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Decreased insulin binding to adipocytes and circulating monocytes from obese subjects.

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Research Article

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Decreased Insulin Binding to Adipocytes and Circulating Monocytes from Obese Subjects

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ABSTRACT Insulin binding to isolated adipocytes from 16 normal and 14 obese patients was studied. The data indicated that, as a group, adipocytes from the obese patients bound significantly less insulin than normal. However, of the 14 obese patients, 5 were not hyperinsulinemic and 4 of these 5 subjects had normal insulin binding. These subjects were also younger, and had the onset of obesity in childhood. When these five patients were separated from the original 14 obese patients, enhanced differences in insulin binding to adipocytes were observed when normals and the remaining 9 obese subjects were compared. Similar findings were obtained with isolated circulating mononuclear cells from these same patients. Presumably the five normoinsulinemic obese patients were not insulin-resistant, and, thus, the data indicate that insulin binding to adipocytes was decreased only in insulin-resistant obese patients. This conclusion was strengthened by finding a highly significant correlation ($r = -0.71$, $P < 0.001$) between insulin binding to adipocytes and fasting plasma insulin level, while a weaker correlation ($r = -0.49$, $P < 0.01$) existed between insulin binding and degree of obesity. Finally, when insulin binding to adipocytes and mononuclear cells from the same individual was compared, a significant positive correlation was found ($r = 0.53$, $P < 0.01$). In conclusion: (*a*) insulin binding to adipocytes and mononuclear cells is decreased in cells from insulin-resistant obese patients; (*b*) a significant inverse relationship exists between fasting plasma insulin level and insulin binding to adipocytes; and (*c*) in obesity, events that affect insulin receptors on adipocytes similarly affect insulin receptors on mononuclear cells.

INTRODUCTION

Insulin resistance and hyperinsulinemia are well-described features of obesity. Recently, decreased insulin binding to tissues from genetically (1–3) and spontaneously (4) obese rodents has been described, and the potential relationships between hyperinsulinemia, decreased insulin receptors, and insulin resistance have been discussed (3, 4). Similar results have been obtained in humans. Thus, Archer et al. (5, 6) have demonstrated decreased insulin binding to circulating mononuclear cells obtained from obese patients, and have also found that insulin binding increases after weight loss. Furthermore, Marinetti et al., in a brief report, described decreased insulin binding to adipocytes isolated from obese subjects (7). However, decreased insulin binding to tissues from obese human subjects has not been found by all workers; Amatruda et al. (8) have reported that adipocytes from lean and obese subjects bind comparable amounts of insulin.

It might be argued that the different results of Archer et al. (6) and Amatruda et al. (8) are due to tissue differences, and that the results with adipocytes are more important, since adipocytes represent a more relevant insulin target tissue (9). However, this seems unlikely, because previous studies have demonstrated that mononuclear cells accurately mirror the changes in insulin receptors occurring at target tissues (3). Thus, it seems possible that differences in study groups, especially in terms of in vivo insulin resistance, may be responsible for these seemingly disparate reports (6–8). Consequently, we have studied insulin binding to adipocyte and mononuclear cell insulin receptors in normal and obese subjects, and have attempted to relate these measurements to in vivo aspects of insulin metabolism. The results indicate that insulin binding is decreased to tissues from obese subjects, if the subjects studied are insulin-resistant.

METHODS

Materials. Porcine monocomponent insulin was generously supplied by Dr. Ronald Chance of the Eli Lilly and Company (Indianapolis, Ind.). Na^{125} was purchased from the New England Nuclear Co. (Boston, Mass.), bovine serum albumin (fraction V) from Armour Pharmaceutical

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Company (Chicago, Ill.), and collagenase from Worthington Biochemical Corp. (Freehold, N. J.).

Subjects. The clinical and metabolic data of the study group are given in Table I. For these studies, a subject was considered obese if his relative weight exceeded 135% of ideal according to Metropolitan Life tables, while subjects were considered normal if their relative weight was less than 110%. All studies were performed while patients were hospitalized in the Stanford General Clinical Research Center. No patient had a fasting plasma glucose level over 110 mg/100 ml or was receiving any drug known to affect glucose or insulin metabolism.

