ations between zygote and gamete. Thus, our conclusions point to the need for even more detailed study of comparative anatomy and the fossil record, because without such study, the accurate calibration of "protein clocks" will be difficult if not impossible.

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Insulin Receptor of Fat Cells in Insulin-Resistant Metabolic States

Abstract. *A diminished response to insulin is exhibited by isolated fat cells obtained from rats that have been either starved, or treated with prednisone, or made diabetic by administration of streptozotocin. This decrease in response is not accompanied by changes in the quantity of insulin receptor of these cells or in the affinity of these receptors for insulin. Similarly, the decreased responsiveness to insulin of fat cells obtained from certain species (hamster, rabbit, mouse, guinea pig) is not explainable in terms of alterations of the insulin receptor.*

Insulin exerts many, if not all, of its metabolic effects on adipose cells by interacting with specific superficial receptors on the membrane (*1*, *2*). The specific binding of [^{125}I]insulin to intact, metabolically responsive cells appears to provide a sensitive method of directly measuring the initial interaction of the hormone with specific cell receptors by means which are independent of complex metabolic parameters (*3*). This binding interaction with intact cells and with cell membranes involves biologically active receptors for insulin (*3*, *4*). We studied the specific binding of [^{125}I]insulin to isolated adiposites of rats in a variety of "insulin-resistant" metabolic states in order to assess the possible contribution of defective or diminished receptor binding in these conditions.

Adiposites obtained from rats that

Table 1. Effect of starvation, prednisone administration, streptozotocin diabetes, and species differences on insulin enhancement of glucose transport in isolated adiposites. Adiposites were prepared (*11*) from epididymal fat pads of Sprague-Dawley rats. The cells (0.5×10^4 to 2×10^4) were incubated for 90 minutes at 37°C in 1 ml of buffer (Krebs-Ringer-bicarbonate) containing 2 percent (weight to volume) albumin and 0.2 m*M* D-[^{14}C]glucose (5.1 μc/μmole) (*2*). Rats treated with prednisone received 1 mg of prednisone intraperitoneally daily for 7 days. Streptozotocin was administered as a single intraperitoneal injection in 0.1*M* sodium citrate buffer, *p*H 4.0, at a dosage of 70 mg/kg, 7 days prior to the experiment. The diabetic state was indicated by a glucosuria greater than 0.5 mg per 100 ml. Separate experiments, each with separate control cells, are also presented. Various concentrations of porcine insulin were used in the range of 10^{-11} to 10^{-8}*M*; only representative values are presented here. The glucose oxidation assay was repeated at least once for each "resistant" state. Standard deviations in these experiments varied from 3 to 6 percent. The specific binding of [^{125}I]insulin to fat cells from the different animals is described in Table 2.

Animal	Weight (g)	Conversion of D-[^{14}C]glucose to $^{14}CO_2$ [moles of $^{14}CO_2$ per cell per minute ($\times 10^{16}$)] at insulin concentrations of:				
		0	8.5×10^{-11}*M*	5.3×10^{-9}*M*	2.6×10^{-8}*M*	Increase* (%)
Fed rat†	320	3.3		15		354
Fed rat†	120	3.0		12		300
Starved rat‡	230	1.0		1.2		20
Control rat	133	2.2	7.9		9.5	332
Prednisone-treated rat	101	1.6	2.0		2.9	81
Control rat	137	5.3	16		17	220
Streptozotocin-diabetic rat	138	3.4	4.6		5.2	53
Control rat	101	1.8	7.7		9.7	438
Hamster	107	1.6	1.5		1.7	6
Guinea pig	457	3.0	3.3		3.7	23
Control rat	127	5.9	9.8		13	120
Mouse	27	7.4	7.8		8.6	16
Rabbit	3000	2.8	2.9		2.0	

* Increase of baseline rate of glucose oxidation observed with the highest insulin concentration studied. † Average diameter of fat cells from 320-g rats, 70 μm; average diameter of cells from 120-g rats, 32 μm (*10*). ‡ Obtained from the control, fed group (320 g), then starved for 5 days; average fat cell diameter, 36 μm.

Table 2. Effect of starvation, prednisone administration, streptozotocin diabetes, and species differences on the specific binding of [^{125}I]insulin to isolated adiposites. Fat cells obtained from the same or similar animals described in Table 1. The adiposites were incubated for 40 minutes in 0.2 to 0.3 ml of buffer (Krebs-Ringer-bicarbonate) containing 2 percent (weight to volume) albumin at ambient temperature. Specific binding of [^{125}I]insulin was determined (*3*). Separate experiments, each with separate control cells, are presented. The concentrations of [^{125}I]insulin in the incubation medium ranged from $2 \times 10^{-10}M$ to $3 \times 10^{-9}M$; representative concentrations are given in italics in the table. The specific binding assay was repeated at least two times for each species or insulin-resistant state studied, and in all cases the results were qualitatively identical. Standard deviations in these experiments averaged ± 9.3 percent.

