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6. Abbreviations: BOC, *t*-butyloxycarbonyl; OBz, *O*-benzyl; CBZ, benzyloxycarbonyl (carbobenzoxy); Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Cys, cysteine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Ileu, isoleucine; Leu, leucine; Lys, lysine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tryosine; and Val, valine. All amino acids are of the L-configuration, except glycine.
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Insulin Insensitivity of Large Fat Cells

Abstract. *Large insulin-insensitive adipocytes from adult rats have normal binding capacities and affinities for insulin. Diminished insulin-like responses to spermine and reduced rates of glucose oxidation are also evident in these cells. The results indicate that the defect responsible for this insulin-resistant state exists in a step subsequent to insulin binding, possibly in transmission of the insulin-receptor "signal" since insensitivity occurs under conditions where glucose transport and oxidative processes are not apparently impaired.*

Adipose tissue from obese humans is less sensitive to the action of insulin on glucose metabolism than fat tissue obtained from normal individuals (*1*, *2*). Similarly, the magnitude of response elicited by insulin in isolated fat tissue from adult rats is below that in tissue from young rats (*3*, *4*). In both instances the insulin insensitivity appears to be related primarily to increased cell size (*2*, *4*) and not to other factors such as age (*5*). The relative resistance of large adipocytes to insulin may explain the abnormally high circulating levels of insulin observed in obesity during the fed and fasting state and may contribute significantly to the glucose intolerance of maturity-onset diabetes mellitus (*6*). Little is known concerning the molecular site(s) responsible for insulin insensitivity in large adipocytes.

Insulin elicits many and possibly all of its biological effects by interacting with specific "receptor" sites located on the surface of the plasma membrane (*7*). In turn, this interaction modulates various cellular systems involved in the mediation of insulin action; thus, an alteration of any step in this sequence may be responsible for the insulin-resistant state. The possibility that insulin-cell association is involved in insulin insensitivity was examined by studies utilizing the insulin-like action of spermine (*8*) and by determining the extent of [^{125}I]insulin binding (*9*) in large (mean volume, 28 μmole of triglyceride per microgram of DNA) and small (mean volume, 9 μmole/μg) rat adipocytes. In addition, the capacity of both cell types to metabolize glucose in the absence of insulin was compared to assess the influence of glucose transport and oxidation on insulin resistance.

Insulin sensitivity in large and small adipocytes was determined by studies of the dose-response relationships for the effect of insulin on glucose oxidation (Fig. 1A). Although basal glucose oxidation was similar in both cell types, the maximal insulin response achieved by large cells was approximately one-half that of small cells. Similar insulin concentrations stimulated one-half maximal glucose oxidation in both cell types, suggesting that affinity of large cell receptors for insulin was not diminished.

Spermine and certain other polyamines mimic the action of insulin on fat cells by apparently acting at membrane sites separate from insulin receptors but which share a common pathway with insulin mediated responses (*8*). By virtue of its site of action, spermine would be expected to stimulate greater glucose oxidation in large cells than insulin if insulin resistance resulted from defective insulin-receptor interaction. However, as shown in Fig. 1B, the maximal spermine response of large cells was similar to the insulin response and was also one-half that of small cells.

More conclusive evidence that alterations of the association between insulin and large fat cells are not involved in their insulin resistance is given in Table 1. With saturating concentrations of [^{125}I]insulin, the total insulin-binding capacities of large and small cells were equal. Furthermore, the affinity between insulin and insulin-binding sites was not diminished in large insulin-insensitive cells since specific binding of [^{125}I]insulin at concentrations below saturation was similar in both groups of cells.

