Insulin causes translocation of the facilitative GLUT4 from intracellular membrane compartments to the plasma membrane of muscle and fat tissues. This phenomenon is a prerequisite for insulin-stimulated glucose uptake (1–3). However, there is an ongoing debate about whether increased GLUT4 translocation to the plasma membrane can fully account for the insulin-stimulated increase in glucose uptake (4). Several studies have shown a discrepancy between the extent of GLUT4 translocation and the stimulation of glucose uptake in response to insulin in skeletal muscle (5–7), rat white adipocytes (8), and brown adipocytes (9,10). In addition, the fold increase of glucose uptake was much greater than the change in GLUT4 content in isolated plasma membrane vesicles isolated from untreated or insulin-stimulated skeletal muscle tissue (11).

To date, the documentation of GLUT4 translocation has largely relied on subcellular fractionation, immunodetection by light, and electron microscopy or GLUT photolabeling. Although greatly informative, none of these methods led to an accurate quantification of GLUT4 translocation (see DISCUSSION). To circumvent this problem, we recently developed an L6 muscle cell line (L6 GLUT4myc) expressing GLUT4 harboring a myc epitope in the first exofacial loop of the transporter. GLUT4, at the cell surface of intact myotubes, was accurately detected by immunofluorescent and immunochemical labeling of the myc epitope (12–14). The intracellular distribution, segregation, recycling, exocytic, and endocytic rates and insulin response of GLUT4myc were virtually identical to those of GLUT4 (14–17). This system allowed us to directly compare the fold GLUT4 translocation with the fold stimulation of glucose uptake caused by insulin and, therefore, to make predictions about the intrinsic activity of GLUT4 (18–20). Using L6 GLUT4myc myotubes, we identified conditions that segregate insulin-stimulated GLUT4 translocation from glucose uptake by different approaches. First, we observed a time-dependent dissociation between insertion of GLUT4myc into the plasma membrane and increase in glucose uptake, such that GLUT4myc translocation precedes the stimulation of glucose transport by insulin (18). Second, we and others observed different sensitivities of GLUT4 translocation and glucose uptake toward the phosphatidylinositol 3-kinase inhibitor wortmannin (19,21). The drug caused significant inhibition of insulin-stimulated glucose uptake at concentrations <10 nmol/L, which did not prevent GLUT4 translocation. Finally, pretreatment of cells with SB203580, an inhibitor of p38 mitogen-activated protein kinase (MAPK), caused a reduction in insulin-dependent glucose uptake without preventing GLUT4 translocation in muscle and adipose cells in culture (20). Since SB203580 did not directly inhibit GLUT function (18), we postulated that activation of the GLUT4 transporter might occur via a signaling event that includes p38 MAPK. All of the above findings suggest
a role for regulation of the intrinsic activity of GLUT4 in insulin-stimulated glucose uptake.

It is essential to understand whether GLUT4 can be activated in primary tissues and whether physiological and pathological situations can cause GLUT4 activation in addition to GLUT4 translocation. To begin to examine this question, we have transferred the GLUT4myc technology to a transgenic mouse model that expresses GLUT4myc in cardiac and skeletal muscle and brown and white fat. Here, we report the characteristics of these GLUT4myc mice and have determined that the stimulation of glucose uptake by insulin in isolated brown adipocytes far surpasses the extent of GLUT4 translocation, lending credence to the possibility that GLUT4 is activated in primary tissues. A link between this activation and the stimulation of the β-isofrom of p38 MAPK is also proposed.

RESEARCH DESIGN AND METHODS

Materials. Human insulin (Humulin R) was obtained from Eli Lilly Canada (Toronto, ON, Canada). SB203580 was purchased from Calbiochem (La Jolla, CA). Wortmannin, DNase I, phosphatic acid dioniester, and α,phenylenediamine dihydrochloride (OPD) were from Sigma (St. Louis, MO). Collagenase (Toronto, ON, Canada). SB203580 was purchased from Calbiochem (La Jolla, CA). Wortmannin, DNase I, phosphatic acid dioniester, and α,phenylenediamine dihydrochloride (OPD) were from Sigma (St. Louis, MO). Collagenase and horseradish peroxidase were a kind gift from Dr. J.E. Pessin, University of Iowa (23).

