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Research Article

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Distribution of Glucose Transporters in Membrane Fractions Isolated from Human Adipose Cells

Relation to Cell Size

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Abstract

We examined insulin's effects on glucose transport and on subcellular transporter distribution in isolated human omental adipocytes of various sizes. Insulin stimulated 3-*O*-methylglucose transport by twofold in small cells, while a smaller and insignificant effect was measured in large cells. In the small cells, basal concentrations of glucose transporters were 2.9 and 17.2 pmol/mg membrane protein in the plasma and the low density microsomal membranes, respectively. Increasing cell size was associated with a 50% decrease in the concentration of transporters in each fraction, with no change in their total number per cell. Insulin stimulated the translocation of transporters from the intracellular pool to the plasma membranes, irrespective of cell size. Thus, insulin resistance at the postreceptor level, observed in human obesity, may be associated with a relative depletion of total transporters per cell together with a reduction in their intrinsic activity at the plasma membrane level.

Introduction

A fundamental action of insulin in promoting glucose metabolism in adipose and muscle cells is its stimulation of glucose transport. Recent studies in rat adipocytes by Wardzala et al. (1), Cushman and Wardzala (2), Karnieli et al. (3), and independent studies by Kono and coworkers (4–7), have established that insulin stimulates glucose transport in the isolated rat adipose cell through a rapid, reversible, and hormone concentration- and energy-dependent translocation of glucose transporters from a large intracellular pool to the plasma membrane. Horuk et al. (8) have provided evidence for a similar translocation mechanism in isolated guinea pig adipose cells, and Wardzala and Jeanrenaud (9, 10) have suggested the existence of such a mechanism in the rat diaphragm. However, a similar mechanism of insulin action has not yet been demonstrated in human adipose cells.

The clinical significance of this novel concept of insulin action has emerged from three experimental models of insulin resistance: (*a*) the streptozotocin-diabetic rat (11), (*b*) the high fat/low carbohydrate–fed rat (12), and (*c*) the aged, obese male rat (13). In each of these experimental models, the marked postreceptor insulin resistance of the adipose cell is associated with a relative depletion of the intracellular pool, from which glucose transporters are translocated to the plasma membrane in response to insulin.

The present studies were undertaken in order to demonstrate that insulin stimulates glucose transport in human omental adipose cells through the same translocation process as previously shown in the rat, and to examine glucose transporter distribution in human obesity.

Methods

Patients and adipose tissue. Human omental adipose tissue was obtained from Caucasian patients, none of whom were known to have any thyroid, renal, or adrenal abnormalities. The patients were undergoing an elective abdominal surgery. Patients followed a weight maintenance diet for 3 d before the operation. After an overnight fast, general anesthesia was induced with a short-acting barbiturate and maintained with a mixture of halothane, nitrous oxide, and oxygen. For each experiment, ~50–100 g of omental tissue were removed from each patient as soon as possible after the operation began. After removal from the patient, the adipose tissue samples were kept in normal saline containing 5 mM glucose at 37°C during the 5-min period required for their transport to the laboratory. All procedures were approved by the Rambam Medical Center and the Technion Institute Human Rights Boards, and informed written consent was obtained from each patient.

Adipose cells and subcellular membrane fractions. Isolated adipose cells were prepared according to the method described by Rodbell (14) and modified by Pederson et al. (15), using 3 mg/ml collagenase. All incubations were carried out in Krebs-Ringer bicarbonate/Hepes buffer (standard Krebs-Ringer bicarbonate buffer reduced to 10 mM HCO_3^- and supplemented with 30 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), pH 7.4, containing 4% crude bovine serum albumin (Fraction V). In each experiment, at least 50 g of omental adipose tissue from each patient were needed in order to acquire 30 ml of 40% cell suspension, which yielded enough membranes for two preparations (basal and insulin-stimulated). In each experiment, cells from one patient were collected and pooled together. Adipose cell size was measured using a cell-counting chamber and eyepiece micrometer, assuming that the cells had spherical shape. The mean cell size was used for further calculations. Small cells were collected from six normal patients (percent of ideal body weight [%IBW][1] ≤ 120) while large cells were collected from four obese ones (%IBW > 120). After isolation, the pooled adipose cells from each patient were equally distributed in 15-ml volumes to two 950-ml polypropylene jars (basal and insulin-stimulated preparations, respectively), each containing 21 ml of incubation medium. The cells were preincubated for 15 min at 37°C, then insulin was added to a final concentration of 0 or 7.0 nM (1,000 μU/ml) and incubations were continued for another 30–45 min. 70-μl replicate samples were then removed from either preparation to determine the basal and the insulin-stimulated rates of 3-*O*-methylglucose transport, according to the method described by Whitesell and Gliemann (16) and modified by Pederson and Gliemann (17), using 0.1 mM of 3-*O*-methylglucose as a substrate. In each experiment (namely, for each patient), plasma and low density microsomal membrane fractions

