Exercise training and the glucose transport system in obese SHHF/Mcc-fa<sup>cp</sup> rats

**Ferrara, Cynthia M., W. Michael Sherman, Nicole Leenders, Sylvia A. McCune, and Karla Roehrig.** Exercise training and the glucose transport system in obese SHHF/Mcc-fa<sup>cp</sup> rats. J. Appl. Physiol. 81(4): 1670–1676, 1996.—The effects of a similar exercise training stimulus on maximal insulin-stimulated (MIS) plasma membrane glucose transporter number and glucose transport were determined in lean and obese SHHF/Mcc-fa<sup>cp</sup> rats. Six-week-old lean and obese male rats were randomly divided into four groups: lean sedentary (LSed), obese sedentary (OSed), lean exercise (LEX), and obese exercise (OEx). An 8- to 12-wk treadmill running program equalized daily muscular work for LEx and OEx. Plasma membranes were isolated from control and MIS muscles of mixed fiber types. MIS significantly increased glucose transport (3.4- and 2.8-fold) in Lsed and Osed, respectively. MIS significantly increased glucose transporter number (2.5-fold) in Lsed, but there was no increase in glucose transporter number in Osed. Peak oxygen uptake and citrate synthase activity were increased a similar amount for LEx and OEx groups, demonstrating a similar training stimulus. MIS significantly and similarly increased glucose transport in LEx and OEx (4.4- and 5.1-fold, respectively). The effects of MIS on plasma membrane glucose transporter number in the exercise-trained rats were similar to the responses observed in the sedentary lean and obese groups. MIS significantly increased glucose transporter number (2.6-fold) in LEx, whereas there was no increase in glucose transporter number in OEx. The reduction in MIS glucose transport in Osed appears to be related to a defect in the processes associated with the translocation of glucose transporters to the plasma membrane. Exercise training of the obese rats apparently did not alter this defect. Similar increases in peak oxygen uptake, citrate synthase, and MIS glucose transport in LEx and OEx groups suggest that insulin resistance does not limit the ability of the glucose transport system to adapt to exercise training in the obese male SHHF/Mcc-fa<sup>cp</sup> rats.

Obesity; non-insulin-dependent diabetes; insulin resistance.

**METHODS**

Six-week-old obese male SHHF/Mcc-fa<sup>cp</sup> rats and their lean littermates were randomly divided into four groups: lean sedentary (LSed), lean exercise-trained (LEX), obese sedentary (OSed), and obese exercise-trained (OEx). Animals were provided rat chow ad libitum (Purina) and were exposed to a thermoneutral environment and a 12:12-h light-dark cycle. This study was approved by the Institutional Animal Care and Use Committee.

Animals assigned to the exercise-trained groups began 8–12 wk of treadmill running on a rodent motor-driven treadmill (Quinton Instruments, Seattle, WA) for 5 days/wk at 18 m/min at a 15% grade (~70% peak oxygen consumption [\(V\text{O}_{2\text{peak}}\)] of obese animals). This intensity of exercise increases glucose transport and aerobic enzyme activity in insulin-resistant skeletal muscle (7, 25). The duration of exercise for the obese animals was gradually increased to 1.5
h/session within the first 2 wk of training. The duration of each exercise session for lean animals was adjusted to equal the amount of work in kilograms times meter performed by a matched obese animal. Thus the daily skeletal muscle "work" was equal for the exercise-trained lean and obese animals. Exercise training took place near the end of the dark cycle, and sedentary animals were exposed to a similar amount of handling and exposure to the treadmill.

At the end of the exercise training period, VO\textsubscript{2peak} was measured using the DREX drum exerciser and the Oxymax open-circuit calorimeter system (Columbus Instruments International, Columbus, OH). After 30 min of rest in the exercise chamber, the initial drum speed was increased to either 18 or 24 m/min for obese and lean animals, respectively; was increased 6 m/min after 2 min; and was increased by 6 m/min every minute thereafter until the animal could not maintain the running wheel speed. The highest oxygen consumption value was identified as VO\textsubscript{2peak}.