Animals. The GLUT4myc transgene was constructed in pHBluescript consisting of a 2.4-kb fragment human GLUT4 promoter (−2,316 to 84) (23), a 2.1-kb rat GLUT4myc cDNA, and a 0.5-kb poly A-tail signal sequence. The GLUT4myc transgenic founder mice were injected by injecting the linear transgene into pronuclei of fertilized mouse embryos of C57Bl/6J mice. Transgenic animals were identified by Southern blot analysis of restriction enzyme digested genomic DNA from tail biopsies using a radiolabeled probe recognizing GLUT4myc. Genotyping of progeny from a selected founder was performed by PCR analysis of tail DNA using a 24-bp 5′ primer (5′GCCCACTGTCCTCGGAGT3′) recognizing a sequence in the GLUT4 promoter and a 21-bp 3′ primer (5′GGTCGGTCTC CCCTCTAAGCAAGTC3′) recognizing part of the myc site of the GLUT4myc cDNA coding region.

All mice were housed in a pathogen-free environment on a 12-h light-dark cycle and fed ad libitum with standard rodent diet. All protocols have been approved by the Animal Care Committee of The Hospital for Sick Children.

GLUT4myc protein expression. Total membranes from a number of tissues were isolated as described earlier (24). A total of 750 μg protein was immunoprecipitated with a monoclonal anti-myc antibody (9E10; 1:150). Cyanine 3–conjugated goat anti-mouse was obtained from Jackson Immunoresearch Laboratories (West Grove, PA), horseradish peroxidase–conjugated goat anti-rabbit antiserum from Pierce (Rockford, IL), and anti–phospho-ATF-2 (Thr 71) polyclonal antibody from Cell Signaling Technology (Beverly, MA). A polyclonal anti-GLUT4 rabbit antibody was raised in our laboratory to a peptide encompassing the last 12 COOH-terminal amino acids (22). ATF-2 fusion protein was purchased from New England Biolabs (Beverly, MA). The human GLUT4 promoter was a kind gift from Dr. J.E. Pessin, University of Iowa (23).

Animals. The GLUT4myc transgene was constructed in pHBluescript consisting of a 2.4-kb fragment human GLUT4 promoter (−2,316 to 84) (23), a 2.1-kb rat GLUT4myc cDNA, and a 0.5-kb poly A-tail signal sequence. The GLUT4myc transgenic founder mice were injected by injecting the linear transgene construct (released by NcoI and KpnI digestion) into the pronuclei of fertilized mouse embryos of C57Bl/6J mice. Transgenic animals were identified by Southern blot analysis of restriction enzyme–digested genomic DNA from tail biopsies using a radiolabeled probe recognizing GLUT4myc. Genotyping of progeny from a selected founder was performed by PCR analysis of tail DNA using a 24-bp 5′ primer (5′GCCCACTGTCCTCGGAGT3′) recognizing a sequence in the GLUT4 promoter and a 21-bp 3′ primer (5′GGTCGGTCTCC CCTCTAAGCAAGTC3′) recognizing part of the myc site of the GLUT4myc cDNA coding region.

All mice were housed in a pathogen-free environment on a 12-h light-dark cycle and fed ad libitum with standard rodent diet. All protocols have been approved by the Animal Care Committee of The Hospital for Sick Children.

GLUT4myc protein expression. Total membranes from a number of tissues were isolated as described earlier (24). A total of 750 μg protein was immunoprecipitated with a monoclonal anti-myc antibody (9E10; 2 μg per condition) overnight at 4°C. The immunocomplexes were then adsorbed to protein A-Sepharose. The immunocomplexes were released with two-times Laemmli sample buffer containing 0.02 mol/l N-ethylmaleimide, heated for 30 min at 2°C, resolved by 10% SDS-PAGE, and immunoblotted for GLUT4 with antibody, and the reading was subtracted from the experimental values. In some experiments, 2-deoxyglucose uptake was measured with 100 nmol/l wortmannin and with or without 100 nmol/l SB203580, respectively. This was followed by incubation with 100 nmol/l insulin for 30 min in the continued presence of the inhibitors. 2-Deoxy-3H-glucose uptake (final 2-deoxyglucose concentration: 10 μmol/l) was determined over a 5-min period. In some experiments, 2-deoxyglucose uptake was measured with 100 nmol/l indomethacin, 0.1% or 0.1% wortmannin, present only during the 5-min transport assay. Glycogen uptake was stopped by separating cells from the medium by centrifugation through an oil mixture (silicone oil DC550 with phthalic acid dinonyl ester, ratio 2:3) and then subjected to liquid scintillation counting. Cell number was determined by electronic Coulter counting. Cellular lipid content was determined by lipid extraction as follows: an aliquot of suspended cells was placed into the extraction solution consisting of isopropyl alcohol:heptane:sulfuric acid (66:1:4.4). After incubation for 1 h at room temperature, heptane and distilled water were added, mixed by vortexing, and centrifuged for 5 min at 1,000 rpm. The heptane layer (containing the lipids) was placed into a glass tube, dried, and weighed.