An assessment of the glucose transport and oxidizing capacities of large and small adipocytes was made by studying the effect of glucose concentration on glucose oxidation (Fig. 2A). The rates of CO_2 production by both cell types were essentially identical when

Table 1. Specific binding of [^{125}I]insulin to large and small adipocytes. Fat cells, prepared as described in the legend of Fig. 1, were suspended (6.1 and 8.6 μg of cell DNA for large and small cells, respectively) in 0.5 ml of Krebs-Ringer-bicarbonate buffer containing 1 percent (w/v) albumin, and the indicated concentration of [^{125}I]insulin (920 mc/μmole). After incubation for 50 minutes at 24°C, specific binding of insulin was determined by Millipore membrane filtrations as described previously (*9*). These data, from a single experiment, are representative of six separate experiments which gave similar results. The effects of insulin on the conversion of [^{14}C]glucose to $^{14}CO_2$ by the same cells used in this experiment were similar to those described in Fig. 1; the maximal oxidative response in small cells was twice that of large cells.

Concentration of [^{125}I]insulin ($10^{-12}M$)	Specific [^{125}I]insulin bound (count/min per μg DNA)	
	Small cells	Large cells
16	130	14,700
48	440	170
140	1,410	520
470	4,140	1,490
1400	10,400	4,370
3300	14,100	11,500

cells were incubated in glucose concentrations of less than 10 m*M*. However, incubation of large cells in glucose concentrations above 10 m*M* did not cause a further increase in CO_2 production, whereas small adipocytes required glucose concentrations above 20 m*M* to saturate glucose oxidation, glucose transport systems, or both. In the presence of 0.3 m*M* glucose, maximal insulin stimulation in both cell types failed to produce responses that approached the maximum possible rate of glucose oxidation (Fig. 2B). These results indicate that the experiments demonstrating insulin and spermine resistance reported in Fig. 1 were conducted under conditions in which glucose transport and oxidation were not rate-limiting in either cell type.

These studies indicate that insulin binding is not the site responsible for insulin resistance. The similar degree of resistance in large adipocytes to both insulin and spermine in conjunction with normal [^{125}I]insulin binding suggests an alteration in the processes which follow insulin-cell association. Large cells evidently have an abnormality in glucose metabolism, as is indicated by their diminished capacity to oxidize glucose. A decrease in hexokinase activity in large adipocytes provides an explanation for this defect (*10*). However, insulin insensitivity was observed under conditions in which maximum insulin stimulation failed to saturate glucose metabolic processes, indicating that diminished glucose metabolism cannot be the sole explanation of insulin insensitivity in large cells. Indeed, the abnormality in glucose metabolism may be a consequence of the insulin-resistant state since insulin is known to affect levels of certain enzymes (for example, hexokinase) involved in glucose metabolism in adipose tissue of rats (*11*).

The primary defect responsible for insulin resistance may involve an abnormality in transmission of the "signal(s)" arising from insulin-receptor interaction. Defective signal transmission may be related to the "dilution" of insulin receptors over the surface of large cells. Although total insulin-binding capacity was similar in both cell types, the increased surface area of large cells (*12*) denotes a diminished number of insulin receptors per unit of membrane area. If the sites of glucose transport are similarly "diluted" by the expanded cell surface, it is conceivable that "signal" transmission from the insulin-receptor complex to the transport system is partially uncoupled by an increase in intervening membrane space or structures and therefore results in insulin resistance. However, a simple reduction in insulin receptor concentration does not necessarily cause insulin resistance. A previous study demonstrated a diminished concentration of receptors in the absence of insulin resistance in adipocytes obtained from 320-g rats (*13*). It appears therefore that the fat cell has the capacity to expand without affecting insulin sensitivity until a critical size is reached beyond which further expansion may result in diminished insulin response.

