exhibited a wide range of tolerance. Plants E-1 and E-2, which were found growing in the least soil arsenic, showed the least degree of tolerance, while D-5 and D-9 from highly arsenical soil continued growing in 50 ppm arsenic in solution.

These data suggest that the ability of *A. scoparius* to tolerate soils contaminated with arsenic is the result of a genetic change wrought by selection. If, as is likely, there is some genetic variation for arsenic tolerance inherent in the control population, it is at low frequency. We initially tested these plants after cultivation for 4 months in the greenhouse, and our results did not differ significantly from those obtained after 18 months of cultivation. Although progeny tests would be more conclusive, the fact that tolerance is not lost in cultivation suggests to us that it is a genetically controlled character and, together with the failure of control tillers to root in low concentrations of arsenic, seems to rule out habituation.

The degree of arsenic tolerance of each of the mine plants is related to the amount of arsenic in the soil. It is not surprising that plants from the most arsenical soils exhibit the greatest tolerance since selection would eliminate less tolerant individuals. However, it seems remarkable that few highly tolerant plants are found in the areas of low soil arsenic. Metal-tolerant plants of several species have been shown to be at a disadvantage on normal soil suggesting that nontolerant individuals are competitively superior on uncontaminated soils (*7*). We believe that competition on the areas of low soil arsenic may be great enough to exclude highly tolerant individuals. To test this hypothesis, we determined the number of plants of *A. scoparius* per square meter at each station. Our results, ranging from sparse (2 plants per square meter) to dense (11 plants per square meter), show that, except for station E-1, the lower the tolerance of each plant, the greater the number of plants per square meter. Thus, given the variation in density of plants in relation to the amount of soil arsenic, selection tends to favor highly tolerant individuals in extremely toxic areas and eliminate them in less toxic areas.

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Insulin Receptor: Role in the Resistance of Human Obesity to Insulin

Abstract. *Large adipocytes from obese subjects have similar receptor numbers and affinities for insulin as small adipocytes from subjects of normal weight. These results indicate that the insulin insensitivity of large fat cells from obese humans occurs after the insulin-receptor interaction and might be explained by either a dilution of receptors over the cell surface or by alterations in intracellular metabolism.*

Hyperinsulinemia in the presence or absence of glucose intolerance is a universal accompaniment of obesity and suggests peripheral resistance to the action of insulin. In obese subjects direct correlations have been made between the degree of adiposity (*1*), the increased size of fat cells (*2*), and the elevated plasma insulin. Impaired action of insulin has been demonstrated in human subcutaneous adipose tissue from obese subjects in vivo in studies of perfused forearms (*3*) and in some (*4–6*) but not all (*7*) in vitro studies of adipose tissue and isolated adipocytes. Following the decrease in plasma insulin and the size of fat cells that accompany reduction in weight, the responsiveness of adipose tissue to insulin is restored (*4*).

Insulin initiates its action on target tissues by interacting with specific receptors on the cell membrane (*8, 9*). The insensitivity of large fat cells to insulin could result from a decrease in the number of insulin binding sites on the cell membrane, a lower affinity of binding, or a defect following the insulin-cell interaction. In a recent study Olefsky *et al.* (*10*) measured insulin binding to adipocytes from subjects of normal weight. The present report describes the first detailed study that compares the number and affinity of insulin receptors in adipocytes from both normal and obese human subjects.

Fat tissue for these studies was obtained from markedly obese subjects and from those of normal weight (Table 1). All obese subjects (five fe-

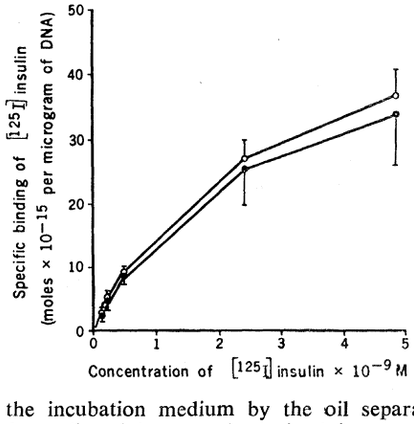

Fig. 1. Specific binding of [^{125}I]insulin to large and small human adipocytes. Following the surgical removal of subcutaneous adipose tissue, large fat cells (●) from obese subjects and small fat cells (○) from normal weight subjects were prepared by gently shaking at 37°C for 45 minutes in Krebs-Ringer bicarbonate buffer (*p*H 7.4) containing crude collagenase (1 mg/ml) and albumin (4 percent). Large (0.3 to 1.4 μg of DNA) and small (0.6 to 2.7 μg of DNA) cells were incubated for 40 minutes at 24°C in 0.3 ml of Krebs-Ringer bicarbonate buffer containing 1 percent albumin and the designated concentrations of [^{125}I]insulin. The fat cells were separated from the incubation medium by the oil separation method of Gliemann *et al.* (*26*), and the radioactivity was determined in a gamma counter. DNA was determined by the diphenylamine reaction (*27*). The [^{125}I]insulin was prepared by iodination with "carrier-free" Na^{125}I with the use of chloramine-T as described by Cuatrecasas (*9*). Specific binding is calculated as the difference between the total binding of [^{125}I]insulin in the absence of native insulin and in the presence of a large excess of unlabeled insulin (50 μg/ml)

males and four males) were undergoing jejunal-ileal bypass for morbid obesity and had normal oral glucose tolerance tests. The subjects of normal weight were females with no history of diabetes mellitus or other underlying illness who were undergoing elective, nonendocrine gynecological surgery and had normal fasting concentrations of insulin and glucose. As shown in Table 1, the mean diameter of fat cells and the calculated volume of these cells for the obese group were significantly larger than those determined for the group with normal weights ($P < .001$). These values for small and large cells are in agreement with those reported by Bray (*11*).