Detection of surface GLUT4myc by indirect immunofluorescence in intact brown adipocytes. Brown adipocytes were isolated as described above. Cells were suspended in RPMI, added to collagen-coated cover slips, and incubated at 37°C to settle for 1 hr. Cells were then incubated with an atmosphere of 5% CO2/95% air. Cells were then incubated for 50 min with DMSO vehicle only, 10 μmol/l SB203580, or 100 nmol/l wortmannin. Insulin (100 nmol/l) was added during the final 30 min of this incubation. Cells were washed in cold PBS and incubated for 1 h at 4°C with a monoclonal antibody against the myc epitope (9E10; 1:150). Cyanine 3–conjugated goat anti-mouse antibody (1:250) was used to label the primary anti-myc antibodies for 30 min at 4°C. Adipocytes were fixed with 4% (vol/vol) paraformaldehyde in PBS for 20 min and then washed with 0.05 mol/l NH4Cl in PBS for 10 min to react with excess paraformaldehyde. Coverslips were mounted on glass slides with Dako(R) Fluorescent Mounting Medium (Dako, Carpenteria, CA). Fluorescence was detected using a Leica DRB Inverted Fluorescence Microscope and quantitated using National Institutes of Health (NIH) Image version 1.61 (NIH, Bethesda, MD), and Adobe Photoshop version 6.0 (Adobe Systems, San Jose, CA) was used to calculate the average pixel density per unit area. Background fluorescence (determined in the absence of myc staining of brown adipocytes of nontransgenic animals) was subtracted to exclude myc staining of brown adipocytes of nontransgenic animals. There was no discernible signal above background fluorescence in these samples.

To detect intracellular myc staining, cells were fixed with ice-cold 4% (vol/vol) paraformaldehyde in PBS for 20 min immediately after incubation with insulin. After quenching with 0.05 mol/l NH4Cl in PBS for 10 min, cells were incubated in peroxidase Triton X-100 wash at room temperature and then blocked for 20 min with 5% goat serum in PBS. Incubation with primary and secondary antibody was performed as described in the preceding protocol. For confocal fluorescence imaging, a Zeiss Axiovert 100M laser scanning confocal microscope 510 was used.

Detection of surface GLUT4myc by an antibody-coupled colorimetric assay in intact brown adipocytes. After incubating isolated brown adipocytes with 100 nmol/l insulin for 30 min at 37°C, subcellular traffic of GLUT4myc was stopped by the addition of 2 mmol/l KCl (26). Cells were incubated with 9E10 antibody (1:200) for 1 h at room temperature. Adipocytes were then washed three times with KRHB containing 4% (wt/vol) fatty acid–free BSA to remove excess antibody and incubated for 30 min with peroxidase-conjugated donkey anti-mouse IgG (1:1,000) at room temperature. Cells were washed with 4% KRHB and then separated from the extracellular fluid by centrifugation through the silicone/phthalate mixture. Cells were resuspended in 1 ml OPD reagent (0.4 mg/ml OPD and 0.4 mg/ml urea hydrogen peroxide). The reaction was stopped after 30 min with 0.25 ml of 3 N HCl. The optical absorbance of the collected supernatant was measured at 492 nm. The linearity of the assay was confirmed using different amounts of basal or insulin-stimulated cells. Background was determined in each experiment by omitting the primary antibody, and the reading was subtracted from the experimental values. In every experiment, each condition was performed in duplicate.

