A comparison of insulin binding by liver plasma membranes of rats fed a high glucose diet or a high fat diet

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Abstract The interaction of ^{125}I-labeled insulin with purified liver plasma membrane from rats fed a high fat (L) diet or a high glucose (G) diet was studied with respect to specific binding, insulin degradation, binding site degradation, and rate of hormone association and dissociation. Scatchard analysis suggested the presence of high and low affinity binding sites for membranes of both G and L diet-adapted rats. However, liver plasma membrane from rats fed the high glucose diet bound 50% more insulin than did membrane from rats fed the high fat diet. Diet did not change insulin binding site degradation. The results suggested that an apparently reduced number of insulin binding sites ($G = 10.2 \pm 2.45 \times 10^{-12}$ mol/mg membrane protein, $L = 4.5 \pm 1.73 \times 10^{-12}$ mol/mg membrane protein) associated with fat feeding as compared to glucose feeding was responsible for the reduced insulin binding by membrane from rats fed the high fat diet. The effects of concanavalin A (Con A) on insulin binding to liver plasma membranes were also investigated. Con A enhanced the specific binding of insulin to liver plasma membranes from rats fed either diet at concentrations lower than 50 μg/ml, whereas at concentrations higher than 50 μg/ml Con A inhibited insulin binding to these membranes. The stimulatory effect of Con A on insulin binding at low concentrations was greater and inhibition of binding at high concentration was less in the case of membrane prepared from L diet-adapted animals. These results suggested that diet can modify the plasma membrane glycoproteins.

Supplementary key words Concanavalin A · insulin degradation · insulin receptor degradation · negative cooperativity

In a previous report from this laboratory (1), it was shown that feeding a high fat, carbohydrate-free diet for 5 days to rats caused a significant decrease in epinephrine- and glucagon-stimulated adenylate cyclase activity of adipocyte ghosts. A shift back to a high carbohydrate diet resulted in a complete recovery of epinephrine-stimulated cyclase activity. In addition, we found that feeding a high fat diet to rats results not only in a decrease in glucose transport in intact adipocytes and in purified adipocyte membranes, but also in decreased insulin sensitivity and insulin binding in isolated dispersed adipocytes (2, 3). Scatchard analysis of the binding assay indicated that cells of fat-fed animals contained an apparently diminished number of insulin binding sites. The first step in insulin action appears to be the binding of the hormone to the cell surface membrane. Therefore, our previous findings strongly suggested that the overall nutritional response of adipocytes in fat diet-adapted animals included a reversible modification of their plasma membranes.

Concanavalin A, a plant lectin, binds selectively to alpha-D mannose and alpha-D glucose residues (4, 5) on the plasma membrane in molar quantities that far exceed insulin binding (4). Previous experiments reported from this laboratory (2) indicated that Con A decreased insulin binding to fat cells of rats fed a glucose diet to a greater extent than was found for fat cells of rats fed a high fat diet. This observation suggested a change in membrane properties involving many surface glycoproteins. Chang, Huang, and Cuatrecasas (6) recently reported that lectin binding studies indicated widespread alteration in liver plasma membrane glycoproteins of genetically obese mice in addition to the decrease in insulin binding that had been previously reported.

The present study was begun in an effort to discover whether the diet-induced alterations in plasma membrane functions described above are limited to adipocyte membrane or whether membranes of other tissues show similar changes. Purified liver plasma membrane was selected for study because a number of other investigators had demonstrated parallel changes in adipocytes and liver membranes in obese mice (7–10) or animals exposed to excessive amounts of glucocorticoids (11).

Abbreviations: Con A, concanavalin A; L diet, high fat (lard) diet; G diet, high carbohydrate (glucose) diet; ^{125}I-insulin, ^{125}I-labeled insulin.

MATERIALS AND METHODS

Animals and diets

Male Sprague-Dawley albino rats, 90–100 g in weight, were obtained from Taconic Farms, Germantown, NY, and kept individually in wire cages with food and water ad libitum. The animals were fed Purina rat chow before they were fed either a high fat (L) or a high glucose (G) diet (isocaloric) for 5 days. As described in a previous report (2), both diets included 33% of calories as casein with 67% of calories in the form of lard in the L diet or glucose for the G diet. Adequate amounts of vitamins, minerals, and cellulose were added. Rats fed either diet gained weight throughout the feeding period with an average gain of 6.8 ± 0.6 g/day for G diet-fed rats and 7.5 ± 0.6 g/day for the L diet animals. Liver weights were not significantly different.