Dietary factors have been shown to influence the responsiveness of fat cells to insulin (*5*). Salans *et al.* (*5*) have recently shown that adipose tissue from obese subjects (with large fat cells) is less responsive to insulin than adipose tissue from normal weight subjects (with small fat cells) when both are maintained on similar diets. However, large fat cells obtained from individuals ingesting high carbohydrate (70 percent), low fat (15 percent) diets can be more sensitive to insulin than smaller cells obtained during periods of ingestion of low carbohydrate (25 percent), high fat (60 percent) diets. These dietary extremes did not apply to the subjects in this study. In addition, nothing is presently known about the relation between diet and insulin binding.

Figure 1 shows [^{125}I]insulin binding, expressed per microgram of DNA, to large and small human adipocytes. Binding to large and small cells was similar over concentrations of [^{125}I]insulin ranging from 1.2×10^{-10} to $5 \times 10^{-9}M$. When expressed as [^{125}I]insulin bound per cell (data not shown), there were again no differences between large and small cells. Cell number was calculated by dividing the volume of packed cells by the mean cell diameter for each subject. To determine the validity of this method, several correlations were examined. Cell number and DNA determinations were positively correlated ($r = .716$, $P < .005$). Also, DNA determinations were linearly related to cell dilution ($r = .9897$) and to specific binding of [^{125}I]insulin ($r = .971$). Specific binding was in turn directly related to cell dilution ($r = .9926$). Based on the number of cells, the number of insulin molecules bound per cell was 16,900

Table 1. Age, weight, plasma insulin, and size of fat cells in obese subjects and in those of normal weight. Ideal body weight was obtained from Metropolitan Life Insurance tables. The diameter of fat cells was measured under the 10-power objective of a monocular microscope fitted with a Filar micrometer eyepiece capable of size estimation of 1 μm. Fat cell volume was calculated from the formula of Goldrick (*25*): volume (ηl) $= \pi/6\ (\bar{x}^2 + 3\text{S.D.}^2)\bar{x}$, where $\bar{x}$ is the mean diameter of the cells. Abbreviations: S.D., standard deviation; S.E.M., standard error of the mean.

	Subjects	
	Normal weight*	Obese†
Mean age (years) plus range	29 (17 to 40)	33 (17 to 45)
Mean percentage of ideal body weight plus range	106 (97 to 113)	263 (185 to 318)
Mean fasting plasma insulin (microunit/ml) plus range‡	12 (7.5 to 15)	45 (30 to 63)
Fat cell diameter (μm, mean ± S.E.M.)	77.6 ± 3.3	124 ± 5.4
Fat cell volume (ηl, mean ± S.E.M.)	0.23 ± 0.03	1.12 ± .14

*Eight subjects. † Nine subjects. ‡ One milligram equals 24 units.

at a [^{125}I]insulin concentration of $4.8 \times 10^{-10}M$. At concentrations of [^{125}I]-insulin from 1.2×10^{-10} to $2.4 \times 10^{-9}M$, insulin degradation, as measured by precipitation with 12 percent trichloroacetic acid (*12*), was equal in large and small cells (< 4 percent) and thus did not influence the interpretation of insulin binding studies. There is no receptor degradation under the conditions of our study (*10*).

The affinity of the insulin receptor for its hormone was examined by measuring the displacement of [^{125}I]insulin by native insulin in adipocytes from obese and normal weight subjects. The concentrations of native insulin necessary to displace 50 percent of the maximum specific binding were $2.17 \times 10^{-9}M$ for small adipocytes and $1.75 \times 10^{-9}M$ for large adipocytes (Fig. 2). These findings indicate that this parameter of adipocyte insulin-receptor interaction is also unaltered in obesity.

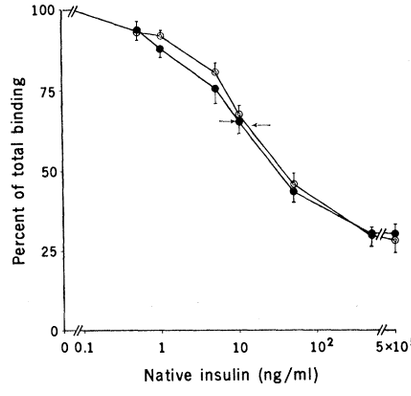

Fig. 2. Displacement by native insulin of [^{125}I]insulin bound to isolated human adipocytes. Large (●) and small (○) human adipocytes prepared as described in the legend to Fig. 1 were incubated for 10 minutes at 24°C with [^{125}I]insulin ($4.8 \times 10^{-9}M$), after which the designated concentrations of unlabeled insulin were added. The cells were then further incubated for 30 minutes and insulin binding was determined as described in the legend to Fig. 1. The arrows indicate the points at which 50 percent of specifically bound insulin is displaced.

