A Possible Mechanism of Insulin Resistance in the Rat Adipose Cell with High-Fat/Low-Carbohydrate Feeding

Depletion of Intracellular Glucose Transport Systems

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SUMMARY

The effects of high-fat/low-carbohydrate feeding on glucose transport activity and on the concentrations of glucose transport systems in the plasma and low-density microsomal membranes in isolated rat adipose cells have been examined. Glucose transport activity was assessed by measuring 3-O-methylglucose transport and the concentration of glucose transport systems estimated by measuring specific D-glucose-inhibitable cytochalasin B-binding. Basal glucose transport activity is not significantly influenced by high-fat/low-carbohydrate relative to low-fat/high-carbohydrate feeding and is accompanied by a constant 10 pmol of glucose transport systems/mg of membrane protein in the plasma membrane fraction. In contrast, maximally insulin-stimulated glucose transport activity decreases from 4.72 to 2.29 fmol/cell/min and is accompanied by a decrease from 44 to 26 pmol of glucose transport systems/mg of plasma membrane protein. These diminished effects of insulin on glucose transport activity and the concentration of glucose transport systems in the plasma membrane fraction are paralleled by a 48% decrease in the basal number of glucose transport systems/mg of membrane protein in the low-density microsomal membrane fraction, the source of those glucose transport systems appearing in the plasma membrane in response to insulin. Thus, the "insulin-resistant" glucose transport of the adipose cell with high-fat/low-carbohydrate feeding may be the consequence of a depletion of glucose transport systems in the intracellular pool. DIABETES *31*:589–592, July 1982.

In several previous reports, Ip et al.,[1] Olefsky,[2] Lavau et al.,[3] and Salans et al.[4] have demonstrated that high-fat feeding, either in the absence or presence of low carbohydrate, is accompanied by a marked reduction in insulin's stimulatory action on glucose transport activity in the isolated rat adipose cell. The latter investigators have further shown that a decrease in the number of functional glucose transport systems appearing in the cell's plasma membrane in response to insulin fully accounts for this phenomenon.

Recent studies in this laboratory,[5,6] and independently by Suzuki and Kono[7] and Kono et al.,[8] have established that insulin stimulates glucose transport in the isolated rat adipose cell primarily through a rapid, reversible, and hormone concentration- and energy-dependent translocation of glucose transport systems from a membrane-associated intracellular pool to the plasma membrane. Salans et al.[4] have proposed that a dietary-induced decrease in this intracellular pool of glucose transport systems might explain the observed reduction in insulin's stimulatory action on glucose transport with high-fat feeding. The present investigations were undertaken to examine this possibility.

METHODS

Experimental design. In each of three experiments, approximately 100 weanling male Sprague-Dawley rats (CD strain, Charles River Breeding Laboratories, Wilmington, Massachusetts) were randomly divided into two groups and housed three per cage. Each group was then fed ad libitum either a low-fat/high-carbohydrate (9%/71% by calories) or high-fat/low-carbohydrate (50%/30% by calories) diet for 21 days.[4] The two diets were of equal caloric density with protein maintained at a constant 20% by calories. As previously reported using these same two diets, the two groups of animals grew at identical rates (final body weights of 157 ± 3 g and 148 ± 5 g, mean ± SEM of 155 and 153 animals, respectively) and were characterized by identical epididymal adipose cell sizes (0.097 ± 0.004 μg and 0.103 ± 0.022 μg lipid/cell, mean ± SD of three isolated cell preparations each, respectively.[4]

Isolated adipose cells, subcellular membrane fractions, and analytical procedures. On day 21, all animals in each

