Mechanism for Enhanced Glucose Transport Response to Insulin in Adipose Cells from Chronically Hyperinsulinemic Rats

Increased Translocation of Glucose Transporters from an Enlarged Intracellular Pool

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Abstract

The mechanism for the increased glucose transport response to insulin in adipose cells from chronically hyperinsulinemic rats was examined. Rats were infused with insulin s.c. for 2 wk. Isolated adipose cells were incubated with and without insulin, 3-*O*-methylglucose transport was measured, and glucose transporters in subcellular membrane fractions were assessed by cytochalasin B binding. Adipose cells from insulin-treated rats showed no change in basal but a 55% increase in insulin-stimulated glucose transport activity compared with those from control rats (7.1±0.8 vs. 4.6±0.5 fmol/cell per min, mean±SEM) and a corresponding increase in the concentration of glucose transporters in the plasma membranes (44±5 vs. 32±6 pmol/mg of membrane protein). In the low-density microsomes, glucose transporter concentrations in both basal and insulin-stimulated states were the same, but the total numbers were greater in cells from the insulin-treated rats because of a 39% increase in low-density microsomal protein. Therefore, chronic experimental hyperinsulinemia in the rat enhances the stimulatory action of insulin on glucose transport in the adipose cell by increasing the concentration of glucose transporters in the plasma membranes. This results from (*a*) an enlarged intracellular pool due to increased intracellular protein and (*b*) enhanced glucose transporter translocation in response to insulin.

Introduction

Although chronic hyperinsulinemia in humans is usually associated with systemic insulin resistance (1–4), in several rat models it is accompanied by insulin hyperresponsiveness at the cellular level (5–10). Kobayashi and Olefsky (5, 6) first demonstrated increased insulin-stimulated glucose transport in the adipose cell in association with hyperinsulinemia induced in normal rats by subcutaneous insulin injections. Subsequently, similar findings were reported by Trimble et al. (8) using intravenous insulin infusion and by Wardzala et al. (9) using subcutaneous insulin infusion via osmotic minipumps.

The stimulatory action of insulin on glucose transport in the rat adipose cell has recently been shown to occur through the translocation of glucose transporters from a large intracellular pool to the plasma membrane (11–14). Furthermore, insulin resistance at the cellular level in rat models of aging/obesity (15), high fat/low carbohydrate feeding (16), and streptozotocin diabetes (17) has been explained by a reduction in the number of glucose transporters translocated to the plasma membrane in response to insulin due to a depleted intracellular pool.

The current study was therefore designed to determine whether the increased glucose transport response to insulin in adipose cells from rats treated chronically with insulin could be attributed to a change in the number or subcellular distribution of glucose transporters. The results suggest that chronic insulin infusion in the rat is associated with an increase in the insulin-stimulated translocation of glucose transporters to the plasma membrane. This increased translocation appears to result from an enlarged intracellular pool due to a generalized increase in net protein synthesis. A recent study of the mechanism of the hyperresponsive insulin-stimulated glucose transport in adipose cells from young, genetically obese, naturally hyperinsulinemic Zucker fatty rats shows similar results (10).

Methods

Animals and experimental design. Male Sprague–Dawley rats (CD strain, Charles River Breeding Laboratories, Wilmington, MA) were received at body weights ranging from 130 to 140 g and maintained with *ad libitum* feeding (standard National Institutes of Health laboratory diet) for several days before insertion of osmotic minipumps (Alzet no. 2001, Alza Corp., Palo Alto, CA) containing purified U-500 porcine crystalline insulin (Eli Lilly and Co., Indianapolis, IN) prepared as described by Bringer et al. (18) to prevent aggregation. Rats were anesthetized with ether and pumps were inserted subcutaneously in the intrascapular area. Infusion was begun at 2 U/d and sequentially increased to 6 U/d at 4–6-d intervals by altering the concentration of insulin in the pumps according to the protocol of Wardzala et al. (9). Both control and insulin-infused rats had free access to granulated sucrose, standard rat laboratory diet, and water. After 2 wk of treatment, animals were killed by cervical dislocation and decapitation between 8 and 10 a.m. Blood was collected and plasma was stored for measurement of plasma glucose and insulin. A total of five experiments were carried out.