In vitro p38 MAPK activity assay. Protein kinase activity was measured as described (27), with the following modifications: anti-p38 MAPK α or anti-p38 MAPK β antibodies (2 μg per condition) were preadsorbed to protein G-Sepharose beads. Suspended brown adipocytes were incubated with either 100 nmol/l insulin for 10 min or 600 nmol/l sorbitol for 20 min. Thereafter, cells were lysed in 1 ml lysis buffer containing phosphatase and protease inhibitors (27). Lysates were transferred to Eppendorf tubes and passed five times through a 25-gauge syringe and then centrifuged for 5 min (1,000 rpm at 4°C). Supernatants were collected, and 250 μg total protein were immunoprecipitated overnight with the preadsorbed Sepharose beads. Immunocom-
RESULTS

GLUT4myc is expressed in a tissue-specific manner.

The expression of GLUT4myc protein in different tissues was assessed by Western blot analysis of GLUT4myc immunoprecipitates prepared from detergent-solubilized membrane extracts (Fig. 1). GLUT4myc protein could be detected in immunoprecipitates from skeletal muscle (quadriceps and gastrocnemius), heart, and white adipose tissue (WAT) and BAT, whereas no GLUT4myc protein could be isolated from kidney, liver, brain, or testis. GLUT4myc expression was highest in brown adipocytes, as shown earlier for a reporter gene driven by the human GLUT4 promoter (23). Compared with endogenous GLUT4 protein levels, GLUT4myc expression was low in all tissues, making up <10% of endogenous GLUT4 levels in brown adipocytes. This amount was calculated from comparisons of the GLUT4 immunoblot signals (using polyclonal antibodies) of immunoprecipitates performed using monoclonal anti-myc or anti-GLUT4 antibodies. The ratio of GLUT4myc expression in the insulin-sensitive tissues was 9.1:4.1 for BAT:WAT:skeletal muscle.

Glucose and insulin tolerance are not altered in heterozygous GLUT4myc mice. Previous studies have shown that a 2- to 10-fold overexpression of GLUT4 in mouse tissues results in elevated glucose and insulin tolerance (28–31). Although GLUT4myc mice only express traces of the transgene, it was important to determine their ability to respond to insulin and to handle glucose. In the present study, the fasting blood glucose levels of 6-week-old heterozygous GLUT4myc mice (n = 11) did not significantly differ from littermate controls (n = 10) (7.4 ± 0.5 and 7.2 ± 0.4 mmol/l, respectively; P = 0.77, Student’s t test). Glucose tolerance tests did not show differences (P = 0.66, repeat-measures ANOVA) between control and GLUT4myc mice (Fig. 2A). Similarly, insulin tolerance tests did not differ significantly between the two groups (P = 0.53, repeat-measures ANOVA). Glucose and insulin tolerance tests performed at 3 months of age also failed to detect differences between control and GLUT4myc mice (P = 0.86 and P = 0.54, for each test) (Fig. 2B).

2-Deoxyglucose uptake into brown adipocytes is blocked by wortmannin and partly inhibited by SB203580. In isolated brown adipocytes of heterozygous GLUT4myc mice, we determined the effect of the phosphatidylinositol 3-kinase inhibitor wortmannin and the p38 MAPK inhibitor SB203580 on glucose uptake (Fig. 3A). Pretreatment with wortmannin (100 nmol/l, 20 min) completely inhibited the insulin-induced increase in glucose uptake (insulin 6.4 ± 0.1-fold, insulin plus wortmannin 0.95 ± 0.25-fold; P < 0.005, Student’s t test). Interestingly, preincubation with 10 μmol/l of the pyridinylimidazole SB203580 reduced insulin-stimulated glucose uptake by

**FIG. 1.** GLUT4myc is specifically expressed in insulin-dependent tissues. Total membrane fractions were collected from different tissues of heterozygous GLUT4myc mice. A total of 750 μg protein was immunoprecipitated (i.p.) with a monoclonal anti-myc antibody and then subjected to immunoblot analysis (i.b.) using a polyclonal anti-GLUT4 antibody specific for the C-terminus. A total membrane fraction from L6 GLUT4myc cells (35 μg) was immunoprecipitated and served as a positive control. NTG, nontransgenic mouse; Sk. m., skeletal muscle; TG, transgenic mouse.