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1. *Abbreviation used in this paper:* %IBW, percent of ideal body weight.

were prepared from the incubated cells (basal or insulin-stimulated) by the differential ultracentrifugation method described elsewhere (2, 3, 18), and modified as follows. Briefly, incubated cells were washed twice by centrifugation at 1,000 *g* for 2 min, and homogenized at 17°C in a buffer containing 20 mM Tris-HCl, 1 mM EDTA, and 255 mM sucrose, pH 7.4. All steps after homogenization were carried out at 4°C using this buffer. Each homogenate, from basal and insulin-stimulated preparations, was then centrifuged at 16,000 *g* for 15 min. The supernatant was aspirated and saved, and the fat cake discarded. The pellet (containing plasma membranes, mitochondria, and nuclei) was washed once, repelleted at 17,000 *g* for 20 min, resuspended in 4.5 ml of buffer, then layered on top of a 1.12-M sucrose cushion containing 20 mM Tris-HCl and 1 mM EDTA, pH 7.4, and centrifuged at 101,000 *g* for 65 min. The plasma membrane band was washed twice by centrifugation at 200,000 *g* and resuspended to a final concentration of 1–2 mg protein/ml. The low-density microsomal membrane fraction was obtained from the initial 16,000 *g* supernatant, first by centrifugation at 48,000 *g* for 15 min, yielding a pellet of high density microsomal membranes,[2] and then by recentrifugation of the 48,000 *g* supernatant at 360,000 *g* for 60 min. The latter pellet, the low density microsomal membrane fraction, was washed once and resuspended to a final concentration of 1–2 mg protein/ml.

Equilibrium D-glucose–inhibitable cytochalasin B binding to the plasma and low density microsomal membrane fractions was then measured in the presence of 4.63 μM cytochalasin E and the concentration of the D-glucose–inhibitable binding sites calculated by a modification of the method previously described (1, 2). In each experiment and for either preparation (basal or insulin-stimulated), cytochalasin B–binding isotherms were determined in the absence or presence of 500 mM D-glucose. [^{14}C]urea added to the final mixture was used to determine trapped unbound [^{3}H]cytochalasin B in the membrane pellets obtained during the binding assay. From each membrane fraction prepared, 12 different concentrations of [^{3}H]cytochalasin B and [^{14}C]urea were used to determine the Scatchard binding curve in the absence of D-glucose, and 4 different concentrations were used to determine the binding curve in the presence of D-glucose. The concentration of glucose transporters in each fraction (D-glucose–inhibitable cytochalasin B binding sites) were then estimated (2) by an IBM personal computer using a CBCAL (APL System, Micosys Ltd., Haifa, Israel) program. This method is of particular advantage in estimating low concentrations of transporters, as found in human adipose cells.

As the Scatchard plots obtained in presence of D-glucose only approached a straight line, this number of cytochalasin B binding sites is only an estimation of the number of glucose transporters.

The reproducibility of the fractionation procedure between experimental points and among experiments was assessed by measuring the specific marker enzyme activities of 5′ nucleotidase, rothenone-insensitive NADH-cytochrome C reductase, and UDP-galactose:*N*-acetylglucosamine galactosyl transferase in each fraction and in the original homogenate (19–21). Protein concentration was determined by the Lowry method as modified by Peterson (22), using albumin (bovine-crystallized) as a standard, and the cellular protein recovery of each fraction was calculated. All chemicals were purchased from Sigma Chemical Co, Petach-Tikva, Israel. Radioisotopes were obtained from New England Nuclear, Boston, MA.