Olefsky *et al.* (*10*) have studied the characteristics of insulin binding to isolated human adipocytes obtained from subjects of normal weight. Our data are in agreement with theirs in showing similar dissociation constants, similar numbers of receptors per cell, and low levels of insulin degradation by human fat cells.

In contrast to studies in humans, insulin receptor interaction has been extensively investigated in several animal models with a variety of metabolic conditions characterized by insulin resistance. Decreased insulin binding to target tissues has been observed in the genetically obese–hyperglycemic mouse (*13*), in rats treated with glucocorticoids (*14*), and following implantation of a pituitary tumor that secretes growth hormone, prolactin, and adrenocorticotropic hormone (*15*). However, there is no defect in insulin binding to the large adipocytes from adult rats, even though such cells are less sensitive to insulin stimulation of glucose oxidation than are the small fat cells from younger animals (*16*). In starvation, insulin binding to adipocytes is also unaltered (*17*). These diverse results suggest that the mechanism underlying insulin insensitivity varies in different animal models and in different resistant states.

To our knowledge there are two reports evaluating insulin binding in human obesity. In the more detailed study (*18*), which compared insulin binding to circulating lymphocytes from obese people and those of normal weight, insulin binding was the same in cell

populations from both groups. Significant differences, however, were observed in the concentration of insulin required to inhibit 50 percent of maximum binding in lymphocytes from obese and normal weight people. At present, we feel that studies in which circulating lymphocytes are used to examine insulin binding in resistant states should be viewed with some caution. Unlike the adipocyte, circulating lymphocytes have extremely low levels of specific insulin binding and, to date, no biological response to insulin has been observed (*19*). Furthermore, preparations of the circulating lymphocytes used in these insulin binding studies were significantly contaminated with other cell types (*18*), some of which bind insulin avidly (*19*). The second report, which was presented at a symposium in 1971 (*20*), alludes to studies of insulin binding to human fat cells from obese and lean individuals. No affinity studies were performed and there is no information on the quantity of insulin bound per cell. Furthermore, saturability, a requisite of specific receptor binding, was not present.

It has been suggested that elevated circulating insulin may contribute to the decrease in insulin receptors seen in some insulin-resistant states (*21*). Studies of cultured human lymphocytes (*19, 21*) which, unlike peripheral circulating lymphocytes (*19*), possess a high density of insulin receptors have shown that the incubation of lymphocytes with very high concentrations of insulin ($> 10^{-8} M$) for five or more hours at 37°C leads to a decrease in insulin receptors (*21*). The concept that elevated concentrations of insulin lead to a decrease in receptors does not appear to apply to human obesity. Despite elevated concentrations of insulin (Table 1), there is no decrease in insulin binding to either human adipocytes as shown in the present study (Fig. 1) or to circulating lymphocytes (*18*).

An impairment of insulin action in large adipocytes from obese human subjects has been demonstrated (*3–6*). Although the present study indicates that this insulin resistance follows the insulin-cell association, it does not provide direct information concerning the cellular alteration (or alterations) responsible for hormonal insensitivity. One possibility is that a "dilution" of insulin receptors over the surface area of large cells may hinder the transmission of the signal arising from the interaction between insulin and the insulin receptor (*16*). This does not appear to be the case in at least one animal model in which large adipocytes have an efficient D-glucose transport system that is responsive to insulin despite reduced insulin-stimulated glucose oxidation (*22*). Numerous studies in which both animal (*23*) and human (*6, 24*) adipocytes were used show that intracellular carbohydrate and lipid metabolism is altered in large fat cells as compared to small ones. It is possible that these metabolic alterations might render large fat cells less sensitive to some of the actions of insulin which would in turn appear as insulin resistance.

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Nuclear Magnetic Resonance of Rotational Mobility of Mouse Hemoglobin Labeled with [2-^{13}C]Histidine

Abstract. *Carbon-13 nuclear magnetic resonance studies were made on mouse hemoglobin specifically labeled at the C-2 histidine position. Measurement of the spin lattice relaxation times of the label before and after hemolysis of the erythrocytes provides information on the intracellular fluid viscosities.*

Suspensions of some cells (*1, 2*) and even tissues (*3*) exhibit ^{13}C nuclear magnetic resonance (NMR) spectra characterized by a high degree of resolution, thus providing an opportunity for studies of the structure and dynamics of molecular constituents in intact cells and tissues. In complex systems containing large numbers of carbon atoms, selective enrichment with ^{13}C offers a number of advantages (*4*), and the introduction of the isotope label does not perturb (*5*) the experimental system to the extent that fluorescent probes or paramagnetic spin labels do. In this study we have used