**FIG. 2.** Glucose and insulin tolerance are similar in heterozygous GLUT4myc mice and littermate controls. A: Six-week-old mice were fasted overnight. Intraperitoneal glucose tolerance test (0.75 g glucose/kg body wt) was performed, and blood glucose was monitored in samples collected from the tail vein of conscious heterozygous GLUT4myc mice (○, n = 11) or nontransgenic mice (▲, n = 10) (left panel). Intraperitoneal insulin tolerance test (0.5 units insulin/kg body wt) was performed in conscious heterozygous GLUT4myc mice (○, n = 6) or nontransgenic mice (▲, n = 7) (right panel). B: Glucose tolerance (0.75 g glucose/kg body wt, left panel) and insulin tolerance tests (0.75 units insulin/kg body wt, right panel) were performed in 3-month-old heterozygous GLUT4myc mice (n = 9 in glucose tolerance test and n = 7 in insulin tolerance test) or nontransgenic mice (n = 8 in glucose tolerance test and n = 6 in insulin tolerance test).
results suggest that the insulin-induced increase in glucose uptake into brown adipocytes is entirely mediated by GLUT4. By comparison, basal glucose uptake was only partly inhibited by indinavir, suggesting that another transporter, possibly GLUT1 (which is insensitive to indinavir) contributes to basal glucose uptake.

**GLUT4myc translocation to the cell surface is blocked by wortmannin but unaffected by SB203580.** A major goal of this study was to assess whether the product of the GLUT4myc transgene can be regulated by insulin. The presence of GLUT4myc at the surface of intact brown adipocytes was measured by two different approaches: indirect immunofluorescence (Fig. 4A) and an antibody-coupled colorimetric assay (Fig. 4C). By either method, the insulin-dependent gain in surface GLUT4myc was only twofold (2.0 ± 0.1-fold and 2.1 ± 0.2-fold, respectively). This result is in sharp contrast to the much larger increase in glucose uptake caused by insulin (Fig. 3). To determine whether the inhibition of insulin-stimulated glucose uptake by either wortmannin or SB203580 was due to a reduction in GLUT4 translocation, we next measured cell surface GLUT4 levels in intact cells pretreated with each compound before and during the insulin stimulation.

To ascertain that the myc signal arose only from the cell surface and not from any spurious penetration of the antibodies into these nonpermeabilized cells, we used laser confocal fluorescence microscopy and carried out optical sections within the labeled cells. There was no discernable signal arising from intracellular spaces at any of the intracellular optical cuts. Figure 4B illustrates the GLUT4myc signal in surface-labeled adipocytes using an optical cut through the center of the cell. Only a rim of fluorescence is observed (upper panel). In contrast, abundant GLUT4myc signal was detected when cells were permeabilized with Triton X-100 before labeling the myc epitope (Fig. 4B, lower panel). These results confirm that GLUT4myc resides preferentially in intracellular stores and that the fluorescence assay used to label cell surface is not prone to contamination by intracellular signal.

**Insulin selectively stimulates the activity of the p38 MAPK β-isoform.** Figure 3A shows that insulin-stimulated 2-deoxyglucose uptake is partly inhibited by the p38 MAPK inhibitor SB203580. We have previously reported that insulin stimulates both the α- and β-isoforms of p38 MAPK in muscle cells in culture as well as in rat skeletal muscle (18,35). In isolated brown adipocytes, insulin stimulated the activity of the β-isoform of p38 MAPK by 1.9 ± 0.15-fold over basal (P < 0.01 compared with basal, Student’s t test). The basal activity of each isoform to a similar extent (α 1.2 ± 0.2-fold, P < 0.01). Insulin-stimulated 2-deoxyglucose uptake was completely abolished by the presence of 100 μmol/l indinavir in the transport solution (insulin 5.2 ± 0.95-fold above basal, insulin plus indinavir 0.52 ± 0.08-fold). These

55 ± 2% (insulin 6.7 ± 0.7-fold, insulin plus SB203580 3.0 ± 0.25-fold; P < 0.005). Basal glucose uptake was not affected significantly by either inhibitor.