Membrane preparation

Purified plasma membrane fractions were prepared from rat liver according to the procedure of Neville (12), and frozen in aliquots at −70°C until use. Electron micrographs of these preparations (not shown) revealed plasma membrane composed mainly of paired membrane sheets with no identifiable organelle contaminants. Routine studies with marker enzymes 5′-nucleotidase (13), NADH oxidase (13), and cytochrome *c* oxidoreductase (14) demonstrated that contamination with mitochondria or microsomes accounted for less than 11% of the total membrane protein. The extent of purification was the same for both diet groups. Protein concentrations were determined by the method of Lowry et al. (15) with serum albumin as a standard. The 5′-nucleotidase specific activity per mg protein was the same for membrane preparations prepared from livers of animals fed the two diets (G diet, 10.87 ± 0.64 μmol ^{3}H-labeled AMP degraded/mg/min; L diet, 11.03 ± 0.57 μmol/mg/min). Results have been referred to membrane protein which, therefore, did not appear to vary with diet.

^{125}I-Labeled insulin preparation

Carrier-free ^{125}I was purchased from New England Nuclear, Boston, MA. Crystalline porcine insulin Lot No. 615-D63-10, single component, and monocomponent insulin, Lot 615-1082B-108-I were gifts from Dr. Mary Root and Dr. Ronald E. Chance of Eli Lily and Company, Indianapolis, IN. Insulin was iodinated to a specific activity of 92–250 μCi/μg by the chloramine T method as adapted from Roth (16). Over 97% of the radioactivity of the ^{125}I-insulin preparation used for binding studies was precipitable by 10% TCA and 95% of the radioactivity was adsorbable by 25 mg of talc. All storage and dilution of ^{125}I-insulin was in plastic disposable tubes. Similar binding results were obtained with both lots of insulin.

Binding conditions

The binding of ^{125}I-insulin to purified liver plasma membrane was performed in triplicate in Beckman microfuge tubes with an incubation period of 2 hr at 20°C. Binding was determined according to the method of Freychet et al. (7) as modified by Soll and Kahn (9) in Krebs-Ringer phosphate buffer, pH 7.5, containing 1% bovine serum albumin. The final concentration of the ^{125}I-insulin is indicated in each experiment. Membrane concentration was 615 μg protein per ml. The total volume of the incubation mixture was 0.15 ml. When the effect of Con A on insulin binding was studied, 0.1 μg/ml to 120 μg/ml of Con A were preincubated with the liver plasma membrane in Krebs-Ringer phosphate buffer for 1 hr at 20°C before addition of the ^{125}I-insulin. The membrane-bound ^{125}I-insulin was sedimented by centrifugation in a Beckman microfuge for 1 min, the supernatant was removed, and the tips of the microfuge tubes were excised. Radioactivity in the pellet was measured with 10 ml of Instabray (Yorktown Research, Hackensack, NJ) in a Nuclear Chicago Isocap 300 liquid scintillation counter at 50% efficiency, and dpm was calculated by the channels ratio method using an external standard. All data were corrected for nonspecific binding measured in the presence of 53 μg/ml of native insulin. The nonspecific binding in all experiments was less than 10% of the total binding.

Hormone degradation

Determination of ^{125}I-insulin degradation was done at the end of each binding experiment. The supernatant of the binding mixture after centrifugation was removed and tested for TCA precipitability (17) and talc adsorbability (18).

Receptor degradation

Insulin receptor degradation was determined according to the method of Soll and Kahn (9) by preincubating the liver plasma membrane in a Beckman microfuge tube with Krebs-Ringer phosphate buffer at different time intervals at 20°C. ^{125}I-Insulin was added and, after 2 hr at 20°C, the tubes were centrifuged; the supernatant was removed and the radioactivity in the pellet was counted.

Rate of association and dissociation

The rates of association and dissociation of insulin with liver membrane were measured according to a method adapted from DeMeyts et al. (19). The rate of dissociation was measured in the presence and absence of an excess of native insulin after 100-fold dilution of the hormone receptor complex.

The methodological details of rate of dissociation, as well as the concentrations of insulin and membrane used in each experiment, are indicated in the figure legends.

