Mechanism for Markedly Hyperresponsive Insulin-stimulated Glucose Transport Activity in Adipose Cells from Insulin-treated Streptozotocin Diabetic Rats

EVIDENCE FOR INCREASED GLUCOSE TRANSPORTER INTRINSIC ACTIVITY*

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The effects of insulin therapy in streptozotocin diabetic rats on the glucose transport response to insulin in adipose cells have been examined. At sequential intervals during subcutaneous insulin infusion, isolated cells were prepared and incubated with or without insulin, and 3-O-methylglucose transport was measured. Insulin treatment not only reversed the insulin-resistant glucose transport associated with diabetes, but resulted in a progressive hyperresponsiveness, peaking with a 3-fold overshoot at 7–8 days (12.1 ± 0.3 versus 3.1 ± 0.1 pmol/cell/min, mean ± S.E.) and remaining elevated for more than 3 weeks. During the peak overshoot, glucose transporters in subcellular membrane fractions were assessed by cytochalasin B binding. Insulin therapy restored glucose transporter concentration in the plasma membranes of insulin-stimulated cells from a 40% depleted level previously reported in the diabetic state to ~35% greater than control (38 ± 4 versus 28 ± 2 pmol/mg of membrane protein). Glucose transporter concentration in the low-density microsomes from basal cells was also restored from an ~45% depleted level back to normal (50 ± 4 versus 50 ± 6 pmol/mg of membrane protein), whereas total intracellular glucose transporters were further increased due to an ~2-fold increase in low-density microsomal membrane protein. However, these increases remained markedly less than the enhancement of insulin-stimulated glucose transport activity in the intact cell. Thus, insulin treatment of diabetic rats produces a marked and sustained hyperresponsive insulin-stimulated glucose transport activity in the adipose cell with little more than a restoration to the non-diabetic control level of glucose transporter translocation. Because this enhanced glucose transport activity occurs through an increase in \( V_{\text{max}} \), insulin therapy appears to be associated with a marked increase in glucose transporter intrinsic activity.

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Streptozotocin diabetes in rats (1–3) and both Type I (4, 5) and II (6, 7) diabetes mellitus in humans have been shown to be associated with insulin resistance at the cellular level. In particular, a marked reduction in insulin's stimulatory effect on glucose transport activity in the isolated adipose cell has been reported in spite of increased binding of insulin to its receptor (1, 2). Although this cellular resistance to insulin appears to be reversible with insulin therapy in human Type II diabetics (6, 7), the mechanism has not been identified. Additionally, the effect of insulin treatment on adipose cell glucose transport activity has not been studied in the streptozotocin diabetic rat or systematically in human Type I diabetics.

Studies from our laboratory (8, 9) and independently by Kono and co-workers (10, 11) have shown that insulin stimulates glucose transport in the rat adipose cell primarily through the translocation of glucose transporters from a large intracellular pool to the plasma membrane. Furthermore, in streptozotocin diabetic rats, resistance to insulin's stimulatory effect on glucose transport has been demonstrated to be a consequence of a depletion of intracellular glucose transporters, resulting in a decrease in the number translocated to the plasma membrane in response to insulin (3). This same mechanism appears to explain the insulin-resistant glucose transport seen in adipose cells from rats which have undergone high fat feeding (12), aging/obesity (13), or fasting (14) and from normal guinea pigs (15) and humans (16, 17) where the typical glucose transport response to insulin is reduced compared to that in rats. Recently, a corollary mechanism has been reported in states of insulin hyperresponsiveness associated with chronic hyperinsulinemia experimentally induced in normal rats (18) or naturally occurring in young, obese Zucker rats (19) or with physical training (20). Under these conditions, insulin stimulation results in the translocation of more glucose transporters to the plasma membrane from an enlarged intracellular pool.

