

Insulin binding and insulin response of adipocytes from rats adapted to fat feeding¹

Clement Ip², Helen M. Tepperman, Peter Holohan, and Jay Tepperman

Department of Pharmacology, State University of New York, Upstate Medical Center, Syracuse, New York 13210

Abstract The effect of fat feeding on adipocyte insulin binding was examined to expand a study of adaptive changes in plasma membrane functions. Cells from rats fed a high fat (L) diet for five to seven days bound less insulin and showed a decreased response to insulin (glucose oxidation) compared to those from rats fed a high glucose (G) diet. Both high and low affinity sites were influenced; the extent of the binding difference increased as increasing concentrations of insulin were present in the assay medium. Diet did not change hormone degradation or the capacity of phospholipase C to increase binding. Concanavalin A effects on fat cells were also decreased by L diet both in inhibition of insulin binding and its insulin-like effect on glucose oxidation. Spermine, which had no effect on insulin binding, also had a smaller insulin-like effect on glucose oxidation by L cells than by G cells. Serum insulin was significantly lower ($30 \pm 3.7 \mu\text{U/ml}$) in L than in G ($43 \pm 3.1 \mu\text{U/ml}$) groups. Dietary fat produces alterations in fat cells that decrease insulin binding as a part of a complex overall adaptation to the diet.

Supplementary key words fat cell size • phospholipase C • serum insulin levels • concanavalin A • spermine

The present study was designed to investigate the possibility that a plasma membrane-associated function, insulin binding capacity, can fluctuate in response to changes in diet composition. Reports that insulin binding capacity is altered by certain hormones (1), and in obesity (2, 3) in association with changes in insulin sensitivity indicate that such adaptation to environmental changes can occur. In a previous report from this laboratory (4) we demonstrated that both a decreased plasma membrane adenylate cyclase response to epinephrine and glucagon in fat cell ghosts and decreased lipolytic response of fat tissue were found in rats fed high fat diets. It seemed of interest, therefore, to study the effect of feeding a high fat diet to rats on the capacity of their fat cells to bind insulin. It has already been reported that fat feeding decreases adipose tissue response to insulin as measured by its effect on glucose uptake (5), glucose oxidation (6) or lipolysis (7). Serum insulin and

glucose concentrations in rats fed high fat or high carbohydrate diets were also measured.

Concanavalin A (8, 9) has been reported to have an insulin-like effect on fat cells and to decrease insulin binding (8). It seemed possible that changes in the function of fat cell membranes induced by diet might influence the availability of the concanavalin A binding sites. Therefore the effects of this agent on glucose oxidation and on insulin binding by fat cells from rats on the various diets were investigated. In preliminary experiments, it was found that spermine, which also mimics the action of insulin on fat cells (10), does not inhibit binding of the hormone. Fat cells from rats fed each of the diets were tested for their response to spermine as a measure of diet effects on glucose oxidation that do not involve insulin binding.

MATERIALS AND METHODS

Animals and diets

Male Sprague Dawley albino rats (Holtzman Co., Madison, Wis., or Charles River Laboratories, Wilmington, Mass.) weighing 100–120 g at the start of the feeding period were used throughout the study. They were fed either a high glucose or a high fat diet for 5–10 days as shown in the captions of figures and tables. The experimental diets contained 33% of the calories as protein (casein) and 67% as either glucose (G diet) or lard (L diet). Vitamin diet fortification mixture (4% by wt) salt mixture (USP XIV, 5% by wt) and cellulose (5% by wt) purchased from Nutritional Biochemicals Corp., Cleveland, Ohio, were included in each diet.

Abbreviation: HEPES, *N*-2-hydroxyethylpiperazine-*N*-ethanesulfonic acid.

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² Present address: Department of Breast Surgery, Cell and Virus Bldg., Roswell Park Memorial Institute, Buffalo, N. Y. 14203.

Preparation of isolated fat cells and measurement of cell size and number

Rats were killed by decapitation and fat cells were isolated from epididymal fat pads according to the method of Rodbell (11). Six to eight rats were usually used for each cell preparation. Adipose tissue (approximately 1 g) was incubated with 0.1% collagenase (Worthington Biochemical Corp., Freehold, N. J.) in 3 ml Krebs-Ringer bicarbonate buffer containing 4% bovine serum albumin (fraction V, Armour Pharmaceutical Co., Omaha, Neb., or Sigma Chemicals, St. Louis, Mo.) at 37°C for 60 min. Cells prepared with the different lots of albumin used responded similarly to insulin.

Adipocyte diameters were measured by means of a micrometer eyepiece. At least 150 adipocytes were measured for each preparation of cells used in each experiment. Mean cell volume was then calculated as described by DiGirolamo, Mendlinger, and Fertig (12).

Triglyceride content of an aliquot of fat cells was determined by the method of Stern and Shapiro (13) using triolein as the standard.

Assay of insulin biological activity

The response of fat cells to insulin was determined by measuring the stimulation of the conversion of [U-¹⁴C]glucose to ¹⁴CO₂ essentially as described by Rodbell (11). Fat cells (ca. 7×10^4 per vessel) were incubated in Krebs-Ringer bicarbonate buffer (with half the recommended concentration of Ca²⁺) with 1% bovine serum albumin containing 0.1 mg/ml of D-glucose (14) and bovine insulin (Sigma) (concentrations in tables and figures) in a final volume of 2 ml. Each incubation vessel contained 0.1 μ Ci of D-[¹⁴C]-glucose (Schwarz/Mann). Incubation was carried out in a Dubnoff metabolic shaker at a speed of 110 oscillations per minute at 37°C for 1 hr. Radioactivity of CO₂ was measured as described by Rodbell (11). Results are expressed as m μ moles of glucose oxidized/hr per 10⁶ cells.