Skeletal muscle plasma membrane glucose transporter concentrations and/or the rate of plasma membrane glucose transport were measured in each animal at rest under basal conditions for muscle obtained from one leg and after maximal insulin stimulation for muscle obtained from the opposite leg. Thus, each animal served as its own control for the insulin-stimulated condition (e.g., one leg was in the basal state and the other leg was in the insulin-stimulated state). This experiment occurred 3–4 h postprandial for all animals and 40–48 h after the last exercise training session for the respective exercise training groups.

After anesthetization (ketamine and rompin), a blood sample was obtained from the tail for measurement of serum glucose and insulin concentrations. Gastrocnemius, plantaris, soleus, and red quadriceps muscles were rapidly removed from the right leg, cleaned of connective tissue, weighed, and then used to prepare plasma membranes. Next, a maximal insulin dose (20 IU) was administered to insulin-stimulated muscles (7–8 passes). A 0.3-to 0.5-ml well-mixed aliquot was removed from both the control and insulin-stimulated homogenates. The resulting crude muscle homogenate was then pooled, weighed, and then used to prepare plasma membranes. Serum and the quick-frozen muscle samples were stored at −80°C until analysis.

The muscles were finely minced in ice-cold 255 mM sucrose, 100 mM tris(hydroxymethyl)aminomethane (Tris), and 0.2 mM EDTA buffer (pH 7.6) and homogenized on ice by using a polytron with a Recod speed controller (Kinematica, Switzerland) for 3 × 60 s homogenizations at 3,000 revolutions/min. The resulting crude muscle homogenate was then centrifuged on ice by using a potter-elvehjem tissue grinder (7–8 passes). A 0.3- to 0.5-ml well-mixed aliquot was removed from both the control and insulin-stimulated homogenates and diluted 1:2 with sucrose, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) buffer (250 mM sucrose, 20 mM HEPES, pH 7.4 at 4°C). These two aliquots were used for measurement of the protein concentration, plasma membrane marker enzyme activity, and GLUT-4 protein concentration. The crude homogenates were centrifuged at 48,000 g for 30 min at 4°C. The resulting pellet was used for purification of plasma membranes essentially according to the procedure described by Hirshman et al. (11).

Protein concentrations were determined for each crude homogenate and each plasma membrane preparation by using the bicinchoninic acid protein assay (Pierce, Rockford, IL) with crystalline bovine serum albumin as the standard.

Potassium-stimulated p-nitrophenylphosphatase (K\textsuperscript+ pNPPase) activity was measured as the marker for plasma membranes in crude homogenates and plasma membrane samples (2). The coefficient of variation of both assays was ≤5%.

α-glucose inhibitable [\textsuperscript{3}H]cytochalasin-B (CB) binding was measured in freshly isolated plasma membrane fractions using the procedures of Cushman and Wardzala (5), as modified by Klip et al. (18) and Greco-Perotto et al. (10). Glucose transporter concentrations were determined using Scatchard analysis of CB binding to the membrane sample at six CB concentrations (14, 45, 77, 139, 202, and 264 nM). Cytochalasin E was added at each CB concentration to reduce nonspecific binding. Scatchard plots were constructed for each plasma membrane preparation (Fig. 1). At least four points per membrane sample were used to construct the Scatchard plots for each sample. The x-intercept is an estimate of the glucose transporter concentration in a particular sample (R\textsubscript{o}). The dissociation constant (K\textsubscript{d}), a measure of the affinity of the glucose transporter for CB, is equal to the negative inverse of the slope of the line derived from Scatchard plot. The coefficient of variation for this assay was ≤9%. A correlation of between −0.90 and −1.0 for the Scatchard plot analysis was established as the criterion to accept the data from the binding experiment.

GLUT-4 concentrations were measured in the crude muscle homogenate and plasma membrane samples using immunonequantitation of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) blots onto nitrocellulose. Samples were prepared in a sample buffer containing 2% SDS, 60 mM Tris-HCl (pH 6.8), glycerol, 0.1% bromophenol blue, and β-mercaptoethanol. Aliquots of the prepared muscle and membrane samples containing 25 μg of protein and biotinylated molecular weight standards (Bio-Rad Laboratories, Richmond, CA) underwent SDS-PAGE on a 10% polyacrylamide gel. Samples from all four groups for control and insulin-stimulated conditions were run on the same gel.

