Decreased Activation Rate of Insulin-Stimulated Glucose Transport in Adipocytes From Obese Subjects

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Recent studies from our laboratory have shown that the rate at which insulin activates glucose disposal in vivo is much slower in obese subjects compared with lean controls. To determine if this was caused by an abnormality in activation of insulin-stimulated glucose transport at the cellular level, we measured the rate at which insulin stimulated glucose transport in human adipocytes from obese volunteers. Basal rates of 3-O-methylglucose transport in the absence of insulin were lower (0.20 ± 0.04 vs. 0.40 ± 0.11 pmol · 10^{-5} cells · 10 s^{-1}, $P < .25$) in adipocytes from obese subjects (n = 10) than in lean control subjects (n = 5), but this did not achieve statistical significance. Maximal insulin-stimulated (4300 pM insulin) glucose transport rates were significantly decreased in obesity (2.81 ± 0.81 vs. 1.15 ± 0.20 pmol · 10^{-5} cells · 10 s^{-1}, $P < .005$). It took longer for adipocytes from obese subjects to achieve half-maximal activation of insulin-stimulated glucose transport than those from lean subjects (15 ± 2 vs. 9.4 ± 1.2 min, $P < .05$). The slower overall rates of activation of maximal insulin-stimulated glucose transport observed in adipocytes from obese subjects mirror the slower rates of stimulation of glucose disposal in vivo, which suggests that the in vivo findings are caused by a cellular abnormality in insulin action at a step beyond the binding of insulin to its receptor. The decreased maximal rates of glucose uptake plus the slower rates of activation demonstrated in these studies combine to form an even greater degree of insulin resistance than might be expected from static measurements alone. *Diabetes* 38:991–95, 1989

Glucose 1 mM = 18 mg/dl	Insulin 1 pM = 0.139 μU/ml

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Insulin resistance is a characteristic feature of obesity (1,2). Insulin resistance is caused by a spectrum of defects in insulin action that range from decreased insulin receptors to a defect in insulin action at one or more postbinding steps (3).

Insulin resistance in vivo is often assessed by measuring the glucose disposal rate during a continuous intravenous insulin infusion under near steady-state conditions (4). However, insulin is normally secreted in a phasic manner after meals, and high, sustained serum levels of insulin, such as those achieved during hyperinsulinemic glucose-clamp studies, are never attained in normal day-to-day life, which makes measurements of insulin resistance with this type of technique relatively unphysiological. Along these lines, recent studies from our laboratory have shown that the rate at which insulin stimulates glucose disposal in vivo is much slower in obese subjects than in lean control subjects (5). Therefore, under physiological conditions of phasic insulin secretion where the hormone level is increased for a limited time, the rate at which insulin stimulates glucose disposal may be an important physiological manifestation of insulin resistance. The in vivo studies mentioned above do not elucidate the mechanisms for the kinetic defect in insulin action in obese subjects. Possible explanations include a delay in delivery of insulin to tissue sites of action or a defect in stimulation of insulin-stimulated glucose uptake at the cellular level. To assess the latter possibility, we obtained human adipocytes from obese volunteers and directly measured the rate at which insulin stimulates glucose transport in vitro.

RESEARCH DESIGN AND METHODS

Materials. Porcine monocomponent insulin was supplied by Lilly (Indianapolis, IN). 3-*O*-methyl-D-[1-^{14}C]glucose (30MG) was purchased from New England Nuclear (Boston, MA), collagenase was purchased from Worthington (Freehold, NJ), bovine serum albumin (BSA, fraction V) was purchased

from Armour (Phoenix, AZ), phloretin was purchased from Biochemical Laboratories (Redondo Beach, CA), and silicone oil was purchased from Union Carbide (New York).

Preparation of human adipocytes. Adipose tissue was obtained by open biopsy of the lower abdominal wall as previously described (6). To avoid potential effects of the local anesthetic, 1% lidocaine was infiltrated along the perimeter of a square field, and the biopsy was taken from the center of the field. All patients were hospitalized in the Special Diagnostic and Treatment Unit of the San Diego Veterans Administration Hospital and maintained on a weight-maintenance diet that consisted of 50% carbohydrate, 20% protein, and 30% fat for 48 h before any study was performed. Tissue was obtained from 10 obese and 5 lean subjects after a 12-h overnight fast. All procedures were approved by the human subjects research review committee, and all subjects gave written informed consent.

