Mechanism of insulin resistance in adipocytes of rats fed a high-fat diet

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Abstract Insulin's ability to stimulate glucose metabolism is severely diminished in the adipose tissue of rats fed a high-fat diet as compared to that of rats fed a low-fat diet. To elucidate the mechanism for this effect we have measured the binding of insulin, the hormone effect on 2-deoxyglucose uptake and the major pathways of [1-^{14}C]glucose metabolism, and the activity of lipogenesis-related enzymes in adipocytes of rats fed a low- or high-fat diet for 7 days. Rats fed high- or low-fat diets bound equal amounts of insulin per adipocyte at all insulin concentrations tested. Basal and maximally insulin-stimulated 2-deoxyglucose uptake per fat cell were reduced in high-fat-fed rats. However, the insulin stimulation over basal was the same in both groups (230%). Submaximal doses of insulin produced equivalent increments of 2-deoxyglucose uptake in both groups, as would be predicted by the binding studies. The effect of both submaximal and maximal insulin concentration on the labeling of CO_2 and fatty acids was markedly decreased by high-fat feeding. The insulin response of the glyceride–glycerol pathway was less severely, though significantly, reduced. Acetyl CoA carboxylase and malic enzyme in adipocytes of high-fat-fed rats were reduced to 13% of the activity in the low-fat-fed rats. Glucose-6-phosphate and 6-phosphogluconate dehydrogenases were decreased to 20% and 34% of their activities in low-fat-fed rats, respectively. These reductions paralleled the changes in insulin-stimulated glucose oxidation and fatty acid synthesis. The data therefore strongly suggest that the blunted response of glucose metabolism to insulin in adipocytes of high-fat-fed rats is a result of a decreased intracellular capacity to utilize glucose for lipogenesis.

Supplementary key words insulin binding · 2-deoxyglucose uptake · lipogenic enzymes · fatty acid synthesis · fat cell size

We have previously shown an altered directional flow of pyruvate (1) and glucose (2, 3) through adipose tissue metabolic pathways in rats fed high-fat as compared to low-fat diets. Furthermore, this tissue exhibited an impaired glucose uptake in response to a maximal dose of insulin (2). We recently confirmed this resistance to insulin in vitro and in vivo, demonstrating that fatty acid synthesis was strongly inhibited by fat feeding and remained negligible even after insulin administration (4). In contrast, glyceride–glycerol labeling was stimulated to the same extent regardless of diet (4). This pathway specificity of the insulin resistance is inconsistent with an impairment of hormone binding, but rather suggests that insulin action on the adipose tissue of fat-fed rats is limited by some intracellular defect. The present study attempted to identify in adipocytes the primary site responsible for the impairment of the insulin effect by quantifying the early events of hormone–cell interaction: insulin binding and its effect on glucose transport. The responsiveness of the major pathways of glucose utilization to insulin and the level of activity of some intracellular enzymes were also measured and discussed in relationship to the pathogenesis of insulin resistance in the adipose tissue of high-fat-fed animals.

METHODS

Rats and diets

Male Wistar rats, 4 weeks old, were obtained from Charles River Breeding Laboratories. They were fed either a low-fat or a high-fat diet (**Table 1**) ad libitum for one week and up to the time of death. Rats were killed by decapitation at 10 AM. Blood was collected and plasma insulin level was subsequently measured (5) using a rat insulin standard.

Preparation of isolated adipocytes

For each experiment the total epididymal adipose tissue obtained from 4–8 rats was pooled, weighed, and then cut into small pieces. The fat cells were isolated by digestion for 1 hr at 37°C in 15 ml of Krebs–Ringer phosphate buffer, pH 7.4, containing 3% serum al-

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bumin and 25 mg of collagenase (6). The fat cells were then filtered through a 250-μm mesh filter. After three washings with collagenase-free buffer, they were suspended in 15 or 30 ml of 3% albumin buffer. Fat cell size was measured utilizing a photomicrographic method previously described (7). The number of fat cells in the fat cell suspension was assessed according to Livingston, Cuatrecasas, and Lockwood (8) by measuring the DNA content (9) of 1 ml of fat cell suspension after removal of most of the infranatant buffer.