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group were killed between 9 and 11 a.m., the epididymal fat pads were removed, isolated adipose cells were prepared,[9] and adipose cell size was determined.[10] All incubations were carried out in Krebs-Ringer-bicarbonate-Hepes buffer, pH 7.4, 37°C, containing 10 mg bovine serum albumin/ml.[5] The cells were then equally distributed in 15-ml volumes to 950-ml polypropylene jars containing 21 ml of incubation medium, and preincubated for 15 min. Insulin (crystalline zinc insulin, courtesy of Dr. Ronald E. Chance, Eli Lilly and Co., Indianapolis, Indiana) was added at final concentrations of 0 or 7.0 nM (1000 μU/ml), incubation was continued for 15 min, and replicate 0.25-ml samples of cells were removed for determination of the rate of 3-O-methylglucose transport and the intracellular water space (calculated from steady-state uptake levels).[6] Plasma, high-density microsomal, and low-density microsomal membrane fractions were prepared by differential ultracentrifugation, equilibrium D-glucose-inhibitable cytochalasin B-binding was measured, and the concentrations of binding sites were calculated.[5,6] The specific 5′-nucleotidase, rotenone-insensitive NADH-cytochrome c reductase, and UDP-galactose/N-acetylglucosamine galactosyltransferase activities of each homogenate and membrane fraction were assayed.[5-8] Protein was determined by the Coomassie brilliant blue method described by Bradford[11] (Bio-Rad Protein Assay, BioRad Laboratories, Richmond, California) using crystalline bovine serum albumin (Sigma Chemical Co., St. Louis, Missouri) as the standard. Comparisons were made using a paired *t* test of statistical significance and differences accepted as significant at the $P \leq 0.05$ level.

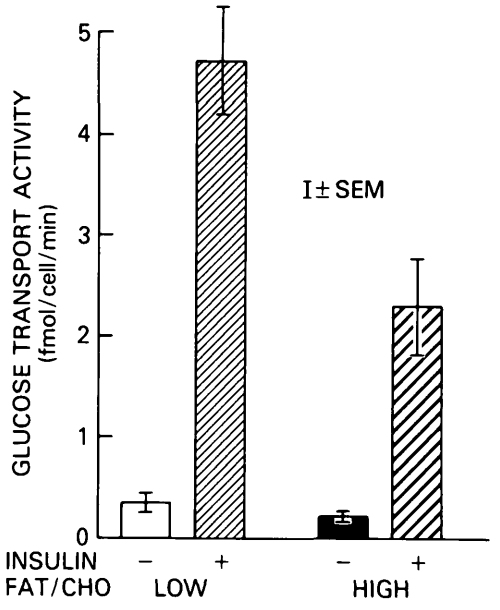

FIGURE 1. Glucose transport activity in the intact adipose cell. Isolated cells were prepared from low-fat/high-carbohydrate-fed and high-fat/low-carbohydrate-fed rats (mean cell sizes of 0.097 and 0.103 μg lipid/cell, respectively), incubated in the presence of 0 or 7.0 nM (1000 μU/ml) insulin, and sampled for measurement of 3-O-methylglucose transport as described in METHODS. Results are the means ± SEM of the individual mean values obtained from triplicate samples in three experiments. (FAT/CHO: ratio of dietary fat to carbohydrate.)

RESULTS

Glucose transport activity. Glucose transport activity in the intact adipose cell in the absence or presence of 7.0 nM (1000 μU/ml) insulin is illustrated in Figure 1. Basal 3-O-methylglucose transport is somewhat, but not statistically significantly, decreased in cells from the high-fat-fed compared with the low-fat-fed rats. Maximally insulin-stimulated 3-O-methylglucose transport, on the other hand, is decreased in cells from the high-fat-fed animals by 51%.

Subcellular distribution of glucose transport systems. The distribution of glucose transport systems among the plasma, high-density microsomal, and low-density microsomal membrane fractions prepared from basal or maximally insulin-stimulated adipose cells is illustrated in Figure 2. While the numbers of glucose transport systems/mg of membrane protein in the plasma membrane fraction are similar in basal cells from the high- and low-fat-fed rats, the increase in their number in response to insulin is decreased by 53% in cells from the high-fat-fed animals (Figure 2A). A similar relationship is observed in the high-density microsomal membrane fraction (Figure 2B) where the observed glucose transport systems are thought to be associated with contaminating plasma membranes (unpublished observations). In contrast, the number of glucose transport systems/mg of membrane protein in the low-density microsomal membrane fraction prepared from basal cells of the high-fat-fed animals is decreased by 48%. Furthermore, the absolute number of glucose transport systems/mg of membrane protein in the low-density microsomal membrane fraction of maximally insulin-stimulated cells from the high-fat-fed rats is decreased by 37% compared with that from the low-fat-fed animals, and the net disappearance of glucose transport systems in response to insulin is decreased by 59% (Figure 2C).