Preparation of tissues. Insulin binding to adipocytes was measured in tissue obtained by open biopsy from the left lower abdominal wall of unanesthetized patients. To obviate any possible effect of the local anesthetic, 2% Xylocaine (lidocaine, Astra Pharmaceutical Products, Inc., Worcester, Mass.) was infiltrated in a square-field fashion, and the biopsy was obtained from the center of the square. As previously described (9), isolated fat cells were prepared by shaking at 37°C for 60 min in Krebs-Ringer bicarbonate buffer containing collagenase (3 ng/ml) and albumin (40 mg/ml), by the method of Rodbell (10). Adipocyte counts were performed by a modification of method III of Hirsch and Gallian (11), in which the cells are fixed in 2% osmium tetroxide in 0.05 M collidine buffer (made isotonic with saline) for 72 h at 37°C, and then taken up in a known volume of 0.154 M NaCl for counting. Counting was performed with a Celluscope Model 112H particle counter with a 400 μM aperture (Particle Data, Inc., Elmhurst, Ill.). Adipocyte size was determined with a calibrated microscope by the method of Di Girolamo et al. (12). With

TABLE I

Clinical and Metabolic Data of the Study Group

Subject	Age	Relative wt	Fasting plasma insulin level	Insulin specifically bound to adipocytes[1]	Insulin specifically bound to mononuclear leukocytes*	Mean adipocyte surface area
	yr	%	*μU/ml*	*%/2 × 10⁵ cells*	*%/50 × 10⁶ cells*	*μm² × 10³*
Obese						
1	39	148	49	.81	—	38.7
2	42	137	31	1.70	—	31.4
3	56	156	35	1.52	—	35.3
4	50	137	28	1.52	—	26.6
5	63	161	40	.90	—	39.4
6	50	152	63	1.16	1.75	40.1
7	50	141	25	1.50	3.16	25.4
8	58	138	22	1.28	3.73	26.6
9	51	143	28	1.26	2.17	23.2
10	51	135	17	1.44	1.57	26.0
11	24	166	9	3.08	4.82	45.2
12	29	138	19	2.58	3.86	38.0
13	33	146	18	2.16	3.78	22.2
14	26	145	9	1.92	3.91	24.3
Mean ±SE	44±3	146±3	28±4	1.63±0.17	3.2±0.37	31.7±2.0
Normals						
15	51	93	4	2.02	4.42	16.7
16	37	110	18	3.30	2.73	24.1
17	47	86	12	1.86	2.93	19.1
18	47	101	6	2.42	3.58	20.1
19	50	110	15	2.28	3.46	24.3
20	26	101	2	2.72	7.11	18.1
21	60	92	16	2.30	4.63	21.1
22	25	94	9	2.08	4.92	22.2
23	26	100	8	2.82	4.52	15.8
24	34	103	8	2.40	4.16	16.2
25	47	92	9	1.88	3.74	21.6
26	58	98	6	1.96	3.10	19.6
27	24	101	8	2.42	5.38	21.1
28	61	103	5	2.32	4.11	20.1
29	27	92	9	2.96	3.67	19.1
30	36	89	8	2.68	4.54	16.2
Mean ±SE	41±3	98±2	9±1	2.4±0.1	4.19±0.27	19.7±0.67

* Data obtained at an insulin concentration of 0.4 ng/ml for adipocytes, and 0.2 ng/ml for mononuclear leukocytes.

this method, the mean adipocyte volume was 261 pl/cell for the 16 normal subjects, and 539 pl/cell for the 14 obese patients.

Isolated circulating mononuclear cells were prepared from heparinized blood obtained after an overnight fast. As previously described by Gavin et al. (13), plasma and buffy coat were removed from 120 ml of whole blood and transferred to a Ficoll-Hypaque gradient for cell fractionation by the method of Böyum (14). Cells prepared in this manner contain a mixed population of monocytes, bone marrow-derived lymphocytes (B cells), thymus-derived lymphocytes (T cells), and a small proportion of granulocytes (1.1±1%). Monocytes were quantitated with the alpha naphthyl esterase stain as described by Yam et al. (15), and the monocyte content of the cell preparations averaged 14±3%. This is higher than previously reported (16, 17), due to this more specific method of monocyte identification. B cells and T cells were determined by previously described methods (18), and the total lymphocyte population was found to consist of 20±3% B cells and 78±10% T cells. Cells were counted in triplicate with a Neubauer chamber and diluted with buffer to the desired concentration for binding studies. Viability, as determined by trypan blue exclusion, was always greater than 97%. No differences in cell viability, or percentages of monocytes, T cells, or B cells were found between the study groups.