Proteins were transferred to Immobilon-P transfer membranes (Millipore, Bedford, MA) at 4°C for 1.5 h at 100 V (0.25–0.30 A). The Immobilon was blocked overnight at 4°C in a primary antibody solution containing 5% nonfat dry milk in a Tris-buffered saline solution with 0.05% Tween-20 (TBBS), incubated for 1 h at room temperature in a primary antibody solution containing a 1:10,000 dilution of a polyclonal GLUT-4 antibody (14) (East Acres Biologicals, Southbridge, MA), TBBS, and 1% nonfat dry milk, and then incubated for 1 h in secondary antibody. An amplified alkaline phosphatase color development detection system (Bio-Rad Laboratories) was used to identify the GLUT-4 bands. The LKB Bromma Ultrascan XL enhanced laser densitometer and GelScan XL

![Fig. 1. Scatchard plot analysis for cytochalasin-B binding assay for a representative plasma membrane sample.](http://jap.physiology.org/)
between time vs. glucose influx (Fig. 2). The coefficient of
established as the criterion for an acceptable relationship

\[ r = 0.99; \text{for L-glucose:} \quad y = 1.868x + 1.331, \quad r = 0.95; \text{for D-glucose:} \quad y = 2.694, \quad r = 0.95. \]

Fig. 2. Plasma membrane vesicle L- and D-glucose transport under control (A) and insulin-stimulated (B) conditions for a representative membrane sample. A: for D-glucose: \( y = 1.868x + 1.331, \quad r = 0.99; \) for L-glucose: \( y = 0.813x + 1.207, \quad r = 0.97. \) B: for D-glucose: \( y = 6.491x + 1.882, \quad r = 0.99; \) for L-glucose: \( y = 0.682x + 2.694, \quad r = 0.95. \)

computer program (LKB Produkter AB, Bromma, Sweden) were used to analyze the GLUT-4 bands and to reduce the densitometer data. GLUT-4 concentrations are expressed as a percentage of the GLUT-4 absorbance for an aliquot of muscle protein that was run on each gel.

D-[14C]- and L-[3H]glucose uptake into isolated plasma membrane vesicles was measured under conditions of equilibrium exchange using a rapid-filtration technique (15, 22). Plasma membrane vesicles were initially treated to the procedures described by Ploug et al. (22) to minimize leakage by the plasma membranes. Glucose uptake was initiated by combining 20 µl of plasma membrane vesicles (~35 µg of protein) with 80 µl of incubation medium (Krebs-Ringer solution with 40 mM L- and D-glucose, plus 6 µCi/80 µl of L-[1-3H]glucose and 1.6 µCi/80 µl of D-[14C]glucose; 16.2 Ci/mmol, and 3.80 mCi/mmol, respectively) (DuPont NEN Research Products, Boston, MA). The time points to determine the initial rate of glucose transport were 0, 1, 3, and 5 s for the control conditions, and 0, 1, 1.5, and 2 s for the insulin-stimulated conditions. Glucose transport was stopped by addition of 1 ml of ice-cold stop solution (265 mM NaCl, 5 mM KCl, 1.2 mM MgCl₂, 20 mM HEPES, pH 7.8, containing 0.2 mM phloretin). The membranes were rapidly filtered (Millipore HA 0.45 µm) and washed. The filter and adhering membranes were analyzed by liquid-scintillation counting using quench correction for the dual label. The initial rates of L- and D-glucose uptake were obtained by calculation of the slope from the linear portion of the graph of time vs. influx (nmol/mg of protein). Facilitated glucose transport was calculated by subtracting the initial rate of L-glucose transport from the rate of D-glucose transport. A correlation \( \geq 0.90 \) was established as the criterion for an acceptable relationship between time vs. glucose influx (Fig. 2). The coefficient of variation for this assay was \( \leq 9.5\% \).

Citrate synthase activity and muscle glycogen concentrations were measured on a mixture of the four muscles used for the plasma membrane isolations. Muscles samples were pulverized under liquid nitrogen, mixed, and divided for measurement of citrate synthase activity and muscle glycogen. Samples were homogenized in a 1:20 dilution of either 100 mM Tris, 0.4% Triton X-100 (pH 8.1) for measurement of citrate synthase activity, or 20 mM Na₂HPO₄, 0.5 mM EDTA, 0.02% bovine serum albumin, and 0.5 mM β-mercaptoethanol (pH 7.4) for measurement of glycogen. Citrate synthase activity was measured spectrophotometrically using the method of Sjöstrand (27). The muscle glycogen concentration was measured fluorometrically using the method of Passoneau and Lauderdale (21). The coefficient of variation for both assays was \( \leq 5\% \).