These dietary-induced alterations in the distribution of glucose transport systems are not accompanied by significant alterations in the relative distributions of membrane

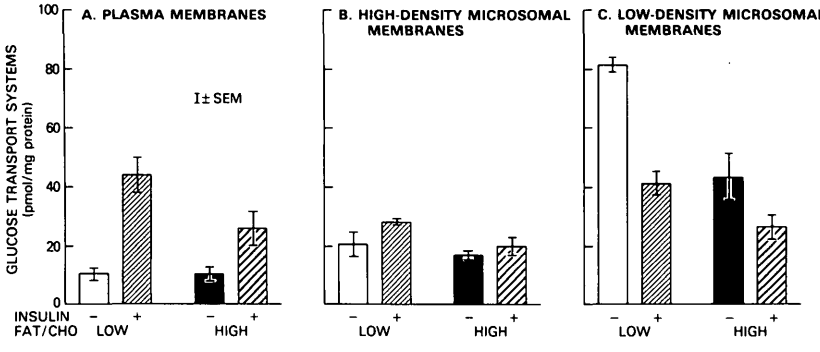

FIGURE 2. Concentration of glucose transport systems in the plasma membrane (A), high-density microsomal membrane (B), and low-density microsomal membrane (C) fractions of the adipose cell. Membrane fractions were prepared from the isolated cells described in Figure 1 and the concentrations of glucose transport systems were determined using a D-glucose-inhibitable cytochalasin B-binding assay as described in METHODS. Results are the means ± SEM of the individual values obtained in three experiments. (FAT/CHO: ratio of dietary fat to carbohydrate.)

marker enzyme activities (not illustrated). Although the yield of protein in the plasma membrane fraction of cells from the high- compared with the low-fat-fed rats is also unchanged, that in the summed high- and low-density microsomal membrane fractions of cells from the high-fat-fed animals is significantly decreased by 21% (from 33.2 ± 7.2 to 26.0 ± 8.2 pg/cell, mean ± SEM, respectively). The intracellular water space of cells from the high-fat-fed rats is decreased by 28% (from 2.93 ± 0.28 to 2.12 ± 0.15 pl/cell, mean ± SEM, respectively).

DISCUSSION

The results of the present investigations demonstrate that the isolated adipose cell's resistance to insulin at the glucose transport level in the high-fat/low-carbohydrate-fed, relative to the low-fat/high-carbohydrate-fed, rat is accompanied by (1) a markedly diminished stimulatory effect of insulin on the concentration of glucose transport systems in the plasma membrane fraction (Figure 2A), closely paralleling the markedly decreased stimulatory effect of insulin on 3-O-methylglucose transport in the intact cell (Figure 1), and (2) a marked reduction in the concentration of glucose transport systems in the low-density microsomal membrane fraction in the basal state, and in a corresponding decrease in their disappearance from this fraction in response to insulin (Figure 2C). The latter represents the cell's intracellular pool from which glucose transport systems are translocated to the plasma membrane. These alterations occur without significant changes in the membrane species comprising those fractions examined here, as reflected in the distributions of marker enzyme activities (not illustrated). Thus, the "insulin-resistant" glucose transport of the adipose cell with high-fat/low-carbohydrate feeding can be explained by a decrease in the number of glucose transport systems appearing in the plasma membrane in response to insulin, perhaps as the consequence of a depletion of these glucose transport systems in the cell's intracellular pool. A similar explanation has recently been proposed for the "insulin-resistant" glucose transport of the adipose cell from the streptozotocin-induced diabetic rat[12] and of the enlarged adipose cell from the aged, obese rat.[13]