Binding of ¹²⁵I-labeled insulin to isolated fat cells

¹²⁵I-Labeled porcine insulin (sp act 114–120 μ Ci/ μ g) was purchased from Cambridge Nuclear Radiopharmaceutical Corporation, Billerica, Mass. The labeled insulin was stated by the manufacturer to contain less than 1 atom of iodine per molecule of protein, to be greater than 95% immunoprecipitable, and to be intact by chromatoelectrophoresis. Each batch of radioactive insulin was routinely purified by talc adsorption according to the method of Cuatrecasas (15). Over 95% of the radioactivity of the purified ¹²⁵I-labeled insulin prepared used for bind-

ing studies was precipitable with 5% trichloroacetic acid and talc. The biological activity of this preparation was found to be equivalent to that of native insulin (on a weight basis) in the stimulation of [¹⁴C]glucose to ¹⁴CO₂ in isolated fat cells.

The binding of ¹²⁵I-labeled insulin to fat cells was determined according to the following procedure adapted from those of Freychet, Roth, and Neville (16) and of El-Allaway and Gliemann (17). Isolated fat cells (ca. 10⁶ cells) in Krebs-Ringer HEPES buffer (pH 7.4) containing 1% albumin and 0.54 mg/ml D-glucose were incubated for 30 min at 24°C with ¹²⁵I-labeled insulin (40–45 microunits or 7 microunits as indicated in tables and figures). The total volume of the incubation mixture was 0.8 ml. Under these conditions equilibrium was attained with cells from both diet groups of animals used in this study (data not shown).

After incubation, the cells were separated from the medium as described by El-Allaway and Gliemann (17) and their radioactivity was measured in a liquid scintillation counter with Instabray (Yorktown, Hackensack, N. J.). A correction for medium radioactivity trapped in the cell layer was made on the basis of data given by Gliemann et al. (18). HEPES buffer was used in these experiments because it was more convenient for binding studies and the results were the same as those obtained with the bicarbonate buffer, which was most suitable for metabolic studies. More concentrated cell-suspensions than those in the metabolic studies were used to increase the accuracy of the binding measurement. Under these conditions approximately 3% of the radioactive insulin was bound at the lowest concentrations used.

All binding data in this study are reported as “specific binding”. This is obtained by subtracting from the total radioactive uptake the amount of labeled insulin that was not displaced by large excesses of unlabeled hormone—50 μ g of native insulin per incubation vial (15). The nonspecific binding in all experiments was 5–10% of the total binding and did not vary with diet.

In experiments designed to study the maximum binding capacity for insulin, fat cells were incubated with a constant quantity of ¹²⁵I-labeled insulin (40–45 μ U or 7 μ U) together with increasing concentrations of unlabeled native insulin. The binding of radioactivity to cells was determined as usual, the total insulin binding was calculated from the calculated specific activity of the total insulin in each tube, and the results were analyzed by Scatchard plots (19). Corrections for insulin degradation were made as described by Kahn, Freychet, and Roth (20), who have pointed out that the interpretation of these plots is complex. Only apparent affinity constants and an approxima-

tion of the numbers of binding sites can be obtained by this type of analysis. Nevertheless it is useful for comparative purposes and has been so employed in the present study.

Association and dissociation

The rates of association of insulin with fat cells were measured as described above; fat cells were incubated with 7 microunits of insulin ($9 \mu\text{U/ml}$) at various time intervals as shown in Fig. 5.

The rates of dissociation were measured in the presence and absence of an excess of unlabeled insulin in order to test for negative cooperativity by the method of DeMeyts et al. (21). The dissociation rate was measured at 15°C since preliminary studies showed that the small negative cooperativity effect was more readily seen at that temperature than at 24°C . Isolated fat cells were labeled with ^{125}I -labeled insulin by incubating approximately 10^7 cells with $5 \times 10^{-11}\text{M}$ ^{125}I -labeled insulin in a final volume of 2.5 ml at 24°C . The infranate was separated from the fat cells by centrifugation. The aqueous fraction was discarded while the adipocytes were resuspended in 2 ml of cold, fresh HEPES buffer, pH 7.4. Aliquots of this labeled cell suspension were then dispensed into plastic beakers containing 10 ml of buffer with or without the presence of $10 \mu\text{g}$ of native unlabeled insulin. The beakers were incubated in a metabolic shaker at 15°C . At intervals, the contents of each beaker were filtered through Millipore filters (EAWP, pore size $1.0 \mu\text{M}$) with the aid of a sampling manifold. The filters were washed with 2 ml of buffer and were then transferred to scintillation vials, and 10 ml of Bray's scintillation fluid added. Radioactivity was measured in a liquid scintillation counter.

Determination of degradation of ^{125}I -labeled insulin by fat cells

Fat cells were incubated with ^{125}I -labeled insulin as described in the insulin binding section. At various intervals after initiation of incubation, the cells were separated from the buffer by the oil flotation technique. Radioactivity remaining in the infranant fraction of the incubation mixture was tested for trichloroacetic acid precipitability and adsorption to talc as described by Freychet et al. (22). Under these conditions, the radioactivity precipitated by 5% trichloroacetic acid and absorbed to talc is a measure of the undamaged ^{125}I -labeled insulin (15).

Measurement of serum insulin and glucose

Insulin was measured by means of insulin immunoassay kits purchased from Amersham/Searle Corporation. The glucose oxidase method (glucostat, Worthington) was used in measuring glucose. The blood for these analyses was collected in the morning when rats were killed for binding studies.

Digestion of cells with phospholipase C

Phospholipase C from *Clostridium welchii* (10 units/mg) was purchased from Sigma Chemical Company. Cells were incubated with the desired phospholipase C concentration for 15 min at 37°C and washed several times before being used in ^{125}I -labeled insulin binding studies.

An estimation of cell lysis caused by treatment with phospholipase C was derived by measuring the DNA content of intact cells remaining after digestion. DNA analysis was performed by the method of Burton (23). (23).