Statistical evaluations were made by Student's t test with differences of $P < 0.05$ being considered significant.

RESULTS

The time course of the binding reaction, when expressed as percent of maximal ^{125}I-insulin binding, was similar for G and L membranes. In both cases the binding reached its maximum at 2 hr. After that time the specific binding of ^{125}I-insulin begins to decrease. The extent of insulin degradation was determined at the end of each binding experiment. It was found that at least 90% of the insulin remaining in the supernatant was intact based on TCA and talc testing. Moreover insulin degradation did not vary with diet. All subsequent binding experiments were performed for 2 hr.

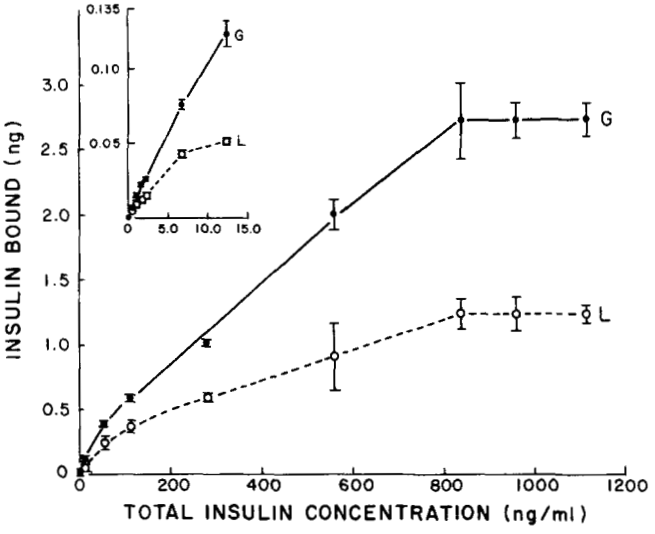

Fig. 1. Specific insulin binding to liver plasma membranes from glucose (G) or lard (L) fed rats. Liver membranes, 615 μg protein/ml, were incubated with 1.1 ng/ml (or less) of ^{125}I-insulin and with unlabeled insulin over the range of 0–1200 ng/ml (total). The binding conditions were described in detail in Methods section. The specific insulin bound is plotted as a function of the total insulin concentration. Data for G and L membranes are the means ± SEM of triplicate determinations in five experiments and $P < 0.05$ for the difference between the two types of membranes at all points.

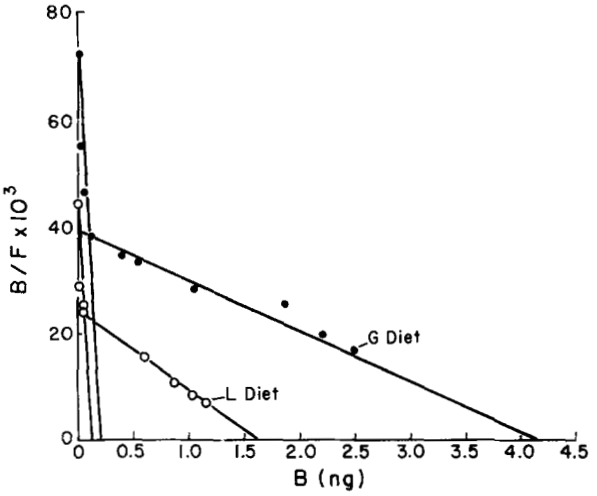

Fig. 2. Scatchard plot of insulin binding to liver plasma membranes from glucose (G) or lard (L) fed rats. Points were calculated from the results given in Fig. 1. All data were corrected for nonspecific binding, percent of hormone degraded, and the contribution from low affinity sites to high affinity sites. Data for G and L membranes are the means of triplicate determinations in five experiments. $P < 0.05$ for all the points. B/F, bound/free.

When insulin binding with liver plasma membrane was investigated in the presence of increasing concentrations of hormone, different binding results were found for G and L membranes (**Fig. 1**). By plotting insulin bound as a function of hormone concentration, it was found that the curve for L membrane was lower than that for G membrane over the range of insulin concentration from 0.1 to 1200 ng per ml. Both binding curves reached a plateau at an insulin concentration of 800 ng/ml, indicating that, at this high concentration, all available receptors were saturated. At every concentration of insulin, L membrane bound approximately 50% as much as G membrane. Changes in either affinity or capacity of the insulin receptor populations could account for these binding differences.