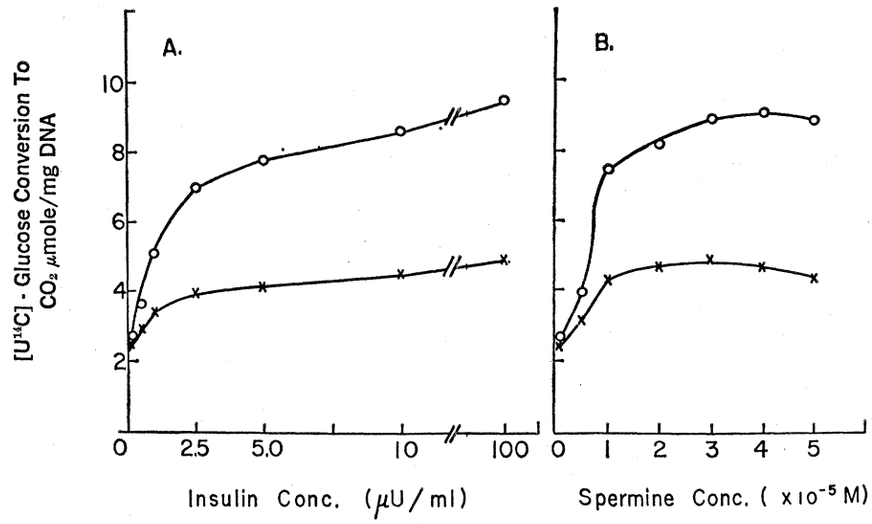

Fig. 1. Effects of various concentrations of insulin (A) and spermine (B) on glucose metabolism in large (crosses) and small (open circles) rat adipocytes. Data are given as the mean of six separate experiments. Large and small adipocytes were isolated by the method of Rodbell (*14*) from the epididymal fat pads of large (375 to 425 g) and small (140 to 175 g) rats, respectively. Isolated adipocytes (0.5 to 1.5 μg of DNA) were incubated at 37°C in 2 ml of Krebs-Ringer-bicarbonate buffer containing albumin (2 g/100 ml), 0.3 m*M* D-[^{14}C]glucose (uniformly labeled) (4.8 mc/mmole), and the indicated concentrations of insulin or spermine. The amount of $^{14}CO_2$ formed after 2 hours' incubation was determined as previously described (*14*). Adipocyte DNA was quantitated by the diphenylamine method (*15*). Triglyceride content was determined by measuring the amount of glycerol released (*16*) after extraction of the lipids (*17*) and saponification with alcoholic potassium hydroxide.

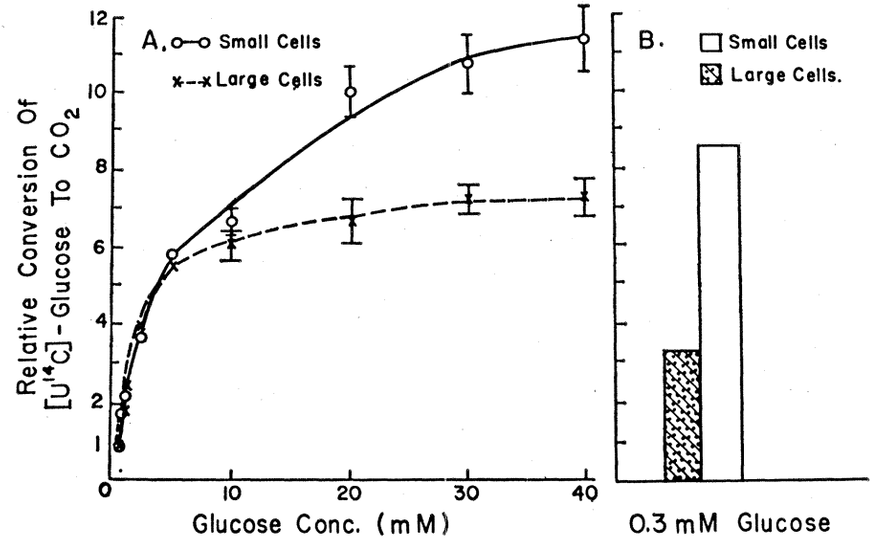

Fig. 2. Effects of glucose concentration and insulin stimulation on glucose oxidation by large and small rat adipocytes. The effects are expressed relative to those obtained when cells were incubated in 0.3 m*M* glucose in the absence of insulin. The values represent means ± S.E. calculated from a total of four separate experiments. (A) Incubations in the absence of insulin; (B) incubations with 0.3 m*M* glucose in the presence of insulin (1000 μU/ml).