TABLE 1. Effect of diet on body weight, fat pad weight, and fat cell volume^a

Group ^b	Body Weight ^c	Weight Gain		Fat Pad Weight	Mean Cell Vol.
		1st Period	2nd Period		
	g	g/day	g/day	mg/100 g	picoliters
G ₅	154 ± 1.9	6.0 ± 0.17		600 ± 13.8	52.1 ± 7.00
L ₅	162 ± 5.7	7.4 ± 0.25		629 ± 17.3	64.5 ± 6.70
P	NS	<0.005		NS	NS
G ₁₀	175 ± 1.9	6.2 ± 0.17	6.3 ± 0.35	631 ± 32.8	65.1 ± 5.35
L ₁₀	191 ± 4.6	7.7 ± 0.23	7.9 ± 0.27	802 ± 28.3	71.2 ± 4.10
P	<0.025	<0.005	<0.025	<0.01	NS
G ₅ -L ₅	189 ± 4.7	5.6 ± 0.23	7.8 ± 0.55	681 ± 16.3	67.4 ± 7.33
L ₅ -G ₅	182 ± 3.1	7.1 ± 0.30	5.3 ± 0.21	689 ± 36.8	75.6 ± 11.40
P	NS	<0.01	<0.01	NS	NS

^a Values are means ± SE for four experiments. (Each experiment included five to six rats).

^b Letters refer to diets, numbers to the number of days rats were fed the diets. G, glucose (fat free) diet; L, high (lard) diet.

^c Values obtained at the time rats were killed.

Effect of concanavalin A on insulin binding

In this experiment adipocytes were incubated at 24°C for 30 min with various concentrations of concanavalin A before being tested for insulin binding as previously described.

Statistics

Results are given as means \pm SE. Statistical comparisons were made by Student's *t* test. In all experiments, differences between means were considered nonsignificant (NS) when the *P* value was greater than 0.05.

RESULTS

Preliminary experiments confirmed a published report that dietary fat decreases adipose tissue glucose oxidation in the presence of insulin (9). Fat cells from rats fed a high fat diet (L) for 5 days oxidized significantly less glucose in the presence of a maximally stimulating concentration of insulin ($10^4 \mu\text{U/ml}$) than cells from rats fed a high glucose, fat-free diet (G), (152 ± 10.4 nmoles/ 10^6 cells in one hr for the L cells vs. 718 ± 161 for the G). Furthermore the stimulation of glucose oxidation by insulin in cells from fat-fed rats was significantly diminished compared to

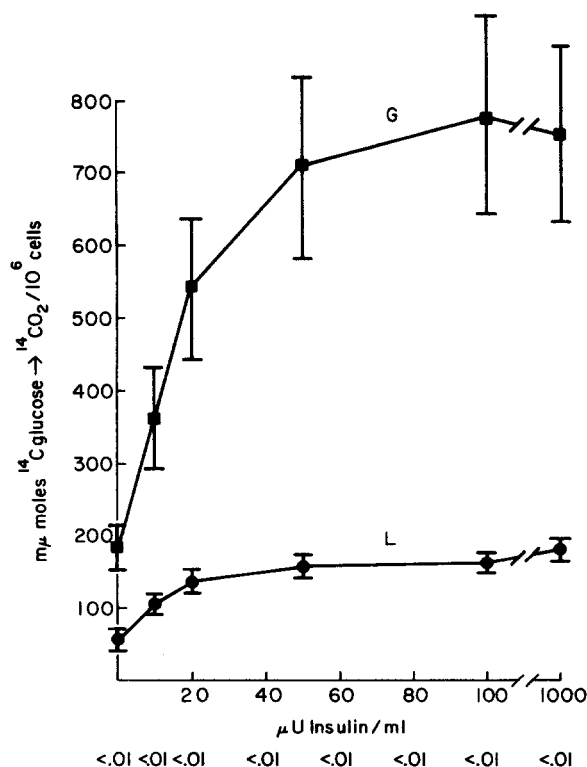


Fig. 1. The effect of insulin on glucose oxidation by adipocytes from rats fed glucose (G) or lard (L) diets. Glucose conc, 10 mg/100 ml. Incubation time, 1 hr.

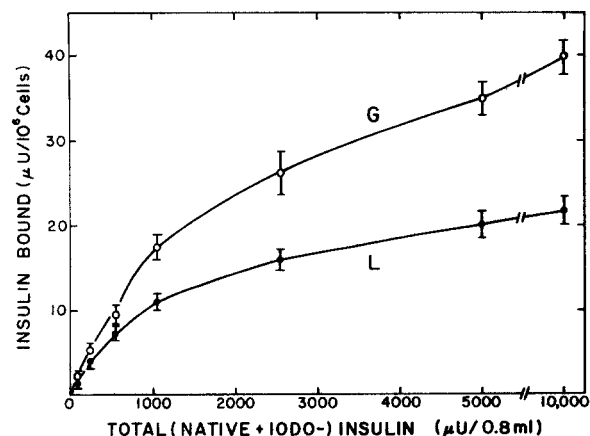


Fig. 2. Specific insulin binding to fat cells of rats fed glucose (G) or lard (L) diets. ^{125}I -Labeled insulin was present at 40–45 $\mu\text{U/tube}$ with increasing amounts of unlabeled insulin (total volume per tube 0.8 ml).

that found in cells from glucose-fed animals ($L = 361 \pm 49.1\%$ insulin/no insulin, $G = 1002 \pm 79.3$, $P < 0.001$).

An experiment was then performed to test the effects of these diets on insulin binding. Groups of rats were fed either the lard (L) or the glucose (G) diet for 5 days and their fat cells tested for insulin binding and insulin response. **Table 1** shows that the rats fed the high fat diet gained weight more rapidly than those fed the glucose diet. The mean final body weight was not significantly greater for this group in the 5-day experiment, possibly because of slight variations in the starting weight of these rats. There was, however, a trend toward higher body weight, fat pad weight and fat cell size in the fat-fed animals. These data are representative of the experiments in which rats were fed these two diets. Food intake was measured in one such experiment. The caloric intake was the same for both groups, suggesting that the efficiency of utilization of calories is greater for high fat than for high carbohydrate diets. **Fig. 1** shows that fat cells from the rats in this experiment showed the expected loss of insulin response as a result of fat feeding.

Although some of the difference in the amount of glucose oxidized in the presence of insulin is related to the difference in basal oxidation, the increment in the presence of maximally stimulating concentrations of insulin is significantly greater for the G cells and it represents a significantly greater percent of basal values.

