Contraction-activated glucose uptake is normal in insulin-resistant muscle of the obese Zucker rat

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BROZINICK, J. T., JR., G. J. ETGEN, JR., B. B. YASPELKIS III, AND J. L. IVY. *Contraction-activated glucose uptake is normal in insulin-resistant muscle of the obese Zucker rat.* J. Appl. Physiol. 73(1): 382–387, 1992.—The rates of muscle glucose uptake of lean and obese Zucker rats were assessed via hindlimb perfusion under basal conditions (no insulin), in the presence of a maximal insulin concentration (10 mU/ml), and after electrically stimulated muscle contraction in the absence of insulin. The perfusate contained 28 mM glucose and 7.5 μCi/mmol of 2-deoxy-D-[^{3}H-(G)]glucose. Glucose uptake rates in the soleus (slow-twitch oxidative fibers), red gastrocnemius (fast-twitch oxidative-glycolytic fibers), and white gastrocnemius (fast-twitch glycolytic fibers) under basal conditions and after electrically stimulated muscle contraction were not significantly different between the lean and obese rats. However, the rate of glucose uptake during insulin stimulation was significantly lower for obese than for lean rats in all three fiber types. Significant correlations were found for insulin-stimulated glucose uptake and glucose transporter protein isoform (GLUT-4) content of soleus, red gastrocnemius, and white gastrocnemius of lean (r = 0.79) and obese (r = 0.65) rats. In contrast, the relationships between contraction-stimulated glucose uptake and muscle GLUT-4 content of lean and obese rats were negligible because of inordinately low contraction-stimulated glucose uptakes by the solei. These results suggest that maximal skeletal muscle glucose uptake of obese Zucker rats is resistant to stimulation by insulin but not to contractile activity. In addition, the relationship between contraction-stimulated glucose uptake and GLUT-4 content appears to be fiber-type specific.

glucose transporter; GLUT-4; 2-deoxyglucose; electrical stimulation; skeletal muscle; muscle fiber type

THE SKELETAL MUSCLE of the obese Zucker rat is highly insulin resistant, as evidenced by a pronounced decrease in insulin-stimulated glucose uptake (3, 4, 15, 17, 30, 35). Despite this resistance, we have demonstrated that the obese rat has a greater reliance on blood glucose for fuel during exercise than its lean littermate (32). This may indicate that the ability of contractile activity to stimulate glucose uptake is not impaired in the obese rat. Alternatively, contraction-stimulated muscle glucose uptake may be depressed similarly to that seen in the presence of insulin. Inasmuch as the abilities of insulin and muscle contraction to stimulate glucose uptake are additive (16, 25, 27, 28), it is possible that the hyperinsulinemia of the obese rat and muscle contraction combine to compensate for each other so that an adequate rate of glucose uptake can be achieved during muscle activity. Therefore, one purpose of this study was to determine whether the maximal capacities of insulin- and contraction-stimulated muscle glucose uptake were impaired in the obese Zucker rat.

It has recently been demonstrated that skeletal muscle expresses a unique glucose transporter protein isoform (GLUT-4) that is responsible for facilitated glucose uptake in response to both insulin and muscle contraction (5–7, 13, 18). In the rat, differences occur in the GLUT-4 concentration of the three basic muscle fiber types, with the highest concentration found in the slow-twitch oxidative muscle fibers and the lowest in the fast-twitch glycolytic fibers (11, 20). GLUT-4 concentration has been shown to be significantly related to maximum insulin- and contraction-stimulated glucose uptake, which suggests that the maximum glucose transport capacities of the various muscle fiber types are due to their GLUT-4 concentrations (11, 20). However, the relationships between GLUT-4 concentration and maximal rate of glucose uptake due to insulin stimulation or contractile activity have not been assessed in insulin-resistant muscle. These relationships have also not been determined for normal muscle in the presence of a saturating concentration of glucose. Therefore the second purpose of this study was to determine the relationships between GLUT-4 content and insulin- and contraction-stimulated maximal glucose uptake capacity in the skeletal muscle of obese and lean Zucker rats.