Preparation of isolated adipose cells and measurement of adipose cell size. Immediately after the animals were sacrificed in the fed state, whole epididymal fat pads were removed, and isolated adipose cells were prepared by the method originally described by Rodbell (19) and sub-

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sequently modified by Cushman (20) using crude collagenase (Cooper Biomedical, Inc., Malvern, PA). All incubations were carried out in Krebs–Ringer bicarbonate buffer reduced to 10 mM HCO_3^- and supplemented with 30 mM Hepes (Sigma Chemical Co., St. Louis, MO), pH 7.4, 37°C, containing 1% untreated bovine serum albumin (bovine serum albumin powder, fraction V, Reheis Chemical Co., Kankakee, IL). Adipose cell size was determined by the osmic acid fixation, Coulter Electronic counter method (method III) described by Hirsch and Gallian (21) for intact tissue fragments, and modified for isolated cell suspensions by Cushman and Salans (22).

Measurement of cellular glucose transport activity and intracellular water space. Isolated adipose cells from a minimum of 15 rats for each experimental group were distributed in 15-ml volumes into two 950-ml polypropylene jars containing 21 ml of incubation medium and incubated at 37°C for 30 min in the presence of 0 or 700 nM (100,000 μU/ml) insulin (courtesy of Dr. Ronald E. Chance, Eli Lilly and Co.). Samples were then taken for determination of 3-*O*-methylglucose transport using a modification described by Karnieli et al. (23) of the L-arabinose uptake method of Foley et al. (24). The intracellular water space was assessed from the steady-state 3-*O*-methylglucose uptake levels.

Preparation of subcellular membrane fractions and determination of glucose transporter concentration. Plasma, high-density microsomal, and low-density microsomal membrane fractions were prepared from the remaining cells by differential ultracentrifugation as previously described (12, 25). Equilibrium D-glucose-inhibitable [^{3}H]cytochalasin B binding was then measured and the concentrations of glucose transporters were calculated (11, 12).

Membrane protein was determined by the Coomassie Brilliant Blue method (Bio-Rad protein assay, Bio-Rad Laboratories, Richmond, CA) described by Bradford (26) and modified by Simpson and Sonne (27) using crystalline bovine serum albumin (Sigma Chemical Co.) as the standard. The specific 5'-nucleotidase (28), rotenone-insensitive NADH-cytochrome *c* reductase (cytochrome *c* reductase)[1] (29), and UDP-galactose:*N*-acetylglucosamine galactosyltransferase (galactosyltransferase) (30) activities of each homogenate and subcellular membrane fraction were also assayed as previously described (25).

Other assays, calculations and statistical analyses. Plasma glucose concentrations were measured by a glucose oxidase method using a Beckman glucose analyzer (Beckman Instruments, Inc., Palo Alto, CA) and plasma insulin concentrations by radioimmunoassay with polyethylene glycol precipitation. All calculations were carried out on the Dartmouth time-sharing system computer facilities. Comparisons between experimental groups were made using a *t* test of statistical significance and differences were accepted as significant at the $P \leq 0.05$ level.

Results

Physiological parameters. Table I compares some of the physiological parameters of the insulin-infused and control rats; the values closely concur with those reported by Wardzala et al. (9). At the end of the experimental period, body weight, weight gain, and adipose cell size were similar in the two groups. However, mean postprandial plasma glucose was 34% lower and mean plasma insulin 15-fold higher in the insulin-treated animals. In addition, the adipose cell intracellular water space, an indirect measure of cytoplasmic mass, was increased 33%.

Glucose transport activity. The effects of chronic insulin infusion on adipose cell 3-*O*-methylglucose transport are illustrated in Fig. 1. Basal transport rates were not significantly affected by insulin treatment of rats, although they tended to increase. Maximally insulin-stimulated transport rates, in contrast, were consistently higher in cells from the insulin-treated rats, with a mean increase of 55%.

