The effects of spontaneous obesity on insulin binding, glucose transport, and glucose oxidation of isolated rat adipocytes.

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J Clin Invest. 1976;57(4):842-851. https://doi.org/10.1172/JCI108360.

Research Article

We have studied insulin, binding, glucose transport, and glucose oxidation, using large adipocytes isolated from older, fatter rats (greater than 12-mo-old, greater than 550 g), and smaller cells obtained from younger, leaner animals (4-5-wk-old, 120-160 g). At media glucose levels less than 5 mM, basal (absence of insulin) rates of glucose oxidation are comparable in both groups of cells. However, in the presence of insulin, the increase in glucose oxidation is much greater in the smaller cells. Maximally effective insulin levels could not overcome the defect in glucose oxidation by larger cells, and thus, even though studies of insulin binding demonstrated a 30-40% decrease in insulin receptors on the larger cells, it is probable that the defect in glucose oxidation is distal to the insulin receptor. Glucose transport was assessed by direct measurement of 2-deoxy glucose uptake. Basal levels of uptake were greater for the larger cells, whereas at maximally effective insulin concentrations, rates of 2-deoxy glucose uptake were the same for both groups of cells. Thus, in the presence of maximally effective levels of insulin, the apparent Km (2.3-2.7 mM) and Vmax values (2.6 and 2.7 nmol/10(5) cells per min) of 2-deoxy glucose uptake were comparable, indicating that the glucose transport system of the larger cells was intact. However, at submaximal levels of insulin, small adipocytes took up more […]

The Effects of Spontaneous Obesity on Insulin Binding, Glucose Transport, and Glucose Oxidation of Isolated Rat Adipocytes

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ABSTRACT We have studied insulin binding, glucose transport, and glucose oxidation, using large adipocytes isolated from older, fatter rats (> 12-mo-old, > 550 g), and smaller cells obtained from younger, leaner animals (4–5-wk-old, 120–160 g). At media glucose levels < 5 mM, basal (absence of insulin) rates of glucose oxidation are comparable in both groups of cells. However, in the presence of insulin, the increase in glucose oxidation is much greater in the smaller cells. Maximally effective insulin levels could not overcome the defect in glucose oxidation by larger cells, and thus, even though studies of insulin binding demonstrated a 30–40% decrease in insulin receptors on the larger cells, it is probable that the defect in glucose oxidation is distal to the insulin receptor. Glucose transport was assessed by direct measurement of 2-deoxy glucose uptake. Basal levels of uptake were greater for the larger cells, whereas at maximally effective insulin concentrations, rates of 2-deoxy glucose uptake were the same for both groups of cells. Thus, in the presence of maximally effective levels of insulin, the apparent K_m (2.3–2.7 mM) and V_{max} values (2.6 and 2.7 nmol/10^5 cells per min) of 2-deoxy glucose uptake were comparable, indicating that the glucose transport system of the larger cells was intact. However, at submaximal levels of insulin, small adipocytes took up more 2-deoxy glucose than large cells. These findings represent a rightward shift in the insulin dose-response curve in the cells from the older, fatter animals, and this is the predicted functional sequelae of the observed decrease in insulin receptors. Finally, when the amount of insulin bound was plotted as a function of 2-deoxy glucose uptake, no difference was seen between both groups of cells. This indicates that coupling between insulin receptor complexes and the glucose transport system is intact in large adipocytes, and is further evidence that a defect(s) in intracellular glucose metabolism is responsible for the decrease in glucose oxidation of adipocytes from older, fatter rats. In conclusion: (*a*) insulin-mediated glucose oxidation is markedly decreased in large adipocytes from older, fatter rats, and since this decrease cannot be corrected by maximally effective insulin levels, the defect is probably distal to the insulin receptor; (*b*) the glucose transport system is basically normal in large adipocytes; (*c*) insulin binding to receptors is decreased in large cells and the functional sequelae of this decrease in insulin binding, i.e., a rightward shift in the insulin dose-response curve for 2-deoxy glucose uptake, was observed, and (*d*) since the decreased rates of insulin-mediated glucose oxidation cannot be attributed to changes in insulin receptors or to changes in glucose transport, an intracellular defect in glucose metabolism is suggested.

INTRODUCTION

Insulin resistance is a well-described feature of obesity (1). In vitro investigations into the mechanisms of this insulin-resistant state have relied heavily upon the isolated adipocyte as a cell model. This approach has seemed appropriate since these cells are relatively easy to obtain, are metabolically active, and bear an obvious morphological relationship to the obese state. Binding to specific cell surface receptors is the initial step in insulin's action, and decreased insulin receptors have been found in a variety of tissues in genetically obese mice (2–4), and

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Received for publication 29 August 1975 and in revised form 21 November 1975.

in man (5, 6). Recently, we have also found decreased insulin binding to adipocytes from spontaneously obese rats (7). However, the potential relationship between decreased insulin binding and insulin action is not straightforward. For example, maximal insulin stimulation of glucose metabolism occurs when only a small fraction of the adipocyte insulin receptors are occupied (8–10), and, thus, the functional sequelae of a decreased number of receptors would be a diminished insulin response only at insulin levels which saturate less than the number of receptors required for a maximal effect, with normal responses to maximally effective insulin levels. Therefore, in the normal adipocyte, if only 10% of the receptors must be occupied to elicit a maximal insulin effect, then if enough insulin is present (maximally effective insulin level) to occupy this same absolute number of receptors in cells from obese animals, one would expect to see a normal insulin response. Indeed, such observations have been reported. Thus, Livingston and Lockwood (11) have shown that maximally effective insulin levels lead to comparable rates of glucose transport in normal vs. enlarged adipocytes. On the other hand, glucose oxidation has been studied more extensively, and several groups have found (12–15) that maximally effective insulin levels have a decreased ability to stimulate glucose oxidation in isolated adipocytes from obese rats when compared to cells from lean animals. Obviously, the glucose transport data of Livingston and Lockwood (11) are compatible with a decreased number of insulin receptors on adipocytes from obese rats, while the data which demonstrate decreased maximal rates of glucose oxidation (12–15) imply additional abnormalities. The aim of the present study was to interrelate insulin binding, glucose transport, and glucose oxidation in normal and enlarged adipocytes. Consequently, adipocyte metabolism was studied over a wide range of insulin and glucose concentrations. These studies have shown that the predicted functional consequence of decreased insulin receptors can be elicited in enlarged adipocytes, and furthermore, that these cells demonstrate an additional major intracellular abnormality preventing normal rates of glucose oxidation.