Fig. 2 represents the specific binding of insulin when 40–45 μU of ^{125}I -labeled insulin (50–58 $\mu\text{U/ml}$) and increasing amounts of unlabeled hormone were present. At each concentration tested, the cells from glucose-fed rats bound more hormone. This binding

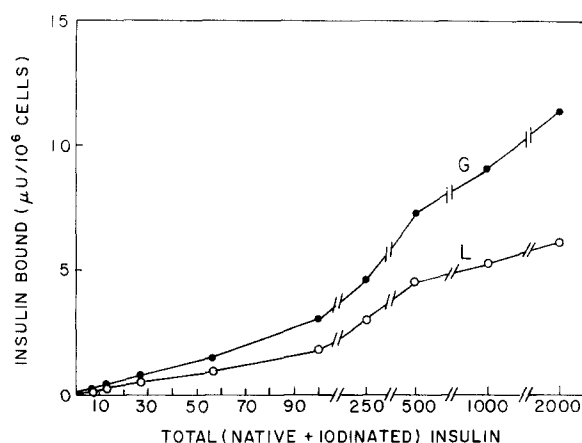


Fig. 3. Specific insulin binding to adipocytes of rats fed glucose (G) or lard (L) diets. $7 \mu\text{U}$ ^{125}I -labeled insulin was present in each tube with increasing amounts of unlabeled insulin. Details are in Methods section.

difference was increased as the concentration of insulin was increased. In order to test the binding to high affinity, low capacity binding sites, which had been reported to be present in adipocytes (24, 25), a second similar experiment was carried out with $7 \mu\text{U}$ of radioactive insulin in each tube ($9 \mu\text{U}/\text{ml}$). The results of that test are shown in **Fig. 3**. Again the fat cells from glucose-fed rats bound more insulin at each concentration. The apparent existence of both types of binding sites is shown by the Scatchard plots in **Fig. 4**, representative of those in six separate experiments. **Table 2** shows that dissociation constants calculated from these figures are not significantly influenced by diet. The total binding capacity, however, was always greater for G cells.

The rates of association and dissociation were also measured directly with fat cells from rats fed each of the diets. **Fig. 5** shows that there was no detectable difference in the rates of association between G and L groups. The rates of dissociation were compared in the presence and absence of an excess of unlabeled insulin in order to test the possible role of negative cooperativity in insulin binding, according to the procedure of DeMeyts et al. (21). The results (not shown) indicated that there was very little if any negative cooperativity with no detectable difference between the diet groups in this respect. Hill plots (not shown) of the data used to calculate the binding capacities summarized in **Table 2** (B) lead to the same conclusion (coefficient for G = 0.96, for L = 0.95). The rates of dissociation appeared to be very similar for fat cells from both groups of animals, confirming the results of calculations from Scatchard plots ($t_{1/2}$ for G = 48 min, L = 54 min).

It was possible that the apparent differences in insulin binding capacity might be explained by a dif-

ference in the rate of degradation of insulin by the two types of cells. To test this hypothesis, ^{125}I -labeled insulin at two concentrations was incubated with fat cells prepared from rats fed the G and L diets for 5 days and the degradation of hormone was measured. An appreciable amount of degradation took place but it was the same for the two types of cells. **Fig. 6** shows the results obtained when cells were incubated with $9 \mu\text{U}$ insulin/ml. At 50 – $58 \mu\text{U}/\text{ml}$ of insulin, degradation was also similar for both groups of cells. When talc adsorption was used as a test of insulin integrity, similar results were obtained (not shown).

To test the reversibility of the effects of the high fat diet on insulin binding and insulin response, groups of rats were fed the high fat diet for 5 days, then changed to the high glucose diet for an additional 5 days before fat cells were prepared. Similarly, the effects of changing from a high glucose to a high fat diet were studied. For comparison, rats fed each diet continuously for 10 days were included in the experiment. Again rats fed the high fat diet gained more weight than those fed the glucose diet with a trend toward increased fat pad weight and cell size (see **Table 1**). **Fig. 7** demonstrates that both the insulin binding capacity and the insulin response effects of fat feeding are reversible. In this experiment the increment over baseline of glucose oxidation at maximally stimulating insulin concentrations was less for L cells

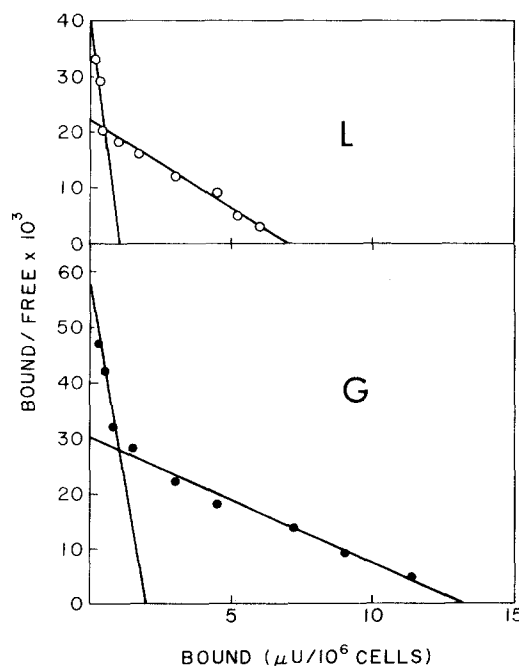


Fig. 4. Scatchard plots of insulin binding to adipocytes of rats fed glucose (G) or lard (L) diets. Upper panel L, lower panel G. These plots are representative of those used to calculate the data in **Table 1**, experiment B.

TABLE 2. Effect of dietary treatment on insulin binding to isolated adipocytes

Experiment	Dietary Group	Maximal Binding Capacity ^a		Dissociation Constant ^a	
		I ^b	II ^c	I	II
		<i>Molecules × 10⁻³/cell</i>		<i>M × 10⁹</i>	
A ^d	Glucose	187.9 ± 5.7		9.1 ± 1.2	
	Lard	91.7 ± 6.1		7.7 ± 0.7	
	<i>P</i>	<0.001		NS ^e	
B ^f	Glucose	58.4 ± 7.3	9.1 ± 0.8	4.1 ± 0.5	0.31 ± 0.02
	Lard	28.6 ± 3.8	5.0 ± 0.5	3.1 ± 0.2	0.28 ± 0.02
	<i>P</i>	<0.02	<0.01	NS	NS

^a Values are mean ± SE.