Results

Patient clinical data are described in Table I. All patients were females, 21–68-yr-old (47±6 yr, mean±SEM), with normal blood glucose (101±2 mg/dl).

The omental adipose cell size significantly correlated with the %IBW calculated for each patient ($r = 0.74$, $P < 0.05$; data not shown). Hence, based upon their body weight, patients were divided into two groups: normal, with %IBW ≤ 120%, and obese, with %IBW > 120%. Thus, small cells were derived from normal patients and large cells were obtained from obese ones.

Table I. Clinical Data of the Patients

Group	Diagnosis/ operation	Sex	Age	IBW	Blood glucose	Cell size
			yr	*%*	*mg/dl*	*μm*
Normal	TAH	FM	68	100	100	55.6
	CC	FM	21	116	95	66.8
	CC	FM	37	114	95	58.3
	CC	FM	36	120	88	51.9
	CC	FM	53	100	110	72.8
	DU	FM	43	94	102	76.0
mean±SEM			43±6	109±4*	98±2	64±4*
Obese	H	FM	59	218	105	83.6
	CC	FM	34	210	100	137.4
	CC	FM	62	161	110	89.5
	CC	FM	54	140	97	84.6
mean±SEM			52±7	196±15	105±2	104±14

TAH, total abdominal hysterectomy; CC, cholecystectomy; DU, duodenal ulcer; H, hernioplasty.
* Difference between normals and obese is statistically significant at the $P < 0.05$ level.

Glucose transport activity. Individual glucose transport rates were measured in intact cells isolated from each patient, and the mean rates of glucose transport for either group of patients, normal or obese, were then calculated per cell (Fig. 1 *A*), or per cellular surface area (Fig. 1 *B*). With either method of calculation, insulin significantly stimulated glucose transport by twofold ($P < 0.05$) in cells isolated from normal patients. A smaller and statistically insignificant increase of transport rate was measured in obesity. When calculated per cell (Fig. 1 *A*), the mean basal

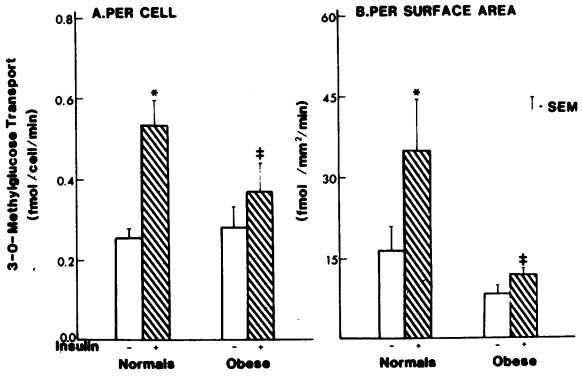

Figure 1. Glucose transport in human adipose cells. 3-*O*-methylglucose transport rate per cell (*A*) and per cellular surface area (*B*) was measured in isolated human omental adipose cells. Small and large cells were isolated from six normal (%IBW ≤ 120) and four obese (%IBW > 120) female patients, respectively. For each patient, the cells were pooled and preincubated with or without 7.0 nM insulin for 30 min at 37°C. Cell sizes are 64±4 μm and 104±14 μm for small and large cells, respectively. Results are presented as mean±SEM. *, Difference between basal and insulin-stimulated states is statistically significant at the $P < 0.05$ level. ‡, Difference between normals and obese is statistically significant at the $P < 0.05$ level.

2. In separate studies, very low and inconsistent cytochalasin B binding sites were found in the high density microsomal membrane fractions, and no significant insulin effect was noticed.

rate of glucose transport in the normal group did not differ significantly from that of the obese one ($P > 0.05$). However, when calculated per cellular surface area, a 30% decrease in the basal transport rate could be noted in obesity (Fig. 1 *B*).