In this study, we have investigated the effects of insulin treatment of the streptozotocin diabetic rat on glucose transport activity in the adipose cell. Insulin therapy results not only in a restoration of insulin responsiveness, but in a marked and sustained hyperresponsive insulin-stimulated glucose transport activity. Furthermore, the mechanism for this altered responsiveness differs from that in all previous models. Because this large increase in insulin-stimulated glucose transport activity is accompanied by little more than a restoration to the non-diabetic control level of the number of glucose transporters translocated, it appears to involve augmentation of glucose transporter intrinsic activity. Whereas
modulation of glucose transporter intrinsic activity by acute in vitro incubation of insulin-stimulated rat adipose cells with adenylate cyclase stimulators and inhibitors has recently been reported (21-23), the present study is the first to demonstrate such modulation by in vivo metabolic alterations.

**EXPERIMENTAL PROCEDURES**

**Animals and Experimental Design—Male Sprague-Dawley rats (CD strain, Charles River Breeding Laboratories) were received at 130-140 g and maintained with ad libitum feeding (standard National Institutes of Health chow) for ~1 week prior to intraperitoneal injection of 80-85 mg/kg anhydrous streptozotocin and citric acid (Zanosar, The Upjohn Co.) reconstituted with 0.9% saline. Seven days later, the effects of fasting blood glucose concentration >20 mM (360 mg/dl) were treated via subcutaneous osmotic minipumps (Alzet 2001, Alza Corp.) with 4 units/day U-500 porcine crystalline insulin (Lilly) prepared as described by Bringer et al. (24) to prevent aggregation. Those rats with a fasting blood glucose concentration <20 mM were not included in the study.

Initial groups of control animals were treated with sham intraperitoneal injections of normal saline and implantation of osmotic minipumps delivering diluting solution without insulin. After sham treatments were shown to have no effect on body weight gain, adipose cell size, blood glucose concentration, or adipose cell glucose transport activity, the control group was treated with streptozotocin but not with insulin and showed no spontaneous improvement in the above parameters relative to the diabetic state.

The first series of experiments was performed to establish the time course of the effects of insulin treatment of diabetic animals on basal and maximally insulin-stimulated adipose cell glucose transport activity. Insulin treatment was continued for up to 39 days, and animals were killed in the fed state on sequential days by cervical dislocation and decapitation between 8 and 10 a.m. A second series of experiments including preparation of subcellular membrane fractions was then undertaken at the point of maximum overshoot in insulin-stimulated glucose transport activity at 7-8 days of insulin treatment to investigate the mechanisms for the alterations observed.

In both series, blood was sampled daily from the tail vein for immediate blood glucose determination. On the days when adipose cell glucose transport activity was to be examined, blood glucose concentration was again determined immediately after decapitation. Rats with a postprandial blood glucose concentration >10 mM at any time after 48 h of insulin treatment were not included in the studies. In all cases, blood glucose was measured in whole blood using Chemstrip reagents (Boehringer Mannheim Diagnostics) read in an Hitachi 912 reflectance meter (Bio-Dynamics). Serum was prepared from blood obtained after decapitation and stored for determination of insulin concentration by radioimmunoassay.

**Preparation of Isolated Adipose Cells and Measurement of Adipose Cell Size—Immediately after the animals were killed, the whole epididymal fat pads were removed, and isolated adipose cells were prepared as originally described by Rodbell (25) and subsequently modified by Cushman (26) using crude collagenase (Cooper Biomedical). All incubations were carried out in Krebs-Ringer bicarbonate buffer reduced to 10 mM HCO₃⁻ and supplemented with 30 mM Hepes (Sigma), pH 7.4, at 37 °C containing 1% untreated bovine serum albumin (bovine serum albumin powder, Fraction V, Reheis Chemical). Adipose cell size was determined by the osmic acid fixation, Coulter Electronic Counter method (Method III) described by Hirsch and Gallian (27) for intact tissue fragments and modified for isolated cells as described by Cushman and Salinas (28).