Serum glucose concentrations were determined spectrophotometrically by using a glucose-HK kit (Boehringer Mannheim Diagnostics, Indianapolis, IN). Serum insulin concentrations were determined by using the RSL 125I-RIA insulin kit (ICN Biomedicals, Costa Mesa, CA) with a rat insulin standard. Before the insulin analysis, all samples were treated with 25% polyethylene glycol to remove endogenous interfering substances from the serum samples (6). The coefficient of variation for the glucose assay was \( \leq 2\% \) and for the insulin assay was \( \leq 5\% \).

Data were analyzed using a two-way analysis of variance (phenotype x activity level) with a priori comparisons, using the appropriate contrasts and Bonferroni adjustments as necessary (Statview SE+ and Graphics, Abacus Concepts, Berkeley, CA, and SigmaStat, Jandel Scientific, San Rafael, CA). The level of significance was set at \( P \leq 0.05 \). All values are expressed as means \( \pm SE \).

RESULTS

Both OSed and OEx (439.8 ± 13.3 and 415.1 ± 14.2 µU/ml, respectively) weighed significantly more than either LSed or LEx (309.9 ± 8.5 and 294.5 ± 6.8 µU/ml, respectively). Fed serum glucose concentrations were significantly higher in both OSed and OEx (23.9 ± 1.6 and 21.6 ± 1.8 mM, respectively) compared with both LSed and LEx (14.2 ± 0.7 and 15.5 ± 1.2 mM, respectively). Fed serum insulin concentrations were significantly higher in both OSed and OEx (340 ± 71 and 262 ± 86 µU/ml, respectively) compared with both LSed and LEx (53 ± 11 and 18 ± 3 µU/ml, respectively). Thus exercise training did not significantly affect the serum glucose and insulin concentrations for neither LEx nor OEx, compared with their respective sedentary counterparts.

The muscles of animals in all groups were exposed to a maximal insulin stimulus of glucose transport as confirmed by the serum insulin concentration 30 min after intraperitoneal injection. Serum insulin concentrations averaged 76 ± 24 µU/ml for all groups, and there were no significant differences between groups (75 ± 30, 61 ± 21, 60 ± 12, and 111 ± 32 µU/ml for LSed, OSed, LEx, and OEx groups, respectively).

Muscle glycogen concentrations were similar for LSed, OSed, and LEx groups (24 ± 3, 24 ± 3, and 31 ± 3 µmol/g, respectively). On the other hand, the glycogen concentration for OEx (46 ± 4 µmol/g) was significantly higher compared with LSed, OSed, and LEx.
Table 1. $V_{\text{O2peak}}$, citrate synthase, and total muscle GLUT-4 concentrations

<table>
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<tr>
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<th>Sedentary Lean</th>
<th>Sedentary Obese</th>
<th>Exercise-Trained Lean</th>
<th>Exercise-Trained Obese</th>
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<tr>
<td>$V_{\text{O2peak}}$, ml kg$^{-1}$·min$^{-1}$</td>
<td>43.3±5.8</td>
<td>41.8±2.5</td>
<td>82.0±3.0*</td>
<td>64.3±5.4*</td>
</tr>
<tr>
<td>Citrate synthase, umol·g$^{-1}$·min$^{-1}$</td>
<td>28.6±2.1</td>
<td>30.4±1.1</td>
<td>45.6±4.0*</td>
<td>50.0±6.1*</td>
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</table>
| Total muscle GLUT-4, %absorban
cence/mg muscle | 2.5±0.4        | 2.6±0.3         | 3.5±0.4                | 2.9±0.3                |

Values are means ± SE, $P \leq 0.05$ vs. sedentary groups. Total muscle GLUT-4 concentrations are expressed as %absorbance of a 100-µg protein aliquot/mg of muscle loaded per well; mg of muscle loaded per well was calculated by the following equation: [(total mg of muscle)/(mg protein yield)] $\times$ 0.025 mg protein. Ns, of animals per group are 3, 8–11, 3–7 for peak oxygen consumption ($V_{\text{O2peak}}$), citrate synthase, and total muscle GLUT-4, respectively.