Iodination of insulin. ^{125}I-insulin was prepared at a specific activity of 100–150 μCi/μg by the modification of Freychet et al. (19) of the method of Hunter and Greenwood (20).

Binding studies. Isolated fat cells were suspended in a buffer containing 35 mM Tris, 120 mM Na Cl, 1.2 mM Mg SO_4, 2.5 mM KCl, 10 mM glucose, 1 mM EDTA, and 1% bovine serum albumin (20), pH 7.6, and incubated with ^{125}I-insulin and unlabeled insulin in plastic flasks in a 24°C shaking water bath, as previously described (4, 9). Mononuclear cells (50×10^6 cells/ml) were incubated with insulin at 15°C in 0.5 ml of 25 mM Tris buffer (pH 7.6)-1% bovine serum albumin for 100 min as described by Gavin et al. (21). Details concerning the measurement and calculation of the amount of insulin bound to adipocytes and mononuclear cells have previously been published (4, 9, 13, 17). Adipocytes and mononuclear leukocytes from the obese patients behaved identically to cells from control subjects in regard to the time-course of insulin binding, insulin degradation, and receptor degradation under these conditions.

It is theoretically possible that in vivo binding of insulin could result in occupation of receptor sites, leading to "masking" of these sites during the in vitro assay. We have previously shown that this is not the case with rat adipose tissue (4), and Archer et al. (5) have demonstrated that this does not occur with mononuclear leukocytes. To be certain that masking of receptor sites was not happening with human adipose tissue, an adipose tissue specimen was divided into two equal portions and incubated with insulin (25 ng/ml) or with insulin-free buffer for 30 min at 37°C. Cells were then prepared from each portion, and no differences in insulin-binding ability between these two groups of cells were noted. The apparent explanation for this is that any insulin bound to the cell in vivo dissociated from the receptor and was washed away during the cell isolation and washing steps.

Calculations. Specific [^{125}I]iodoinsulin binding was calculated by subtracting the amount of [^{125}I]iodoinsulin nonspecifically bound from the total amount of [^{125}I]iodoinsulin bound at each insulin concentration (2, 4, 17, 21). Nonspecific binding is defined as the amount of [^{125}I]iodoinsulin remaining "bound" in the presence of a large excess—200 μg/ml (30 μM)—of unlabeled insulin (2, 4, 17, 21). The number of receptor sites per cell has been estimated as the number of insulin molecules specifically bound per cell at an insulin concentration of 100 ng/ml (2,400 μU/ml or 17 nM) for reasons previously discussed (4). The number of molecules bound at this insulin concentration can be accurately measured with an intra-assay coefficient of variation of 5% in individual experiments. Scatchard analysis (22) of the mean group data revealed that 70% of the total number of receptors were occupied at an insulin concentration of 100 ng/ml (for both groups of cells) and, consequently, to calculate maximal binding, the amount bound at 100 ng/ml should be multiplied by 1.43. This amount can be converted to total sites per cell by multiplying the amount bound per cell (in moles) by 6.03×10^{23}.

Since mononuclear cell preparations contain a mixed population of cells (monocytes and lymphocytes), and since Schwartz et al. (23) have recently shown that monocytes account for most of the insulin binding in these preparations, we have quantitated the monocyte content of the cell preparations and have normalized the binding data to monocyte content as well as to total mononuclear cell content.

Analytical methods. Plasma insulin concentration was determined according to the method of Desbuquois and Aurbach (24), and plasma glucose level was measured by the glucose oxidase method with a Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, Calif.).

RESULTS

Fig. 1 presents the data on insulin binding to isolated adipocytes. Results are given at two insulin concentrations: Fig. 1A represents the amount of insulin bound at tracer concentrations (0.4 ng/ml or 10 μU/ml), and Fig. 1B gives the amount of insulin bound at a near-saturating insulin concentration (100 ng/ml), which closely reflects the number of insulin receptors per cell (4). The clear bars represent the data from 16 normal subjects, while the shaded bars give the data for the entire group of 14 obese subjects. As can be seen, adipocytes from obese subjects bind less insulin than cells from normals, and this difference is significant at the $P < 0.05$ and 0.01 levels. However, closer examination of the individual data revealed that five of the obese subjects (patients 10–14, Table I) did not have overt hyperinsulinemia (> 20 μU/ml), and from the close correlation between insulin resistance and fasting insulin concentration (25), these patients were probably not insulin-resistant. These patients had fasting plasma insulin levels not significantly greater than normal (14±3 vs. 9±1), but significantly lower (14±3 vs. 37±4) than in the remaining nine obese subjects. In four of these five patients, insulin binding was not decreased, and these subjects were also relatively young and had been obese since early adolescence. Fig. 1 demonstrates that the mean insulin binding data for these five subjects (hatched bars) is indistinguishable from normal. Furthermore, when the remaining nine obese subjects were analyzed as a group (solid bars), the decrease in insulin binding to adipocytes is enhanced. This compari-