**Measurement of Cellular Glucose Transport Activity and Intracellular Water—**For experiments examining the time course of insulin treatment of diabetic rats, isolated adipose cells from a minimum of three rats for each experimental groups were incubated at 37 °C for 30 min in the presence of 0 or 7 mM (1000 micromolars/ml) insulin (crystalline porcine zinc insulin, courtesy of Dr. Ronald B. Chance, Lilly). 3-O-Methylglucose transport was then measured by a modification described by Karmeli et al. (29) of the t-arabininase uptake method of Foley et al. (30). For kinetic experiments carried out at the point of maximal overshoot in insulin-stimulated glucose transport activity, cells from a minimum of five rats for each experimental group were incubated at 37 °C for 60 min in the presence of 7 nM insulin and 0.5-40 mM unlabeled 3-O-methylglucose prior to the measurement of 3-O-methylglucose transport as just described. For experiments in which subcellular membrane fractions were to be prepared, samples of cells were taken for the determination of 3-O-methylglucose transport from larger volume incubations (see below). The intracellular water space was assessed as the steady-state 3-O-methylglucose uptake levels.

**Preparation of Subcellular Membrane Fractions and Determination of Glucose Transporter Concentration—**Isolated adipose cells from a minimum of 15 rats for each experimental group were distributed in 15-ml samples into each of 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 mM insulin. Following removal of small samples of cells for the determination of 3-O-methylglucose transport (see above), plasma, high-density microsomal, and low-density microsomal membrane fractions were prepared from the remaining cells by differential ultracentrifugation as previously described (9, 31). Equilibrium d-glucose-inhibitable [3H]cytochalasin B binding was then measured, and the concentrations of glucose transporters were calculated (8, 9).

Membrane protein was determined by the Coomassie Brilliant Blue method (Bio-Rad protein assay) described by Bradford (32) and modified by Simpson and Sonne (33) using crystalline bovine serum albumin (Sigma) as the standard. The specific activities of 5'-nucleotidase (34), cytochrome c reductase (rotenone-insensitive NADH-cytochrome c reductase) were no. (35), and galactosyltransferase (UDP-galactose:N-acetylgalactosamine galactosyltransferase) (36) in each homogenate and subcellular membrane fraction were also assayed. These marker enzyme activities were used to estimate the relative cross-contamination and the recoveries of the three specific membrane species (31).

**Calculations and Statistical Analyses—**All calculations were carried out on the Dartmouth Time-Sharing System computer facilities. Maximum velocities (Vmax) and Michaelis-Menten constants (Km) for 3-O-methylglucose transport were determined using Eadie-Hofstee plots. Comparisons between experimental groups were made using a t test of statistical significance, and differences were accepted as significant at the p < 0.05 level.

**RESULTS**

**General Characteristics of Control and Insulin-treated Streptozotocin Diabetic Rats—**Several physiological parameters were measured during the study period. Nonfasting blood glucose concentrations ranged from 6.4 to 8.9 mM in the control rats. In the diabetic rats, blood glucose was >22 mM prior to insulin therapy, decreased to the lower limit of the control range during the first 3 days of insulin treatment, and remained ~30% below the mean control concentration throughout the remainder of the study. During this same period, insulin treatment of the diabetic rats was accompanied by: 1) a slightly greater growth rate than that observed in the control rats, but not sufficient to restore fully to normal, even after 39 days, the initially 25% reduced body weight observed with diabetes; 2) a faster rate of increase in adipose cell size compared to the controls, but also not sufficient to restore fully to normal the initially almost 50% reduced cell size; and 3) a markedly changing adipose cell intracellular water space which rapidly increased to ~2-fold that in cells from the control rats at 7-8 days and then gradually declined to the unchanging control level. Table I shows these physiological characteristics of the 7-8-day insulin-treated diabetic rats compared to the corresponding control rats. Body weight and blood glucose were significantly lower; serum insulin was ~11-fold greater. Adipose cell size measured as micrograms of lipid/cell was also lower, but intracellular water space was greater by ~1.8-fold.