Insulin binding studies

^{125}I-Labeled insulin was either prepared as previously described (10) so as to obtain biologically active monoiodo-hormone (11) or was purchased from Cambridge Nuclear Radiopharmaceutical Corporation, Billerica, MA (sp act 117–120 mCi/mg; less than 1 atom of iodine per molecule of insulin). Binding was assayed within one week after the iodination of insulin. Aliquots of the fat cell suspension (0.4 ml containing about 10^6 cells) were incubated in duplicate in siliconized flasks for 40 min at 24°C in Krebs–Ringer phosphate buffer, pH 7.5. The buffer contained 5 mM glucose, 2% albumin, and 0.07 nM ^{125}I-labeled insulin, with or without the addition of unlabeled insulin (final concentration was in the range of 0.07 nM–17 nM, in a final volume of 0.8 ml). Separation of bound hormone from free hormone was achieved either by filtration of the incubation mixture on cellulose acetate EAWP (1 μm) Millipore filters (10, 12) or by centrifugation of the incubation mixture in plastic microtubes through 200 μl of silicone oil (Arthur Thomas Co.) (13). Data are reported as specific binding which was obtained by subtracting from total binding that amount of radioactivity that remained bound to the cells in the presence of a large excess (17 μM) of unlabeled insulin.

Degradation of ^{125}I-labeled insulin by fat cells was assessed by trichloroacetic acid precipitation or talc adsorption of the radioactivity remaining in the infranatant fraction of the incubation mixture (14). Radioactivity was measured in a gamma spectrometer.

Glucose transport studies

Transport studies were performed using labeled 2-deoxyglucose as described by Livingston and Lockwood (15). Triplicate aliquots (0.1 ml) of the fat cell suspension were incubated at 24°C in plastic tubes with 0.30 mM 2-deoxy[1-^{14}C]glucose (sp act 2 mCi/mmol, 0.2 μCi/tube) and 1.25 μCi [^{3}H]inulin in 0.2 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing 2% serum albumin. The tubes were shaken by hand.

TABLE 1. Composition of diets

	Low-fat	High-fat
	g/100 g	
Casein	20	29
Lard	3	44
Corn oil	1	1
Wheat starch	68	14
Vitamin mixture[a]	2	3
Salt mixture[b]	4	6
Cellulose	2	3
	kcal/100 kcal	
Protein	21	20
Lipid	9	70
Carbohydrate	70	10

[a] Vitamin diet fortification mixture, ICN Corp., Cleveland, Ohio.
[b] Salt mixture XIV, ICN Corp.

At the appropriate times (see Fig. 2), the assay was terminated by centrifugation of the assay mixture through silicone oil (15). The counts in the cell layer were corrected for sugar trapped in the extracellular water as determined by using [^{3}H]inulin in each assay.

To investigate the insulin effect on glucose transport, the cells were preincubated with insulin concentrations ranging from 0 to 2.78 nM for 45 min at 24°C.

Glucose metabolism studies

Triplicate aliquots (0.5 ml) of the fat cell suspension were incubated in plastic vials at 37°C with 2 ml of Krebs–bicarbonate buffer, pH 7.4, containing 2% albumin, 5 mM of [1-^{14}C]glucose (0.2 μCi/flask), and insulin concentration ranging from 0 to 2.78 nM. The flasks were gassed with 95% CO_2–5% O_2 for 45 sec and capped with a stopper equipped with a center well (Kontes Glass). After a 2-hr incubation 0.3 ml of Hyamine was added to the center well and 0.5 ml of 6N H_2SO_4 was added to the medium. CO_2 was collected during an additional 90-min incubation and the center wells were then transferred into a liquid scintillation vial. The contents of the incubation flasks were extracted and washed for lipids according to the procedure of Folch, Lees, and Sloane Stanley (16). An aliquot of the lipid extract was dried under nitrogen and used for the measurement of ^{14}C incorporation into total lipids. The remainder of the lipid was saponified with ethanolic KOH, acidified, and the fatty acids were extracted by washing three times with petroleum ether. The radioactivity was measured in both the organic (fatty acid moiety) and the aqueous phases (glycerol moiety). Blanks were run in parallel with each experiment by incubating cells in the presence of acid (6N H_2SO_4) in order to correct

TABLE 2. Experimental animals[a]