METHODS

Materials. Porcine monocomponent insulin was generously supplied by Dr. Ronald Chance of Eli Lilly Co., Indianapolis, Ind. ^{125}I-Na was purchased from New England Nuclear, Boston, Mass., bovine serum albumin (fraction V) from Armour & Co., Chicago, Ill., collagenase from Worthington Biochemical Corp., Freehold, N. J., guinea pig anti-insulin antibody from Pentex Biochemical, Kankakee, Ill., and [1-^{14}C]glucose, 2-deoxy[1-^{14}C]glucose, and [^{14}C]inulin from New England Nuclear.

Preparation of isolated adipocytes. Male Sprague-Dawley rats were used for all experiments. All studies were performed in the morning on animals who had free access to standard rat chow. Young, lean animals weighed 120–160 g and were 4–5-wk-old, while the older, fatter animals weighed > 550 g and were > 12-mo-old. Animals were stunned by a blow to the head, decapitated, and epididymal fat pads removed. Isolated fat cells were prepared by shaking at 37°C for 60 min in Krebs-Ringer bicarbonate buffer containing collagenase (3 mg/ml) and albumin (40 mg/ml), according to the method of Rodbell (16). Cells were then filtered through a 250-μm nylon mesh, centrifuged at 400 rpm for 4 min, and washed three times in buffer (7). Adipocyte counts were performed according to a modification of method III of Hirsch and Gallian (17), in which the cells are fixed in 2% osmium tetroxide in 0.05 M collidine buffer (made isotonic with saline) for 72 h at 37°C and then taken up in a known volume of 0.154 M NaCl for counting. Counting was performed using a Celloscope model 112H particle counter with a 400-μm aperture (Particle Data, Inc., Elmhurst, Ill.). Adipocyte size was determined using a calibrated microscope according to the method of Di Girolamo et al. (18).

Iodination of insulin. ^{125}I-Insulin was prepared at a sp act of 100–150 μCi/μg according to Freychet et al. modification (19) of the method of Hunter and Greenwood (20) as previously described (21).

Binding studies. Isolated fat cells were suspended in a buffer containing 35 mM Tris, 120 mM NaCl, 1.2 mM $MgSO_4$, 2.5 mM KCl, 10 mM glucose, 1 mM EDTA, 1% bovine serum albumin (22), pH 7.6, and incubated with ^{125}I-insulin and unlabeled insulin in plastic flasks in a 24°C shaking water bath as previously described (7, 23, 24). Optimal steady-state binding conditions are achieved at 24°C after 45 min of incubation (7). The incubations were terminated as described by Gammeltoft and Gliemann (9) by removing 200-μl aliquots from the cell suspension, and rapidly centrifuging the cells in plastic microtubes to which 100 μl of dinonyl phthalate oil had been added. Dinonyl phthalate has a specific gravity intermediate between buffer and cells, and therefore, after centrifugation, three layers resulted: cells on top, oil in the middle, and buffer on the bottom. The cells were then removed and the radioactivity was determined. All studies were done in triplicate.

Glucose oxidation studies. The ability of adipocytes to oxidize glucose was determined according to the method of Rodbell (16). Adipocytes were incubated at 37°C with [1-^{14}C]glucose at a total glucose concentration of 2 mM in Krebs-Ringer bicarbonate buffer, pH 7.4, containing bovine serum albumin (40 mg/ml). After 1 h of incubation the generated $^{14}CO_2$ was collected and counted in a liquid scintillation counter.

Glucose transport studies. Transport studies were performed using the same cell centrifugation technique as described for the binding studies; the details of this method have been previously described (10). Unless otherwise stated, isolated adipocytes were incubated with 2-deoxy-D-[1-^{14}C]glucose (sp act 2 mCi/mM) at a concentration of 0.125 mM in Krebs-Ringer bicarbonate, pH 7.4, containing bovine serum albumin (10 mg/ml) at 24°C. This assay measures the total uptake of the radiolabeled 2-deoxy glucose and is based on the principle that while 2-deoxy glucose is transported and phosphorylated by the same process as D-glucose, it cannot be further metabolized (25). The assay was terminated at the end of 3 min by transferring 200-μl aliquots from the assay mixture to plastic microtubes containing 100 μl of dinonyl phthalate oil. The tubes were centrifuged for 30 s in a Beckman microfuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.); the assay was considered terminated when centrifugation began. In experiments in which the stimulatory effect of insulin on

TABLE I
*Experimental Animals**

	Younger, leaner animals, 4–5-wk-old	Older, fatter animals, >12-mo-old
Body weight, *g*	142 (120–160)	575 (510–700)
Adipocyte volume, *pl*	57 (42–82)	377 (263–557)
Plasma insulin level, *uU/ml*	30 (25–40)	65 (47–109)
Plasma glucose level, *mg/100 ml*	120 (92–137)	121 (90–134)

* Data represent means; numbers in parentheses are ranges.

uptake was measured, the cells were preincubated with insulin for 45 min at 24°C. The amount of sugar trapped in the extracellular water space of the cell layers was determined using [^{14}C]inulin according to the method of Gliemann et al. (26). Extracellular water space was measured in each experiment, and all data of sugar uptake were corrected for this factor. The percent of the total amount of sugar available trapped in the extracellular water space was a linear function of cell concentration, and averaged 0.033± 0.001% for small adipocytes and 0.054±0.002% for large adipocytes at a concentration of 2×10^5 cells/ml. The amount of trapped sugar ranged from 2 to 10% of the total sugar uptake, depending upon the conditions of incubation.

Analytical methods. Plasma glucose was measured by the glucose oxidase method using a Beckman Glucose Analyzer (Beckman Instruments, Inc., Clinical Instruments Div., Fullerton. Calif.).

RESULTS

Experimental animals. Some characteristics of the animals studied are given in Table I. Two groups were studied: young, lean rats, and older fatter rats. As can be seen, fat cell size was approximately sixfold greater in the older fatter animals and a.m. plasma insulin levels were also greater. Plasma glucose values were comparable in both groups.