^b I refers to the population of high-capacity, low-affinity insulin receptor sites.

^c II refers to the population of low-capacity, high-affinity insulin receptor sites.

^d In experiment A, insulin binding studies were carried out using 40–45 μ U of ¹²⁵I-labeled insulin.

^e Not significant, *P* > 0.05.

^f In experiment B, insulin binding studies were carried out using 7 μ U of ¹²⁵I-labeled insulin. In computing the high affinity sites (II), the contribution of the low affinity sites (I) has been accounted for. The binding capacity of both (I) and (II) were calculated from the data in Fig. 3 by Scatchard plot analyses as described in reference 20.

than for G cells, but the percentage increase was similar for both.

The decrease in fat cell insulin binding capacity in response to fat feeding might reflect a change in affinity of binding sites for the hormone, a decrease in the total number of binding sites per cell, or a change in membrane structure that reduced the availability of binding sites to the hormone. It has been reported (26) that treatment of fat cells with phospholipase C increases their capacity to bind insulin, presumably by making accessible previously unavailable, or "masked", binding sites. It therefore seemed possible that, if feeding the lard diet resulted merely in occluding fat cell insulin binding sites, phospholipase C treatment might increase insulin binding to a greater extent in fat cells from the lard diet-fed rats than in those obtained from rats on a high glucose diet. Preliminary experiments revealed that phospholipase C treatment resulted in cell lysis in a dose-dependent relationship as did the increase in insulin binding resulting from such treatment. For the studies on cells from glucose and lard diet-fed rats, 10 μ g/ml phospholipase C was used since it increased insulin binding with the least cell lysis. The results of the experiment, shown in Table 3, indicated that a similar increase in insulin binding was produced in both types of cells.

Several studies reported by Roth and his collaborators (summarized in ref. 27) suggested that decreased insulin binding capacity of various types of cells was associated with exposure to increased concentrations of insulin over a period of time. To explore the possibility that a change in insulin concentration was involved in the change in insulin binding capacity of fat

cells from fat-fed rats, blood samples were collected from some of the rats used in the experiments shown in Figs. 2 and 7. Table 4 shows that significantly less circulating insulin was present in the rats fed the lard diet for 5 or 10 days than in those fed the glucose diet. Feeding the carbohydrate diet for 5 days to rats previously fed the high fat diet did not, however, increase the insulin level. No significant effects on circulating glucose were observed.

Results of experiments with concanavalin A are given in Figs. 8 and 9. Concanavalin A decreased insulin binding to both types of fat cells. This concanavalin A effect was much more marked on the cells from rats fed the glucose diet than on those from lard

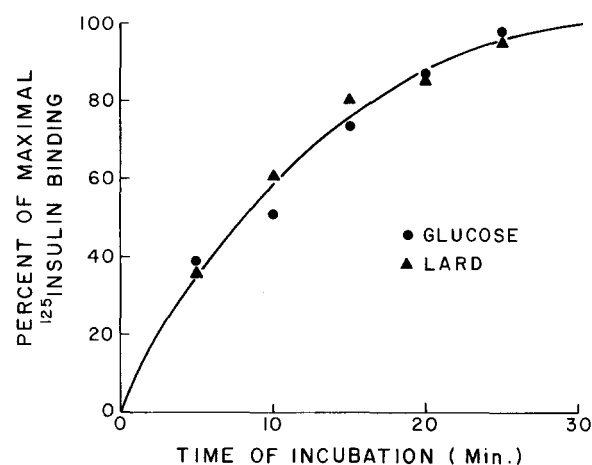


Fig. 5. Time course of uptake of ¹²⁵I-labeled insulin by fat cells from rats fed either a glucose or lard diet. The specific ¹²⁵I-labeled insulin binding was determined (Methods section) and plotted as percentage of maximal binding. Each point is the mean of triplicate determinations.

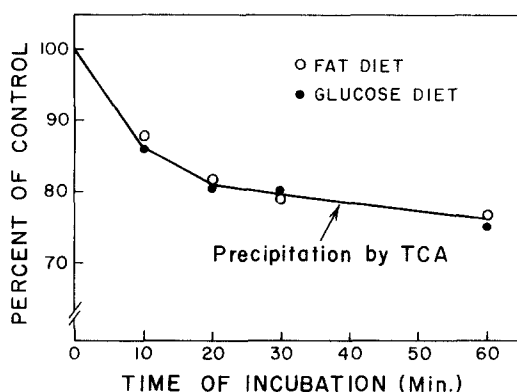


Fig. 6. Degradation of insulin by adipocytes from rats fed a glucose (G) or lard (L) diet. After incubating ^{125}I -labeled insulin ($9\text{ }\mu\text{U/ml}$) for various lengths of time with fat cells as described in the Methods Section, the cells and medium were separated by centrifugation and the labeled insulin in the medium tested for precipitability by trichloroacetic acid.

diet-fed rats (Fig. 8). The insulin-like responses to concanavalin A were also significantly greater in the more insulin-responsive cells (Fig. 9). The maximum responses as percentage of control were $G = 450 \pm 23.9$, $L = 253 \pm 15.5$ ($P < 0.005$).

It was found that spermine did not decrease binding of labeled insulin to fat cells prepared from any group of rats. Its insulin-like effect on fat cell glucose oxidation was, however, similarly related to the diet of donor rats, although the differences were less marked. The maximum responses as percentage of control values were $G = 337 \pm 37.2$, $L = 230 \pm 15.5$ ($P < 0.05$). This finding suggests that dietary changes in response of fat cells to insulin depend in part on adaptations in addition to those directly related to hormone binding.

It is known that high fat diets produce adaptive decreases in the activity of various adipocyte intracellular enzymes involved in glucose metabolism (28). When the concentration of glucose in the medium is increased, intracellular processes become limiting for glucose oxidation at high glucose concentrations. The difference in glucose oxidation by the cells from rats fed different diets (see Fig. 10) is probably related to adaptive changes in intracellular metabolism. In this experiment, the decreased glucose oxidation by L cells at the lowest concentration of glucose used (0.1 mg glucose/ml) did not reach statistical significance, but the response to insulin was significantly decreased in fat cells from fat-fed rats compared to those from the glucose-fed group as in previous experiments.