METHODS

Experimental animals. Eighteen female obese Zucker rats (*fa/fa*) and 18 lean littermates (*Fa/?*), 14 wk of age, were randomly assigned to a basal (no insulin), insulin, or muscle stimulation group. All rats were housed three to a cage and provided laboratory chow and water ad libitum. The temperature of the animal room was maintained at 21°C, and an artificial 12:12-h light-dark cycle was set.

Surgical preparation and hindlimb perfusion. Rats were anesthetized with pentobarbital sodium (6.5 mg/100 g body wt ip) after a 12-h fast. The surgical procedure for hindlimb perfusion of the rats and the perfusion apparatus used were similar to those previously described by Ruderman et al. (29). Additionally, both sciatic nerves of the muscle-stimulated rats were surgically exposed and attached to miniature electrodes. After completion of the surgical preparation, cannulas were inserted into the abdominal aorta and vena cava of the rats and their hindlimbs were washed out with 50 ml of Krebs-Henseleit buffer (pH 7.4). Immediately thereafter, the cannulas were placed in line with the perfusion system and the hindlimbs were allowed to stabilize during a 15-min nonrecirculating washout period. The perfusion medium consisted of Krebs-Henseleit buffer (pH 7.4) containing 4% dialyzed bovine serum albumin (Cohn fraction V, US Biochemical), 30% time-expired washed human red blood cells, 0.3 mM pyruvate, and 0.5 mM glucose. Perfusate flow rate during the washout period was 15 ml/min. After the washout, perfusate samples were taken from the venous line for determination of insulin (24), and the rat was killed with an intracardiac injection of pentobarbital sodium. Insulin analysis indicated that the washout procedure effectively lowered the insulin concentration of the hindlimbs below detection (<2.5 μU/ml).

For determination of basal and insulin-stimulated glucose uptake, the perfusate was changed to one containing 28 mM glucose, 2-deoxy-D-[^{3}H(G)]glucose (2-DG, 7.5 μCi/mmol glucose), 2 mM mannitol (60 μCi D-[1-^{14}C]mannitol/mmol), and 0.3 mM pyruvate. This concentration of glucose was selected because it was approximately fourfold higher than the average Michaelis constant for insulin-stimulated muscle glucose uptake (23), thereby ensuring that maximum velocity of uptake was measured. When insulin-stimulated glucose uptake was determined, the perfusate also contained insulin (10 mU/ml). Perfusion times were 10 and 7 min for basal and insulin-stimulated rats, respectively, and the perfusate flow rate was maintained at 15 ml/min.

For contraction-stimulated glucose uptake, the skin was reflected from both hindlimbs of the rat during the initial 15-min perfusion period. A section of the calcaneus, with the Achilles tendon still attached, was then clipped from the foot. Next, the triceps surae of both hindlimbs were reflected away from the tibia, and jeweler chains were clipped to both calcaneus-Achilles tendon interfaces. The chains were attached to an apparatus that allowed for adjustment of muscle tension, with one chain in line with an isometric force transducer (Harvard Instruments, Millis, MA), which was in turn connected to a chart recorder. Both hindlimbs of the muscle-stimulated rats were then immobilized in a specially designed acrylic cradle, and the muscles of the triceps surae group were adjusted to achieve maximum twitch tension.

After the 15-min washout period, muscle contraction was initiated by stimulating the sciatic nerves with 200-ms trains of 100 Hz, with each impulse in a train being 0.1 ms. The trains were delivered at a rate of 1/s at a supramaximum voltage (8–12 V) for two 5-min periods separated by a 1-min rest period. During contraction the perfusate flow rate was increased to 25 ml/min to ensure proper oxygenation of the hindlimb muscles. Once the second stimulation period was completed, the perfusate was changed to that used for the measurement of basal glucose uptake and the flow rate was reduced to 15 ml/min. Time of perfusion was set for 7 min. All perfusions were performed at 37°C.

Immediately after each perfusion, the hindlimbs of the rats were washed out with 30 ml of Krebs-Henseleit buffer containing 0.3 mM pyruvate to remove intravascular erythrocytes. The soleus and red and white portions of the gastrocnemius from the right leg were then removed and blotted on gauze dampened with Krebs-Henseleit buffer. The muscles were equally divided into two sections, freeze-clamped in tongs cooled in liquid N_2, and stored at −80°C until analyzed for 2-DG uptake and GLUT-4 content. These muscles were chosen because they represent the three basic muscle fiber types of the rat (1).