Glucose transporter distribution in relation to cell size. In each group of patients (based upon cell size), insulin enhanced the concentration of transporters in the plasma membranes by 30–50%, as compared with the basal state (Fig. 2 *A*). In the basal state, the concentration of glucose transporters in the low density microsomes of each patient examined (Fig. 2 *B*), was six to sevenfold higher than that observed in the plasma membranes ($P < 0.05$). Insulin decreased the concentration of these transporters by about 20–30%.

In the human omental adipose cell, an increase in cell size was accompanied by a decrease in concentration of glucose transporters either in basal or in insulin-stimulated states, in both membrane fractions examined.

Glucose transporter distribution in normal versus obese patients. Glucose transporter concentration was measured in subcellular membrane fractions prepared from basal and insulin-stimulated adipose cells. For each group of patients, the individual values for every fraction examined were averaged, and the mean concentrations of glucose transporters were compared, as shown in Fig. 3. In the insulin-stimulated state, a marked enhancement in the concentration of the transporters in plasma membranes prepared from adipose cells isolated from normal patients could be demonstrated. This enhancement was accompanied by a concomitant decrease in the number of transporters in the low density microsomes.

Qualitatively, the same concentration relationship also holds for the transporters in membrane fractions prepared from adipocytes of obese patients. However, in the latter, insulin exerted a smaller effect (insignificant at the $P < 0.05$ level) both in increasing the level of transporters in the plasma membranes, from 1.5±0.2 to 2.0±0.3 pmol/mg membrane protein, and in decreasing it in the low density microsomes, from 9.1±1.4 to 6.5±2.0 pmol/mg membrane protein.

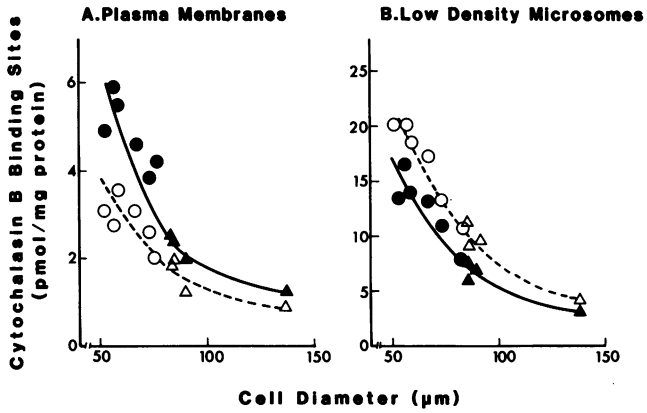

Figure 2. Relationship between cell size and glucose transporter concentration in subcellular membrane fractions of human adipose cells. Adipocytes were isolated from omental adipose tissue obtained from six normal (●, ○) and four obese (▲, △) patients. For each patient, the cells were preincubated with (●, ▲, ——) or without (○, △, - - -) 7.0 nM insulin for 30 min at 37°C. Subcellular membrane fractions were prepared as explained in Methods, and the concentration of glucose transporter in the plasma (*A*) and the low density microsomal (*B*) membrane fractions was determined using the cytochalasin B binding assay.

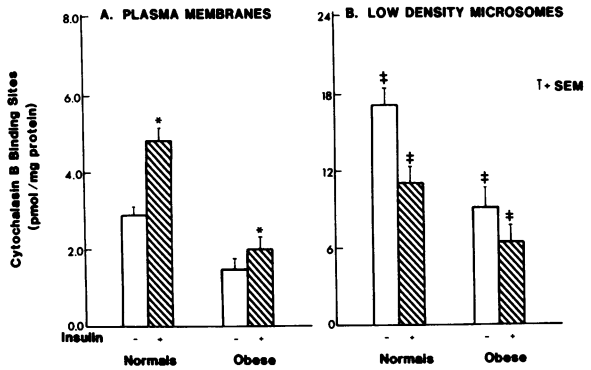

Figure 3. Mean concentration of glucose transporters in small and large human adipose cells. Small (diameter, 64±4 μm) and large (104±14 μm) adipose cells were isolated from the patients, and glucose transporter concentration determined as previously described in Methods. For each group, normal or obese, the individual results were averaged, and the mean concentrations of glucose transporters in plasma membranes (*A*) and low density microsomes (*B*) are shown. Results are preented as mean±SEM. *, Difference between basal and insulin-stimulated states is statistically significant at the $P < 0.05$ level. ‡, Difference between normals and obese is statistically significant at the $P < 0.05$ level.