Isolated adipocytes were prepared according to the method of Rodbell (7). Tissue was minced into plastic flasks that contained collagenase (3 mg/ml) and a buffer that consisted of 150 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM $CaCl_2$, 2.5 mM NaH_2PO_4, 10 mM HEPES, 2 mM pyruvate, and 5% BSA (pH 7.4). All subsequent steps were performed with this buffer. Minced tissue was incubated 70 min at 37°C in a shaking water bath. The cell mixture was filtered through 500-μm nylon mesh, washed twice, then filtered again through fresh 500-μm mesh. After two additional washes, cells were resuspended (~10^6 cells/ml) in buffer. Cell counts were performed by a modification of method III of Hirsch and Gallian (8) in which cells were fixed in 2% osmium tetroxide and counted with a model ZB Coulter counter with a 400-μm–aperture tube. Cell size was estimated by measuring the diameter of osmium-fixed cells and assuming that they are spherical; therefore, cell volume is

$$\frac{4}{3} \times \pi r^3$$

30MG transport. Adipocyte glucose transport was assessed by measuring initial rates of uptake of tracer amounts of 30MG, which is a nonmetabolizable analogue of D-glucose.

The washed cells were incubated for 30 min at 37°C in an air atmosphere, and an aliquot was removed for determination of basal transport in the absence of insulin. Insulin was added to a final concentration of 4300 pM, and the cells were incubated for up to 60 min at 37°C. Aliquots of cells were removed at various times after the addition of insulin for determination of 30MG transport by a modification of the method of Whitesell and Gliemann (9). The reaction was started by addition of 50 μl of the cell incubation mixture to plastic test tubes that contained [^{14}C]30MG (0.4 μCi, final conc 15 μM). Transport was terminated by addition of 400 μl of BSA-free buffer that contained 0.1 mM phloretin, which also blocks release of 30MG from cells. A 400-μl aliquot was placed in a 550-μl microcentrifuge tube containing 100 μl silicone oil. Tubes were spun in a microcentrifuge for 30 s and cut through the oil layer, and cell plugs were added to a liquid-scintillation cocktail for determination of cell-associated radioactivity. The intracellular water space at equilibrium was measured in each subject by incubating a separate aliquot of cells with 30MG and insulin for 30 min, then cell-associated radioactivity was determined. To correct for trapping of water, an aliquot of cells was added to a tube that already contained 30MG and phloretin. In this case, because phloretin was present at the outset, cells did not take up labeled glucose, so any cell-associated radioactivity was caused by trapping of 30MG in the extracellular water space. This correction was made at each time point for each subject. Preliminary studies showed that 30MG transport into adipocytes from obese subjects was less than that of normal subjects at each time point. To maximize the radioactivity present in adipocytes the reaction time was extended in the assays performed with adipocytes from obese subjects. Basal transport into adipocytes was measured for 30 s for normal and 40 s for obese subjects. Aliquots of cells were assayed for glucose transport by exposure to 30MG after exposure to insulin for up to 1 h. Reaction times in normal subjects after exposure to insulin for 5, 10, 20, 30, 45, and 60 min were 20, 15, 10, 10, 10, and 10 s, respectively. Because transport rates into adipocytes from obese subjects were slower, the reaction times were extended slightly to to 25, 20, 20, 18, 15, and 15 s, respectively, for the obese subjects. Earlier studies in cells from normal subjects (10) showed that uptake represented initial rates of glucose transport when uptake was <25% of the equilibrium space. All transport rates reported represent initial rates and are normalized as 30MG transport · 10^{-5} cells · 10 s^{-1}.

Data are expressed as means ± SE and were compared by Student's one-tailed *t* test for unpaired data. Significance was accepted at $P < .05$.

RESULTS

Patient characteristics. The characteristics of the subjects studied are given in Table 1. Age and fasting plasma glucose levels were similar in lean and obese subjects. All of the lean subjects and 7 of the 10 obese subjects had normal oral glucose tolerance tests (OGTTs). Two of the obese subjects had nondiagnostic OGTTs because 2-h plasma glucose level was 7.8 mM, and 1 obese subject had impaired glucose tolerance as defined by the National Diabetes Data Group (11). The obese subjects had significantly higher fasting insulin levels (115 ± 22 vs. 37 ± 5 pM, $P < .015$). The average volume of adipocytes from obese subjects was 2.5 times greater than that of adipocytes from lean subjects, but the volume of intracellular water per cell was the same (8.3 ± 1.3 vs. 12.1 ± 1.7 pl/cell, *P* NS).