Determination of 2-DG uptake. The perfused muscle samples were weighed, homogenized in 1 ml of 10% trichloroacetic acid (TCA) at 4°C, and centrifuged in a microcentrifuge (Fisher Scientific, Houston, TX) for 10 min. Duplicate 0.3-ml samples of the supernatant were transferred to 20-ml scintillation vials containing 10 ml of Scintiverse E (Fisher Scientific, Pittsburgh, PA) and vortexed. For determination of perfusate specific activity, well-mixed samples of the arterial perfusate were obtained during perfusion. The samples were deproteinized in 10% TCA and treated in the same way as the muscle samples. The samples were counted for radioactivity in a liquid scintillation spectrophotometer (model LS-350, Beckman, Fullerton, CA). Efficiency and channel crossover were determined by counting ^{3}H and ^{14}C standards of known disintegrations per minute. The accumulation of intracellular [2-^{3}H]DG, which is indicative of muscle glucose uptake, was calculated by subtracting the concentration of [2-^{3}H]DG in the extracellular space from the total muscle [2-^{3}H]DG concentration. The [2-^{3}H]DG in the extracellular space was quantified by measuring the concentration of [^{14}C]mannitol in the TCA supernatant.

GLUT-4 analysis. Muscle samples were homogenized in HES buffer [20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, 1 mM EDTA, and 250 mM sucrose; pH 7.4; 1:20 wt/vol] with a Polytron homogenizer (Brinkman Instruments, Westbury, NY) using three 15-s bursts at high speed. A 100-μl sample of the tissue homogenate was diluted 1:1 with Laemmli sample buffer. Sample protein (75 μg), along with molecular weight markers (Bio-Rad, Richmond, CA), was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis run under reducing conditions with a 12% resolving gel. Protein determinations were performed on each homogenate via the method of Bradford (2). The red and white gastrocnemius and soleus from lean and obese rats were run in adjacent lanes. Resolved proteins were transferred to polyvinyldene difluoride (PVDF) sheets (Bio-Rad) by use of a Bio-Rad SD semidry transfer unit. All subsequent incubations were carried out in tris(hydroxymethyl)aminomethan (Tris) buffered saline (TBS) composed of 20 mM Tris and 500 mM NaCl (pH 7.5 at 25°C).

After transfer, the PVDF sheets were blocked in TBS and 5% nonfat dry milk (pH 7.5). Next, the sheets were washed in TBS with 0.05% Tween-20 (TTBS) for 10 min and then incubated for 1 h with the polyclonal GLUT-4 antibody R349 (donated by Dr. Mike Mueckler, Washington University, St. Louis, MO) at a titer of 1:500 in TTBS, 1% milk, and 0.02% sodium azide. The PVDF was then washed in TTBS for 20 min, followed by incubation with ^{125}I-labeled goat-anti-rabbit immunoglobulin G (New England Nuclear, Boston, MA) at a concentration of 0.3 mCi/ml for 1 h. Antibody binding was visualized by autoradiography, which was performed at −70°C for 36 h. Labeled bands were traced, cut out, and counted in a gamma counter (model 5500, Beckman). Each band was corrected for background radiation, and the results were expressed relative to an internal standard (75 μg rat heart homogenate protein) run on each gel.

Statistical analysis. The data were analyzed by a two-way analysis of variance to test the effects of phenotype and treatment (basal, contractile activity, and insulin) on muscle glucose uptake. When a significant F ratio was obtained, Newman-Keuls post hoc test was employed to identify statistically significant differences ($P < 0.05$) between the means.