Curvilinear Scatchard plots (20), as shown in **Fig. 2**, were obtained for both G and L membranes. This result would be expected with negative cooperativity as has been previously demonstrated for insulin receptors by several laboratories (4, 9, 21, 22), or it may also indicate two or more populations of receptors with different affinities. For comparative purposes calculations were made assuming binding sites with two affinities, although this procedure can provide only a rough approximation of the actual relationship. The slope of the Scatchard plots for both high ($G = 1.84 \pm 0.53 \times 10^8$ M^{-1}, $L = 1.73 \pm 0.52 \times 10^8$ M^{-1}) and low ($G = 0.72 \pm 0.21 \times 10^7$ M^{-1},

TABLE 1. Effect of diet on affinity constants and binding capacity of purified liver plasma membrane[a]

		G	L
Affinity Constant (M^{-1})	High Affinity	$(1.8 \pm 0.53) \times 10^{8}$	$(1.7 \pm 0.52) \times 10^{8}$
	Low Affinity	$(0.7 \pm 0.21) \times 10^{7}$	$(1.1 \pm 0.27) \times 10^{7}$
Binding Capacity (mol/mg protein)	High Affinity	$(6.8 \pm 2.15) \times 10^{-13}$	$(3.3 \pm 0.99) \times 10^{-13}$
	Low Affinity	$(9.5 \pm 2.27) \times 10^{-12}$	$(4.1 \pm 1.66) \times 10^{-12}$
	Total	$(10.2 \pm 2.45) \times 10^{-12}$	$(4.5 \pm 1.73) \times 10^{-12}$

[a] These results were calculated from Scatchard plots similar to those in Fig. 2. Each value is the mean ± SEM of experiments with five membrane preparations, each preparation made from the livers of eight rats. G = liver membranes isolated from glucose diet-adapted rats; L = membranes from lard-fed animals.

L = $1.15 \pm 0.27 \times 10^{7}$ M^{-1}) affinity sites as indicated in **Table 1** showed no significant difference between the two membranes, indicating that the apparent affinity was unchanged in L diet membrane. Hill plots (23) (not shown) for both membranes constitute further support for this point by showing two superimposable straight lines with a slope of 0.97 for L membrane and 0.94 for G membrane. The apparent number of binding sites calculated from the intercept of the Scatchard plot at the abscissa for high affinity low capacity and low affinity high capacity sites (Table 1) indicates that the decreased insulin binding capacity observed in the L membrane can be accounted for by decreases in concentration of both populations of insulin receptors. The total number of binding sites for L membrane, $(4.51 \pm 1.73 \times 10^{-12}$ mol/mg protein) as compared to that of G membrane $(10.21 \pm 2.45 \times 10^{-12}$ mol/mg protein) was decreased more than 50%.

In order to determine whether the binding difference between the G and L membranes may have been partially attributable to an increase in negative cooperativity of the L membrane in comparison to G membrane, the rate of dissociation of the hormone receptor complex in the presence and absence of unlabeled insulin was investigated. The result is shown in **Fig. 3**. In both cases, dilution or dilution plus unlabeled insulin, dissociation reached a plateau after 100 min of incubation. At that time 50% of ^{125}I-insulin was dissociated when 1 μg/ml of unlabeled insulin was added. The times required to reach half of the maximal dissociation in the case of dilution only and of dilution plus unlabeled insulin were 27 min and 19 min, respectively. The markedly accelerated dissociation rate found in the latter case suggests that negative cooperativity of site–site interaction existed in both G and L membranes. The result also revealed a similarity of dissociation rate in G and L membranes with both dilution and dilution plus unlabeled insulin. The failure to demonstrate a difference in dissociation rate between the membranes under these conditions ruled out the possibility that increasing negative cooperativity was responsible for decreasing the insulin binding found in L membrane.