son is seen in more detail in Fig. 2. Here the percentage of insulin bound over a wide range of insulin concentrations is given, and it can be seen that adipocytes from the group of nine obese subjects bind significantly less insulin at each insulin concentration than normals. On the other hand, increasing concentrations of unlabeled insulin compete for binding with the ^{125}I-insulin (0.4 ng/ml) in a similar fashion in cells from both groups. Thus, at an unlabeled insulin concentration of 1 ng/ml (24 μU/ml), 10–13% of the ^{125}I-insulin binding

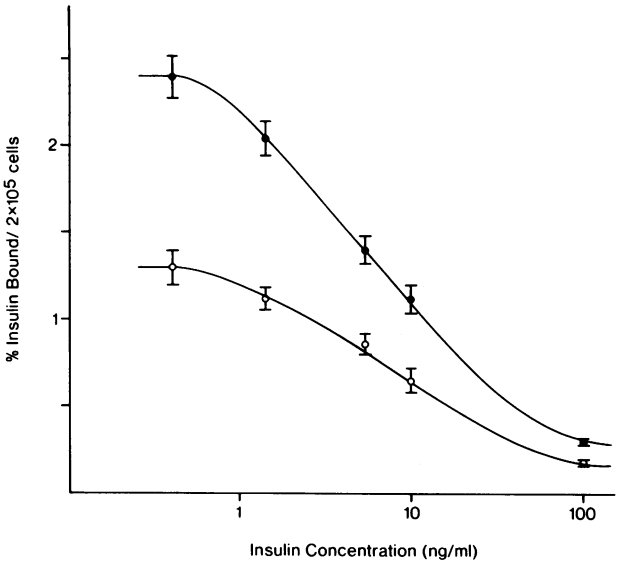

FIGURE 2 Inhibiting effect of unlabeled pork insulin on ^{125}I-insulin binding to isolated adipocytes from normal (●—●) and obese (○—○) subjects. Incubations are performed in the presence of 0.4 ng/ml ^{125}I-insulin and brackets represent ±SE. Data are corrected for nonspecific binding, and the vertical axis is the percent of the ^{125}I-insulin specifically bound at the indicated total insulin concentration. Binding is a linear function of cell concentration up to 3×10^5 cells/ml and all data are normalized to 2×10^5 cells/ml, the average cell concentration used in these studies.