RESULTS

Basal and insulin- and contraction-stimulated glucose uptakes are shown in Fig. 1. Under basal conditions, there were no significant differences between lean and obese rats or among fiber types. Likewise, contraction-stimulated glucose uptake was not different between lean and obese rats across fiber types. However, fiber-type differences were evident, with the highest glucose uptake occurring in the red gastrocnemius (RG) and no difference in uptake occurring between the white gastrocnemius (WG) and the soleus. In contrast to contraction-stimulated uptakes, insulin-stimulated glucose uptakes were approximately four to fivefold higher in lean than in obese rats in each of the three basic muscle fiber types. The fiber-type response for insulin was different from that for contraction-stimulated glucose uptake. Insulin-stimulated glucose uptakes of soleus and RG did not differ and were approximately fourfold higher than that of the WG. This pattern was apparent in both lean and obese rats.

There were no significant differences in GLUT-4 content in the RG and soleus of the lean and obese rats (Fig. 2). However, GLUT-4 content was significantly lower in the obese WG than in the lean WG. These results were the same even when GLUT-4 was subdivided according to treatment groups. For both lean and obese rats, levels of GLUT-4 were highest in the soleus, lowest in the WG, and intermediate in the RG.

A significant positive correlation was found for insulin-stimulated glucose uptake and GLUT-4 content in muscle of both lean ($r = 0.79$) and obese ($r = 0.65$) rats (Fig. 3). In contrast, electrically stimulated muscle did not demonstrate a significant correlation between glucose uptake and GLUT-4 content in either lean or obese rats when all muscle fiber types were included in the analysis (Fig. 4*A*; $r = 0.23$ for lean and $r = 0.12$ for obese).

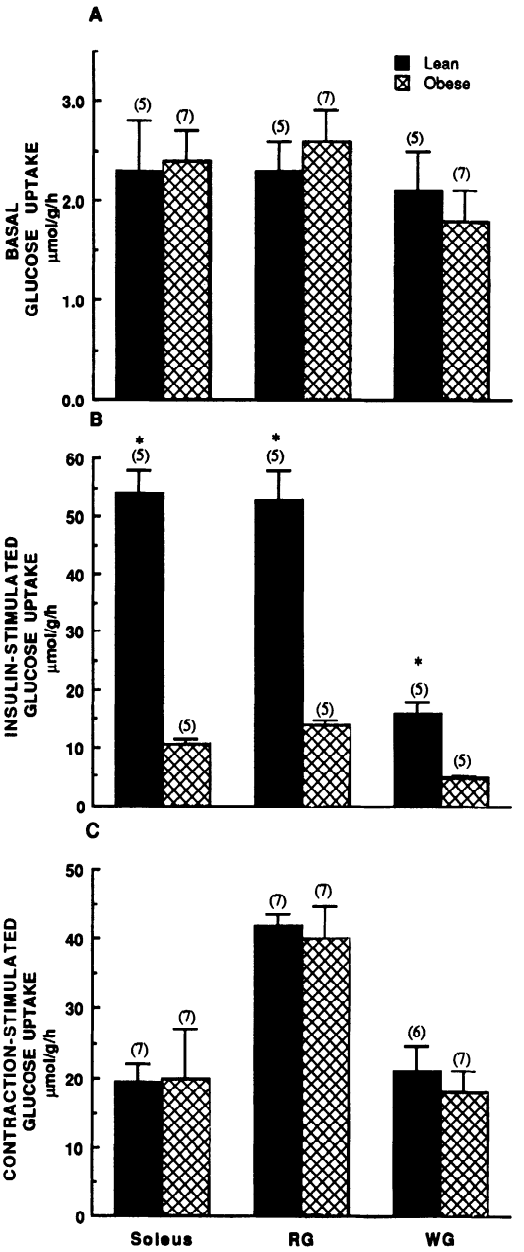

FIG. 1. Glucose uptake in soleus, red gastrocnemius (RG), and white gastrocnemius (WG) of lean and obese Zucker rats during basal conditions (*A*) and insulin stimulation (*B*) and after muscle contraction (*C*). Bars, means ± SE; nos. in parentheses, no. of observations. *Significantly different from corresponding lean value.

When only the fast-twitch muscles were analyzed, however, the correlations between glucose uptake and GLUT-4 were $r = 0.62$ and 0.66 for lean and obese rats, respectively (Fig. 4*B*).