Basal and maximal glucose transport. Initial rates of 30MG transport (Fig. 1) in the absence of insulin were higher in adipocytes of lean ($n = 5$) than obese ($n = 10$) subjects (0.40 ± 0.11 vs. 0.20 ± 0.04 pmol · 10^{-5} cells · 10 s^{-1}, $P < .25$), although this did not achieve statistical significance. Maximal insulin-stimulated (4300 pM insulin) glucose transport rates were significantly decreased with obesity (2.81 ± 0.81 vs. 1.15 ± 0.20 pmol · 10^{-5} cells · 10 s^{-1}, $P < .005$). These results are consistent with previous reports (6,12). Earlier studies showed that the half-maximally effective insulin concentrations for insulin-stimulated glucose transport were 79 ± 17 and 147 ± 24 pM, and maximally effective levels were 0.87 and 1.74 nM in normal and obese subjects, respectively (6). Obesity is often associated with a decrease in insulin receptors. To overcome differences in insulin binding in these experiments, we used an insulin concentration of 4300 pM to far exceed the maximally stimulating concen-

TABLE 1
Patient characteristics

	n	Age (yr)	Body mass index (kg/m²)	Fasting glucose (mM)	Fasting insulin (pM)	Cell size (pl/cell)	Cellular water space (pl/cell)
Normal	5	36 ± 2	22.9 ± 0.7	5.5 ± 0.2	37 ± 5	254 ± 45	12.1 ± 1.7
Obese	10	42 ± 4	31.5 ± 2.0	5.2 ± 0.2	115 ± 22	788 ± 193	8.3 ± 1.3

tration, so that any decrease in glucose transport reflects a decrease in responsiveness as a result of a postbinding defect in insulin action.

Activation of glucose transport. To study the rate of stimulation of glucose transport, we measured glucose transport at various times after the addition of insulin (4300 pM). At this concentration, a maximally stimulating amount of bound insulin is achieved in <1 min (13); therefore, the rate of stimulation of glucose transport represents the rate at which insulin activates postbinding steps in insulin action. Figure 2 depicts the time course of insulin-stimulated activation of glucose transport measured in adipocytes from 5 lean and 10 obese subjects. Because the maximal rates of glucose transport differed among individuals and between the two groups, the form of the resultant activation curves can be better appreciated by plotting the data as a percentage of the maximal insulin effect for each subject (Fig. 1). As can be seen, the curve for the obese subjects is shifted to the right, which indicates a slower rate of activation of insulin-stimulated glucose transport. From the mean data, we calculated the average time necessary to achieve half-maximal activation (A_{50}) of glucose transport (Fig. 2). It took longer for the adipocytes from obese subjects to reach half-maximal activation than those from lean subjects (with an apparent $t_{1/2}$ value of 18 vs. 10 min). To further quantitate this difference, we used the A_{50} for each subject and calculated the mean A_{50} for both groups. This was accomplished by plotting the activation curve for each subject, with transport expressed as a percentage of the maximal insulin effect. A_{50} was read from the graph. Activation was significantly slower in obese than in lean subjects (9.4 ± 1.2 vs. 15 ± 2 min, $P < .05$).

DISCUSSION

After ingestion of glucose, ~33% of the glucose is removed by the liver, and the rest enters the peripheral circulation, where it is predominantly removed by skeletal muscle through a marked rise in glucose uptake that is mediated by a rise in plasma glucose and insulin levels (14). Obesity is characterized by peripheral insulin resistance, and most quantitative in vivo estimates of insulin resistance rely on steady-state measurements of insulin's ability to stimulate glucose disposal (4). However, during glucose-clamp studies, when insulin is given as a continuous intravenous infusion, it takes several hours before steady-state whole-body insulin effects are achieved, and this is not representative of the rapid rise and fall in insulin levels that occur after ingestion of food. Indeed, given the phasic way in which insulin is normally secreted, it is unlikely that steady-state insulin effects are ever reached in vivo. Furthermore, we recently demonstrated marked kinetic defects in insulin action in obesity, with a slower rate of activation and faster rate of deactivation of insulin's in vivo effects (5). Because of the dynamic way insulin is secreted after meals, we postulated that the kinetic defects in insulin action are a major functional