DISCUSSION

These experiments show that changes in the composition of the diet can influence the insulin binding

capacity of fat cells and therefore provide an additional example of adaptive change in plasma membrane function. The association of reduced binding capacity with decreased response to insulin is similar to that reported for obese animals (2), for rats bearing hormone secreting tumors (1), and for aging rats (29). It is not known in any of these situations to what extent the reduced insulin binding capacity contributes to the diminished response to insulin. Additional factors must also be involved in our experiments since the maximum response to insulin is diminished in the cells from fat-fed rats. Whether the decreased response to insulin of adipocytes from fat-fed rats is in part explained by decreased hormone sensitivity is difficult to decide. In all of our experiments the insulin-stimulated increment in glucose oxidation

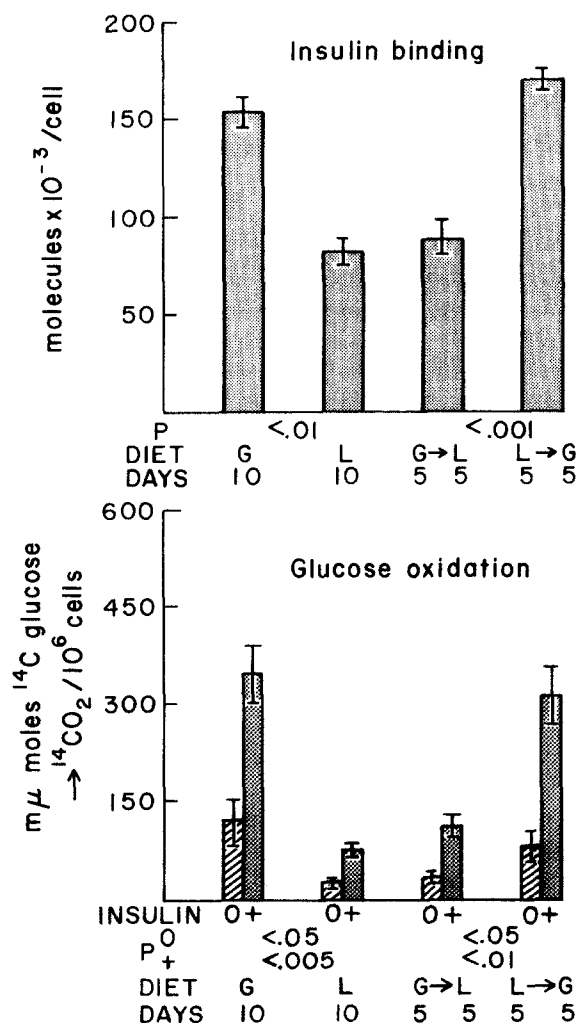


Fig. 7. Reversibility of effect of fat feeding on insulin binding and insulin stimulation of glucose oxidation by adipocytes. Maximum insulin binding was calculated from curves similar to those in Fig. 2. Glucose oxidation was measured at a glucose concentration of 10 mg/100 ml ; insulin when present was $10^4\text{ }\mu\text{U/ml}$. P values are given for comparison of data represented in adjacent bars (or pairs of bars).

TABLE 3. Effect of phospholipase C on insulin binding to fat cells^a

Dietary Group	Treatment ^b	Total Binding Capacity ^c	Dissociation Constant
		molecules $\times 10^{-3}$ /cell	M $\times 10^9$
G ₅ ^d	Control	192	7.7
	Phospholipase C	445	8.8
L ₅	Control	96	6.8
	Phospholipase C	278	7.9

^a Insulin binding was measured as described in the Methods section with 42 μ U of ¹²⁵I-labeled insulin per tube and increasing amounts of unlabeled insulin.

^b Cells were incubated with 10 μ g/ml of phospholipase C prior to insulin binding studies.

^c The values obtained from phospholipase C-treated cells have been corrected for cell lysis by measuring the DNA content of remaining intact cells. According to this method of estimation, approximately 17% of fat cells was lysed by digestion with phospholipase C.

^d Letters refer to diets, numbers to the number of days rats were fed the diets.

over basal values was significantly smaller when cells from fat-fed rats were tested. In some (but not all) experiments there was also a significant decrease in the stimulated value expressed as percent of basal oxidation, suggesting that sensitivity to the hormone was somewhat decreased. That cellular events distal to insulin binding contribute importantly to the decreased response to insulin is indicated by the smaller response to spermine and by the decreased glucose oxidation at high substrate concentrations when adipocytes from fat-fed rats were studied.

In this study adipocytes from rats fed a fat-free diet containing glucose as the carbohydrate were compared with those from rats fed a carbohydrate-free, high fat diet. In preliminary experiments it was found that fat cells from the rats fed the glucose diet bound insulin to approximately the same extent as those from rats fed laboratory chow, a high carbohydrate diet with little free sugar. The insulin effect on glucose oxidation was somewhat less for cells from chow-fed rats than for those from glucose-fed rats but greater than that for cells from fat-fed animals. Whether the presence of fat or the absence of carbohydrate is more important in the behavior of cells from rats fed the lard diet is not possible to determine from these experiments.

In the experiments reported here insulin receptors with different apparent affinities for the hormone were detected and each demonstrated decreased binding capacity in insulin-resistant cells. Dissociation constants for the low affinity sites have been reported by several investigators who obtained values from approximately 3 to 10 $\times 10^{-9}$ M (e.g. refs. 30, 31) depending on experimental conditions. Our experiments A and B in Table 2 were performed at different

times with different rats and with different amounts of ¹²⁵I-labeled insulin so the small differences in K_D values are not surprising. Our values for the dissociation constant of the higher affinity sites also agree with those reported by others (24, 25).