DISCUSSION

Muscle of the obese Zucker rat is highly insulin resistant, which is due in part to a reduced insulin responsiveness (15, 30, 35) that occurs in all three basic muscle fiber types of the obese rat (15, 30). In the present study, we confirmed that the maximal capacity of insulin-stimulated glucose uptake is impaired in the muscle of the obese Zucker rat. However, we did not find an impairment in contraction-stimulated muscle glucose uptake.

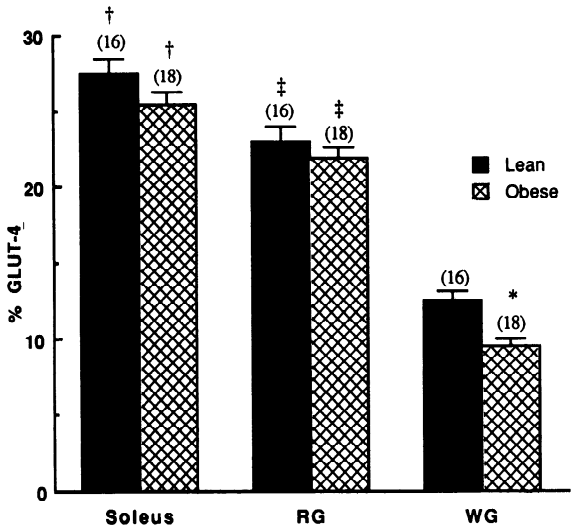

FIG. 2. Soleus, RG, and WG GLUT-4 concentrations expressed as percentage of a rat heart standard. Bars, means ± SE; nos. in parentheses, no. of observations. *Significantly different from corresponding lean value. †Significantly different from corresponding RG and WG values. ‡Significantly different from corresponding WG value.

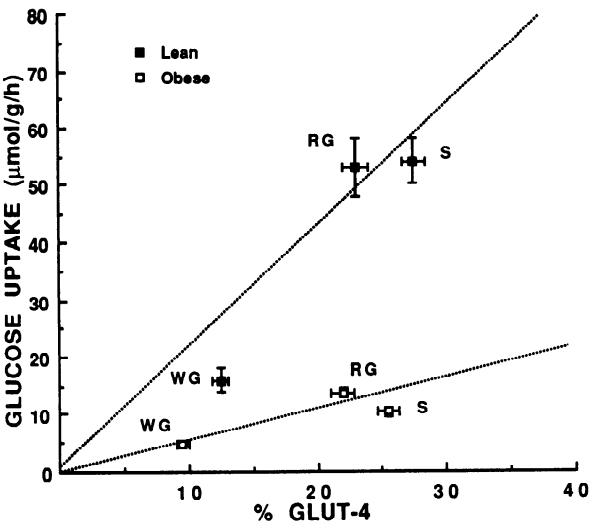

FIG. 3. Correlation between GLUT-4 and insulin-stimulated glucose uptake. Data shown in Figs. 1 and 2 were analyzed by linear regression to correlate values for glucose uptake and GLUT-4 content. Values are means ± SE. Correlation coeff: lean $r = 0.79$, obese $r = 0.65$. S, soleus.

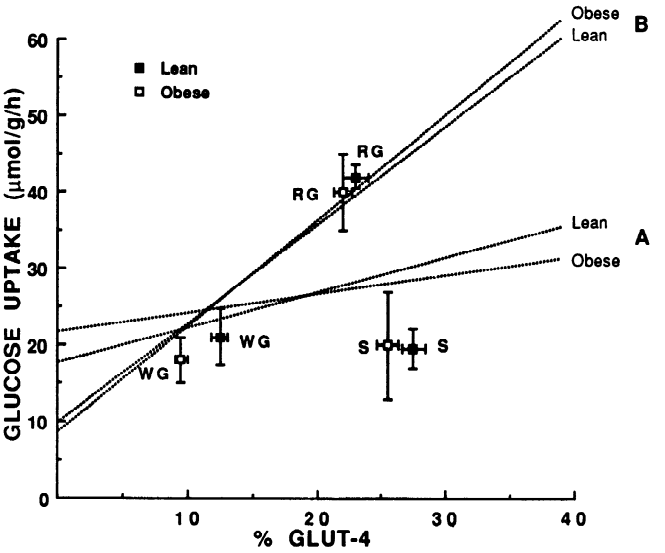

FIG. 4. Correlation between GLUT-4 and contraction-stimulated glucose uptake. Results shown in Figs. 1 and 2 were analyzed by linear regression to correlate values for glucose uptake and GLUT-4 content. Values are means ± SE. *A*: correlation with soleus included in regression analysis; *B*: correlation with soleus removed from regression analysis. Correlation coeff: *A* (lean $r = 0.23$, obese $r = 0.12$); *B* (lean $r = 0.62$, obese $r = 0.66$).