Animal	Specific binding of [^{125}I]Insulin [(mole/cell) × 10^{20})]	
	8.1×10^{-11}M	
Control rat	0.2	
Starved rat	0.2	
	6.3×10^{-10}M	*2.5×10^{-9}M*
Control rat	1.2	4.6
Prednisone-treated rat	1.0	4.6
	3.0×10^{-10}M	*1.5×10^{-9}M*
Control rat	0.4	1.3
Streptozotocin-diabetic rat	0.4	1.4
	2.2×10^{-10}M	*9.0×10^{-10}M*
Control rat	0.8	1.4
Mouse	1.1	3.0
Hamster	1.4	3.1
	2.2×10^{-10}M	*1.1×10^{-9}M*
Control rat	1.1	1.8
Guinea pig	1.9	4.2
Rabbit	1.7	2.8

were either starved (*5*), were treated with prednisone (*6*), or were diabetic (*7*), and cells obtained from certain species such as hamsters, guinea pigs, and rabbits (*8*), exhibit a very poor enhancement of the rate of glucose oxidation in response to insulin (Table 1). Under the conditions of these experiments, the rate of glucose transport across the cell membrane appears to be the limiting step in the oxidative pathway (*9*). Cell size alone is probably not responsible for diminished insulin responses of fat cells as no significant difference is observed in cells with an average diameter of 70 μm compared to cells with a diameter of 32 μm (Table 1) (*10*).

Despite the diminished metabolic effects of insulin in fat cells from rats in these "resistant" states, no significant diminution in the specific binding of [^{125}I]insulin to these fat cells was detectable (Table 2). There was no reduction in the total insulin-binding capacity of the cells, as determined by measurements which use saturating concentrations (10^{-9} to $10^{-10}M$) of [^{125}I]-insulin. The specific binding of insulin to the fat cells of mice, guinea pigs, hamsters, and rabbits was greater than the binding to fat cells of rats. There also appeared to be no reduction in the affinity of the complex of insulin and cell in these conditions as no differences in binding were observed at concentrations of [^{125}I]insulin that were below saturation. The apparent absence of a defect in affinity during formation of the complex indicates that binding equilibrium had been achieved in all measurements and that differences in the rates of association of insulin and cell cannot explain the differences in metabolic responses.

This indicates that, in the insulin-resistant states studied here (*10*), no serious defect exists in the binding function of the insulin receptor. The resistance to insulin observed in these conditions must therefore result from changes in processes which occur after the initial interaction of insulin and cell. Our results also demonstrate that the metabolic differences in insulin response are not explained by possible differences in the capacity of these cells to inactivate insulin; this would have been detected as a decrease in insulin binding.

It is unlikely that fat cells from various species or from rats with altered states of metabolism are more susceptible to damage by proteolytic enzymes to which they may be exposed during cellular isolation (*12*); the primary effect of digesting fat cells with various proteases is a decrease in the affinity of the cell receptor for insulin (*13*). Also a similar resistance to insulin has been observed in studies with other species with adipose tissue slices rather than with isolated fat cells (*14*). It is possible, however, that different metabolic states may affect the susceptibility of the fat cells to other enzymes, such as neuraminidases; the action of such enzymes on the fat cell surface can result in abolition of the insulin response without significantly affecting the interaction of insulin and receptor (*15*). It has recently been demonstrated that insulin can directly modulate the activity of adenylate cyclase (*16*); the effects of insulin on this enzyme in insulin-resistant metabolic states has yet to be determined.

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Crystal Structure of Ethylene Di-11-bromoundecanoate

Abstract. *Ethylene di-11-bromoundecanoate, $C_{24}H_{44}Br_2O_4$, was synthesized as a model for the hydrophobic moiety of saturated phospholipids. The crystals are monoclinic, space group* $P2_1/a$, *with two molecules per unit cell. Unlike folded configurations proposed for phospholipids in biological membranes, the hydrocarbon chains of this diester are fully extended in the crystalline state.*

Several analyses of low-angle x-ray data from biological membranes and oriented lipid bilayers (*1*) reaffirm many of the earlier x-ray studies (*2*), which proposed membrane lipid packings based on the Danielli-Davson model (*3*). This model, which implies a polar interaction between phospholipid and protein, predicates a folding of the phospholipid hydrocarbon chains