Glucose oxidation studies. Fig. 1 shows the effects of insulin to promote glucose oxidation in adipocytes from lean and obese rats. Basal rates of glucose oxidation are the same in both groups of cells. However, in the presence of insulin, marked differences in oxidation are seen. Thus, normal cells oxidize more glucose at all insulin levels compared to enlarged adipocytes. This difference is consistent with the work of others (12–15), and exists if the data are expressed in absolute terms or as the percent rise above basal values. The fact that maximally effective concentrations of insulin cannot overcome this defect in glucose oxidation suggests that a step distal to the insulin receptor is primarily responsible for the decrease in glucose metabolism.

To establish this, and to further define the differences between large and small adipocytes, cells from both groups of animals were incubated over a wide range of glucose concentrations in the presence and absence of maximally effective insulin levels (5 ng/ml). The rates

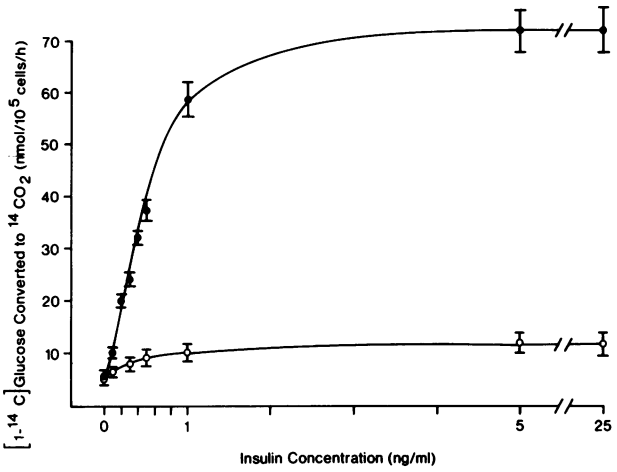

FIGURE 1 Ability of small adipocytes from young, lean animals (●) and large adipocytes from older, fatter animals (○) to oxidize glucose. Data represent the mean (±SE) of 24 separate experiments from different animals using small adipocytes and 8 experiments using large cells.

of glucose oxidation under these conditions are illustrated in Fig. 2. As can be seen, basal rates of oxidation are similar for both types of cells at glucose concentrations below 5 mM. Above this glucose concentration small cells oxidize more glucose than large cells, which further suggests a defect(s) located distal to the insulin receptor in the ability of large cells to generate CO_2. Again, as was seen in Fig. 1, differences between these cells are more striking in the presence of insulin, and maximal rates of glucose oxidation (20 mM glucose, 5 ng/ml insulin) are sevenfold greater for small adipocytes. The individual data for the experiments presented in Fig. 2 can be seen in Table II.

Insulin-binding studies. Glucose oxidation involves a number of steps (insulin binding, glucose transport, and intracellular metabolism), and as discussed above, the data in Figs. 1 and 2 suggest an abnormality distal to the insulin receptor in large cells. Nevertheless, a decrease in the number of insulin receptors per cell has been described both in man and genetically obese rodents, and we have recently found that adipocytes from older, fatter rats (spontaneous obesity) also have fewer insulin receptors per cell (7). This decrease is quantitated in Fig. 3 which demonstrates that adipocytes from the older, fatter animals bind significantly less insulin at all insulin concentrations. Thus, at the lowest insulin concentration used (0.2 ng/ml) the small adipocytes (2×10^5 cells) specifically bind 2.1±0.2% of the insulin, whereas large adipocytes bind only 1.2±0.1%.[1] As previ-

[1] These data are normalized to 2×10^5 cells, since this was the average cell concentration used in the insulin-binding studies.

ously discussed (7), this decrease in insulin binding can be accounted for by a decreased number of receptors per cell rather than a change in affinity of the receptor for insulin. Since maximal insulin action occurs when only a small fraction of the available insulin receptors are occupied (8–10), decreases in insulin's biological effects will only occur at insulin concentrations low enough to occupy less than the absolute number of receptors needed for maximal action. Thus, until the concentration of insulin is high enough to saturate this absolute number of receptors, insulin-mediated cell function should be less in cells with fewer receptors, and a shift in the dose-response curve to the right should be observed.

Glucose transport studies. To better assess the insulin dose-response curve, and to further localize the defect in glucose metabolism observed in the large adipocytes, studies of glucose transport were performed in both groups of cells. Glucose transport was assessed by measuring the uptake of 2-deoxy glucose in both groups of cells, and these data are seen in Fig. 4. Basal rates of 2-deoxy glucose uptake are higher in the large adipocytes, whereas maximal insulin-stimulated rates of uptake are comparable in both types of cells. However, at submaximal stimulatory levels of insulin, adipocytes from the younger, leaner rats take up more 2-deoxy glucose than cells from the older, fatter rats, and, thus, the predicted functional consequence of a decrease in insulin receptors, i.e., a shift in the insulin dose-response curve to the right, is seen. If one calculates the data as the percent rise above basal uptake rates, then, because the smaller cells have lower basal uptake rates, they show a

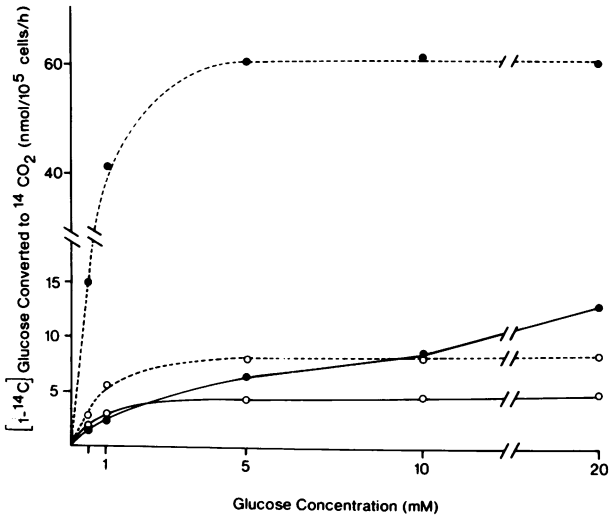

FIGURE 2 Effect of glucose concentration on glucose oxidation by large (○) and small (●) adipocytes. Cells were incubated with increasing glucose concentrations in the absence (——) and presence (····) of 5 ng/ml (120 μU/ml) insulin. Data represent the mean of three experiments for each group.

