88; slopes of less than 1 are consistent with negative cooperativity. When the untransformed binding data for all subjects were examined, the insulin concentration causing 50% inhibition of specific [^{125}I]-insulin binding was 1.26±0.10 nM (mean±SEM).

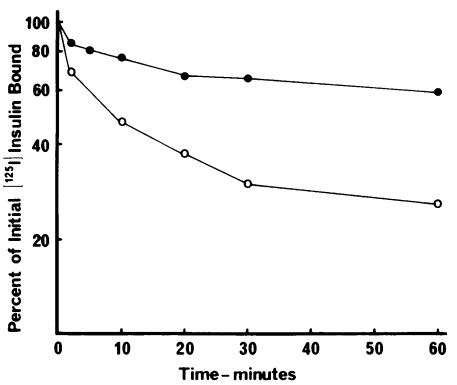

FIGURE 3 Time course of dissociation of [^{125}I]insulin from isolated fat cells in the presence of dilution alone (●—●) and dilution plus 83 nM of unlabeled insulin (○—○). A 20-ml magnetically stirred suspension of isolated fat cells (1.2×10^5 cells/ml) was incubated with [^{125}I]insulin (0.13 nM) for 30 min at 24°C. Duplicate 400-μl aliquots were removed for measurement of cell-bound radioactivity (time 0 min in figure) as described in the legend to Fig. 1. The cells were then allowed to float to the surface and their infranatant medium aspirated via a thin catheter. The cells were washed twice with cold (4°C) buffer and the suspension divided into two 10-ml lots. Cold buffer was aspirated from each and replaced with 20 ml of buffer at 24°C, containing in one case 83 nM of unlabeled insulin. Duplicate 400-μl aliquots were then removed from each of the stirred suspensions at 2, 5, 10, 20, 30, and 60 min for measurement of cell-bound radioactivity. Nonspecific binding, determined in identical experiments in which the incubation mixture also contained 16.5 μM of unlabeled insulin, amounted to 20% of the total binding. Approximately one-half of the nonspecifically bound counts dissociated within the first 2 min; the remainder showed no tendancy to dissociate with time in the presence or absence of unlabeled insulin.

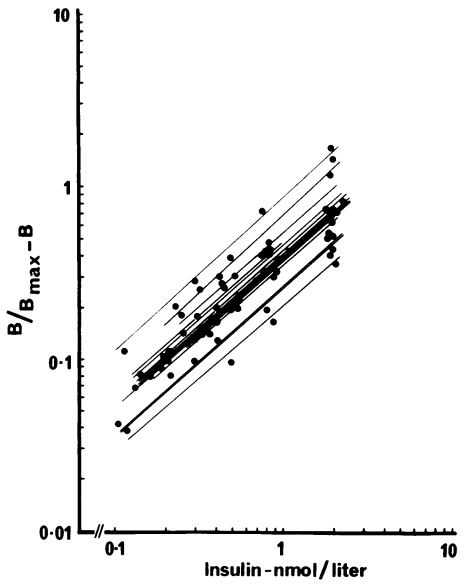

FIGURE 4 Hill analysis of the insulin binding data. Insulin specifically bound to isolated fat cells was measured as described in the legend to Fig. 1. B_{max} was taken to be the insulin specifically bound at an insulin concentration of 16.7 nM. Log $B/(B_{max}-B)$ was plotted as a function of log-free insulin, where B is the insulin specifically bound at the given free insulin concentration. The slope of all plots (Hill coefficient) was 0.88, and the majority were superimposable indicating similar average binding affinities.

The binding capacity (receptor number) for each subject was estimated: (*a*) as maximal binding capacity, R_0, by extrapolation of the Scatchard plot to the abscissa, (26), and (*b*) as insulin specifically bound, B_{max}, at an insulin concentration of 16.7 nM (Fig. 1) (Table I).[3]

Relationships of fat cell insulin binding to insulin sensitivity in vivo. There was an inverse correlation between fasting plasma insulin and both R_0 ($r = -0.65$, $P < 0.01$, $n = 16$) and B_{max} ($r = -0.66$, $P < 0.01$, $n = 16$), and a direct correlation between K_{itt} and both R_0 ($r = 0.93$, $P < 0.001$, $n = 15$) and B_{max} ($r = 0.82$, $P < 0.001$, $n = 15$). These relationships for B_{max} are shown in Fig. 5. Neither of the in vitro binding parameters was correlated with the insulin area under the oral glucose tolerance curve or with the insulinogenic index. Fat cell volume

[3] B_{max} should be roughly proportional to the maximal binding capacity. It was chosen as an operational value because of the difficulty in precisely estimating R_0. The contribution of nonspecific binding to total binding at high concentrations of unlabeled insulin is fractionally greater, and specific binding is difficult to determine accurately above an insulin concentration of 16.7 nM. Furthermore, it is not possible to be sure that the terminal points of the Scatchard plot have been accurately extrapolated to the abscissa.

was inversely related to both R_0 ($r = -0.62$, $P = 0.01$, $n = 16$) and B_{max} ($r = -0.63$, $P < 0.01$, $n = 16$). There was no relationship between age or relative weight and cell binding. The in vivo indices of insulin sensitivity (fasting plasma insulin, K_{itt}, insulin area, and insulinogenic index) showed no relationship to the concentration of insulin required to produce 50% inhibition of specific [^{125}I]insulin binding.