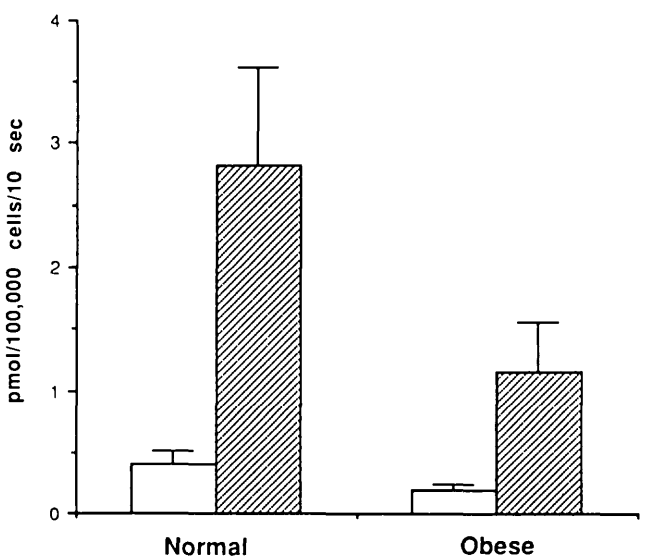

FIG. 1. Basal (*open bars*) and maximal (*hatched bars*) insulin-stimulated glucose transport. 3-O-methylglucose transport in absence or presence of 4.3 nM insulin in adipocytes from lean (n = 5) and obese (n = 10) subjects. Values are means ± SE.

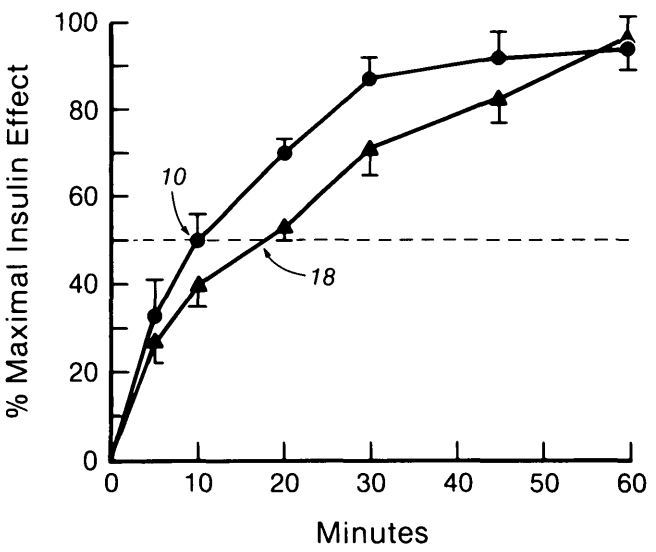

FIG. 2. Time course of insulin-stimulated glucose transport in adipocytes from lean (●, n = 5) or obese (▲, n = 10) subjects. Subjects were exposed to maximally effective concentration of insulin (4.3 nM) at time 0, and 3-O-methylglucose uptake was measured at indicated time points. Data expressed as percentage of maximal insulin effect for each subject and are means ± SE.

component of in vivo insulin resistance. We recently tested this idea by measuring insulin-stimulated glucose disposal during euglycemic clamp studies, in which insulin was infused in a phasic manner to mimic the time course of mean insulin levels during OGTTs (15). These results demonstrated that in obesity the deficit in insulin-stimulated glucose disposal observed when insulin is infused in a phasic manner is far greater than the degree of insulin resistance measured during steady-state insulin infusions. Thus, previous steady-state measures of insulin action underestimate the functional significance of insulin resistance in obesity. This is because in the physiological setting of the postprandial rise and fall in serum insulin levels defects in the rate of insulin action will contribute importantly to the functional abnormalities, whereas rate defects will be of little significance under steady-state conditions when insulin is continuously infused for several hours.