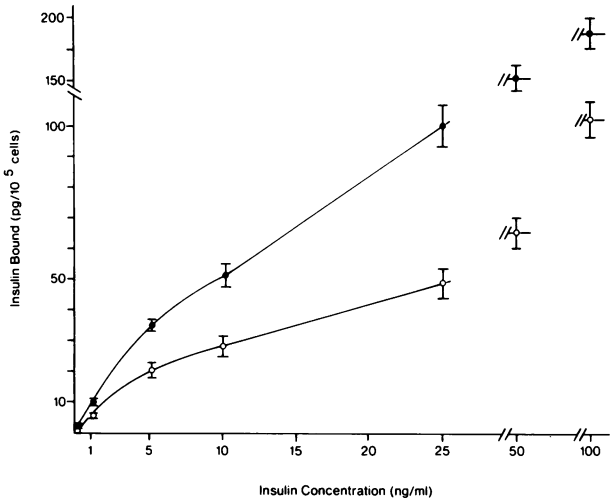

FIGURE 3 Ability of small (●) and large (○) adipocytes to specifically bind ^{125}I-insulin. Cells were incubated for 45 min at 24°C with 33 pM (0.2 ng/ml) ^{125}I-insulin plus unlabeled insulin to give the indicated total insulin concentrations. The amount of insulin bound (pg/10^6 cells) is plotted on the vertical axis and insulin concentration on the horizontal axis. Note breaks in scales. Data represent the mean (±SE) of 10 separate experiments for each group, and all data are corrected for nonspecific binding by subtracting the amount of radioactivity remaining bound at an insulin concentration of 200 μg/ml from the amount of radioactivity in the cell pellet at all other insulin concentrations (2, 7, 24). In these studies nonspecific binding was 4–10% of the total amount bound. The differences in amount of insulin bound between large and small cells are significantly different at each insulin concentration.

greater percent rise in uptake at maximal insulin levels. However, even with this method of calculation, the small cells show a greater sensitivity to insulin at submaximal insulin levels as compared to maximal insulin levels. Thus, as seen in Fig. 5A, if one computes the ratio of the insulin-induced percent increase in 2-deoxy glucose uptake (above basal rates) between small and large cells, this ratio is higher at the lower insulin levels, again indicating a rightward shift in the insulin dose-response curve, for large cells, even with this method of data expression. Lastly, if one calculates the data as a percent of the maximal insulin effect (Fig. 5B), then again a shift in the curve is seen.

Since maximal insulin-stimulated rates of 2-deoxy glucose uptake are the same in both groups of cells, the data in Fig. 4 suggest that the glucose transport system of large adipocytes is normal. To test this hypothesis we studied 2-deoxy glucose uptake over a wide range of hexose concentrations in the presence and absence of maximal insulin levels (5 ng/ml). These data are seen in Fig. 6. Basal rates of 2-deoxy glucose uptake are higher for the large fat cells, and this difference tends to decrease at the higher sugar concentrations. In the presence of insulin, 2-deoxy glucose uptake is compara-

TABLE II

Individual Data of Glucose Oxidation Experiments at Increasing Glucose Concentrations

	Experiment no.	Glucose concentration, *mM*									
		Basal					Insulin (25 ng/ml)				
		0.4	1.0	5	10	20	0.4	1.0	5	10	20
Small cells	1	0.81*	2.0	6.9	8.4	13.6	14.6	30.1	42.5	39.7	39.1
	2	0.93	2.7	7.2	8.0	13.5	12.4	33.4	46.7	50.3	50.5
	3	1.29	2.5	5.3	9.2	12.8	16.4	60.0	84.4	85.9	85.1
	Mean	1.0	2.4	6.5	8.5	13.3	14.4	41.1	57.9	58.6	58.2
	(±SE)	±0.14	±0.2	±0.6	±0.4	±0.3	±1.2	±9.5	±13.3	±14.0	±13.8
Large cells	1	1.4	2.8	3.3	3.4	3.3	2.0	3.8	4.1	4.8	4.8
	2	1.0	2.1	4.0	4.5	4.6	1.8	3.8	6.7	5.9	5.8
	3	1.8	4.0	6.5	6.7	6.8	4.0	8.8	12.2	11.6	12.1
	Mean	1.4	3.0	4.6	4.9	4.9	2.6	5.5	7.7	7.4	7.6
	(±SE)	±0.2	±0.6	±1.0	±1.0	±1.0	±0.7	±1.7	±2.4	±2.1	±2.3

* nmol glucose oxidized/10^5 cells per h.

ble in small and large adipocytes at all hexose concentrations. The shape of all four curves in Fig. 6 suggests two uptake components: a saturable component seen at 2-deoxy glucose concentrations < 5 mM, and a nonsaturable, linear component seen at higher sugar concentrations. This phenomenon has been previously described for 2-deoxy glucose uptake by other cell systems (27, 28), and we have reported similar observations using isolated adipocytes (10). It is likely that this nonsaturable component represents simple inward diffusion of labeled hexose (10, 27, 28), and the data can be corrected for this diffusion component by subtracting the contribution of this linear process (10, 27, 28). When these corrected data are submitted to Lineweaver-Burk analysis, apparent K_m and V_{max} values can be calculated. With this approach (Fig. 7, Table III), the apparent K_m of 2-deoxy glucose uptake is comparable in both groups of cells in the presence or absence of insulin (2.3-2.7 mM). The apparent V_{max} value in the basal state is somewhat higher for larger cells (1.2 vs. 0.9 nmol/10^5 cells per min) and insulin causes the V_{max} to increase to the same level in both groups of cells (2.5 and 2.6 nmol/10^5 cells per min).

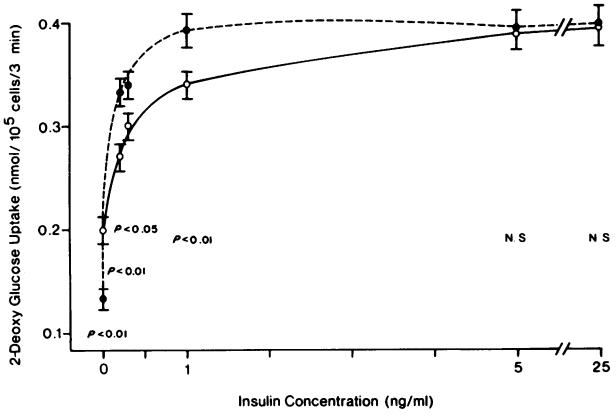

FIGURE 4 Ability of small (●) and large (○) adipocytes to take up 2-deoxy glucose. Cells were preincubated with or without insulin (at the indicated concentrations) for 45 min at 24°C. Uptake was then measured at the end of a 3-min incubation with [2-^{14}C]deoxy glucose (0.125 mM). Data represent the mean (±SE) of 11 experiments for each group and P values were obtained by use of the nonpaired t test.