Table I. Clinical Characteristics of Control and Chronically Insulin-infused Rats

	Control	Insulin-infused
Rat weight (*g*)	285±13	273±19
Weight gain* (*g*)	95±25	95±13
Adipose cell size (*μg of lipid per cell*)	0.17±0.02	0.17±0.01
Adipose cell water space (*pl per cell*)	2.50±0.13	3.32±0.26‡
Plasma glucose (*mg/dl*)	143±1	95±7‡
Plasma insulin (*μU/ml*)	36±2	548±78‡

Results are means±SEM for 76 control and 72 insulin-treated rats.
* During 2-wk treatment period.
‡ Significant difference from corresponding control value.

Subcellular distribution of glucose transporters. Fig. 2 illustrates the subcellular distributions of glucose transporters in the same adipose cells studied in Fig. 1. In the plasma membranes (Fig. 2 *A*), the concentrations of glucose transporters in the basal state were similar between the two groups of rats. However, in the insulin-stimulated state, the concentration was 38% higher in cells from the insulin-treated rats, paralleling the 55% increase in insulin-stimulated glucose transport activity in the intact cell. In contrast, the concentrations of glucose transporters in the high-density (Fig. 2 *B*) and low-density (Fig. 2 *C*) microsomes were similar in the two groups of rats in both the basal and insulin-stimulated states.

Differences in the amount of membrane protein present in the cell or in the recoveries or relative enrichments of the various membrane fractions may result in changes in the number of glucose transporters per cell when the concentrations per milligram of membrane protein are unchanged. For this reason, we measured the protein recoveries for each membrane fraction from adipose cells of the control and insulin-infused rats (Table II) and the percent recoveries and relative enrichments of several specific markers enzymes (Tables III and IV, respectively): 5'-nucleotidase, characteristic of plasma membranes (28); cytochrome *c* reductase, characteristic of the endoplasmic reticulum

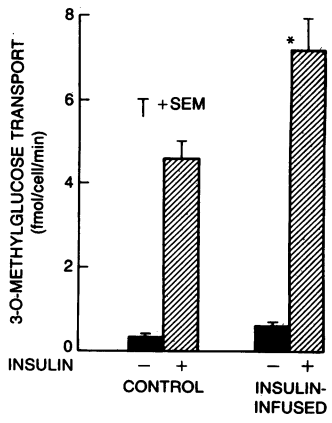

Figure 1. Glucose transport activities in basal and maximally insulin-stimulated adipose cells from control and chronically insulin-infused rats. Isolated cells were prepared from epididymal fat pads from a minimum of 15 rats in each group, preincubated for 30 min at 37°C in the absence or presence of 700 nM (100,000 μU/ml) insulin, and sampled for the determination of the rates of 3-*O*-methylglucose transport as described in Methods. Results are means±SEM of the mean values obtained from at least quadruplicate samples in each of five separate experiments. *Significant difference from corresponding control value.

1. *Abbreviations used in this paper:* cytochrome *c* reductase, rotenone-insensitive NADH-cytochrome *c* reductase; galactosyltransferase, UDP-galactose:*N*-acetylglucosamine galactosyltransferase.

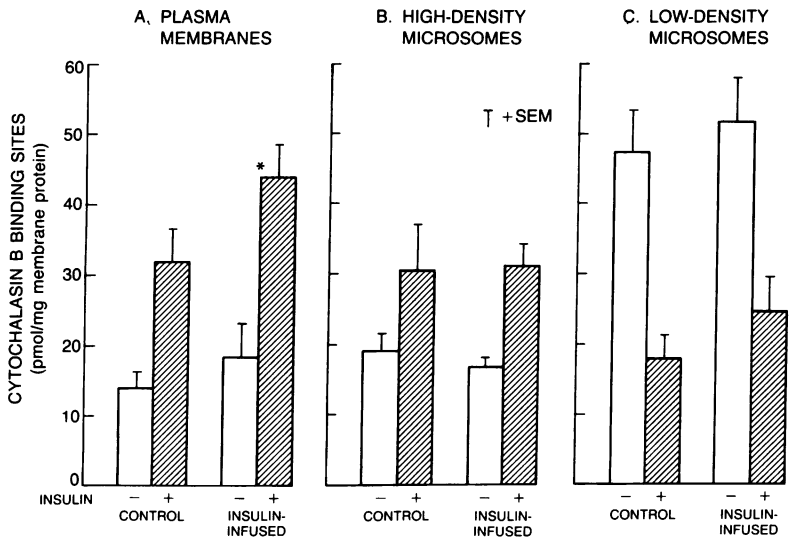

Figure 2. Concentrations of glucose transporters in (*A*) the plasma membranes, (*B*) high-density microsomes, and (*C*) low-density microsomes of basal and maximally insulin-stimulated adipose cells from control and chronically insulin-infused rats. Subcellular membrane fractions were prepared from the remaining isolated cells described in Fig. 1 and the concentrations of glucose transporters determined as described in Methods. Results are means±SEM of the individual values obtained in each of the five separate experiments. *Significant difference from corresponding control value.