**Time Course of Effects of Insulin Treatment of Streptozotocin Diabetic Rats on Adipose Cell 3-O-Methylglucose Transport—**Fig. 1 illustrates that both the basal and insulin-stimulated glucose transport activities in cells from the control rats remain relatively stable over the entire 39-day experi-

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1 The abbreviation used is: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
TABLE I

General characteristics of non-diabetic control and 7-8 day insulin-treated streptozotocin diabetic rats

Rats were made diabetic and treated with insulin as described under "Experimental Procedures." Body weights and blood glucose were obtained at the time of death on 20 randomly chosen non-diabetic control and all 84 insulin-treated diabetic rats and serum insulin on 24 randomly chosen control and insulin-treated diabetic rats over four experiments. Adipose cell size, intracellular water, and protein content were determined on pooled cells from ≥15 rats in each of the four experiments described for Fig. 2. Results of cell size and intracellular water are means ± S.E. of individual preparations of basal and insulin-stimulated cells in each of the four separate experiments.

| Experimental group          | Non-diabetic control | Insulin-treated diabetic |
|----------------------------|----------------------|-------------------------|
| Body weight (g)            | 302 ± 6              | 271 ± 4*                |
| Blood glucose, postprandial (mM) | 8.0 ± 0.2         | 5.4 ± 0.2*              |
| Serum insulin, postprandial (microunits/ml) | 64 ± 4           | 727 ± 181*             |
| Adipose cell size (µg lipid/cell) | 0.145 ± 0.007 | 0.104 ± 0.005*         |
| Adipose intracellular water (pl/cell) | 2.46 ± 0.05  | 4.36 ± 0.17*           |
| Adipose cell protein content (µg/cell)       | Homogenate         | 353 ± 23                |
|                                           | Plasma membranes    | 44 ± 3                  |
|                                           | High-density microsomes | 15 ± 3             |
|                                           | Low-density microsomes | 22 ± 2               |

* Significant difference from control value.

Fig. 1. Time course of effect of insulin treatment of streptozotocin diabetic rats on adipose cell glucose transport activity. U-500 crystalline insulin was infused subcutaneously at 4 units/day via osmotic minipumps. Isolated cells were prepared from the epididymal fat pads of a minimum of three rats and incubated for 30 min at 37 °C in the absence (○, □, and □) or presence (▲, ○, and □) of 7 nM (1000 microunits/ml) insulin, and 3-O-methylglucose transport was measured as described under "Experimental Procedures." Results are the means ± S.E. of the individual quintuplet values obtained in single experiments or of the mean values obtained from quintuplet samples in each of two to four separate experiments. ▲ and ▲, control; ○ and ○, streptozotocin diabetic, insulin-treated; □ and □, streptozotocin diabetic, no insulin treatment.

mental period. Furthermore, as previously reported (3), basal glucose transport activity is not significantly changed in cells from diabetic rats compared to cells from control rats, but insulin-stimulated glucose transport activity is decreased by 60%. However, following initiation of insulin treatment of the diabetic rats, basal glucose transport activity remains unchanged, but cellular insulin resistance is dramatically reversed. Although insulin-stimulated glucose transport activity does not change over the first 2 days of therapy, it rises thereafter, reaching the control level at 4 days and then surpassing the control and peaking with a 3-fold overshoot at 8 days. Insulin-stimulated glucose transport activity then remains elevated at 14 days of insulin treatment, but gradually returns toward control values by 39 days. In cells from rats which remain diabetic without insulin treatment for 14 days, insulin-stimulated glucose transport activity remains ~60% reduced (Fig. 1). Kinetic experiments performed at the time of maximum overshoot in insulin-stimulated glucose transport activity show a marked increase in Vₐₘₐₓ with little change in Kₘ in cells from the insulin-treated diabetic rats compared to the controls (Table II).

Effects of Insulin Treatment of Streptozotocin Diabetic Rats on Subcellular Distribution of Glucose Transporters—The effects of 7-8 days of insulin treatment of diabetic rats on adipose cell glucose transport activity were determined in a separate series of experiments designed to examine the subcellular distribution of glucose transporters. Over the four experiments carried out, basal glucose transport activity is slightly, but significantly increased (0.29 ± 0.04 versus 0.15 ± 0.03 fmol/cell/min, mean ± S.E.), whereas the insulin-stimulated activity is markedly increased (5.98 ± 0.68 versus 2.47 ± 0.06 fmol/cell/min). When reexpressed per unit cellular surface area because the cells from the insulin-treated streptozotocin diabetic rats are smaller (Table I), the differences from the control cells are even greater (basal, 0.25 ± 0.03 versus 0.10 ± 0.02 amol/µm²/min; insulin-stimulated, 5.18 ± 0.47 versus 1.73 ± 0.02 amol/µm²/min).