	Low-fat	High-fat	P value
Body weight, g	127 ± 4.4 (30)	136 ± 5.1 (30)	>0.05
Epididymal adipose tissue weight, g	0.71 ± 0.033 (8)	0.89 ± 0.064 (8)	<0.05
Fat cell diameter, μm	45.6 ± 0.76 (5)	48.0 ± 0.50 (5)	<0.05
Plasma insulin, nM	0.197 ± 0.018 (17)	0.223 ± 0.019 (15)	>0.05

[a] Four-week-old rats were fed either a low-fat or a high-fat diet for 7 days. Mean ± SEM, number of observations in parentheses.

all the measured parameters for nonmetabolic ^{14}C labeling.

Preliminary experiments were performed to check that the metabolism of [1-^{14}C]glucose was a linear function of time over a 2-hr incubation period under all our experimental conditions (data not shown).

Enzyme activity studies

Aliquots of the fat cell suspension (1 ml) were homogenized in 2 ml of 0.25 M sucrose containing 1 mM dithiothreitol and 1 mM EDTA at pH 7.4. Homogenates were centrifuged at 105,000 *g* at 0°C for 60 min. The clear supernatants were used for enzymatic assays of acetyl CoA carboxylase (EC 6.6.1.2) (17), citrate cleavage enzyme (EC 4.1.3.8) (18), glucose-6-phosphate dehydrogenase (EC 1.1.1.4.9) (19), 6-phosphogluconate dehydrogenase (EC 1.1.1.4.4) (19), and malic enzyme (EC 1.1.1.4.0), which was measured by the method of Ochoa (20), but with 10 mM malate. All enzyme activities were linear with respect to time and sample concentration. Enzyme activities were expressed as nanomoles of substrate utilized/10^6 cells per minute.

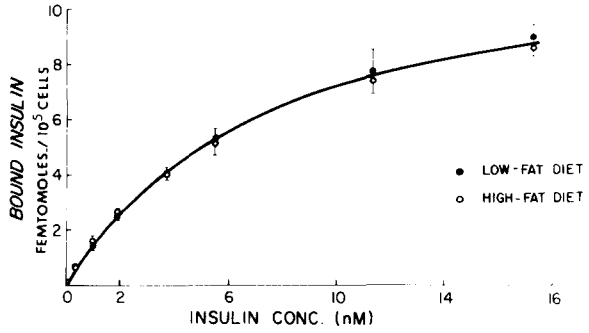

Fig. 1. Insulin binding to isolated fat cells from 5-week old rats fed either a low-fat diet or a high-fat diet for 7 days. Cells were incubated for 40 min at 24°C with 0.078 nM ^{125}I-labeled insulin plus unlabeled insulin to give the indicated total insulin concentrations. The amount of insulin bound (femtomol/10^5 cells) is plotted on the vertical axis and insulin concentration on the horizontal axis. Data represent the mean (±SEM) of four experiments, each done on fat cells from six to eight rats on each diet. All data are corrected for nonspecific binding by substracting the amount of radioactivity remaining bound at an insulin concentration of 17 μM from the amount of radioactivity in the cell pellet at all other insulin concentrations.

RESULTS

Experimental animals

No significant difference between the body weights of the two groups of rats was found (**Table 2**). However, the weight of the epididymal adipose tissue was significantly increased after one week of high-fat diet as compared to low-fat diet. The fat-cell size also was increased by fat feeding. Although the difference was small, it was detectable and significant. There was no detectable change in DNA content per fat pad suggesting, as previously shown (7), that in short-term fat feeding no hyperplasia occurs.

Plasma insulin, as measured in the morning, was not changed by feeding the rats a high-fat diet. However, studies of diurnal variations have shown that high-fat-fed rats have lower insulin levels during the night hours (21).