It is possible that the decreased insulin binding capacity found in L membrane might also be explained by increased insulin degradation, thereby resulting in a decrease in functionally active insulin for binding to L membrane. To test this hypothesis, insulin degradation was determined at the end of 90 binding experiments by the talc adsorption test. It was found, over a range of insulin concentration from 0.11 ng/ml to 1200 ng/ml, that the average intact insulin after 2 hr was 89.9 ± 4.1% for L membrane and 89.0 ± 4.5% for G membrane. The fact that

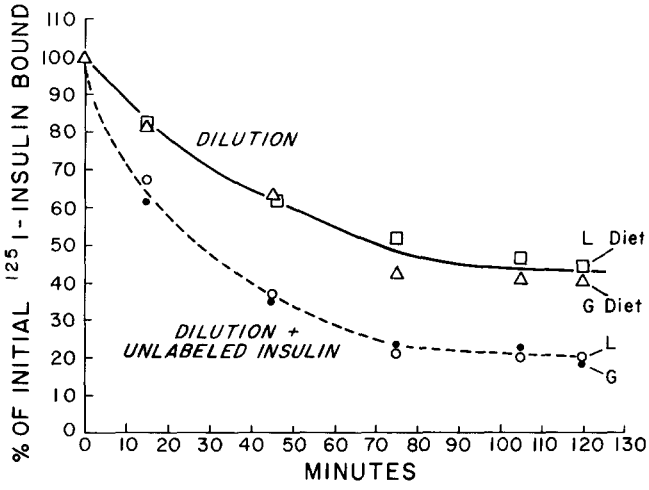

Fig. 3. Effect of native insulin on the dissociation of ^{125}I-insulin from liver plasma membranes from glucose (G) and lard (L) fed rats. 1.1 ng/ml of ^{125}I-insulin was incubated with 615 μg/ml membrane for 2 hr at 20°C. Triplicate 50-μl aliquots were then diluted in 5 ml of Krebs-Ringer phosphate buffer with 0.3% albumin, pH 7.5, or 5 ml of buffer containing 1 μg/ml of unlabeled insulin, pH 7.5. All tubes were incubated at 20°C for the indicated time. At the end of each incubation, membrane-bound ^{125}I-insulin was separated by 0.22 μm Millipore filters and washed once with 10 ml of cold buffer. The filters were counted and the total ^{125}I-insulin bound, after subtracting a filter paper blank, was determined and plotted as the percentage of initial ^{125}I-insulin binding. The data are the means of triplicate determinations in three separate experiments.

both membranes degraded insulin to the same extent makes it unlikely that a difference in binding could be explained on the basis of different rates of insulin degradation.

It has been pointed out by Soll and Kahn (9) in studying the insulin binding to liver membranes of *ob/ob* mice that the insulin receptor is defined only by its functional characteristics. We cannot exclude the possibility that the L diet may have caused an alteration in the receptor by influencing sites other than those responsible for the binding of hormone in quantity. An apparently decreased insulin receptor concentration might occur if there were an alteration in receptor stability during incubation. Degradation of membrane receptor was examined to test this possibility. The result indicates a slow receptor-degrading process in both membranes. Ninety percent of the receptors remained intact after a 2 hr preincubation and there was no appreciable difference in receptor degradation between G and L membranes.

The effect of Con A on insulin binding (**Fig. 4**) depended largely on the concentration of Con A used. Con A in the concentration range of 1–50 μg/ml stimulated insulin binding up to 14%. Concentrations of Con A greater than 50 μg/ml markedly reversed the stimulating effect and caused an inhibitory effect of up to 32% for G membrane and 11% for L membrane. At a concentration of 75 μg/ml the inhibitory effect reached a plateau for both membranes. The enhancement effect at low Con A concentration was greater and the inhibitory effect at high concentrations was smaller in the case of L membranes.

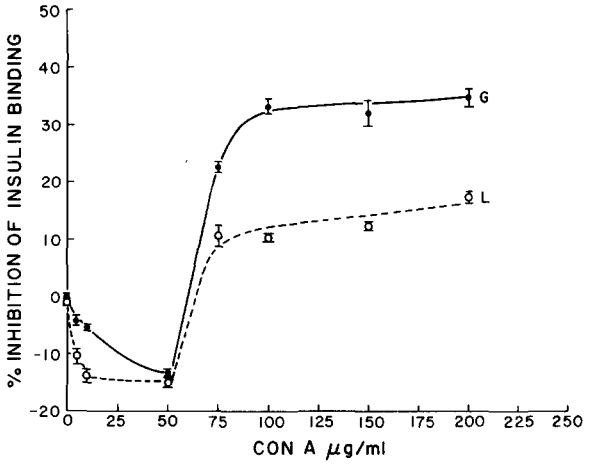

Fig. 4. Effect of concanavalin A on the specific binding of ^{125}I-insulin to liver plasma membranes from glucose (G) or lard (L) fed rats. Various concentrations of Con A (0.1 μg/ml–120 μg/ml) were preincubated with liver membrane for 1 hr before adding the ^{125}I-insulin. Insulin binding was then measured at a concentration of 1.1 ng/ml as described in the legend for Fig. 1. Means ± SEM of triplicate determinations from five experiments are shown. Differences between the means of diet groups are all significant ($P < 0.05$) except the values at 50 ng/ml.