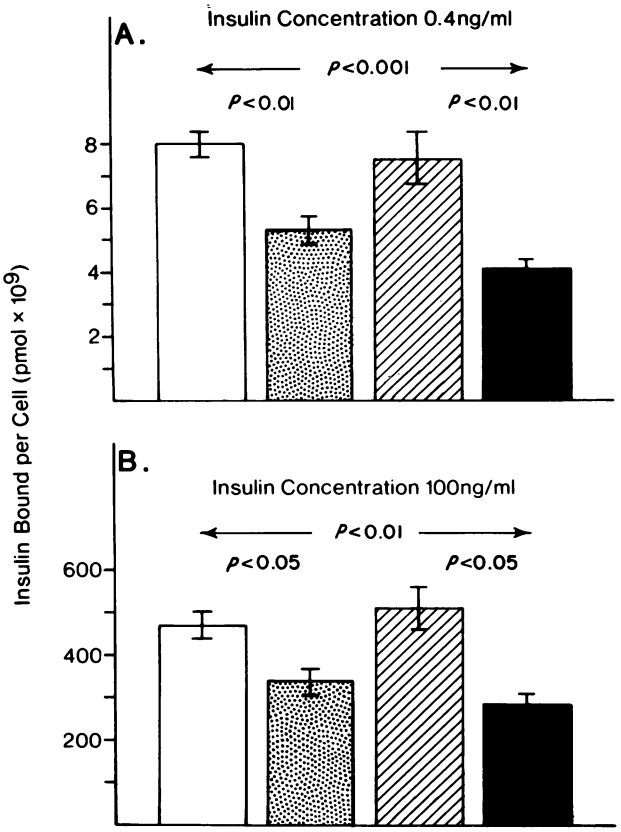

FIGURE 1 Insulin binding to adipocytes from the entire group of 16 normal (clear bars) and 14 obese (shaded bars) subjects. Hatched bars represent the data from the subgroup of five nonhyperinsulinemic obese subjects and the solid bars represent the remaining nine obese subjects. All data are corrected for nonspecific binding. In A, the data represent the mean (±SE) amount of insulin bound per cell at a subsaturating insulin concentration of 0.4 ng/ml (10 μU/ml or 67 pM). In B, the data represent the mean (±SE) amount of insulin bound per cell at an insulin concentration of 100 ng/ml (16.7 nM). For reasons discussed previously (ref. 4, and Methods section), this amount of insulin bound closely reflects the number of insulin receptor sites per cell.

is inhibited, and binding can be 50% inhibited at an insulin concentration of 8–9 ng/ml. All data are corrected for nonspecific binding, which averaged 0.27±0.015 and 0.30±0.017% of the total available insulin for the normal and obese groups, respectively. Since the ability of unlabeled insulin to inhibit the ^{125}I-insulin binding is a reflection of the binding affinity (4, 16), the inhibition curves in Fig. 2 indicate that the affinity of adipocytes for insulin is comparable for both groups (4, 16).[1] Thus, as is also seen in Fig. 1B (see Methods), the decreased insulin binding to cells from obese patients is primarily due to decreased numbers of available receptor sites per cell. Scatchard analysis of these group data reveals that cells from the control subjects contain 290,000 receptor sites/cell (15 sites/μm^2), while adipocytes from the obese subjects contain 180,000 sites/cell (6 sites/μm^2).

Insulin binding to isolated mononuclear cells was also measured in a subset of the above subjects, and these

[1] It should be pointed out that the decrease in percent binding that occurs as the media insulin concentration increases is probably a function of both saturation of receptors and negative cooperativity (26). However, by the method suggested by DeMeyts and Roth (27), one can calculate that only 18% of the decrease in percent binding at an insulin concentration of 100 ng/ml would be due to negative cooperativity.

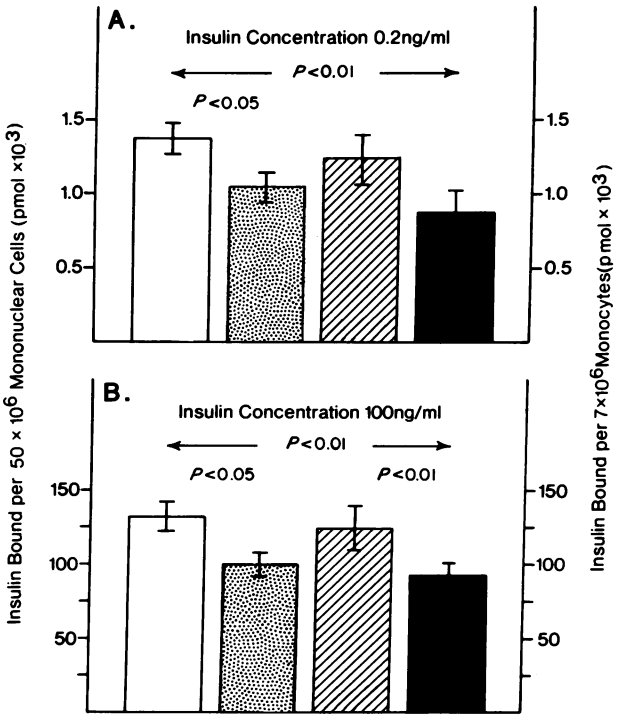

FIGURE 3 Insulin binding per 50×10^6 total mononuclear cells (left-hand vertical axis) or per 7×10^6 monocytes (right-hand vertical axis) in 16 normal (clear bars) and 14 obese (shaded bars) subjects. Hatched bars represent the data from the subgroup of five nonhyperinsulinemic obese subjects and solid bars represent the remaining four obese subjects (five of the older, hyperinsulinemic obese patients did not have mononuclear cell-binding studies). See legend to Fig. 1 for further details.