Insulin binding studies

Fig. 1 summarizes the results of four experiments measuring insulin binding to fat cells from rats fed either a low-fat or high-fat diet for 7 days. The data have been corrected to show the specific binding. There was no significant difference in the ability of the adipocytes of the two groups of rats to bind insulin at the steady state, at any concentration of insulin tested. The data from the binding studies have been plotted according to Scatchard's analysis (22). It has been pointed out that the Scatchard plot gives only an approximation of the number and affinity of insulin binding sites (23, 24). It was used in this study solely for comparative purposes. Over the range of insulin concentrations tested in this study, only the second component of the curvilinear plot was substantiated and used for calculations. Using this analysis no significant changes were found in the apparent number or affinity of low-affinity binding sites in rats fed the high-fat as compared to the low-fat diet. The apparent number of binding sites per cell and the apparent dissociation constant of the receptor–insulin complex were, respectively: 71,912 ± 9,191 and (6.9 ± 1.8) × 10^{-9} M for rats fed the low-fat diet versus 62,160 ± 5,121 and (5.6 ± 0.65) × 10^{-9} M for rats fed

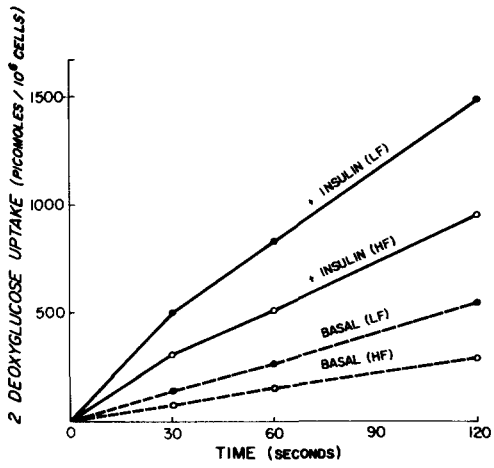

Fig. 2. Time course of 2-deoxyglucose uptake in the absence of (basal) and presence of insulin (2.78 nM) by fat cells from 5-week-old rats fed a low-fat diet (LF) (●) or a high-fat diet (HF) (○) for 7 days. Cells were preincubated at 24°C for 45 min, with or without insulin. Glucose transport was then assessed by measuring the uptake of 2-deoxy[1-^{14}C]glucose (0.3 mM) at the end of 30-, 60-, or 120-sec incubations at 24°C. Data represent the mean of two experiments for each group. For each experiment the pooled fat cell suspension of four rats was used. All data are corrected for sugar trapped in the extracellular water as determined by using [^{3}H]inulin in each assay.

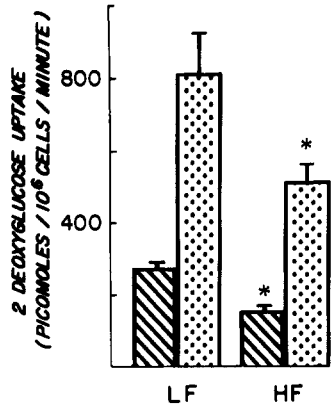

Fig. 3. 2-Deoxyglucose uptake in the absence of (hatched bars) and presence of insulin (stippled bars) by fat cells from 5-week-old rats fed a low-fat (LF) diet or a high-fat (HF) diet for 7 days. Cells were preincubated at 24°C for 45 min with or without insulin (2.78 nM). 2-Deoxyglucose (0.3 mM) uptake was then measured at the end of 2-min incubations at 24°C. Data represent the mean (±SEM) of seven experiments, each done on fat cells from four rats fed each diet. All data are corrected for sugar trapped in the extracellular water as determined by using [^{3}H]inulin in each assay. *$P < 0.05$ vs. the appropriate control.

the high-fat diet. In addition, there were no differences between the two groups of rats in the ability of their fat cells to degrade insulin in the incubation medium. Precipitable insulin remaining after the binding assay at 0.07 nM insulin was 95 or 97% of the control assay (without cells) regardless of diet.

It should be pointed out that the binding isotherms did not contain enough data at very low insulin concentrations to allow an accurate analysis of the first component of the curvilinear plot. Therefore we cannot be certain that changes did not occur in high-affinity binding sites.

Glucose transport studies

Glucose transport was assessed by using the glucose analogue 2-deoxyglucose. This sugar is transported by the same carrier as glucose and is phosphorylated, but is not further metabolized. It has been shown to be a good tool to study glucose transport in fat cells (25). Time-course experiments shown in **Fig. 2** established that 2-deoxyglucose uptake by the adipocytes was a linear function of time over a 2-min incubation period in both basal and insulin-stimulated conditions and regardless of diet. In order to further investigate the glucose transport capacity and its response to insulin, we performed 2-min incubations with and without the addition of submaximal and maximal doses of insulin. The data reported in **Fig. 3** demonstrate that the absolute rates of 2-deoxyglucose uptake were significantly decreased in fat cells from rats fed a high-fat as compared to a low-fat diet in both basal and maximally insulin-stimulated conditions.