Fig. 2 illustrates the subcellular distributions of glucose transporters in these same adipose cells. In the plasma membranes, the concentrations of glucose transporters per milligram of membrane protein are only modestly increased with insulin treatment of diabetes compared to controls in both the basal and insulin-stimulated states (Fig. 2A). In fact, the modest increase in glucose transporter concentration in the insulin-stimulated state markedly contrasts with the ~3-fold increase in glucose transport activity in the intact cells discussed above (also Fig. 1). Furthermore, no differences are observed between the insulin-treated and control groups when either the low-density microsomes containing relatively high concentrations of glucose transporters (Fig. 2C) or the high-density microsomes containing relatively low concentrations of glucose transporters (Fig. 2B) are examined in either the basal or insulin-stimulated state. Relative to the initial diabetic state, however, where glucose transporter concentration in the low-density microsomes from basal cells is reduced by ~45% and in the plasma membranes from insulin-stimulated cells by ~40% compared to the controls, insulin treatment of the diabetic rats restores these values to the normal level or even somewhat higher. In addition, the Kₘ of the glucose transporters for cytochalasin B binding in any of the membrane fractions from cells from the insulin-treated diabetic

TABLE II

Kinetic parameters of 3-O-methylglucose transport in adipose cells from non-diabetic control and 7-8 day insulin-treated streptozotocin diabetic rats

Isolated adipose cells prepared from the epididymal fat pads of five rats in each group were incubated for 1 h at 37 °C in the presence of 7 nM insulin and 0.5-40 mM unlabeled 3-O-methylglucose. Results are means of triplicate samples from two separate experiments.

| Experimental group          | Vₐₘₐₓ (fmol/cell/min) | Kₘ (µM) |
|----------------------------|----------------------|---------|
| Non-diabetic control        | 76, 109              | 2.5, 3.5|
| Insulin-treated diabetic    | 315, 411             | 3.7, 5.3|
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Fig. 2. Concentrations of glucose transporters in plasma membranes (A), high-density microsomes (B), and low-density microsomes (C) of basal (D) and maximally insulin-stimulated (E) adipose cells from non-diabetic control and 7-8-day insulin-treated streptozotocin diabetic rats. Isolated cells were prepared from the epididymal fat pads of a minimum of 15 rats in each group, incubated for 30 min at 37 °C in the absence or presence of 700 nM (100,000 microunits/ml) insulin, and sampled for determination of 3-0-methylglucose transport. Subcellular membrane fractions were then prepared from the remaining cells, and the concentrations of glucose transporters were determined as described under "Experimental Procedures." Results are the means ± S.E. of the individual values obtained in each of the four separate experiments. The asterisk indicates significant difference from corresponding non-diabetic control value.

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 of glucose transporter 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-treated rats (Table I) and the percent recoveries and relative enrichments of several specific marker enzymes (Table III and IV): 5'-nucleotidase, characteristic of plasma membranes (34); cytochrome c reductase, characteristic of endoplasmic reticulum (35); and galactosyltransferase, characteristic of the Golgi apparatus in many cell types (36), but possibly less specific in the rat adipose cell (31, 37). Total homogenate protein per cell is increased 104% in cells from the insulin-treated diabetic rats and is comprised primarily of intracellular protein as reflected in 77, 53, and 109% increases in intracellular water and high- and low-density microsomal protein, respectively, with no change in plasma membrane protein. Insulin treatment of diabetic rats is not associated with significant changes in the recovery (Table III) or relative enrichment (Table IV) of 5'-nucleotidase or cytochrome c reductase, although the absolute specific activity of cytochrome c reductase is reduced in the high-density microsomes. However, the recovery (Table III) of galactosyltransferase is decreased in the low-density microsomes, and its relative specific activity (Table IV) is increased in the plasma membranes and high-density microsomes.