An important objective of this study was to expose both lean and obese exercise-trained animals to a similar level of muscular stress. Thus animals in LEx and OEx groups were required to perform a similar amount of work on a daily basis. During the sixth through tenth weeks of exercise training, animals in these groups undertook between 72 and 114 kg·m of work per day. To confirm that the similar physical work resulted in similar cardiovascular and peripheral adaptive responses, $V_{\text{O2peak}}$ and the activity of citrate synthase were measured in animals from all groups. Both $V_{\text{O2peak}}$ and the activity of citrate synthase were significantly higher in both LEx and OEx compared with LSed and OSED rats (Table 1). The $V_{\text{O2peak}}$ and the activity of citrate synthase were similar for LEx and OEx rats ($P > 0.05$). These results indicate that the lean and obese exercise-trained animals were exposed to a similar degree of muscular work that produced similar adaptive responses in $V_{\text{O2peak}}$ and muscle aerobic activity. Because there were similar adaptive responses in aerobic capacity for both lean and obese exercise-trained animals, it is possible to determine whether a similar aerobic adaptive response in skeletal muscle produces a similar or reduced adaptive response in the glucose transport system in obese animals compared with lean animals.

Table 2. Muscle weights and total protein yields for crude homogenates and plasma membrane fractions

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<th>Exercise-Trained Lean</th>
<th>Exercise-Trained Obese</th>
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<tr>
<td>Muscle weight, g</td>
<td>2.4±0.1</td>
<td>2.1±0.1*</td>
<td>2.4±0.1</td>
<td>1.9±0.1*</td>
</tr>
<tr>
<td>Protein yields Homogenate, mg</td>
<td>517±24.7</td>
<td>461.9±22.7*</td>
<td>545.1±27.2</td>
<td>342.3±17.4*</td>
</tr>
<tr>
<td>Plasma membrane, mg</td>
<td>1.1±0.1</td>
<td>1.2±0.1</td>
<td>1.0±0.1</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>Animals/group</td>
<td>23</td>
<td>18</td>
<td>20</td>
<td>18</td>
</tr>
</tbody>
</table>

Values are means ± SE. Weights of the soleus, gastrocnemius, plantaris, and red quadriceps muscles were combined.* $P \leq 0.05$ vs. lean groups; † $P \leq 0.05$ obese exercise-trained (OEx) vs. obese sedentary (OSED) group.

GLUT-4 concentrations were measured in muscle homogenates representing a mixture of the four muscles that were used in the isolation of plasma membranes under basal, non-insulin-stimulated conditions. There was no significant differences in muscle homogenate GLUT-4 concentrations among LSed, OSED, LEx, and OEx groups (Table 1).

Four muscles were combined for the isolation of plasma membranes from the animals in the respective groups. The muscle weights of the obese animals were significantly lower than those of the lean animals (Table 2). This produced lower crude homogenate protein yields and thus contributed to lower plasma membrane protein yields for the obese groups. However, the protein yields were similar to those protein yields reported by other investigators for both crude homogenates and plasma membrane preparations (3, 4, 16). Crude homogenate K$^+$·pNPPase activity was significantly higher in OEx compared with OSED rats (Table 3). Plasma membrane K$^+$·pNPPase activity was also significantly higher in the lean compared with the obese animals and for the exercise-trained compared with the sedentary animals. Other investigators have also observed lower K$^+$·pNPPase activity plasma membranes isolated from diabetic skeletal muscle compared with nondiabetic muscle (4, 17). There were no differences among groups for the percent of plasma membranes recovered. Fold enrichments were significantly higher for lean vs. obese animals and for exercise-

Table 3. K$^+$-stimulated p-nitrophenol phosphatase activities, %recoveries, and enrichments for crude homogenate and PM fractions from sedentary and exercise-trained rats