results are seen in Fig. 3. It should be noted that Fig. 3 gives the data from all 5 of the obese subjects with fasting plasma insulin levels under 20 μU/ml (hatched bars), all of the 16 normals (clear bars), but only 4 of the 9 overtly hyperinsulinemic obese individuals. Nevertheless, the results are quite comparable to those obtained with adipocytes. As discussed in the Methods section, the mononuclear cell preparations contain a mixed population of cells, and Schwartz et al. (23) have recently suggested that of the total mononuclear cells, the monocytes account for most (80–90%) of the insulin binding. Consequently, the data in Fig. 3 are expressed both on the basis of 50×10^6 total monounclear cells (left-hand vertical axis) and 7×10^6 monocytes (right-hand vertical axis). As can be seen, however, since monocyte content was the same for all groups, the comparisons remain unchanged.

These data suggest that insulin binding is decreased in obese subjects, but only if the subjects are specifically defined. Thus, five of the obese subjects had fasting insulin levels that did not exceed the range of normal (under 20 μU/ml). In four of these five subjects insulin binding was not decreased, and these subjects were all young with the onset of obesity in childhood. On the other hand, relative weight was 146% and mean adipocyte volume was 529 pl for the group of five, vs. 146% and 542 pl for the group of nine. Thus, in terms of these two measurements of the degree of obesity, the two groups of obese patients were comparable. To examine more closely the relationships between insulin binding, degree of obesity, and plasma insulin level, the data were analyzed as seen in Fig. 4. In this figure, the amount of insulin bound per adipocyte is plotted as a function of fasting insulin concentration (4A), and relative weight (4B). As can be seen, the correlation between insulin

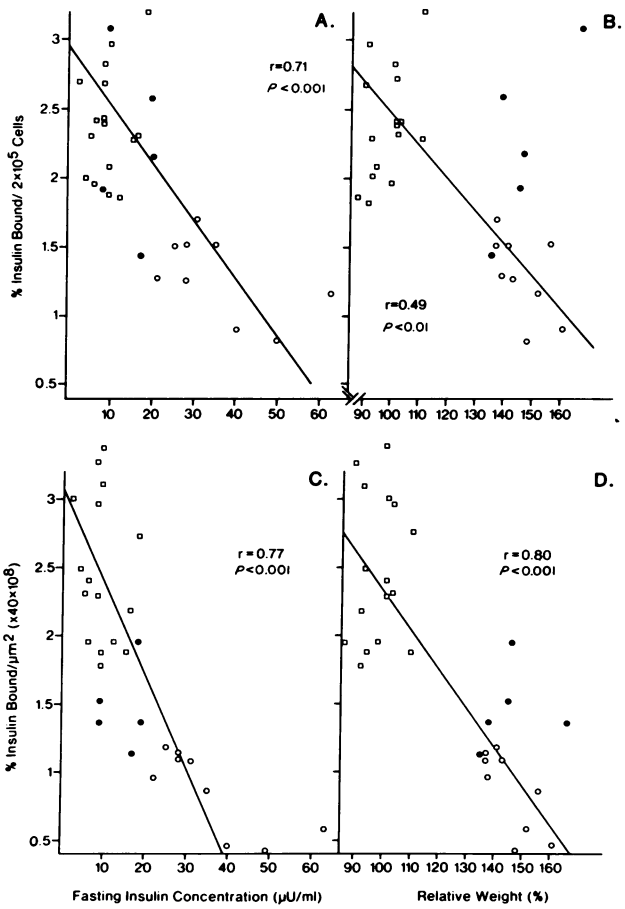

FIGURE 4 Relationship between the ^{125}I-insulin specifically bound to adipocytes (2×10^5 cells) and fasting plasma insulin level (A), or relative weight (B). Symbols: □, normals; ●, five nonhyperinsulinemic obese; ○, nine hyperinsulinemic obese. In C and D, the insulin-binding data have been normalized to unit surface area rather than per 2×10^5 cells. This is done by calculating the total surface area per 2×10^5 cells (surface area/cell $\times 2 \times 10^5$) and normalizing all individual data to 20,000 $\mu M^2 \times 2 \times 10^5$ (mean surface area of the cells from the normal subjects).