DISCUSSION

This study demonstrates that insulin treatment of streptozotocin diabetic rats not only reverses the marked insulin resistance of the adipose cell associated with untreated diabetes, but results in a striking, although transient enhancement of insulin's stimulatory effect on glucose transport activity compared to the non-diabetic state. Maximally insulin-stimulated glucose transport activity increases progressively from a 67% depressed level in untreated diabetes to an ~3-fold overshoot with 8 days of insulin treatment and remains markedly elevated for at least an additional 3 weeks. By 5 weeks, this activity has returned close to base line. This overshoot occurs entirely through an increase in the glucose transport $V_{max}$, with little change in $K_m$.

In previously reported states of altered insulin responsiveness, both insulin-resistant (3, 12-17) and hyperresponsive (18-20) glucose transport activity in the intact adipose cell could be explained by changes in the number of glucose transporters translocated to the plasma membrane in response to insulin from a depleted or expanded intracellular pool, respectively. However, with insulin treatment of the diabetic rat, the ~3-fold overshoot in insulin-stimulated glucose transport activity observed in the intact cell is accompanied by little more than a restoration to the non-diabetic control level of the number of glucose transporters translocated in response to insulin. Thus, insulin therapy of diabetes in the rat appears also to be accompanied by a marked increase in the intrinsic activity of those glucose transporters present in the plasma membrane in the insulin-stimulated state. A recent preliminary report by Karnieli et al. (38) shows similar findings.

In the streptozotocin diabetic rat compared to the non-

| Marker enzyme activity | Experimental group | Plasma membranes | High-density microsomes | Low-density microsomes | % of homogeneity activity |
|------------------------|--------------------|------------------|-------------------------|------------------------|--------------------------|
| 5'-Nucleotidase        | Non-diabetic control | 67.8 ± 6.9       | 5.5 ± 0.5               | 3.0 ± 0.4              |
|                        | Insulin-treated diabetic | 60.1 ± 6.9       | 6.4 ± 0.8               | 3.4 ± 0.2              |
| Cytochrome c reductase | Non-diabetic control | 14.6 ± 0.8       | 17.0 ± 1.0              | 13.3 ± 0.3             |
|                        | Insulin-treated diabetic | 13.8 ± 0.2       | 14.6 ± 2.2              | 11.6 ± 1.0             |
| Galactosyltransferase  | Non-diabetic control | 26.5 ± 2.6       | 13.7 ± 3.2              | 43.7 ± 6.9             |
|                        | Insulin-treated diabetic | 19.8 ± 1.9       | 14.0 ± 0.6              | 26.2 ± 1.9*            |

* Significant difference from corresponding control value.
Table IV

| Marker enzyme activity and experimental group | Plasma membranes | High-density microsomes | Low-density microsomes | % of highest specific activity |
|---------------------------------------------|------------------|------------------------|------------------------|-----------------------------|
| 5'-Nucleotidase (μmol/mg/h)                  |                  |                        |                        |                             |
| Non-diabetic control                         | 100 (0.58 ± 0.02) | 24.7 ± 2.7             | 8.7 ± 2.0              |                             |
| Insulin-treated diabetic                     | 100 (0.68 ± 0.12) | 27.0 ± 4.2             | 6.0 ± 0.7              |                             |
| Cytochrome c reductase (μmol/min)            |                  |                        |                        |                             |
| Non-diabetic control                         | 21.9 ± 1.9       | 100 (3.11 ± 0.01)      | 44.3 ± 5.3             |                             |
| Insulin-treated diabetic                     | 36.9 ± 10.4      | 100 (1.91 ± 0.01)      | 30.4 ± 1.6             |                             |
| Galactosyltransferase (μmol/mg/2 h)          |                  |                        |                        |                             |
| Non-diabetic control                         | 28.1 ± 1.8       | 45.0 ± 3.4             | 100 (97.5 ± 8.4)       |                             |
| Insulin-treated diabetic                     | 76.8 ± 8.1       | 137.3 ± 21.8           | 100 (80.3 ± 4.5)       |                             |
| *Actual specific activity value set at 100% (mean ± S.E.).
| ^Significant difference from corresponding value.