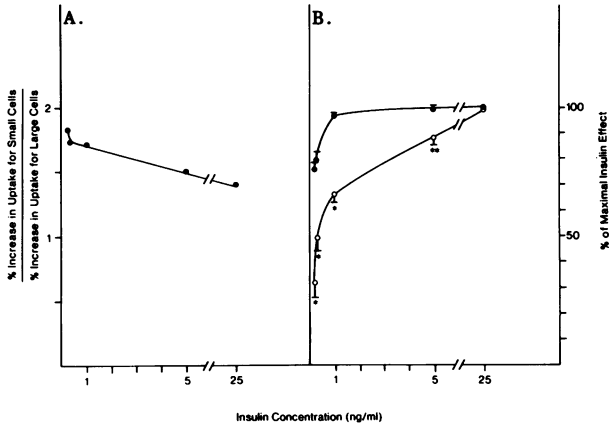

FIGURE 5 Comparison of insulin's effects on 2-deoxy glucose uptake in small and large adipocytes. (A) Horizontal axis is insulin concentration and the vertical axis represents the ratio of the insulin-induced percent increase above basal rates of 2-deoxy glucose uptake for small and large cells. (B) Vertical axis represents the percent of the maximal insulin effect. This is calculated by dividing the increment in 2-deoxy glucose uptake (absolute value − basal) at the indicated insulin concentrations by the maximum increment in uptake (at 25 ng/ml insulin). Data are derived from the data in Fig. 4, and represent the mean (±SE) for small (●) and large (○) cells. (*) indicates $P < 0.01$ and (**) indicates $P < 0.05$.

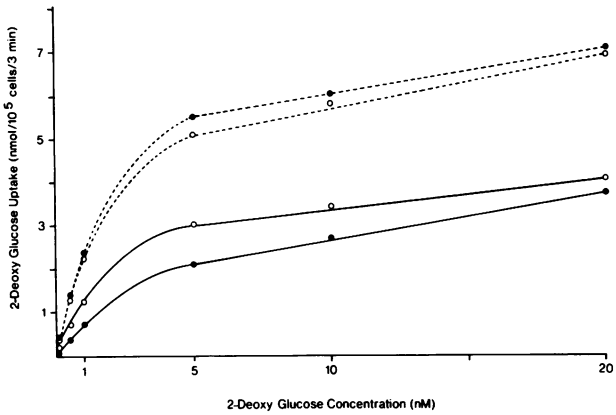

FIGURE 6 Effect of substrate concentration on 2-deoxy glucose uptake by small (●) and large (○) adipocytes in the basal (——) and in insulin stimulated (- - - -) state. At 2-deoxy glucose concentrations > 5 mM the slopes of the curves represent simple inward diffusion of the sugar into the cells (10, 27, 28); the data can be corrected for this linear diffusion component by subtracting the contribution of this linear process from the absolute value (10, 27, 28). Data represent the mean of eight separate experiments for each group.

Relationship between insulin binding and insulin function. Thus, the data presented suggest that the glucose transport system of adipocytes from both groups of animals is comparable. Furthermore, in the large cells, the decrease in transport at submaximal insulin levels (which is probably due to decreased insulin receptors) is quantitatively unlikely to account for the decrease in glucose oxidation. Consequently, a major defect in glucose oxidation distal to the glucose transport system seems likely. This hypothesis is examined in Fig. 8. In Fig. 8A the

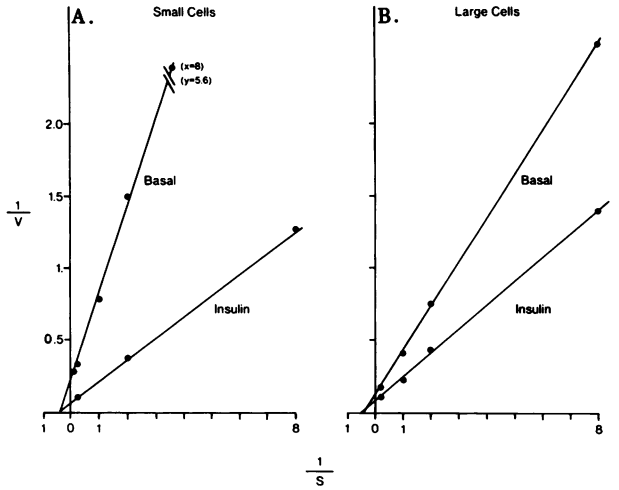

FIGURE 7 Lineweaver-Burk plot of the corrected data from Fig. 6 for small (A) and large (B) adipocytes. The calculated "apparent" K_m and V_{max} values are given in Table III.

TABLE III

K_m and V_{max} Values for 2-Deoxy Glucose Uptake*

	Small cells		Large cells	
	Basal	Insulin	Basal	Insulin
K_m, *mM*	2.5	2.3	2.3	2.7
V_{max}, *nmol/10⁵ cells per min*	0.9	2.6	1.2	2.5

* These values represent the "apparent" K_m and V_{max} values calculated from the data in Fig. 6. Small cells are those obtained from the younger, leaner animals (120–160 g, 4–5-wk-old) and large cells from the older, fatter rats (>550 g, >12-mo-old).

amount of insulin bound per cell is plotted as a function of 2-deoxy glucose uptake. As can be seen, the relationship is similar for both groups of cells, indicating that the decrease in 2-deoxy glucose uptake at submaximal insulin levels in large adipocytes (Fig. 4) is quantitatively accounted for by the decrease in insulin binding seen in Fig. 3. Furthermore, these data indicate that the coupling between insulin-receptor complexes and the glucose transport system is normal in large adipocytes. A similar graph is seen in Fig. 8B with glucose oxidation