binding and fasting insulin level is highly significant ($r = 0.71$, $P < 0.001$), suggesting that the lower the degree of insulin binding, the higher the fasting insulin level, and, as reported previously, the greater the degree of insulin resistance (25). It should be noted, however, that the data generally segregate into two clusters and more data, spanning the entire spectrum of fasting insulin levels, will be necessary to be certain of the relationship. On the other hand, the correlation between the amount of insulin bound and relative weight is somewhat weaker ($r = 0.49$), reflecting the influence of the four obese subjects with normal insulin binding. However, since insulin binding is a plasma membrane function, it could be argued that these insulin binding data should be normalized to unit cell surface area, and in Fig. 4C and D, this has been done. As can be seen, the correlation between fasting insulin level and binding is unchanged by this maneuver, whereas the correlation between insulin binding and relative weight is greatly enhanced. This latter change is primarily because the four obese subjects with normal insulin binding on a per cell basis have larger adipocytes than controls, and consequently have decreased insulin binding when the data are expressed on the basis of unit surface area.

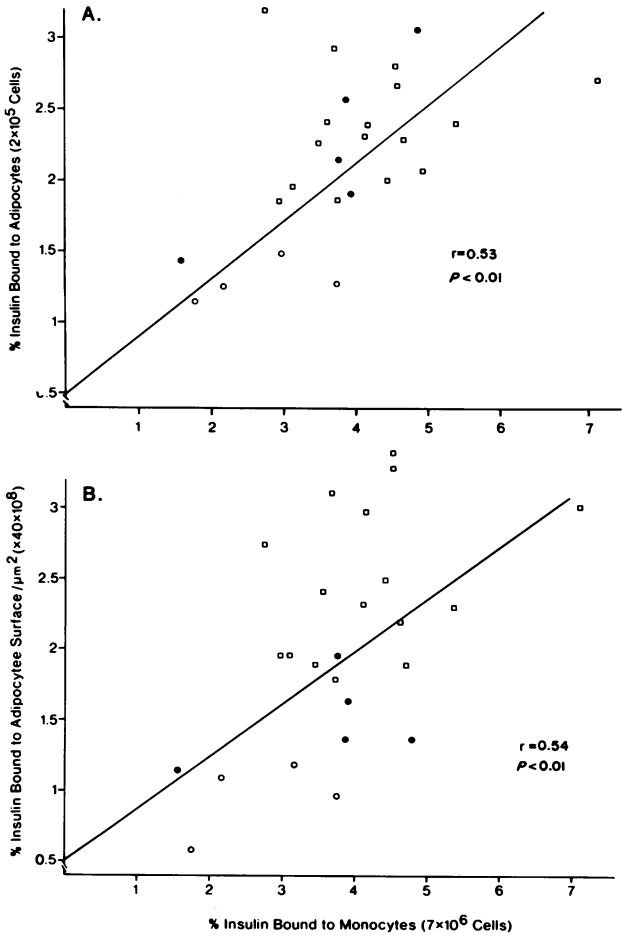

FIGURE 5 Relationship between the percent ^{125}I-insulin bound to adipocytes and monocytes obtained from the same individuals. □, normals; ●, five nonhyperinsulinemic obese; ○, four hyperinsulinemic obese. In A the insulin-binding data are normalized to 2×10^5 cells, and in B the data are normalized to unit surface area (see legend to Fig. 4 for details).

Finally, in the subjects from whom both adipocytes and mononuclear cells were obtained, the correlation between the amount of insulin bound to both tissues was examined. This data is presented in Fig. 5 and indicates that while adipocytes bind much more insulin per cell, changes in insulin binding in both tissues are closely correlated. When the percentage of insulin bound to adipocytes is expressed per unit surface area (5B), no change in this relationship is observed. Thus, it would appear that at least some events that regulate insulin receptors on adipocytes also influence insulin receptors on monocytes.