The differences between subjects and the relationships described above were present when binding was expressed not only on a cell basis (per 10^9 cells), but also on the basis of cell surface area or cell volume.

Insulin degradation vs. insulin binding. Insulin degradation assessed by binding to excess anti-insulin antibody was consistently greater than when assessed by TCA precipitation, and averaged 11.8% (range 0–20%). Binding data were not corrected for insulin degradation. Differences in insulin binding between subjects could not be accounted for by differences in insulin degradation.

Acute effect of ambient insulin levels of fat cell binding. To ascertain whether the differences in binding capacity between patients could be due to occupation of receptors by insulin in the plasma at the time of biopsy, the following experiment was performed. Adipose tissue pieces were divided into two lots and preincubated with and without unlabeled insulin (0.83 nM) for 1 h at 37°C. The tissues were washed, isolated cells were prepared, and binding studies were performed in the routine manner. In three such experiments there was no significant difference in binding capacity between cells from tissues preincubated with and without insulin.

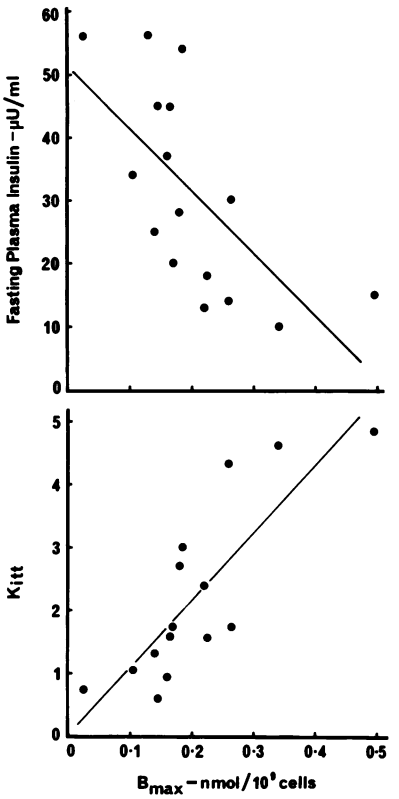

FIGURE 5 Relationship in obese subjects between B_{max} the insulin specifically bound to fat cells at an insulin concentration of 16.7 nM, and fasting plasma insulin ($r = -0.66$, $P < 0.01$, $n = 16$) and K_{itt}, the rate constant for the fall in blood glucose after 0.05 U/kg intravenous insulin ($r = 0.82$, $P < 0.001$, $n = 15$).

DISCUSSION

A number of studies in animals (see review, [3]) indicate that a decrease in the number of insulin receptors per cell is a characteristic feature of insulin resistance in obesity. In human obesity, Archer et al. (10) reported that insulin binding to circulating monocytes was decreased; this has recently been shown by Bar et al. (11) to be due to a decrease in insulin receptor concentration. A brief report by Marinetti et al. (14) had earlier described decreased insulin binding to fat cells from obese subjects, but Amatruda et al. (13) were unable to confirm this. The present study has shown that the number of insulin receptors on fat cells is directly related to insulin sensitivity in obese humans. This finding is not surprising, since the changes in insulin binding in obese animals are present not only in circulating monocytes but also in liver, fat, and muscle (3). The insulin receptor was here defined purely in terms of specific physicochemical binding. It is reasonable, though, to assume that we are dealing with a site which mediates the biological action of insulin, since K_{itt} correlated with insulin binding and since K_{itt} has previously been shown (28) to correlate with the insulin sensitivity of glucose oxidation in adipose tissue from obese subjects. Our findings support the emerging concept that a decreased cell concentration of receptors is related to a decreased effect of insulin in obese subjects and are consistent with the hypothesis that plasma insulin modulates the concentration of its own receptors in target tissues (3, 6).

Our results contrast with those of Amatruda et al. (13) who failed to demonstrate a difference in fat cell insulin binding between groups of normal and obese subjects. Their measurement of binding by competition of increasing amounts of unlabeled insulin with [^{125}I]insulin is similar in principle to ours, except that they reacted cells with a much higher concentration of tracer [^{125}I]insulin (4.8 nM) before the addition of unlabeled insulin. Fat cell insulin sensitivity (29) and insulin binding (6, 10) are closely

related to diet. Thus, receptor deficiency in obesity can be partially corrected by chronic calorie restriction with a fall in plasma insulin (6, 10); by contrast, receptor affinity rather than number is increased in obese subjects by an acute 72-h fast (11). It is therefore possible that antecedent dietary conditions, not specifically stated by Amatruda et al. (13), could account for these discrepant results with human fat cells. The presentation of their data does not allow direct comparison with our results and we therefore cannot reconcile them. However, both studies indicate that the number of insulin receptors per unit of surface area is reduced in large cells, the more so in the present study where the number of receptors per cell or per unit volume of cell lipid is also reduced.