In the presence of a saturating concentration of glucose, insulin-stimulated glucose uptake was approximately equal for the slow-twitch oxidative fibers of the soleus and the fast-twitch oxidative-glycolytic fibers of the RG. These fibers had rates of glucose uptake that were four- to fivefold greater than that of the fast-twitch glycolytic fibers of the WG. In contrast, contraction-stimulated glucose uptake was highest in the RG, which was approximately twofold greater than that observed for the soleus or WG.

Activation of glucose uptake by insulin or contractile activity is associated with the rapid reversible translocation of specific transporter proteins from low-density microsomes to the plasma membrane (5–7, 10, 13, 14). In muscle the two predominant isoforms of the glucose transporter are GLUT-1 and GLUT-4 (5, 6, 18). The GLUT-1 isoform is found in small amounts on the plasma membrane, and its concentration is not significantly increased in the presence of insulin (5, 6). However, insulin and contractile activity increase the plasma membrane concentration of GLUT-4, which suggests that the GLUT-4 isoform of the transporter is principally involved in the regulation of insulin- and contraction-stimulated glucose uptake in muscle (5, 6, 13). Recently, it was demonstrated that maximal activation of muscle glucose uptake by insulin or contractile activity was associated with the muscle GLUT-4 concentration (11, 20). Furthermore it was observed that insulin resistance due to streptozocin treatment (26, 31) or muscle denervation (12) was associated with a reduced muscle GLUT-4 concentration.

In the present study, no difference in GLUT-4 concentration was found between the lean and obese rats for the soleus and RG. A significant difference, however, was noted between the GLUT-4 concentration of the WG of lean and obese rats. These results indicate that the insulin resistance of the soleus and RG of the obese Zucker rat was not due to a deficient GLUT-4 concentration. It is also unlikely that a significant percentage of the insulin resistance of the WG could be attributed to a reduced GLUT-4 concentration, inasmuch as the difference in glucose uptake between the lean and obese rats far exceeded their difference in GLUT-4 concentration. Friedman et al. (8) reached a similar conclusion after they had determined that the gastrocnemius of lean and obese Zucker rats had similar GLUT-4 concentrations. Similar observations have also been made for the insulin-resistant *db/db* mouse (22) and for obese non-insulin-dependent diabetes patients (9). The present results extend the findings of Friedman et al. (8) and others (9, 22) by demonstrating that a deficient GLUT-4 content can be fiber-type specific and, therefore, suggest that, in evaluating

the cause of muscle insulin resistance, fiber-type specificity should be taken into account.

The present results should not be interpreted to suggest that GLUT-4 concentration does not influence the insulin responsiveness of the obese Zucker rat. As was determined for the lean rats, we found a strong positive relationship between insulin-stimulated glucose uptake and muscle GLUT-4 concentration of the obese rats. However, the slope of the regression line for the lean rats was significantly greater than that of the obese rats. The difference was such that, for the same concentration of GLUT-4, it would be predicted that an obese rat would have a maximal insulin response one-fourth that of its lean littermate. The reason for this difference in insulin responsiveness is not known but could be related to a defective insulin signal transduction process, which could prevent normal translocation of the transporters, a defect in the transporter translocation process, or an inability of insulin to activate the transporter once it is translocated to the plasma membrane.