In the plasma membranes (Fig. 3 *A*) the concentration of transporters in the basal state in normal patients was significantly higher than that measured in obese ones, 2.9±0.2 and 1.5±0.2 pmol/mg membrane protein, respectively ($P < 0.05$).

In the basal state, concentration of glucose transporters in the low density microsomes (Fig. 3 *B*) was about sixfold higher both in normal and in obese patients than that observed in the plasma membranes of the same patients, respectively. Furthermore, concentration of glucose transporters in the intracellular pool was significantly higher in normals, compared with obese patients, being 17.2±1.2 and 9.1±1.4 pmol/mg membrane protein, respectively ($P < 0.05$).

Total yields of membrane protein and total number of transporters per cell in each membrane fraction are shown in Table II. The total number of transporters was calculated from the estimated cytochalasin B binding sites and the fractional protein yields. A fourfold increase in the adipose cell's volium was associated with a significant threefold increase in total membrane protein, calculated per cell. On the other hand, a similar total number of transporters was found in the cells obtained from either normal or obese patients. Exposure of the intact cells to a maximally stimulating concentration of insulin (7 nM) did not influence either the protein recoveries in any of the fractions examined or the total number of transporters per cell (differences between the basal and the insulin-stimulated states are insignificant at the $P < 0.05$ level).

Total recoveries of specific marker enzyme activities among the different membrane fractions are shown in Table III, and include 5′-nucleotidase, rothenone-insensitive NADH-cytochrome C reductase, and UDP-galactose:*N*-acetylglucosamine galactosyltransferase, which characterize the plasma membranes, the endoplasmic reticulum, and the Golgi apparatus, respectively (23). In spite of the cross-contamination observed, enzyme distribution demonstrates that major 5′-nucleotidase activity was found in plasma membranes, while galactosyltransferase activity was maximal in the low density microsomes. Furthermore, no insulin effect on the distribution of these enzymes could be noted.

Table II. Subcellular Distribution and Total Number of Cytochalasin B Binding Sites in Isolated Human Omental Adipocytes

Group	Membrane fractions	Insulin (7nM)	Protein recovery	CB binding sites	
				Fraction	Intact cell
			pg/cell	*pmol/mg*	*no./cell × 10⁴*
Normal	Plasma membrane	−	16.8±2.8	2.9±0.2*	2.6±0.4*
		+	18.0±2.9	4.8±0.3	4.9±0.8
	Low-density microsomes	−	10.1±1.0	17.2±2.4*	9.4±1.0*
		+	10.7±1.3	12.8±1.0	7.2±0.8
	Total	−			12.0±1.1
		+			12.1±1.2
Obese	Plasma membrane	−	26.3±4.1‡	1.5±0.2‡	1.8±0.2*
		+	33.4±7.5‡	2.0±0.3‡	3.2±0.5
	Low-density microsomes	−	27.1±4.6‡	9.1±1.4‡	11.9±1.0*
		+	27.2±4.5‡	6.6±1.0‡	8.6±0.7
	Total	−			13.7±1.1
		+			11.8±1.0

The individual values obtained from six normal and four obese patients were averaged, and are shown on the upper and the lower parts of the table, respectively. For each fraction, cellular protein recovery (picogram per cell) was calculated from the measured protein concentration and the number of cells in the original homogenate. Total number of cytochalasin B binding sites was estimated from the cytochalasin B binding data in Fig. 3 and the calculated cellular protein recovery. * Difference between basal and insulin-stimulated states is statistically significant at the $P < 0.05$ level. ‡ Difference between normals and obese is statistically significant at the $P < 0.05$ level.

Discussion

The present data add support to the hypothesis that insulin stimulates glucose transport in the intact human adipose cell by a mechanism similar to that postulated for isolated rat and guinea pig adipose cells and rat diaphragm, namely, through the translocation of glucose transporters from a large intracellular pool to the plasma membrane.