In diabetic control, the concentrations of glucose transporters in the plasma membranes from basal and maximally insulin-stimulated adipose cells directly correlate with the glucose transport activity expressed per unit cellular surface area, probably reflecting the fact that the recovery of plasma membrane protein is approximately proportional to the surface area of the original intact cells (3). Fig. 3 illustrates the reduced cellular glucose transport activity and correspondingly reduced plasma membrane concentration of glucose transporters in the insulin-stimulated state. Conversely, 2 weeks of hyperinsulinemia experimentally induced in non-diabetic rats has recently been reported to result in a 55% increase in maximally insulin-stimulated glucose transport activity per unit cellular surface area, with a proportional increase in the concentration of glucose transporters in the plasma membranes from the insulin-stimulated cells (Fig. 3). However, this increase in insulin-stimulated cellular glucose transport activity is not nearly as great as the overshoot reported here with insulin treatment of diabetic animals even though the latter has slightly decreased by 14 days of treatment compared to the peak at 7-8 days. Similar to chronic experimental hyperinsulinemia in normal rats, proportional increases in insulin-stimulated cellular glucose transport activity and the concentration of glucose transporters in the corresponding plasma membranes have been observed with naturally occurring hyperinsulinemia in the young, genetically obese Zucker fatty rat (19) and with physical training in normal rats (20).

Insulin treatment of the diabetic rat studied here restores the concentration of glucose transporters in the plasma membranes from maximally insulin-stimulated adipose cells to ~35% above that observed in the non-diabetic control which approximates the level observed with 2 weeks of experimental hyperinsulinemia in the normal rat (Fig. 3). However, maximally insulin-stimulated glucose transport activity per unit cellular surface area in the intact cells is increased ~3-fold. Because an effect of insulin therapy on the cell fractionation procedure could explain such a discrepancy, the recoveries of membrane protein and the recoveries and relative distributions of several specific marker enzyme activities were carefully examined (31). In fact, the plasma membranes from the cells of the insulin-treated diabetic animals do appear to be more contaminated with low-density microsomal membrane protein than those from the cells of the control rats as reflected in a similar recovery of plasma membrane protein per cell in spite of a smaller cell size (Table I) and a corresponding shift in the relative galactosyltransferase-specific activity from the low-density microsomes to the plasma membranes (Table IV), closely correlating with an ~2-fold increase in the recovery of low-density microsomal protein per cell (Table I). However, taking this apparent increase in cross-contamination of the plasma membranes with low-density microsomes into account would actually reduce the concentration of glucose transporters in the plasma membranes in the insulin-stimulated state and increase the disproportionality between glucose transport activity in the intact cell and the concentration of plasma membrane glucose transporters even further.

A similar increase in plasma membrane cross-contamination with low-density microsomal membrane protein has been
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reported and discussed in detail with adipose cells from the young Zucker fatty rat (19) and appears also to occur with experimental hyperinsulinemia in normal rats (18). In the former, this shift in membrane distribution was invoked as a likely explanation for the disproportionately elevated basal concentration of glucose transporters in the plasma membranes and probably explains the smaller but similarly disproportionate elevation of plasma membrane glucose transporters observed here in the basal state (Fig. 2).

Kinetic studies of 3-O-methylglucose transport in intact adipose cells in the maximally insulin-stimulated state demonstrate that the marked increase in cellular glucose transport activity with insulin therapy of diabetic rats occurs primarily through an increase in the transport $V_{max}$ with little change in $K_m$ (Table II). In fact, the tendency for a slight increase in the $K_m$ would result in decreased insulin-stimulated transport activity and could not contribute to the hyperresponsive glucose transport activity observed. Thus, insulin treatment of the streptozotocin diabetic rat appears not only to restore, or slightly enhance, the appearance of glucose transporters in the plasma membranes in response to insulin, but also to markedly increase their intrinsic activity. Whereas other studies from this laboratory (21-23), have recently demonstrated that adenylate cyclase stimulators and inhibitors can modulate insulin-stimulated glucose transport activity in the rat adipose cell through an apparent regulation of plasma membrane glucose transporter intrinsic activity, the present report is the first to suggest that such a mechanism occurs in vivo.