The explanation for the decreased binding of insulin to adipocytes from fat-fed rats is not clear. The difficulties of quantitative analysis of the complex interaction of insulin with its receptor sites have been emphasized by Kahn et al. (20). Scatchard plots of binding data when heterogeneity of binding sites and/or negative cooperativity is present can provide only approximations of apparent numbers of binding sites and dissociation constants. Nor can dissociation constants be calculated accurately from direct measurements when dissociation does not follow first order kinetics. However these methods of analysis are useful for comparative purposes, provided that their limitations are recognized, and they have been so used by other workers. Scatchard plots of our data and the negative cooperativity experiment described above suggest that fat feeding modifies the fat cell so that decreased numbers of binding sites for insulin are available. No significant changes in affinity for the hormone or in negative cooperativity were demonstrable. However the differences in insulin binding to fat cells from the two diet groups are consistently smaller at the very lowest insulin concentrations tested than at higher levels. Data from several experiments showed that the L cells bound 86% as much insulin as the G cells (mean of eight experiments) at the two lowest insulin concentrations but only 47% as much at higher concentrations (mean of six experiments). This suggests that the diet effect is complex and that fat feeding produces some change in binding properties not measurable by techniques available to us. There appears to be a trend to smaller dissociation constants for fat cells from lard-fed rats whether calculated from Scatchard plots (Table 2) or suggested by the slight difference in rate of dissociation.

TABLE 4. Effect of diet on serum insulin and glucose

Group ^a	Number of Rats	Insulin	Glucose
		μ U/ml	mg/100 ml
G ₅	16	43 \pm 3.7	129 \pm 5.0
L ₅	16	30 \pm 3.1	129 \pm 4.9
P		<0.01	NS
G ₁₀	16	60 \pm 6.4	138 \pm 12.3
L ₁₀	15	41 \pm 5.1	135 \pm 8.7
P		<0.05	NS
G ₅ \rightarrow L ₅	15	41 \pm 7.7	138 \pm 5.9
L ₅ \rightarrow G ₅	16	42 \pm 2.9	143 \pm 8.5
P		NS	NS

^a Letters refer to diets, numbers to the number of days rats were fed the diets.

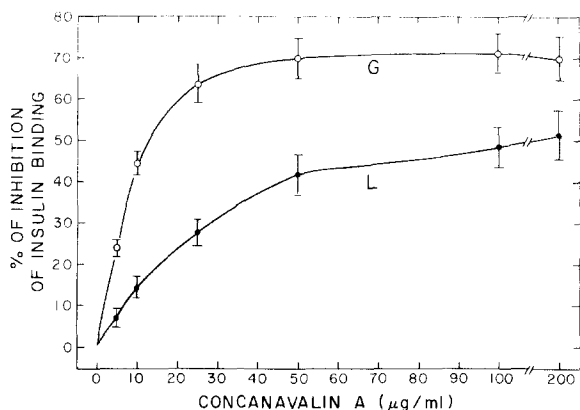


Fig. 8. Inhibition of insulin binding to adipocytes from rats fed glucose (G) or lard (L) diets by concanavalin A. 40–45 μ U of 125 I-labeled insulin was present in each tube. Cells were preincubated for 30 min with concanavalin A before insulin binding was tested.

Whether there is a real, though small, difference in affinity contributing to the difference in binding capacity cannot be established by the experiments reported here. In any case the results show that fat feeding results in decreased fat cell insulin binding capacity.

There are many reports in the literature that fat cell hormone response decreases with increasing cell size (32–35). That cell size is not the only influence on hormone response was suggested by previous work from this laboratory (36) and more recently by Smith, Kral, and Bjorntorp (7) in studies on the effect of fat feeding in response to epinephrine. Salans et al. (37) reported recently that diet composition as well as cell size also influenced fat cell response to insulin. Similarly, the difference in fat cell insulin binding and insulin response reported here cannot be explained by an effect of diet on cell size. The cells from fat-fed rats tended to be larger than those from glucose-fed rats in most experiments but were smaller in the diet reversal experiment (Table 1). None of these differences was significant. Whether the slight increase in weight gain of rats in the fat diet could have contributed to the decreased insulin binding and response of their adipocytes cannot be determined from these experiments. Both groups of rats were gaining weight rapidly.

It has been reported (27) that exposure of cells to high concentrations of insulin results in decreased insulin binding. That other factors can be involved in changing insulin binding capacity is suggested by the experiments reported in this paper, since decreased insulin binding of cells from fat-fed rats was associated with significantly smaller or similar circulating insulin concentrations at the time the rats were killed. The finding that fat feeding results in decreased levels

of circulating insulin is in agreement with the observations of Blazquez and Oujada (5) but in contrast with those of Smith et al. (7). The latter group of investigators reported a tendency to higher insulin levels in larger rats fed a 72% fat diet for two weeks compared to those on a high carbohydrate diet. Possibly the age of the rats, the length of feeding the diets, or the time of day at which measurements were made could explain this difference. Since insulin levels were measured only in the morning when the rats were in the fed state, it is possible that greater fluctuations in insulin levels throughout the 24 hours in glucose-fed rats compared to those fed lard might result in higher insulin concentrations in L animals during some part of the day.

While these experiments were in progress, Cushman and Salans (38) reported that no difference in insulin binding could be detected when adipocytes from fat-fed rats were compared with those fed a high carbohydrate diet. The identity of the carbohydrate was not stated. They tested cells from rats weighing 300 g that had been fed the experimental diets from