Since the basal value differed between the two groups of rats, we elected to analyze the response of glucose transport to insulin either in terms of a percent increase above basal, i.e., (insulin-stimulated minus basal) × 100/basal (**Fig. 4**) or as a percent of the

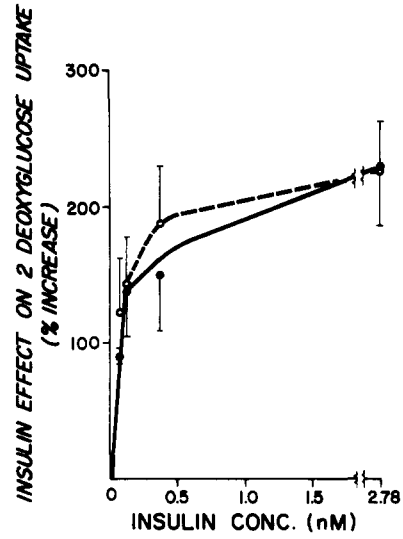

Fig. 4. Insulin effect = (insulin-stimulated minus basal)/basal × 100 on 2-deoxyglucose uptake by fat cells from 5-week-old rats fed a low-fat diet (●) or a high-fat diet (○) for 7 days. Cells were preincubated at 24°C for 45 min without or with insulin at the indicated concentrations. Uptake was then measured at the end of a 2-min incubation. All data are corrected for sugar trapped in the extracellular water as determined by using [^{3}H]inulin in each assay. Data represent the mean (±SEM) of three experiments, each done on fat cells from 4–6 rats on each diet.

maximal insulin effect, i.e., (insulin-stimulated minus basal) × 100/maximum increment (at 2.78 nM) (plot not shown). The results of these computations indicate that the insulin effect was similar in the two groups of rats at both submaximal and maximal insulin concentrations. These superimposable curves (Fig. 4) demonstrate that insulin–receptor interaction amplified the fat cell glucose transport to the same extent regardless of diet. This suggests that the coupling function between the insulin–receptor complex and the effector system (glucose transport and/or phosphorylation) in the adipocytes of fat-fed rats is functionally intact.

[1-^{14}C]Glucose metabolism studies

Since glucose transport was stimulated by insulin to the same extent in both groups, it was important to determine whether this resulted in an equivalent stimulation of glucose metabolism, irrespective of diet. We therefore measured the insulin responsiveness of some major pathways of glucose utilization in isolated fat cells from rats fed a low- or a high-fat diet for seven days. The absolute rates of basal and maximally insulin-stimulated glucose metabolism are shown in **Fig. 5.** High-fat feeding exerted a selective effect on glucose metabolism under basal conditions; CO_2 and fatty acid labeling were significantly decreased, whereas glyceride–glycerol labeling was not affected. Consequently, total lipid labeling was not detectably changed. In the presence of a maximal insulin concentration, fat feeding dramatically reduced the rate of glucose incorporation into CO_2, total lipids, and fatty acids. Again, glyceride–glycerol labeling was not significantly different between dietary groups.

Since the basal rates of glucose metabolism differed between the two groups, we elected to express the dose response curves as insulin effect, i.e., (insulin-stimulated minus basal/basal) × 100 as is shown in **Fig. 6**. It is clear that high-fat feeding markedly decreases the adipocyte's responsiveness to insulin. Moreover, the discrepancy between the insulin dose response curves of the two groups was greater for total lipids and fatty acids than for CO_2. Differences in insulin responsiveness were less pronounced for glyceride–glycerol. These curves also show that the maximal insulin effect occurred at approximately 0.3 nM (which is in the range of serum insulin concentrations in these rats, see Table 1). At this insulin concentration, there were 0.4 femtomoles of insulin bound/10^5 cells (see Fig. 1). Thus, only 2,400 insulin binding sites, i.e., 3% of the total number, needed to be occupied to get the maximum insulin effect. This result is in good agreement with that of other investigators (25–27).