(29); and galactosyltransferase, characteristic of the Golgi apparatus in many cell types (30), but found to be less specific in the rat adipose cell (25, 31). Table II demonstrates that in parallel with the intracellular water space (Table I), total homogenate protein per cell was 49% greater in cells from the insulin-infused animals compared with the controls, with a corresponding 61% increase in high-density microsomal protein and 39% increase in low-density microsomal protein. In contrast, the recoveries of plasma membrane protein were equivalent.

In general, chronic insulin infusion was not associated with significant changes in either marker enzyme recoveries (Table III) or relative enrichments (Table IV), although small changes were observed in the recoveries of 5′-nucleotidase activity in the high-density microsomes and of cytochrome *c* reductase and galactosyltransferase activities in the plasma membranes (Table III), and in the relative enrichment of 5′-nucleotidase activity in the low-density microsomes. However, a substantial increase in the relative enrichment of the plasma membranes and high-density microsomes with galactosyltransferase was observed (Table IV). In addition, the absolute specific activities of 5′-nucleotidase and cytochrome *c* reductase were moderately decreased in their respective predominant membrane fractions (Table IV).

Table II. Protein Recoveries from Adipose Cells of Control and Chronically Insulin-infused Rats

	Recovered protein	
	Control	Insulin-infused
	pg/cell	*pg/cell*
Homogenate	316±31	471±16*
Plasma membranes	38±3	39±1
High-density microsomes	13±1	21±2*
Low-density microsomes	19±2	26±2*

Protein recoveries were measured in the experiments described in Fig. 2 as described in Methods. Results are means±SEM of the mean values obtained from triplicate determinations on individual preparations of basal and insulin-stimulated cells in each of the five separate experiments.

* Significant difference from corresponding control value.

Discussion

This study demonstrates that the hyperresponsive insulin-stimulated glucose transport activity in the adipose cell observed with experimental hyperinsulinemia in the rat (Fig. 1) (5, 6, 8, 9) is accompanied by an increased concentration of glucose transporters in the plasma membranes in the insulin-stimulated state. This, in turn, appears to be the consequence of (*a*) an enlarged intracellular pool in the basal state due to a generalized increase in the net synthesis of intracellular protein, and (*b*) a corresponding increase in glucose transporter translocation in response to insulin. This mechanism is complementary to that recently reported for states of insulin resistance such as aging/obesity in the male rat (15), high fat feeding (16), and streptozotocin diabetes (17). In the latter cases, insulin-resistant glucose transport could be explained by a depletion of intracellular glucose transporters in the basal state resulting in fewer being translocated to the plasma membrane in response to insulin.

In the hyperresponsive model reported here, increased insulin-stimulated glucose transport activity in the intact adipose cell is accompanied by a corresponding increase in the concentration of glucose transporters in the plasma membranes compared to control. This is associated with no change in the recovery of plasma membrane protein per cell (Table II) which is consistent with similar cell sizes (Table I) and therefore, cellular surface areas. Additionally, equivalent recoveries of 5′-nucleotidase (Table III) imply comparable fractional recoveries of plasma membrane protein from the original homogenates.