DISCUSSION

These data clearly demonstrate that adipocytes from obese human subjects have a decreased ability to bind insulin. These findings are most striking when obese subjects are subclassified according to age of onset of obesity and fasting plasma insulin level, with a highly significant correlation present between fasting insulin level and amount of insulin bound. Since we have previously demonstrated a close relationship between the height of the fasting insulin level and the degree of insulin resistance, as measured in vivo (25), we conclude that insulin binding is decreased only in insulin-resistant obese subjects. These observations and conclusions are consistent with a number of existing reports (1–6). Thus, decreased insulin binding has been described in a variety of tissues from genetically obese (1–3) and spontaneously obese (4) rodents. Human obesity has also been studied, and Archer et al. (5, 6) have reported that isolated mononuclear cells from obese subjects bind decreased amounts of insulin. Additionally, in a brief note, Marinetti et al. (7) reported decreased insulin binding to adipocytes from obese subjects. Finally, in a series of in vitro studies, Gavin and co-workers have found that high media insulin concentrations lead to decreased numbers of insulin receptors on cultured lymphocytes (28). Although Huang and Cuatrecasas ascribe this effect to proteolysis (29), Gavin et al. have suggested that this is a physiologic regulatory process and that this same phenomenon occurs in vivo, i.e. hyperinsulinemia leading to decreased insulin receptors on cells. These latter observations may reconcile the apparent contradictory report of Amatruda et al. (8), who were unable to demonstrate any change in insulin re-

ceptors in adipocytes from obese subjects. Thus, we have found that some obese patients are not hyperinsulinemic (and presumably not insulin-resistant) and their adipocytes do not bind decreased amounts of insulin. These patients tend to be younger, with the onset of obesity in childhood. The nine obese patients studied by Amatruda et al. (8) had a mean age of 33 and, thus, were decidedly younger than our nine hyperinsulinemic obese subjects (mean age, 51 yr), but closely resembled our five nonhyperinsulinemic obese patients (mean age, 32 yr). Information as to the age of onset of obesity was not given in the report by Amatruda et al. Furthermore, it is possible that the data of Amatruda et al. (8) are only quantitatively, but not qualitatively different from these current studies. Thus, it can be argued (4) that since insulin binding is a membrane phenomenon, the data should be normalized to unit surface area rather than per cell. If this were done, since adipocytes from obese patients have a greater surface area than cells from normals, the number of insulin receptors per unit surface area would be decreased in adipocytes from obese patients with the data of Amatruda et al. (8) and even further decreased with our own data (as seen in Figs. 4C and D). Other differences between the two studies that may be relevant are: (*a*) five of their nine patients were female, whereas all of our subjects were male; (*b*) their patients were much more obese than ours (mean relative weight 263% vs. 146%), and thus also had larger cells with greater surface area; and (*c*) we obtained our tissue via open biopsy in an area distant from the local anesthetic, whereas they obtained their tissue during general anesthesia. Whether any of these latter differences are critical cannot be determined at this time.

In a previous report we have found that the characteristics of insulin binding to human adipocytes (including affinity constants for the binding reaction) were similar to the characteristics of insulin binding to mononuclear cells (9). This concept is strengthened by these current studies, since a close relationship was found between insulin binding to both adipocytes and monocytes from the same subject. Per cell, adipocytes can bind about 125 times as much insulin as total mononuclear cells, or 17.5 times as much as monocytes. However, as demonstrated in Fig. 3 and 5, changes in insulin binding to adipocytes are reflected by changes in insulin binding to mononuclear cells. Thus, although one can argue that the adipocyte data are more relevant since adipocytes are a more important target cell for insulin than mononuclear cells, these studies and the work of Soll et al. (3) show that changes in insulin receptors of mononuclear cells seem to mirror the events in the more traditional insulin target tissues. It should be cautioned, however, that these results are limited to obesity, and before similar conclusions can be made for other diseases, similar studies will be necessary.

The role of decreased insulin binding in human obesity is not clear from these data. For one, no studies of insulin's cellular action were performed, so the significance of decreased adipocyte insulin receptors in terms of insulin-mediated adipocyte function cannot be stated. However, as we have previously shown (30), the relationship between changes in insulin binding and changes in insulin action are not always straightforward. For example, large adipocytes from spontaneously obese rats have a decreased ability to bind insulin (4), but this decrease in insulin binding only accounts for a relatively small portion of the metabolic abnormalities in these cells, since the major defect involves the intracellular pathway(s) of glucose metabolism (30). Furthermore, even if decreased insulin receptors lead to decreased insulin action, it is not clear what sequence prevails in vivo. For example, does obesity in some manner lead to decreased insulin receptors which, in turn, cause insulin resistance and hyperinsulinemia? Or is obesity first associated with hyperinsulinemia, which then leads to decreased insulin receptors (28) and insulin resistance? Clearly further studies will be necessary to chose between the above possibilities.

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