DISCUSSION

The fact that adaptive changes can occur in plasma membranes in the form of a decrease in insulin binding has been well documented in obesity (7–10), hyperinsulinemia (7–10), aging (24), and excess glucocorticoids (11). The present study and those reported earlier from this laboratory demonstrate a difference in insulin binding in tissues of rats fed high glucose or a high fat diet. Decreased binding capacity found in L membranes as compared to G preparations was attributed to a decrease in the apparent number of insulin receptors. Analysis of this hormone–receptor interaction by Scatchard plots (Fig. 2) must be considered descriptive of apparent site number rather than an exact measurement. According to Kahn, Freychet, and Roth (25) the method of manual curve fitting results in an underestimation of the "high affinity constant" by about 10% and an overestimation of the "low affinity constant" by 150–200%. Nevertheless, the data reported here are consistent with the result of House (26) for liver plasma membrane of rats. The fact that negative cooperativity exists in both membranes, demonstrated by the DeMeyts et al. (19) experiment, was not fully expressed by Hill analysis (slope = 0.94 for G membrane and 0.97 for L membrane). This could be due to the fact that the former is a more sensitive method of analysis than the latter. However, Hill plot analysis does indicate that the extent of cooperative interaction is the same for the two types of membrane. This was further confirmed by dissociation experiments.

The diet effects on insulin binding described in this report and in a previous paper from our laboratory (2) appear to differ in their relationship to plasma insulin level from many reported alterations in binding described by Roth and his collaborators (7–11). Rats fed the lard diet had somewhat lower insulin levels than those fed glucose (2) whereas the decreased insulin binding in obese animals and in rats with elevated glucocorticoids was associated with hyperinsulinism. Thus there appear to be several possible mechanisms involved in regulating plasma membrane insulin binding capacity.

The decreased insulin binding by liver plasma membrane of rats fed a lard diet compared to those from glucose-fed rats may contribute to a decreased response to insulin by the livers of fat-fed rats. Ogundipe and Bray (27) reported decreased insulin sensitivity of fat-fed rats as shown by their serum glucose response to injected insulin. Since it is be-

lieved that a large part of the blood glucose-lowering effect of insulin is attributable to its effects on the liver (28), this observation suggests that fat feeding decreases the hepatic response to insulin. In addition, preliminary experiments in our laboratory[1] suggested that isolated hepatocytes prepared from L diet-fed rats respond less to insulin added in vitro than do those from rats fed the G diet. Adaptive intracellular changes also probably contribute to the alteration in liver response.

Cuatrecasas has shown (29) that Con A at concentrations higher than 100 μg/ml depresses insulin binding to fat and liver cell membrane. The results of our studies of the Con A effect on insulin binding are consistent with those of Cuatrecasas with respect to the inhibitory effect. The stimulatory effect of Con A at concentrations of less than 50 μg/ml may be explained by the recent finding that Con A, at a concentration of less than 20 μg/ml, interferes with the negative cooperativity induced by insulin (30). The observation that plasma membranes of lard-fed rats respond differently to Con A from those of glucose-fed rats suggests that many glycoproteins of the cell surface are modified by diet in the liver as well as in adipose tissue. In this respect fat feeding as compared to glucose feeding produces membrane effects qualitatively similar to those described by Chang et al. (6) in the genetically obese mouse.

In conclusion, these studies demonstrate that fat feeding of rats for 5 days, as compared with glucose feeding, caused a 50% decrease of insulin binding capacity in purified liver plasma membranes. Measurement of the rates of association, the rates of dissociation, insulin degradation, and receptor degradation indicated no obvious difference between membranes prepared from animals adapted to the two diets. The decreased binding capacity is thus attributable to an apparently diminished number of insulin binding sites in the L membranes. This result is consistent with our previous finding in adipocytes and suggests that an alteration in plasma membrane properties as a result of feeding a high fat diet may occur in liver cells as well as in adipocytes.

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[1] Unpublished experiments.

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