Further assessment of marker enzyme recoveries (Table III) and relative specific activities (Table IV) generally show minor changes, suggesting that fractionation is comparable in cells from control and insulin-infused rats. An increase in the relative galactosyltransferase specific activity in the plasma membranes may suggest increased contamination with low-density microsomes, but this would primarily affect the basal concentration of glucose transporters in the plasma membrane because the low-density microsomes from basal cells are relatively enriched

Table III. Marker Enzyme Recoveries from Adipose Cells of Control and Chronically Insulin-infused Rats

Marker enzyme activity	Experimental condition	Subcellular membrane fraction		
		Plasma membranes	High-density microsomes	Low-density microsomes
		% of homogenate activity	*% of homogenate activity*	*% of homogenate activity*
5′-nucleotidase	Control	45.7±5.0	5.1±0.9	2.1±0.5
	Insulin-infused	57.2±7.3	10.3±2.9*	2.3±1.0
Rotenone-insensitive NADH-cytochrome *c* reductase	Control	15.2±0.8	16.1±1.6	10.7±1.3
	Insulin-infused	12.0±1.4*	16.2±1.2	9.3±1.0
UDP-galactose:*N*-acetylglucosamine galactosyltransferase	Control	24.0±2.0	12.4±0.9	35.6±5.4
	Insulin-infused	15.5* (17, 14)‡	10.8 (10, 12)‡	24.0 (27, 21)‡

Marker enzyme activities were measured in the original homogenates and membrane fractions prepared in the experiments described in Fig. 2 using the procedures described in Methods. Within each experiment, total marker enzyme activities for each membrane fraction from basal and insulin-stimulated cells were expressed as a percentage of the respective homogenate activity and the percent activity for the basal and insulin-stimulated cells were averaged. Results are means±SEM of the mean values obtained from quadruplicate samples of individual preparations of basal and insulin-stimulated cells from each of three separate experiments. * Significant difference from corresponding control value. ‡ Results of two individual experiments.

with glucose transporters (Fig. 2). Indeed, a tendency towards increased glucose transporter concentration in the plasma membranes in the basal state in the cells from the insulin-infused rats is observed, although this increase is not statistically significant. In the insulin-stimulated state, the plasma membranes are twofold more enriched with glucose transporters than the low-density microsomes and so the possible contamination would have minimal impact. Despite a generalized increase in intracellular protein, absolute specific activities of some of the marker enzymes are reduced in cells from insulin-treated rats (Table IV). However, only the enzyme recoveries and the relative specific activities are important in assessing the fractionation procedure. Therefore, thorough analysis of the marker enzymes excludes the possibility of major changes in the fractionation of cells from control and insulin-treated rats that could affect the apparent number of glucose transporters. Hence, the increased concentration of glucose transporters in the plasma membranes in the insulin-stimulated state can explain the hyperresponsive insulin-stimulated glucose transport activity in the intact cell.

In previously reported states of altered insulin responsiveness, changes in the insulin-stimulated concentrations of glucose transporters in the plasma membranes have reflected similar

Table IV. Relative Marker Enzyme Specific Activities in Subcellular Membrane Fractions from Adipose Cells of Control and Chronically Insulin-infused Rats

Marker enzyme activity	Subcellular membrane fraction		
	Plasma membranes	High-density microsomes	Low-density microsomes
	% of predominant membrane fraction		
5-nucleotidase (*μmol/mg per h*)			
Control	100 (0.79±0.08)*	32±4	11±2
Insulin-infused	100 (0.58±0.09‡)*	31±4	5±2‡
Rotenone-insensitive NADH-cytochrome *c* reductase (*μmol/mg per min*)			
Control	34±2	100 (2.48±0.10)*	49±3
Insulin-infused	39±5	100 (1.88±0.12‡)*	44±5
UDP-galactose/*N*-acetylglucosamine galactosyltransferase (*nmol/mg per 2 h*)			
Control	29±1	44±4	100 (102±11)
Insulin-infused	48‡ (41, 55)§	62‡ (54, 70)§	100 [80(92, 68)§]*

Marker enzyme activities were measured in the membrane fractions prepared in the experiments described in Fig. 2 using the procedures described in Methods. Within each experiment, marker enzyme specific activities were obtained for each membrane fraction prepared from basal and insulin-stimulated cells and expressed as a percentage of the respective highest activity observed. Results are means±SEM of the mean values obtained from quadruplicate samples of individual preparations of basal and insulin-stimulated cells from each of three separate experiments. * Actual specific activity value set at 100% (mean±standard error of the mean). ‡ Significant difference from corresponding control value. § Results of two individual experiments.