The total number of glucose transport systems in the intact adipose cell cannot presently be determined since methods are not available for measuring their number in the original homogenate and, therefore, the extent of their recovery in each fraction. Furthermore, while the recovery of marker enzyme activities could, in principle, be used for estimating this number, neither the specific membrane species comprising the intracellular pool nor a marker enzyme activity specific for the intracellular pool has been identified (unpublished observations).[8] However, the observed recoveries of protein in the plasma membrane fraction of cells from both the high- and low-fat-fed animals are similar, paralleling the lack of an effect of dietary composition on cell size and cellular surface area. Since the concentrations of glucose transport systems in the plasma membrane fraction of basal cells from the high- and low-fat-fed rats are also similar (Figure 2A), then their total number per cell in the plasma membrane of basal cells from the high-fat-fed rats would appear to be unchanged. The concentration of glucose transport systems in the plasma membrane fraction of insulin-stimulated cells from the high-fat-fed animals, on the other hand, is decreased by 41% (Figure 2A), and thus their total number per cell in the plasma membrane of insulin-stimulated cells would also appear to be decreased by 41%. These estimates closely correlate with the unchanged basal and decreased insulin-stimulated glucose transport activities per cell actually observed (Figure 1).

In contrast, the observed recovery of protein in the summed high- and low-density microsomal membrane fractions of cells from the high- compared with the low-fat-fed animals is decreased by 21%. While this decrease roughly parallels the 28% decrease in the intracellular water space of cells from the high-fat-fed rats, the nature of the relationship between a cell's intracellular membrane content and its cytoplasmic mass is presently unknown. Nevertheless, a 20% correction has been assumed in estimating the effects of dietary composition on the total number of glucose transport systems in the intracellular pool of the intact cell. Since the concentration of glucose transport systems in the low-density microsomal membrane fraction of basal cells from the high-fat-fed rats is decreased by 48% (Figure 2C), then their total number per cell in the intracellular pool of basal cells would appear to be decreased by roughly 58%. Their total number per cell in the intracellular pool of insulin-stimulated cells and their net total number per cell lost from the intracellular pool in response to insulin would appear to be similarly decreased (Figure 2C). These decreases closely correlate with the 55% decrease in the estimated total number of glucose transport systems per cell appearing in the plasma membrane in response to insulin. While the mechanism of the translocation process and the factors that determine the residual number of glucose transport systems in the intracellular pool following attainment of the steady-state maximal response to insulin remain to be determined, the lack of an effect of dietary composition on the mechanism of insulin action itself is suggested by the failure of dietary composition to significantly influence insulin's effect on the intracellular pool when the loss of glucose transport systems is expressed as a percent of the basal level.

The specific process through which the intracellular pool of glucose transport systems in the adipose cell from high-fat/low-carbohydrate-fed rats is reduced remains to be explored. Nevertheless, a selective reduction in the net synthesis of glucose transport systems is suggested by their markedly decreased number in the intracellular pool under conditions where the quantity of membranes in the subcellular fraction with which this pool is associated is decreased only moderately. This selective reduction in net intracellular glucose transport system synthesis may parallel the well-established decrease in the cell's maximal capacity for glucose metabolism[1–4] and, especially, the marked decrease in two of the enzyme activities specifically involved in de novo fatty acid synthesis.[3] Retention of a relatively intact mechanism of insulin action, on the other hand, is supported by an unchanged antilipolytic response to insulin.[3]

The adipose tissue mass per se probably plays a minor role in total systemic glucose homeostasis. Nevertheless, the alteration in the adipose cell's intracellular pool of glucose transport systems reported here may reflect a more general systemic impairment in insulin action, and thus a potential explanation for the apparent in vivo insulin resistance observed with high-fat/low-carbohydrate feeding in both rat and man.

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