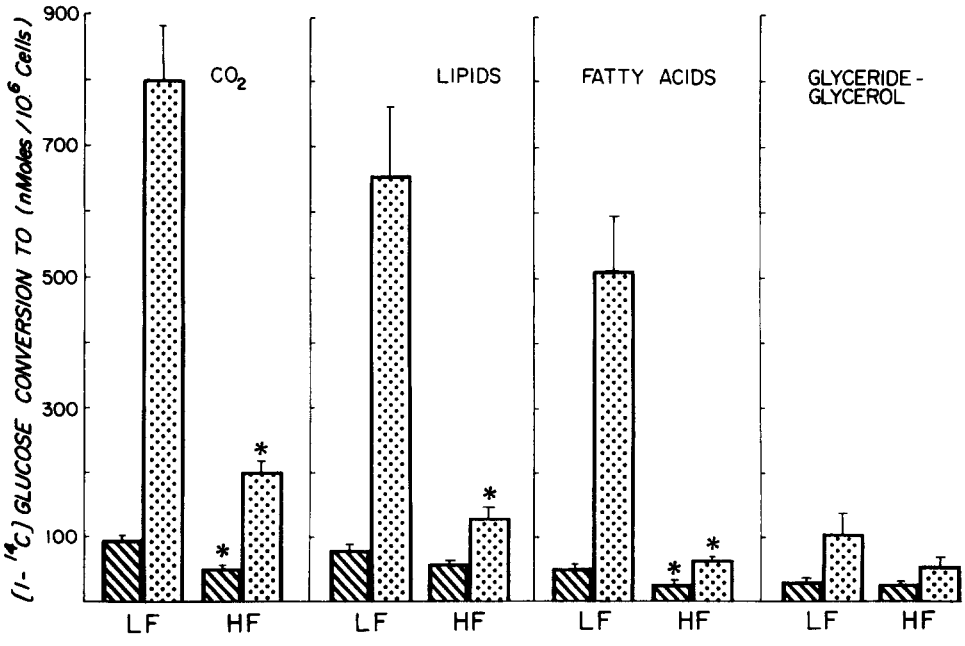

Fig. 5. [1-^{14}C]Glucose metabolism in fat cells from 5-week-old rats fed a low-fat diet (LF) or high-fat diet (HF) for 7 days, in the presence (stippled bars) or absence (hatched bars) of insulin (2.78 nM). Fat cells were incubated at 37°C in Krebs–Ringer bicarbonate buffer containing 2% albumin and 5 mM glucose for 2 hr. Data represent the mean (±SEM) of five experiments, each done on fat cells from six rats on each diet. *$P < 0.05$ vs. the appropriate control.

Enzyme activity studies

Comparison of the dose response curves for 2-deoxyglucose uptake and the metabolic parameters points to an abnormality in the fat cells of high-fat-fed rats that occurs beyond the glucose transport step. The fact that the insulin effect on glucose incorporation into fatty acids is severely diminished suggests that an intracellular defect in lipogenesis could be responsible for the restricted hormone action. The enzyme activity data (**Table 3**) strongly support this hypothesis. After 7 days of the high-fat diet, all of the lipogenic enzyme activities were greatly reduced. Malic enzyme and acetyl CoA carboxylase were reduced to 13% of their activity in the fat cells of low-fat-fed rats. This reduction was comparable to the reduction in the absolute rate of insulin-stimulated fatty acid synthesis from glucose. The glucose dehydrogenases of the hexose monophosphate shunt were somewhat less dramatically altered. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were reduced to 20% and 34% of their activities in control rats, respectively. This is consistent with the fact that the high-fat diet in the presence of insulin had less effect on CO_2 production from [1-^{14}C]-glucose than it did on fatty acid synthesis (see Fig. 6).