changes in the low-density microsomes in the basal state (15–17). In the cells from insulin-treated rats reported here, however, the basal concentration of glucose transporters per milligram of membrane protein in the low-density microsomes, is the same as in the control cells (Fig. 2). Nevertheless, because the low-density microsomal protein is substantially increased (Table II), the actual total number of glucose transporters in this fraction is greater. Homogenate and high-density microsomal protein are also increased (Table II) as is the intracellular water space (Table I), suggesting a more generalized increase in the net synthesis of intracellular protein. Thus, both insulin-stimulated plasma membrane and basal low-density microsomal glucose transporters are increased in cells from insulin-infused rats, the former because of an increase in the concentration per milligram of membrane protein and the latter because of an increase in the amount of protein.

A similar mechanism for hyperresponsive glucose transport activity has recently been described in the physically trained rat (32) and in the chronically hyperinsulinemic, young, obese Zucker fatty rat (10) in which changes in membrane protein recoveries and marker enzyme distributions were essential in correlating the concentrations of glucose transporters assessed by cytochalasin B binding with glucose transport activity in the intact cell. Only after taking into account the marked increase in intracellular protein was it evident that the enhancement of the stimulatory effect of insulin on glucose transport could be explained by the translocation of a greater number of glucose transporters to the plasma membrane from an enlarged intracellular pool.

The possible factors contributing to this alteration in the number of glucose transporters in cells from rats that are naturally (10) or experimentally (5, 6, 8, 9) hyperinsulinemic is of interest. Hyperinsulinemia alone is unlikely to be responsible because in association with obesity (3), impaired glucose tolerance (3), and insulin treatment of type I diabetes (33) in humans, it is accompanied by insulin resistance in vivo and at the cellular level. In addition, two studies of experimental hyperinsulinemia in rats (34, 35) and two in humans (36, 37) failed to show hyperresponsiveness. In all studies where experimental hyperinsulinemia has been associated with cellular hyperresponsiveness, animals have been mildly to markedly hypoglycemic (5, 6, 8, 9), and hyperresponsiveness has not been observed in the absence of hypoglycemia with the exception of the young, Zucker fatty rat (10). Hypoglycemia may play a role in eliciting hyperresponsiveness by causing chronic elevation of counterregulatory hormones which lead to decreased beta receptor sensitivity (38–40) and result in increased insulin action at target tissues (41). Elevations in epinephrine and norepinephrine and increased in vivo insulin effectiveness have been reported in a hyperinsulinemic model achieved by intravenous insulin infusion (8).

Hypoglycemia could also play a role by directly or indirectly regulating glucose transporter number. Studies examining the effects of glucose starvation in cultured cells underscore the potential role of hypoglycemia in modulating the rate of glucose transporter net synthesis, resulting in changes in the total number of transporters per cell. Glucose starvation in cultured fibroblasts (42–44) or transformed 3T3-L1 adipocytes (45, 46) brought about by removal of glucose from the growth medium leads to a dramatic increase in hexose uptake. This is reversible upon refeeding the cells with both metabolizable (42–44) and nonmetabolizable (45, 46) hexoses. The enhancement appears to result from an increased number of glucose transporters in the plasma membrane (47), which is not accompanied by changes in the level of in vitro translatable glucose transporter messenger RNA or the rate of glucose transporter polypeptide synthesis (48), thus suggesting decreased turnover of the glucose transporter. Systemic hypoglycemia resulting in cellular glucose starvation could be instrumental in increasing the net synthesis of glucose transporters in the rats studied here and by previous investigators (5, 6, 8, 9). Further evidence for the role of glucose is seen in a recent preliminary report in diabetic rats that demonstrates that normalizing plasma glucose concentration alone, without changing plasma insulin, can restore in vivo insulin responsiveness (49).

In conclusion, chronic hyperinsulinemia accompanied by a modest decrease in plasma glucose concentration in rats is associated with an increase in insulin-stimulated glucose transport in adipose cells. This can be explained by a corresponding increase in glucose transporter concentration in the plasma membranes that appears to be the consequence of a greater translocation of glucose transporters from an enlarged intracellular pool. The latter, in turn, results from a net increase in intracellular protein synthesis. Whether the enlarged intracellular pool is due to hyperinsulinemia alone or in combination with hypoglycemia remains to be elucidated.

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