<table>
<thead>
<tr>
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<th>Sedentary Obese</th>
<th>Exercise-Trained Lean</th>
<th>Exercise-Trained Obese</th>
</tr>
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<tbody>
<tr>
<td>Homogenate, nmol/mg·30 min</td>
<td>170.2±7.5</td>
<td>144.9±5.6</td>
<td>172.6±8.1</td>
<td>192.5±8.0*</td>
</tr>
<tr>
<td>PM, nmol/mg·30 min</td>
<td>5,875.9±321.1</td>
<td>4,411.6±291.4*</td>
<td>7,489.5±281.1†</td>
<td>6,396.1±285.7†</td>
</tr>
<tr>
<td>%Recovery</td>
<td>7.5±0.6</td>
<td>7.5±0.5</td>
<td>8.5±0.7</td>
<td>7.6±0.6</td>
</tr>
<tr>
<td>Enrichment</td>
<td>35.1±2.3</td>
<td>30.9±1.9*</td>
<td>45.2±2.8†</td>
<td>33.9±1.8*†</td>
</tr>
<tr>
<td>Animals/group</td>
<td>23</td>
<td>18</td>
<td>20</td>
<td>18</td>
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Values are means ± SE. PM, plasma membrane. Recovery calculated as ([PM(protein yield)]/[homogenate(protein yield)]); enrichment calculated as (PM)/(homogenate). * $P \leq 0.05$ vs. lean groups; † $P \leq 0.05$ vs. sedentary groups; ‡ $P \leq 0.05$ vs. OSED group.
DISCUSSION

The present study assessed the effects of maximal insulin stimulation and exercise training on aspects of the glucose transport system in the SHHF/\(Mcc\)-fa\(^{cp}\) rats, an animal model of skeletal muscle insulin resistance and diabetes. The results suggest that there is a defect in skeletal muscle's ability to increase the number of plasma membrane glucose transporters in response to maximal insulin stimulation in obese male SHHF/\(Mcc\)-fa\(^{cp}\) rats. This defect does not appear to be corrected by exercise training. The results also indicate that there are similar adaptive responses to exercise training of insulin-stimulated plasma membrane glucose transport in both lean and obese male SHHF/\(Mcc\)-fa\(^{cp}\) rats. This result suggests that, compared with non-insulin-resistant skeletal muscle, insulin-resistant skeletal muscle has a similar adaptive capacity to exercise training in the processes associated with insulin-stimulated plasma membrane glucose transport.

The rate of plasma membrane glucose transport increased with insulin stimulation in both OSed and LSed rats, despite the fact that there was no insulin-stimulated increase in the plasma membrane glucose transporter number for OSed. The absence of an increase in plasma membrane glucose transporter number after insulin stimulation in obese male SHHF/\(Mcc\)-fa\(^{cp}\) rats is similar to the response observed in the insulin-resistant skeletal muscle of obese Zucker rats (4, 16).

The lower rate of plasma membrane glucose transport in OSed group is presumably related to the lower number of glucose transporters in the plasma membrane after insulin stimulation. Either a reduction in the total number of glucose transporters in the skeletal muscle or a reduction in the number of glucose transporters in the intracellular pool would reduce the number of glucose transporters available for translocation to the plasma membrane in response to insulin stimulation and may contribute to the reduced insulin-stimulated plasma membrane glucose transport in OSed. However, the present results demonstrate that there is no difference in the total GLUT-4 concentration per gram of muscle between obese and lean SHHF/\(Mcc\)-fa\(^{cp}\) rats. Furthermore, there is apparently no difference in the number of glucose transporters in the

![Graph](image-url)

Fig. 3. Total no. of glucose transporters determined by cytochalasin-B binding in skeletal muscle plasma membranes. LSED, lean sedentary; OSed, obese sedentary; LEx, lean exercise trained; OEx, obese exercise trained; * \(P \leq 0.05\) vs. corresponding control condition; \(n = 4–7\) animals/group.

trained vs. sedentary animals. The percent recoveries and enrichments are similar to those previously reported using this method to isolate plasma membranes (11).