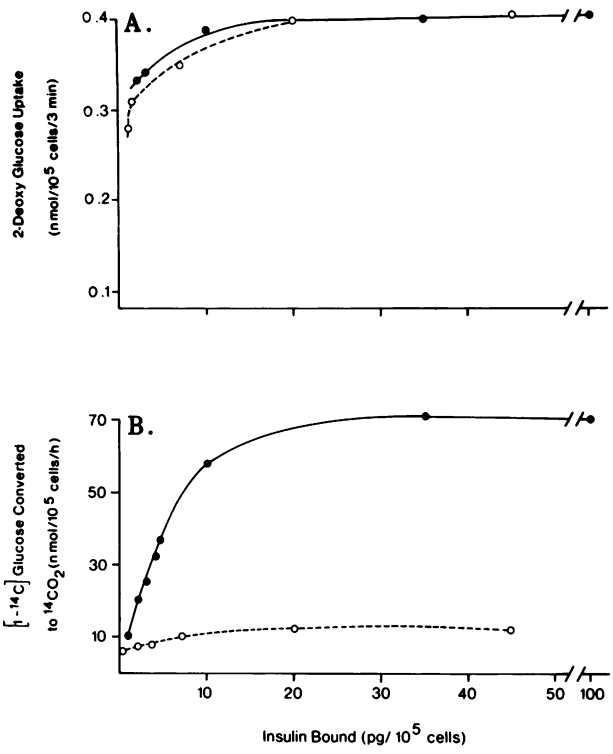

FIGURE 8 Relationship between the amount of insulin bound and insulin action. (A) The amount of insulin bound (at the insulin concentrations used in Fig. 4) is plotted (horizontal axis) as a function of the amount of 2-deoxy glucose uptake at that insulin concentration (vertical axis) for small (●) and large (○) adipocytes. (B) Amount of insulin bound as a function of the amount of glucose oxidized.

TABLE IV

Total Glucose Consumption by Large and Small Adipocytes

	Large cells		Small cells
Glucose consumed,* *nmol/10^5 cells per h*	130±31	$P < 0.01$	218±30
Glucose oxidized‡, %	6±1	$P < 0.001$	27±3

* Adipocytes were incubated at 37°C for 2 h in the presence of 5 mM glucose and 5 ng/ml insulin. Glucose concentration was measured before and after the incubations and the data represent the mean of four experiments.

‡ This was calculated by dividing the amount of glucose oxidized at 5 mM glucose and 5 ng/ml insulin (Fig. 2) by the total amount of glucose consumed. It should be recalled, however, that in these studies glucose oxidation refers to the amount of glucose converted to CO_2 as estimated by oxidation only of the first carbon atom of the glucose molecule. Thus, these calculations are estimates and are most useful for comparative purposes.

plotted on the vertical axis. In this plot the curves are much different, demonstrating that for a given amount of insulin bound, large adipocytes oxidize less glucose. Thus, a defect in the glucose oxidation process distal to the 2-deoxy glucose uptake system is indicated.

Studies of glucose consumption. These studies have shown that at any given hexose concentration, maximal rates of 2-deoxy glucose uptake are the same for both groups of cells, while maximal rates of glucose oxidation are decreased in large cells. Thus, it seemed probable that the distribution of transported glucose was altered in the large cells. To investigate this possibility we measured the total glucose consumption by large and small adipocytes. These data are presented in Table IV and demonstrate that in the presence of maximally effective insulin concentrations, total glucose consumption is less for large cells. However, at the same glucose (5 mM) and insulin (5 ng/ml) concentrations (Fig. 2) the decrease in [1-^{14}C]glucose oxidation[2] by large adipocytes is much greater than the decrease in total glucose consumption seen in Table IV. This is emphasized by comparing the amount of glucose oxidized via the C-1 oxidative pathways (Fig. 2) and the total amount of glucose consumed (Table IV) at 5 mM glucose and 5 ng/ml insulin. Using this data it can be calculated that C-1 oxidation accounts for 26% of the glucose consumed by small cells, but only 6% of the glucose consumed by large cells. This indicates that a defect of the magnitude seen in Fig. 2 cannot exist in all other pathways of glucose metabolism (including other oxidative and nonoxidative pathways), and that some pathway(s) of glucose metabolism must be relatively increased to compensate for the decrease in glucose disposition via C-1 oxidative steps.

DISCUSSION

In these studies we have shown that at glucose concentrations > 5 mM, basal rates of glucose oxidation were higher in adipocytes from younger, leaner rats than from older, fatter animals. Furthermore, insulin could not correct this defect, and the decrease in the maximal rate of glucose oxidation by large adipocytes[3] was much greater in the presence of maximally effective insulin concentrations due to insulin's greater ability to enhance oxidation in small cells. Comparable findings have been previously reported (12–15), and these observations suggest several possible explanations. Since maximal insulin-stimulated glucose oxidation occurs when only a minority (10–15%) of the available insulin receptors are occupied (8–10), a decrease in insulin action observed at insulin concentrations great enough to saturate this absolute number of receptors (maximally effective insulin level) cannot be due to a decreased number of insulin receptors. This line of reasoning implies that the defect in glucose oxidation in large adipocytes is distal to the insulin receptor; i.e., in the glucose transport system or one or more of the intracellular steps in glucose metabolism. This hypothesis is supported by the fact that, in the large cells, maximal rates of oxidation (i.e., at glucose concentrations >5 mM, Fig. 2) are also decreased in the absence of insulin (basal state).

The above suggestions, however, rely on the assumption that all available insulin-binding sites are fully functional (spare receptor theory), and that a separate, unique class of functional receptors which comprise a small minority of available binding sites (10-15%) does not exist. When the number of insulin receptors are decreased, the functional sequelae in the first situation (spare receptor theory) would be a shift in the insulin dose-response curve to the right; that is, decreased insulin action at submaximal insulin levels, and normal insulin action at maximally effective insulin levels. On the other hand, if a unique loss of functional receptors existed, then decreased insulin action at all insulin levels would be observed. The results of the glucose transport studies (Fig. 4) indicate that all available insulin receptors are fully potentially functional. Thus, a shift in the insulin dose-response curve was seen, with small cells able to take up more 2-deoxy glucose than large cells only at submaximal insulin concentrations. This differ-

[2] It should be noted that [1-^{14}C]glucose is oxidized primarily via the pentose shunt.

[3] It should be pointed out that labeling these cells as large adipocytes does not mean that the increased size of these cells is responsible for these differences. Obviously, the larger cells are obtained from animals which differ in regards to degree of obesity, age, plasma insulin levels, etc., and any of these factors may influence adipocyte metabolism.

ence is most likely due to the decreased number of insulin receptors observed in the large adipocytes. This idea is further supported by comparing the amount of insulin bound per cell to the amount of 2-deoxy glucose uptake per cell. This comparison demonstrated that for any given amount of insulin bound, large and small adipocytes take up similar amounts of 2-deoxy glucose. This indicates that the decrease in insulin receptors of large cells quantitatively accounts for the decreased sugar uptake at submaximal insulin levels, and that coupling between insulin-receptor complexes and 2-deoxy glucose uptake is normal in large adipocytes.