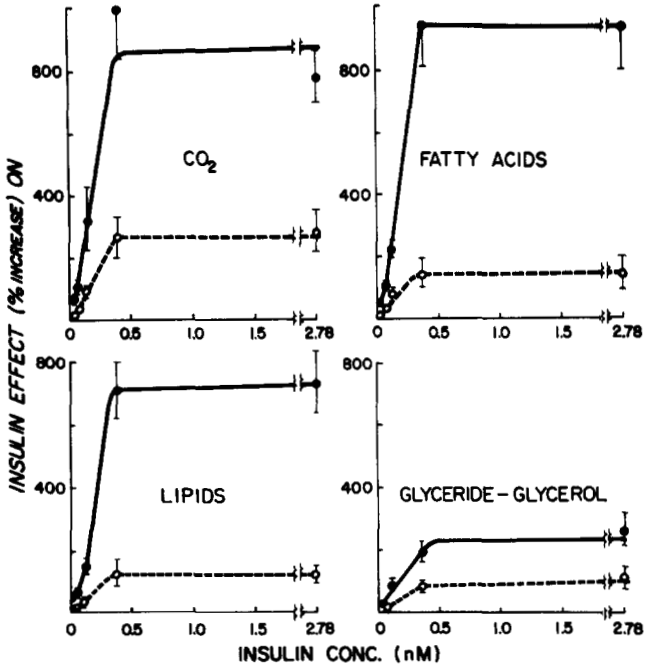

Fig. 6. Insulin effect = (insulin-stimulated minus basal)/basal × 100 on the metabolism of [1-^{14}C]glucose in fat cells from 5-week-old rats fed a low-fat diet (●) or a high-fat diet (○) for 7 days. Fat cells were incubated at 37°C in Krebs–Ringer bicarbonate buffer containing 2% serum albumin and 5 mM glucose with or without insulin at the indicated concentrations. Data represent the mean (±SEM) of five experiments, each done on fat cells from six rats on each diet.

DISCUSSION

These studies demonstrate that the impairment of insulin action on glucose utilization in fat cells from rats fed a high-fat diet cannot be explained by a defect in the insulin receptor. Since insulin binding to the cell membrane is the first step of cellular insulin action, insulin receptor deficiency, which has been documented in several insulin-resistant conditions (28, 29), has been proposed as a possible mechanism of the insulin resistance (30). The model described here is therefore inconsistent with this theory. Recently, the causal relationship between the decrease in the number of insulin receptors on the fat cell membrane and insulin resistance (impaired insulin action on glucose metabolism) has been reevaluated (31, 32) on the ground that only a small fraction of insulin receptors need to be filled to obtain a maximal activation of glucose utilization (25–27). Consequently, a decrease in the number of insulin receptors would be expected only to lead to a rightward shift in the insulin–dose response curve, with normal responses to maximally effective insulin levels. However, as pointed out by Czech, Richardson, and Smith (31), fat cells that exhibit an impaired responsiveness to insulin do so at both submaximal and maximal concentrations of the hormone. Two reports on insulin binding to fat cells

TABLE 3. Enzyme activities in fat cells of 5-week-old rats fed either a low-fat diet or a high-fat diet for 7 days

	CCE	ME	G6PD	6PGD	AcCoAC
	nmol substrate utilized/10^6 cells/min				
Low-fat diet	106 ± 19	411 ± 37	150 ± 33	77 ± 18	5.7 ± 1.63
High-fat diet	18 ± 5	53 ± 8	29 ± 5	26 ± 6	0.75 ± 0.19

Enzyme activities were measured in the 105,000 *g* supernatants of fat cell homogenates in 0.25 M sucrose containing 1 mM EDTA and 1 mM dithiothreitol at pH 7.4.

Mean ± SEM of three experiments done on fat cell suspensions of four pooled rats each. CCE, citrate cleavage enzyme; ME, malic enzyme; G6PDH, glucose-6-phosphate dehydrogenase; 6PGDH, 6-phosphogluconic dehydrogenase; AcCoAC, acetyl CoA carboxylase.

from fat-fed rats, which appeared while these studies were in progress, presented conflicting results. In agreement with the present study, Cushman and Salans (33) did not detect any variation of insulin binding in adipocytes from 300g rats fed a high-fat diet since weaning. However, Ip et al. (34) observed a greater than 50% decrease in fat cell insulin binding capacity after 7 days of fat diet. There is no clear explanation of this discrepancy; the type of carbohydrate, and possibly the essential fatty acid content of the diet which has been shown to alter insulin binding (35) may contribute to the result differences. If, as considerable data indicate, insulin regulates its own receptor number (30, 36), insulin binding in fat-fed rats should not be decreased since these rats show no hyperinsulinemia (Table 2) but rather a tendency to lower insulin levels during some parts of the day (21).