Whether or not a defect or defects exist in the mediation of a "signal(s)" from the insulin-receptor complex to biological effectors of insulin actions as suggested by this investigation remains to be proved. However, this study has shown that insulin resistance in large adipocytes is not a problem arising from defective insulin binding but that, as in other insulin-resistant states (*13*), the defect or defects occurs in processes subsequent to insulin-cell association.

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Retrieval Failure Induced by Electroconvulsive Shock: Reversal with Dissimilar Training and Recovery Agents

Abstract. *Amnesia was obtained following electroconvulsive shock in rats trained at one-trial passive avoidance of immersion in ice water. Avoidance behavior was restored when noncontingent foot shock was administered outside the training apparatus. The qualitative differences between ice water and foot shock demonstrate that the agent inducing recovery of memory need not be physically similar to the reinforcer used during training. These findings are interpreted as supporting a retrieval failure view of experimental amnesia.*

Considerable research has focused on the problem of the permanence of retrograde amnesia induced by electroconvulsive shock (ECS). The traditional view maintains that ECS produces an irreversible loss of memory (*1*). In accordance with this view, most studies looking for spontaneous recovery of memories impaired by ECS have been unsuccessful (*2*).

Several recent experiments (*3*) suggest that recovery of memory after ECS is possible with the aid of a noncontingent "reminder" foot shock (FS) presented between ECS and the retention test. More recently, it was demonstrated that the recovered memory was not due to a change in activity level caused by the interaction of training FS, ECS, and noncontingent "reminder" FS (*4*). Furthermore, these experimenters found that the ability to recover memory was not time dependent, inasmuch as ECS-reminder intervals of 2, 4, 8, 16, 48, and 336 hours all restored memory to a similar degree. Consequently, it appears that ECS may not destroy recently acquired information, but may, instead, interfere with its retrieval.

Similar experiments have observed recovery from cycloheximide-induced amnesia provided the recovery agent was administered within a few hours of training (*5*). Moreover, it was found that the training and recovery stimuli need not be similar, since both amphetamine and corticosteroids were effective in restoring cycloheximide-impaired memories after training with FS. However, both these pharmacological agents were effective in attenuating cycloheximide-induced amnesia only if administered while the short-term memory trace still persisted.

The present experiment was designed to determine whether, as is suggested by "reminder," the training stimulus and the recovery agent must have similar physical properties as well as equivalent internal consequences to produce recovery of memory after ECS. In the present study, stimulus specificity of the recovery agent was investigated by training rats using ice water immersion as the aversive training stimulus (*6*) and using FS as the recovery stimulus. The rationale for training with ice water and "reminding" with FS and not vice versa is that we have found it necessary to manipulate both the intensity and duration of the recovery agent in order to successfully obtain recovery of memory. Inasmuch as it is considerably easier to vary the parameters of FS than those of water, the former was used as the reminder agent and the latter as the training agent.

Subjects for the present experiment were Sprague-Dawley descended, male, albino rats (Carworth Farms) weighing 155 to 180 g at the outset of the experiment. All subjects were individually housed in continuous light and were maintained on 10 g of powdered rat food per day; water was freely available. A two-chambered, V-shaped, step-through device was used for training (*7*). The ceiling and partition between the light and dark compartments were slit to permit suspended and counterweighted wires for delivery of ECS to follow the subject through the gate from one chamber to another. The floor in the dark punishment chamber was hinged and spring-loaded. Activation of a solenoid caused the two parallel metal plates which comprised the floor to swing open. A 30-gal (114-liter) plastic container fitted with an insert was situated directly beneath the apparatus. The insert consisted of a cylinder of sheet metal 30 cm high and 46.5 cm in diameter with an open top and carpenter's cloth screen bottom. The plastic can was partially filled with chopped ice surrounding the insert, and was then filled with tap water to a height of 23 cm above the screen floor and 13 cm below the floor of the step-through apparatus. The temperature of the water was maintained within 2°C of freezing for the entire duration of the experiment. Electroconvulsive shock (54 ma, 60 hertz, 0.3 second) was administered through earclips, while FS (0.25 ma, 60 hertz, 10 seconds) was delivered by a constant-current shock source in an