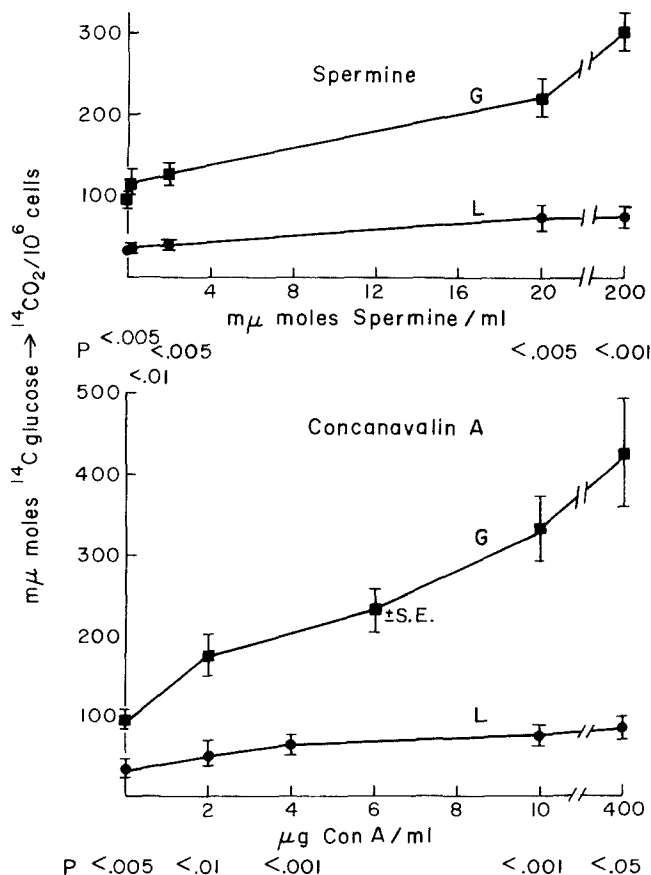


Fig. 9. Effects of spermine and concanavalin A on glucose oxidation by adipocytes from glucose (G) or lard (L) fed rats. Glucose was present at 10 mg/100 ml, incubation time 1 hr.

weaning. Differences in the duration of the diet period, the age of the animals, and possibly the type of carbohydrate may explain the discrepancy between their results and ours.

These experiments suggest that fat feeding alters the availability of the cell surface glycoprotein concanavalin A receptor sites to the lectin. It is not known how concanavalin A interferes with insulin binding. Cuatrecasas (8) and Katzen (39) have suggested that the insulin receptor is a glycoprotein and, in that case, concanavalin A might compete directly with insulin for the hormone receptor site.

The experiments of Czech, Lawrence, and Lynn (40), on the other hand, indicate that the receptors for insulin are not glycoproteins since they are destroyed by trypsin treatment, leaving the major glycoproteins of the cell intact. The concanavalin A effect on insulin binding then might depend on some change in the membrane, induced by concanavalin A binding to its receptors, which would interfere with the accessibility of the hormone to its specific receptor site. In either case, the results suggest that diet fat alters the adipocyte plasma membrane glycoproteins in some way. The possibility that concanavalin A has effects on some intracellular process that may influence insulin binding is not excluded by the experiments reported. If such effects occur, they could possibly vary with diet and provide an alternative explanation of the results.

Recently Chang, Huang, and Cuatrecasas (41) reported that the decreased insulin binding capacity of liver membranes from obese mice compared to that of their lean littermates is associated with a decreased capacity to bind the lectins concanavalin A and wheat germ agglutinin. Membrane glycoproteins appear to be altered in that type of insulin binding deficit under conditions that make intracellular influences less likely.

These experiments and those reported earlier from this laboratory (4) indicate that diet fat can change the properties of adipocytes in several ways. Insulin binding capacity, but not fluoride stimulated adenylate cyclase activity (36), is reduced. Some change in response to concanavalin A, in the response of glucose oxidation to several stimuli and in the response of adenylate cyclase to epinephrine and glucagon all result from altering the composition of the diet. Whether these diets influence the response of fat cells to other effects of insulin remains to be determined.

Although we have demonstrated that the comparative insulin resistance of adipocytes from fat-adapted rats cannot be accounted for entirely by a difference in insulin binding, we believe that the binding dif-

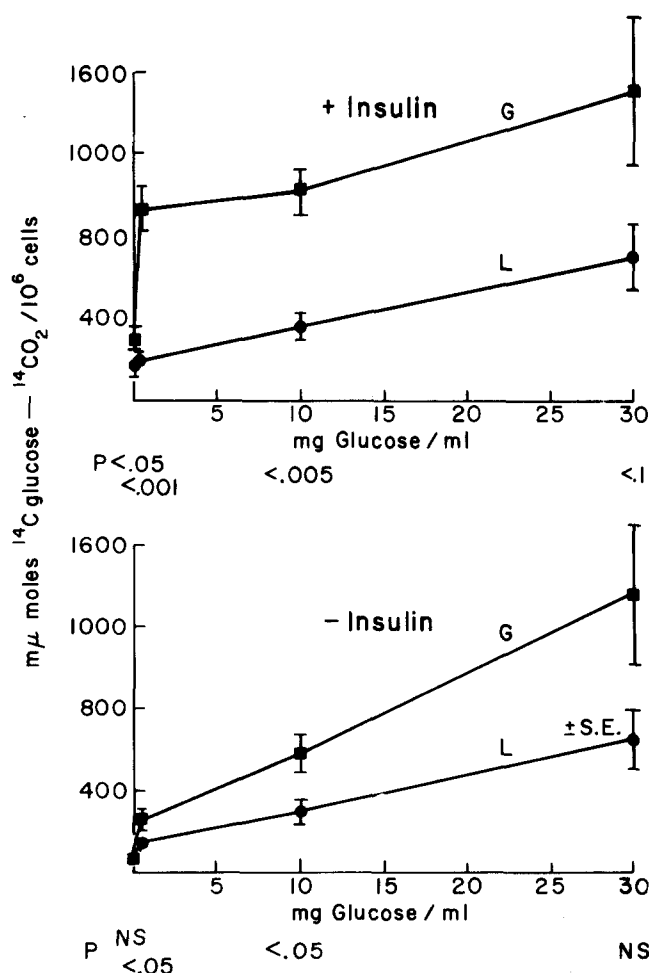


Fig. 10. The effect of glucose concentration on glucose oxidation in the presence and absence of insulin by adipocytes from glucose (G) or lard (L) fed rats. Insulin when present was 10^4 μ U/ml. Incubation time 1 hr. *P* values are given for the difference between the diet groups at each concentration of glucose.

ference may contribute to the insulin resistance. Further work will be required to describe the temporal relationships between adaptive changes in hormone binding and those that appear in the "interior" of the cell. It is conceivable (a) that the binding changes may occur before enzyme adaptations appear within the cell, or (b) that binding changes are secondary to "interior" adaptive events, or (c) that both binding adaptations and intracellular alterations represent coordinate and complementary responses to the same set of environmental stimuli. ■

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