The use of 2-deoxy glucose to assess glucose transport is based on the principle that this hexose is transported and phosphorylated the same as D-glucose but cannot be further metabolized (25). The phosphorylated sugar is then trapped inside the cell, thus allowing prolonged periods of linear uptake which are readily measurable. The validity of this concept in this system has been previously demonstrated (10). However, 2-deoxy glucose uptake reflects glucose transport only if transport, not phosphorylation, is the rate-limiting step. This has been established in a variety of systems (27–29), including epididymal adipocytes (10), and for reasons discussed previously (10), we believe that 2-deoxy glucose uptake closely reflects glucose transport. This idea is further supported by the data of Livingston and Lockwood who have found normal insulin-stimulated rates of 3-*O*-methyl glucose uptake[4] in enlarged adipocytes (11). This, even though a decrease in adipocyte hexokinase has been reported in large adipocytes (15), this decrease is apparently not great enough to cause a decrease in 2-deoxy glucose uptake (Fig. 6). Regardless of the precise step 2-deoxy glucose uptake best reflects, it is obvious that the uptake system for this hexose is basically normal in adipocytes from older, fatter animals. This, combined with the striking decrease in glucose oxidation displayed by large cells, localizes the major abnormality responsible for the decrease in glucose oxidation as being distal to the hexokinase step.

Some basic problems in interpreting this type of data should be mentioned. For example, a persistent dilemma in analyzing insulin function data in which basal and stimulated values are obtained, is whether to consider the insulin effect as the percent rise above basal or as the absolute value. When basal values differ, and maximal insulin-stimulated values are the same, then insulin's effect will be comparable or different, depending upon the method of data analysis one chooses. This problem remains unresolved, with proponents of both views. We have elected to analyze the data in terms of absolute rates of metabolism, since the maximal capacity of the glucose transport system was found to be the same in both groups of cells. However, the complexity of this issue can be illustrated by a close examination of Fig. 6. For example, basal rates of uptake are higher for larger cells, especially at sugar concentrations < 5 mM. If one equalized basal uptake rates by increasing the media 2-deoxy glucose concentration for small cells (i.e., 0.5 mM for large cells and 1 mM for small cells) then, at these sugar concentrations, insulin-stimulated uptake is now twofold greater for small cells. Another problem of data interpretation concerns the method of normalization. In these studies we have normalized the data on a per cell basis. We believe this approach provides a more representative adipocyte characteristic than total DNA or protein measurements for reasons previously discussed (7). However, insulin binding and glucose transport are basically membrane processes, and, thus, one could argue that since these groups of adipocytes differ in size, one should normalize the data to unit surface area, cell volume, or unit intracellular water content (30). No data are available which will clarify these possibilities, and further studies are obviously necessary to resolve this aspect of data calculation.

The K_m and V_{max} values for 2-deoxy glucose uptake for large and small cells are remarkably similar. The uptake of 2-deoxy glucose represents only two processes (transport and phosphorylation), with transport most likely being the rate-determining step (10). Additionally, this measurement is made after a very brief incubation (3 min) and, thus, initial uptake rates are approximated. For these reasons, the apparent K_m and V_{max} values calculated for 2-deoxy glucose uptake are reasonable estimates of the kinetic characteristics of the adipocyte glucose transport system. The effect of insulin in both groups of cells is to increase the V_{max} of 2-deoxy glucose uptake without changing the K_m. Additionally, the similarity of the apparent K_m and V_{max} values for both groups of cells (especially in the presence of insulin), further demonstrates that the glucose transport systems of large and small adipocytes are comparable.

In conclusion, we have found that adipocytes from older, fatter rats have a decreased number of insulin-binding sites compared to cells from the younger, leaner animals. Moreover, the predicted functional sequelae of this decrease in insulin binding was also found, i.e., a rightward shift in the insulin dose-response curve for 2-deoxy glucose uptake, and this provides strong evidence for the idea that all available specific insulin-binding sites are fully potentially active. Furthermore, the data presented have indicated that the major abnormality responsible for the decrease in insulin-mediated glucose oxidation in adipocytes from older, fatter rats is located intracellularly in one or more of the steps of glucose

[4] This sugar is taken up by cells, but is not phosphorylated, and, thus, represents only the transport process.

oxidation, and this idea is consistent with the suggestion of Livingston and Lockwood (11) and Czech.[5] Since the measured capacity of the 2-deoxy glucose uptake system is normal in large cells, two possibilities exist to explain these findings: (*a*) glucose uptake is comparable in large and small cells, but a smaller proportion is oxidized by the large cells. This implies that other pathways of glucose metabolism are enhanced in larger adipocytes, and, indeed, Di Girolamo et al. (12, 31) have demonstrated increased conversion of glucose to glyceride-glycerol in these cells. (*b*) An additional factor which has been suggested by Czech [5] is that while the potential activity of the glucose transport system is normal in large cells, glucose metabolism is blunted, leading to a buildup of glucose metabolites, including free intracellular glucose. Thus, at higher glucose concentrations, in the presence of insulin, an accumulation of free intracellular glucose in large cells could lead to increased rates of glucose efflux, with a decrease in net glucose uptake, resulting in an overall decrease in the amount of glucose consumed by large adipocytes. Our data, which shows a modest decrease in glucose consumption by large cells are consistent with this latter idea; but since the defect in glucose oxidation is quantitatively much greater than the decrease in glucose consumption, we feel that a combination of the above two events best explains all of the data.

ACKNOWLEDGMENTS

The author wishes to thank Ms. Christine Feliton and Mrs. Helen Chang for their expert technical work. I would also like to thank Dr. Michael Czech of Brown University for providing a copy of a manuscript before publication, and for critically reading this paper.

This work was supported by grants from the National Institutes of Health, HL 08506, and from the Veterans Administration.

[5] Czech, M. Submitted for publication.