The overall capacity of the transport (and/or phosphorylation) system, as assessed by 2-deoxyglucose uptake, is decreased in fat cells from rats fed a high-fat diet in both basal and insulin-stimulated conditions. 2-Deoxyglucose uptake reflects glucose transport only if transport, not phosphorylation, is the rate-limiting step. This is true in white fat cells under several experimental conditions (25, 37, 38), but a possible role of phosphorylation cannot be ruled out in the case of the fat-fed rats because Bernstein, Marshall, and Carney (39) have shown that the level of hexokinase II was decreased after 3 weeks of high-fat feeding in rats. Regardless of the cause of the decreased absolute rate of 2-deoxyglucose uptake, these studies clearly demonstrate that the sensitivity of the glucose transport system to insulin is not affected by the amount of dietary fat. This result is in good agreement with the fact that insulin binding is not changed by high-fat feeding. Therefore, the coupling function between insulin binding to its receptor and the activation of glucose transport (and/or phosphorylation) is not altered in fat cells from high-fat-fed rats.

The glucose metabolism studies show that there are large differences in the ability of insulin to stimulate the flow of glucose through the metabolic pathways in the two dietary groups. In addition, the extent of impairment differs with each pathway of glucose utilization. Fatty acid synthesis in adipocytes from high-fat-fed rats is much less responsive to insulin, while the insulin responsiveness of [1-^{14}C]-D-glucose oxidation is somewhat less affected by the amount of fat in the diet. Differences in insulin responsiveness are smallest for glyceride–glycerol. There are some problems in interpreting decreases in intracellular metabolism when transport itself is decreased. In adipocytes from rats fed a high-fat diet, the absolute rates of intracellular metabolism are more affected than is transport. Nevertheless it is possible to suggest that differences in relative saturability of the various intracellular pathways of glucose metabolism would lead to disproportionate changes in different pathways when rates of transport decline. For example, if the glyceride–glycerol pathway were usually saturated, whereas pentose oxidation were not, then a decrease in transport would lead to a fall in pentose oxidation with no changes in glyceride–glycerol flux. The matter is further complicated by the lack of information on the kinetic characteristics of the glucose transport system in adipocytes from rats fed a high-fat diet. If high-fat feeding caused an increase in the transport K_m but no changes in V_{max} then the absolute rates of transport might be normal at the higher glucose concentrations that were employed in the metabolic studies.

The data on enzyme levels document the biochemical alterations by which fat cells adapt to the high fat diet and provide evidence that additional defects beyond glucose transport exist. The lipogenic capacity is reduced to less than 15% that of the low-fat fed group. The ratio of the lipogenic enzyme activities of the two groups of rats is very close to the ratio of the maximal rates of fatty acid synthesis. Thus, the decreased enzyme activity seems to account for the reduction of lipogenesis that was observed in the presence of maximal doses of insulin. This clearly demonstrates that the unresponsiveness of glucose metabolism to insulin originates in the reduction of the intracellular capacity to handle the glucose entering the cell in response to the hormone. Such a modulation of insulin action via alterations of intracellular metabolism suggests an exquisite adjustment that allows a much more selective response than would be accomplished by a modification of the insulin receptor. It allows the fat cell to remain fully sensitive to such insulin effects as the antilipolytic one.[2]

The concept that the intracellular capacity to utilize glucose plays a major role in the modulation of hormone action in adipocytes of high-fat-fed rats is supported by the fact that glucose utilization responds normally to epinephrine in this tissue.[1] When stimulated by epinephrine, the glucose that is taken up into the cell is channeled into the Krebs cycle, lactic acid, and glyceride–glycerol. The capacity of these pathways in adipose tissue is not reduced by high-fat feeding (1, 2) and this allows the effects of epinephrine to be fully expressed.

Since the typical diet in western countries is high in fat (more than 40% fat by calories), the rat

[2] Susini, C., M. Lavau, and J. Herzog. Unpublished data.

fed a high-fat diet provides a model that is much closer to normal human physiology than the rat fed the usual low-fat diet. Impaired insulin responsiveness of fat cells described here could be highly relevant to certain states of insulin resistance in man in which the insulin-binding step has relatively little effect in determining the overall rate of insulin action (32, 40).

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