We previously observed that the obese rat has a greater reliance on blood glucose than its lean littermate during exercise of the same relative intensity (32). This suggests that contraction-activated glucose uptake is not impaired in the obese Zucker rat in vivo. However, the obese rat remains hyperinsulinemic even during exercise (32), and it is well established that contractile activity and insulin have an additive effect on muscle glucose uptake (16, 25, 27, 28). Therefore the direct effect of muscle contraction on glucose uptake could not be discerned from these in vivo results. In the present study, maximal contraction-stimulated glucose uptake was assessed during hindlimb perfusion in the absence of insulin. No differences between lean and obese rats were noted for any of the muscle fiber types investigated. Thus we have clearly demonstrated that contractile activity, independent of other modulating stimuli found during exercise, can maximize muscle glucose uptake to the same degree in lean and obese Zucker rats. The finding that differences in muscle glucose uptake between lean and obese rats occurred only during insulin-stimulated and not during contraction-stimulated glucose uptake indicates that at least a portion of the insulin- and contraction-stimulated glucose pathways are mutually exclusive. This conclusion is supported by the previous findings that the maximal effects of insulin and contractile activity on muscle glucose uptake are additive (25) and that insulin and contractile activity appear to recruit from two distinct pools of transporters (6, 7).

Similar to the obese Zucker rat, rats with 2 days of hindlimb immobilization demonstrate insulin resistance without a significant change in contraction-stimulated glucose uptake (33). Rats made insulin resistant by streptozocin injection, however, demonstrate a reduced contraction-stimulated glucose uptake (25, 27, 34). The reduction in rate of uptake has generally been reported to be ~50%. Recently, Klip et al. (21) and Nishimura et al. (26) reported a reduced insulin responsiveness in streptozocin-treated diabetic rats. A reduced number of glucose transporters in the plasma and microsomal membranes of the streptozocin-treated rats was also reported. Because the skeletal muscle of the obese Zucker rat has a near normal concentration of glucose transporters, it can be postulated that the difference in contraction-stimulated glucose uptake between these insulin-resistant models is due to a difference in total number of muscle glucose transporters. However, other possibilities cannot be ruled out, inasmuch as the insulin resistance caused by streptozocin treatment can precede a decline in GLUT-4 concentration (19). Regardless of the causes of insulin resistance, these results indicate that a reduced insulin-stimulated glucose uptake is not always accompanied by a reduced contraction-stimulated glucose uptake.

Contrary to insulin-stimulated glucose uptake, the relationship between contraction-stimulated uptake and muscle GLUT-4 concentration for both lean and obese rats was negligible. The reason for this finding appeared to be the low contraction-stimulated glucose uptake of the soleus. When the soleus was removed from the regression analysis, a significant correlation between glucose uptake and GLUT-4 concentration was observed. These results suggest that muscle contraction was not as effective in the activation of glucose uptake in slow- as in fast-twitch muscle, despite the high concentration of GLUT-4 in slow-twitch muscle. If it is accepted that insulin and contractile activity recruit GLUT-4 transporters to the plasma membrane from two separate pools (6, 7), then our finding can be explained by the slow-twitch fibers possessing a relatively small contraction-recruitable pool. An alternative explanation is that only one pool of GLUT-4 exists but that the stimulus necessary for contraction-stimulated translocation and/or activation of the GLUT-4 transporter is less effective in slow- than in fast-twitch fibers.

Finally, basal glucose uptake was similar for lean and obese rats across all muscle fiber types. As previously stated, basal glucose uptake appears to be under the control of the GLUT-1 isoform of the glucose transporter. This glucose transporter is believed to be located primarily on the plasma membrane and to be unresponsive to insulin stimulation (5, 6). The present results suggest that basal glucose uptake is not impaired in the muscle of the obese rat and that there is little difference in basal glucose uptake across muscle fiber types. Furthermore, these findings indicate that differences in basal muscle glucose uptake of lean and obese rats could not explain the results obtained for insulin- and contraction-stimulated glucose uptake.

In summary, the maximum contraction-stimulated glucose uptake was not impaired in insulin-resistant muscle of the obese Zucker rat. Contraction-stimulated glucose uptake was not associated with the total muscle GLUT-4 concentration, which appeared to result from a difference in contraction-activated glucose uptake between slow- and fast-twitch fibers. Maximum insulin-stimulated glucose uptake, unlike contraction-stimulated uptake, was found to be closely associated with the muscle GLUT-4 content in both lean and obese rats. However, the muscle insulin resistance of the obese rat could not be attributed to a reduced muscle GLUT-4 concentration.

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