REFERENCES

1. Rabinowitz, D., and K. L. Zierler. 1962. Forearm metabolism in obesity and its response to intra-arterial insulin. *J. Clin. Invest.* **41**: 2173–2181.
2. Kahn, C. R., D. M. Neville, Jr., and J. Roth. 1973. Insulin-receptor interaction in the obese-hyperglycemic mouse. A model of insulin resistance. *J. Biol. Chem.* **248**: 244–250.
3. Freychet, P., M. H. Laudat, P. Laudat, G. Rosselin, C. R. Kahn, P. Gorden, and J. Roth. 1972. Impairment of insulin binding to the fat cell membrane in the obese hyperglycemic mouse. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **25**: 339–342.
4. Soll, A. H., I. D. Goldfine, J. Roth, and C. R. Kahn. 1974. Thymic lymphocytes in obese (ob/ob) mice: a mirror of the insulin receptor defect in liver and fat. *J. Biol. Chem.* **249**: 4127–4131.
5. Archer, J. A., P. Gorden, J. R. Gavin, III, M. Lesniak, and J. Roth. 1973. Insulin receptors in human circulating lymphocytes: application to the study of insulin resistance in man. *J. Clin. Endocrinol. Metab.* **36**: 627–633.
6. Archer, J. A., P. Gorden, and J. Roth. 1975. Defect in insulin binding to receptors in obese man. Amelioration with caloric restriction. *J. Clin. Invest.* **55**: 166–174.
7. Olefsky, J. M., and G. M. Reaven. 1975. Effects of age and obesity on insulin binding to isolated adipocytes. *Endocrinology.* **96**: 1486–1498.
8. Kono, T., and F. W. Barham. 1971. The relationship between the insulin-binding capacity of fat cells and the cellular response to insulin: studies with intact and trypsin-treated fat cells. *J. Biol. Chem.* **246**: 6210–6216.
9. Gammeltoft, S., and J. Gliemann. 1973. Binding and degradation of ^{125}I-insulin by isolated rat fat cells. *Biochim. Biophys. Acta.* **320**: 16–32.
10. Olefsky, J. M. 1975. The effects of dexamethasone on insulin binding, glucose transport, and glucose oxidation by isolated rat adipocytes. *J. Clin. Invest.* **56**: 1499–1508.
11. Livingston, J. N., and D. H. Lockwood. 1974. Direct measurements of sugar uptake in small and large adipocytes from young and adult rats. *Biochem. Biophys. Res. Commun.* **61**: 989–996.
12. Di Girolamo, M., and D. Rudman. 1968. Variations in glucose metabolism and sensitivity to insulin of the rat's adipose tissue, in relation to age and body weight. *Endocrinology.* **82**: 1133–1141.
13. Salans, L. B., and J. W. Dougherty. 1971. The effect of insulin upon glucose metabolism by adipose cells of different size. Influence of cells lipid and protein content, age, and nutritional state. *J. Clin. Invest.* **50**: 1399–1410.
14. Gliemann, J. 1965. Insulin-like activity of dilute human serum assayed by isolated adipose cell method. *Diabetes.* **14**: 643–649.
15. Bernstein, R. S., and D. M. Kipnis. 1973. Regulation of rat hexokinase isoenzymes. I. Assay and effect of age, fasting and refeeding. *Diabetes.* **22**: 913–922.
16. Rodbell, M. 1964. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* **239**: 375–380.
17. Hirsch, J., and E. Gallian. 1968. Methods for the determination of adipose cell size in man and animals. *J. Lipid Res.* **9**: 110–119.
18. Di Girolamo, M., S. Medlinger, and J. W. Fertig. 1971. A simple method to determine fat cell size and number in four mammalian species. *Am. J. Physiol.* **221**: 850–858.
19. Freychet, P., J. Roth, and D. M. Neville, Jr. 1971. Monoiodoinsulin: demonstration of its biological activity and binding to fat cells and liver membranes. *Biochem. Biophys. Res. Commun.* **43**: 400–408.
20. Hunter, W. M., and F. C. Greenwood. 1962. Preparation of iodine-131 labeled human growth hormone of high specific activity. *Nature (Lond.).* **194**: 495–496.
21. Olefsky, J., and G. M. Reaven. 1974. The human lymphocyte: a model for the study of insulin-receptor interaction. *J. Clin. Endocrinol. Metab.* **38**: 554–560.
22. Gavin, J. R., III, P. Gorden, J. Roth, J. A. Archer, and D. N. Buell. 1973. Characteristics of the human lymphocyte insulin receptor. *J. Biol. Chem.* **248**: 2202–2207.
23. Olefsky, J. M., J. Johnson, F. Liu, P. Jen, and G. M. Reaven. 1975. The effects of acute and chronic dexamethasone administration on insulin binding to isolated rat hepatocytes and adipocytes. *Metab. Clin. Exp.* **24**: 517–527.
24. Olefsky, J. M., P. Jen, and G. M. Reaven. 1974. Insulin

binding to isolated human adipocytes. *Diabetes.* **23**: 565–571.
25. Wick, A. N., D. R. Drury, H. I. Nakada, and J. B. Wolfe. 1957. Localization of the primary metabolic block produced by 2-deoxyglucose. *J. Biol. Chem.* **224**: 963–969.
26. Gliemann, J., K. Østerlind, J. Vinten, and S. Gammeltoft. 1972. A procedure for measurement of distribution spaces in isolated fat cells. *Biochim. Biophys. Acta.* **286**: 1–9.
27. Renner, E. D., P. G. W. Plagemann, and R. W. Bernlohr. 1972. Permeation of glucose by simple and facilitated diffusion by Novikoff rat hepatoma cells in suspension culture and its relationship to glucose metabolism. *J. Biol. Chem.* **247**: 5765–5776.
28. Weber, M. J. 1973. Hexose transport in normal and in Rous sarcoma virus-transformed cells. *J. Biol. Chem.* **248**: 2978–2983.
29. Kletzien, R. F., and J. F. Perdue. 1974. Sugar transport in chick embryo fibroblasts. I. A functional change in the plasma membrane associated with the rate of cell growth. *J. Biol. Chem.* **249**: 3366–3374.
30. Di Girolamo, M., and J. L. Owens. 1974. Fat cell size and intracellular water space. *Diabetes.* **23**(Suppl. 1): 370. (Abstr.)
31. Di Girolamo, M., and S. Mendlinger. 1972. Glucose metabolism and responsiveness to bovine insulin by adipose tissue from three mammalian species. *Diabetes.* **21**: 1151–1161.