The subcellular fractionation procedure used here for human adipose cells seems to be analogous to that described for the rat adipocyte. In the human omental adipose cell, basal and insulin-stimulated concentrations of glucose transporters are shown to decrease with increasing cell size. Furthermore, insulin's effect in stimulating the translocation of transporters is preserved in all cell sizes, even in the largest cell measured.

Our results are consistent with those of Hissin et al. (13) on the adipose cell isolated from the aged, obese rats, regarding the relative depletion of the transporter pool. At variance with the latter investigator regarding the rat adipose cell, and with Kashiwagi et al. (24) regarding human subcutaneous adipose cells, we have not been able to show a significant increase in the basal rate of glucose transport in the intact cell, but, rather, we have shown a trend towards a decline in the transport activity calculated per cellular surface area. This low transport activity in the human cell is in accordance with the number of transporters found in the plasma membranes.

Table III. Recovery of Marker Enzyme Activities from Human Adipose Cells

Enzyme activity	Insulin (7 nM)	Membrane fraction		
		Homogenate	Plasma membranes	Low density microsomes
5′-nucleotidase	−	16±4*	111±11*	71±7*
(*μmol/mg/h*)	+	17±4	126±20	67±9
Cytochrome C reductase	−	67±15*	209±48*	596±96*
(*μmol/mg/min*)	+	119±34	247±63	572±14
Galactosyltransferase	−	1.1±0.3*	1.5±0.4*	12±0.5*
(*nmol/mg/2 h*)	+	1.1±0.3	0.8±0.1	11±1.0

Marker enzyme activities were measured in the membrane fractions prepared as described in Methods. For each patient, specific marker enzyme activities were determined in the membrane fractions prepared from basal and insulin-stimulated cells, and the individual values were averaged. Results are presented as mean±SEM of the individual values.

* Difference between basal and insulin-stimulated states is statistically insignificant at the $P < 0.05$ level.

A discrepancy exists between the number of transporters in the plasma membrane and glucose transport activity in large cells. Insulin had a 30–50% effect on translocation of transporters in all cell sizes, but only a small and insignificant effect on promotion of glucose transport could be demonstrated in large cells. The fact that only an estimated number of transporters can be reached while using the cytochalasin B binding assay raises the question of expression of transporters present in the plasma membrane. As glucose transport activity depends not only on the number of transporters but also on their intrinsic activity, changes in the processes of insertion/fusion with the plasma membrane and/or the fluidity of the plasma membrane may be associated with enlargement of the cell and could thus lead to a discrepancy between the magnitude of insulin's translocating effect and the resultant enhancement of glucose transport.

The latter possibility gains further support from works done by us (25), by Kahn and Cushman (26) in insulin-treated diabetic rats, and by Smith et al. (27), which evaluate the effect of adenosine on glucose transport activity and glucose transporters in rat adipose cells. We have shown a large discrepancy between the glucose transport activity in either basal or insulin-stimulated states in the intact cell and the concentration of transporters after 8-d insulin therapy. After 14-d insulin therapy, this discrepancy partially disappeared (25). In all of the above cases, in the presence of a low concentration of transporters in the plasma membranes, glucose transport was increased disproportionately.

Thus, the discrepancy we found between the number of transporters and glucose uptake rate may be due to the presence of a regulator for glucose transport that may, at least in part, act

by modulating the intrinsic activity of the transporter. Such a regulator can exist in different amounts in various pathophysiological states, and may be almost absent in obesity. Further studies are needed to elucidate this hypothetical mechanism.

The down-regulation of glucose transporters found in plasma membrane in basal and insulin-stimulated states may represent a negative feedback mechanism regulating the rate of cellular growth in such a way that, as the cell enlarges, glucose uptake is correspondingly reduced and further growth is thus controlled. The various factors regulating the intracellular pool of transporters are as yet not clearly understood. The size of the pool of transporters can be regulated by insulin itself, can be genetically determined, or can depend on differences in sex and species (28, 29).

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