Our study design does not allow us to examine independently the effects of fat cell size and plasma insulin on insulin binding. Recent evidence, however, suggests that cell size is not a primary determinant of insulin sensitivity (29) and that insulin binding is more a function of the prevailing plasma insulin level than body weight or fat cell size (6, 9).

There are obvious difficulties in determining the reproducibility of binding data in individual human subjects. A repeat experiment was performed in only one patient (J. A.) and showed, with the same preoperative conditions and plasma insulin level, an identical binding curve (data not shown). In any case, lack of reproducibility of binding curves would tend to obscure relationships, rather than produce them artificially. On the other hand, a number of factors could have caused artifactual differences between patients. Anesthetic agents, by their very nature, alter certain membrane properties and are concentrated in fat; they should be considered as a possible influence on binding results. Our subjects, however, were exposed to the same anesthetics for a similar period. Indirect evidence for the absence of a significant anesthetic effect is that the insulin-stimulated glucose oxidation rate in fat obtained under the present conditions was similar to that in fat obtained under local 1% lignocaine skin anesthesia (22). Differences in cell populations (apart from cell size) were excluded by always using subcutaneous adipose tissue from the anterior abdominal wall. Adipose tissue was digested with the same batch of collagenase and treated identically in all cases. Cell viability was checked routinely when sizing cells (22). Although receptor degradation was not measured in each case, previous studies (unpublished) showed that this was not significant for 1–2 h after washing collagenase-liberated cells, and then progressed with a half-time of degradation of 4–6-h. Olefsky et al. (12) obtained similar results when first describing the characteristics of insulin binding to human fat cells. On this basis the timing of the binding experiments would have excluded any significant effect of receptor degradation, although differences between individual subjects could still be present. ^{125}I-insulin degradation was measured in each case and was shown to bear no relationship to differences in binding between subjects. The absence of an acute effect of insulin on binding has been shown previously (5, 6, 8, 10). The lack of effect of insulin preincubated with adipose tissue in vitro on the subsequent binding by isolated fat cells suggests that receptor occupancy by insulin in the plasma at the time of biopsy does not account for intersubject differences. The similarity of the slopes of the Scatchard plots and the fact that the majority of the Hill plots were superimposable implies also that initial degrees of receptor occupancy were similar.

This study does not preclude the possibility that, in some situations, obese individuals may be characterized by different insulin binding affinities. In the presence of negative cooperativity the binding affinity depends upon the fraction of receptors occupied. The problem of expressing affinity in these circumstances has been mentioned and recently dealt with by De Meyts and Roth (26). With respect to the concentration of insulin required to cause 50% inhibition of [^{125}I]insulin binding, differences between patients were small and were not systematically related to any of the other parameters measured. It therefore seems unlikely that receptor affinity is a significant determinant of the differences in insulin sensitivity in these obese subjects. It is also of interest that our estimate of 1.26 nM for receptor affinity compares favorably with that of 1.43 obtained by Olefsky et al. (12) for normal fat cells.

It has been proposed (3) that a decrease in insulin receptor concentration may be an example of negative feedback regulation by circulating insulin, and evidence is accumulating to suggest that hormone modulation of hormone receptors is a general physiological phenomenon (3, 30). Our results, while consistent with this hypothesis, only show that the number and concentration of insulin receptors per fat cell are closely related to insulin sensitivity or plasma insulin, and do not demonstrate causality. Whether high insulin levels lead to low receptor concentrations or vice versa, or whether both mechanisms might apply in different circumstances, is yet unclear. Furthermore, the significance of the reduced receptor concentrations in terms of insulin-mediated metabolism remains unknown. In any case, since adipose tissue even in obesity accounts for only a small percentage of glucose turnover (31), a decrease in fat cell insulin receptor concentration could not be the cause of insulin resistance in vivo. The changes in fat cells would appear, therefore, to reflect those in more significant insulin target tissues such as the liver.

Note added in proof. At the time this manuscript was submitted for publication Olefsky (32) reported decreased insulin binding to fat cells and circulating monocytes from obese subjects. Decreased binding was present only in those subjects with hyperinsulinemia. There was a significant inverse correlation between the fasting plasma insulin level and fat cell insulin binding. The present study confirms this relationship and also shows that binding, in particular receptor number, is closely correlated with a direct index of tissue insulin sensitivity, the K_{itt}.

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