A preliminary attempt has been undertaken to examine the possible mechanism through which such a change in glucose transporter intrinsic activity might come about. Kuroda et al. (22) and Joost et al. (23) have reported that plasma membranes prepared from insulin-stimulated adipose cells treated with adenylate cyclase stimulators and inhibitors do not exhibit altered glucose transporter intrinsic activity when assayed directly for glucose transport unless they have been prepared from cells homogenized in the presence of KCN. A preliminary examination of plasma membrane glucose transport in insulin-stimulated cells from diabetic rats treated with insulin suggests a direct correlation with the concentration of glucose transporters present in the plasma membranes regardless of the homogenization conditions. Thus, the alteration in glucose transporter intrinsic activity associated with insulin therapy is lost with cell disruption. In addition, Western blots of the plasma membranes from insulin-stimulated cells using an antiserum prepared against the purified human erythrocyte glucose transporter (39, 40) fail to detect changes in either the intensity of the cross-reaction, which correlates with the concentration of glucose transporters assayed by cytochalasin B binding, or in the $M_r$ of the band between insulin-treated diabetic rats and non-diabetic controls. Furthermore, insulin therapy is not accompanied by alterations in the $K_m$ of the plasma membrane glucose transporter for cytochalasin B binding. Finally, in a parallel series of experiments demonstrating a similar overshoot in insulin-stimulated glucose transport activity in the adipose cell with fasting and refeeding in the rat, the concentration of cytochalasin B required to produce half-maximal inhibition of glucose transport activity in the intact cell is unchanged (14).

The translocation hypothesis of insulin's stimulatory action on glucose transport in the rat adipose cell is based on evidence that the appearance of glucose transporters in the plasma membranes in response to insulin correlates with their loss from a large low-density microsomal pool (8-11). Metabolic states associated with resistance to insulin's stimulatory effect on glucose transport uniformly show a reduced concentration of glucose transporters in the low-density microsomes in the basal state (3, 12-17). In fact, in all of these states except aging/obesity, the total number of glucose transporters in the intracellular pool in the basal state may be even further reduced due to a decreased quantity of low-density microsomal protein per cell. Conversely, three metabolic states reported to date resulting in insulin hyperresponsiveness are associated with normal basal concentrations of glucose transporters in the low-density microsomes but increased quantities of low-density microsomal protein per cell, resulting in an increased total number of intracellular glucose transporters (18-20).

As reported here, insulin treatment of the diabetic rat is accompanied by a restoration to normal of the concentration of glucose transporters in the basal low-density microsomes (Fig. 2) from the 45% decreased level seen with untreated diabetes (3). An ~2-fold increase in low-density microsomal protein (Table I) results in an increased total number of intracellular glucose transporters which can account for the 36% increase in glucose transporters in the plasma membranes after insulin stimulation (Fig. 2). These findings are remarkably similar both qualitatively and quantitatively to those observed with chronic insulin treatment of normal rats (18). However, insulin treatment of diabetic rats is accompanied by a striking increase in glucose transport activity, at least 2-fold that seen in insulin-treated normal rats, which is unexplainable by an increase in the translocation of glucose transporters (Fig. 3). We have recently observed a similar insulin hyperresponsiveness in the adipose cells of rats refed after fasting (14). Although the magnitude of the effect is not as great, the mechanism also appears to involve a small increase in the number and a marked increase in the intrinsic activity of the plasma membrane glucose transporters. Therefore, we postulate that the increase in glucose transporter intrinsic activity may result from rapid reversal of a catabolic state.

Finally, the persistent enhanced insulin responsiveness observed here with insulin treatment of diabetic rats may bear some relationship to the "honeymoon" phenomenon of prolonged increased insulin effectiveness seen in some newly diagnosed human diabetics shortly after starting insulin therapy. Yki-Järvinen and Koivistö (41) recently showed that normalization of insulin action, in association with some residual insulin secretion, is important for the development of the honeymoon phenomenon in Type I diabetics. Other investigators have demonstrated that both in vivo glucose disposal (42, 43) and in vitro maximally insulin-stimulated glucose transport activity in the adipose cell (6, 7) can be normalized with a brief period of insulin therapy in Type II diabetics, and this improvement persists even after stopping insulin treatment (43). The present report suggests that alterations in both glucose transporter concentration and intrinsic activity may contribute to the early therapeutic effects of insulin in human diabetes.

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