The glucose transport system of LSed and OSed responded differently to maximal insulin stimulation (Fig. 3). Maximal insulin stimulation produced a significant 2.5-fold increase in the plasma membrane glucose transporter number in LSed. In contrast, maximal insulin stimulation produced no significant increase in the plasma membrane glucose transporter number in OSed. This pattern of response in plasma membrane glucose transporter concentration to maximal insulin stimulation was not altered by exercise training in either lean or obese animals. Maximal insulin stimulation produced a significant 2.6-fold increase in the plasma membrane glucose transporter number in LEx. In contrast, maximal insulin stimulation produced no significant increase in the plasma membrane glucose transporter number in OEx. These responses in the numbers of plasma membrane glucose transporters were confirmed by SDS-PAGE and western blotting for GLUT-4 (0.88 ± 0.20 to 1.38 ± 0.34, 0.48 ± 0.07 to 0.54 ± 0.10, 0.78 ± 0.15 to 1.14 ± 0.21, and 0.53 ± 0.16 to 0.54 ± 0.18% absorbance units for LSed, OSed, LEx, and OEx, respectively). The \(K_d\) values, an estimate of the affinity of the glucose transporters to CB, were not affected by obesity or exercise training. The \(K_d\) did increase significantly with insulin stimulation (91.4 ± 11.6 to 217.9 ± 43.8, 115.7 ± 35.3 to 217.6 ± 47.9, 107.1 ± 22.1 to 180.8 ± 40.6, and 102.1 ± 15.1 to 154.6 ± 31.6 nM for LSed, OSed, LEx, and OEx rats, respectively).

The responses in plasma membrane glucose transport to maximal insulin stimulation were similar in LSed and OSed groups (Fig. 4). Maximally insulin-stimulated plasma membrane glucose transport was similarly and significantly increased 3.4-fold in LSed and 2.8-fold in OSed. The responses in plasma membrane glucose transport to maximal insulin stimulation after exercise training were similar in LEx and OEx. Maximally insulin-stimulated plasma membrane glucose transport was similarly and significantly increased 4.4-fold in LEx and 5.1-fold in OEx.

![Graph](image-url)

Fig. 4. Glucose transport in skeletal muscle plasma membrane vesicles. * \(P \leq 0.05\) vs. corresponding control condition; \(n = 4–7\) animals/group.
skeletal muscle intracellular pool of obese and lean SHHF/Mcc-fa\textsuperscript{α} rats (23). Collectively, these results suggest that reduced glucose uptake in the obese SHHF/Mcc-fa\textsuperscript{α} rat (8) is most likely related to defects in the processes associated with the translocation of glucose transporters to the plasma membrane in response to insulin stimulation. Specific defects may include 1) defects in the transduction of the hormone signal from the insulin receptor; 2) defects in the signal for translocation; or 3) defects in the movement, incorporation, and/or activation of GLUT-4 in the plasma membrane.

Exercise training did not alter the effects of insulin stimulation on the plasma membrane glucose transporter number in either lean or obese rats. Insulin stimulation produced a similar 2.5- and 2.6-fold increase in plasma membrane CB-binding in LSed and LEx rats, respectively. Also, there was no insulin-stimulated increase in plasma membrane CB binding for either OEx or O Sed. Thus, in obese animals, exercise training did not modify the defect(s) in the glucose transport system that prevents an increase in the number of plasma membrane glucose transporters in response to insulin stimulation as has also been observed in studies of Zucker rats (3) and Sprague-Dawley rats (9). These results imply that the signal for glucose transporter translocation is not affected by exercise training in either lean or obese animals.

Insulin-stimulated plasma membrane glucose transport increased in response to exercise training in both lean and obese animals but without an increase in the number of glucose transporters in the plasma membrane for OEx. These results differ from those of both Goodyear et al. (9) in Sprague-Dawley rats and Brozinick et al. (3) in obese Zucker rats, who observed a concomitant increase in plasma membrane glucose transport and GLUT-4 concentration. The present study, however, utilized a daily training intensity that was 70% of \( \dot{V}_O^2 \text{peak} \), whereas the other studies with other animal models (3, 9) utilized significantly higher intensity of exercise (85% \( \dot{V}_O^2 \text{peak} \)) may increase glucose transport by increasing the activity of plasma membrane glucose transporters or by increasing the number of cell surface glucose transporters able to transport glucose into the cell. A higher intensity of exercise (85% \( \dot{V}_O^2 \text{peak} \)), similar to that used by other investigators in studies involving obese Zucker rats (3), may be needed to induce an increase in total muscle and plasma membrane GLUT-4 and insulin-stimulated glucose transport.