The in vivo studies do not elucidate the basic mechanisms underlying the kinetic defect in insulin action. For example, it is possible that this defect is because of an impairment in the access of plasma insulin to tissue sites of action; however, the rates of increase in serum levels were the same in hyperinsulinemic glucose-clamp studies performed to measure activation rates of insulin-stimulated glucose disposal in obese and normal human subjects (5). On the other hand, the kinetic defects in insulin action may have a cellular origin, which represents an abnormality in the rate at which insulin bound to cells activates the processes responsible for glucose uptake. In these studies, we directly tested the latter hypothesis by measuring the rate at which insulin stimulates in vitro glucose transport in adipocytes isolated from normal and insulin-resistant obese subjects. Although muscle accounts for the majority of total body glucose usage (16), this does not preclude the use of adipocytes to study glucose uptake in vitro; animal studies have shown a good agreement between transport into adipocytes and muscle cells (17–19). Other studies have shown that glucose transport into human adipocytes is well correlated to whole-body glucose disposal rates (20,21). Furthermore, Dohm et al. (22) demonstrated that maximally insulin-stimulated 30MG transport in vitro into human skeletal muscle from obese subjects is reduced by 55%, which is in good agreement with our finding of a 60% decrease in maximal insulin-stimulated 30MG transport into human adipocytes from obese subjects. Finally, the similarity between the slower rate of activation of insulin-stimulated glucose transport in adipocytes from obese subjects demonstrated here and the slower rates of activation of whole-body glucose disposal previously reported from our laboratory lends credence to our belief that isolated adipocytes are useful in studies designed to uncover the pathophysiological mechanisms of human insulin resistance.

The rate of stimulation of glucose transport is proportional to initial receptor occupancy until a maximally effective insulin concentration is reached (13). In a previous study with rat adipocytes (13), we showed that the insulin concentration employed in this study results in the attainment of a maximally stimulating amount of bound insulin in <1 min. The similarity between the time course of insulin binding to rat (13) and human adipocytes (23) suggests that the same is true in humans. In rat adipocytes, full stimulation of glucose transport is achieved in 10 min with an A_{50} of 3 min (13,24,25). This is considerably faster than the values reported here for human adipocytes, which may be because of species differences and does not preclude the comparison of activation rates between normal and obese human subjects. Thus, these experiments were performed at an insulin concentration much higher than the maximally effective concentration for stimulation of glucose transport, so that a maximally stimulating amount of bound insulin was achieved almost immediately. Therefore, the slower rate of activation of insulin-stimulated glucose transport observed in adipocytes from obese subjects demonstrates the existence of a cellular abnormality in activation of glucose transport at a step beyond the binding of insulin to its receptor.

It is thought that insulin stimulates glucose transport into adipocytes by recruiting glucose transporters from an intracellular pool to the plasma membrane (24,26). Recent evidence suggests that there is also an insulin-dependent enhancement of the intrinsic activity of these glucose transporters after insertion into the plasma membrane (27). Human obesity is associated with a decrease in the number of low-density microsomal glucose transporters that translocate to the plasma membrane with insulin stimulation. However, the decrease in basal and maximal insulin-stimulated glucose transport activity in obesity cannot entirely be explained by a decrease in the number of glucose transporters, which suggests that there is an impairment in the intrinsic activity of the transporters (28).

Expressing the data as a percentage of the maximal insulin effect for each subject controls for differences in maximal glucose transport (Fig. 2). Thus, the slower rate of activation in adipocytes from obese subjects is not caused by a decrease in maximal glucose transport rates. From a mechanistic standpoint, these findings probably represent an additional cellular defect, possibly in the insulin-signaling mechanism, that leads to the slower activation rates in obesity. The nature of this cellular defect is unknown, but several possibilities are under investigation. One area of interest is the role of adenosine. As we have shown (29), treatment of rat adipocytes with adenosine deaminase to deplete cells of adenosine results in a slowing of the activation of glucose transport similar to that seen in cells from obese subjects.

The slower overall rates of activation of insulin-stimulated glucose transport observed in isolated adipocytes from obese subjects parallel the slower rates of stimulation of glucose disposal in vivo, which suggests that the in vivo findings in obesity are caused by a cellular abnormality in insulin action. Furthermore, this study indicates that the defect lies at a step beyond the binding of insulin to its receptor. The decreased maximal rates of glucose uptake plus the slower rates of activation demonstrated in these studies combine to form an even greater degree of insulin resistance than might be expected from static measurements alone.

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