To determine whether the inhibition of glucose uptake observed inFig. 3A might be due to a direct interaction of SB203580 with GLUT4, we measured 2-deoxyglucose uptake when the compound was present only during the transport assay (Fig. 3B). Neither basal nor insulin-stimulated 2-deoxyglucose uptake were affected by the presence of 10 μmol/l SB203580 in the transport solution (SB203580 0.96 ± 0.07-fold above basal, P = 0.56 compared with basal, Student’s t test; insulin 5.6 ± 0.15-fold; insulin plus SB203580 5.4 ± 0.37-fold, P = 0.63 compared with insulin alone). These results suggest that the effect of the pyridinylimidazoles is one of preventing insulin action rather than of inhibiting the transporters.

**Insulin-stimulated 2-deoxyglucose uptake into brown adipocytes is blocked by the HIV-protease inhibitor indinavir.** It was recently shown that the HIV-protease inhibitor indinavir binds to GLUT4 and thereby inhibits glucose uptake through this transporter but does not affect other GLUT isoforms (32–34). Here, we used indinavir to analyze the contribution of GLUT4 in insulin-mediated increase in glucose uptake in brown adipocytes (Fig. 3C). Insulin-stimulated 2-deoxyglucose uptake was completely abolished by the presence of 100 μmol/l indinavir in the transport solution (insulin 5.2 ± 0.95-fold above basal, insulin plus indinavir 0.52 ± 0.08-fold). These
isoform was virtually identical (α assigned an arbitrary value of 1, β 0.9 ± 0.1-fold; P = 0.47).

**DISCUSSION**

Insulin causes a rapid translocation of GLUT4 from intracellular membrane compartments to the plasma membrane of muscle and fat tissues, and this phenomenon is required for insulin-stimulated glucose uptake in skeletal muscle and adipose tissue (2,3). However, in spite of the use of different approaches to measure GLUT4 translocation, most studies report a smaller gain in GLUT4 at the surface of insulin-stimulated muscle and fat cells than the gain in glucose uptake. The documentation of GLUT4 translocation has largely relied on subcellular fractionation, immunodetection, or affinity photolabeling. Although greatly informative over the years, the first technique does not afford a calculation of the number of transporters present in the plasma membrane of intact tissues due to the low yield of membrane and incomplete separation of the individual membrane compartments. In addition, fractionation cannot distinguish GLUT4 vesicles incorporated into the plasma membrane from those docked but unfused or occluded. Immunodetection of GLUT4 epitopes on its cytoplasmic domains requires cellular permeabilization, membrane lawn generation, or tissue sectioning. These approaches cannot distinguish tethered/docked vesicles from fused ones and, moreover, can rarely reconstruct the entire cell surface. Affinity photolabeling with impermeant sugar-site binding ligands coupled to selective immunoprecipitation was designed as an alternative to these methods. However, the photolabels used (e.g., ATB-BMPA) react with the exofacial glucose binding site (36) and, therefore, labeling is compounded by the degree of activity of the exposed surface transporters (8,37,38). Not surprisingly, this method detects changes in transporter number that often parallel changes in glucose uptake, perhaps by excluding detection of transporters with low activity. Therefore, none of the existing methodologies allow for accurate quantification of GLUT4 translocation. Yet, an accurate quantification of GLUT4 translocation would be crucial to analyze the extent by which GLUT4 number explains the glucose transport.
response. The implications of this question to human health and disease are significant, since insulin resistance could conceivably arise not only from defects in GLUT4 translocation but also from defects in its activation.

To establish valid comparisons with glucose uptake measurements, it is necessary to quantify GLUT4 translocation in intact cells and tissues. From such a comparison, it will be possible to assess the contribution of changes in the intrinsic activity of GLUT4 if there is no contribution to glucose uptake from other transporters. Although the GLUT family comprises 13 members, only 6 of these are demonstrated GLUTs, and muscle and fat cells express predominantly GLUT4 (39). In rodents, BAT has the highest amount of GLUT4 per milligram protein (40,41). Moreover, in these cells, GLUT1 does not appear to carry any significant portion of the insulin-stimulated glucose uptake (10 and Fig. 3C).

We present here a new transgenic mouse model that has enabled us to accurately quantify GLUT4 translocation in intact brown adipocytes, along with measurements of glucose influx. In this animal model, GLUT4myc is expressed in a tissue-specific manner reflecting the endogenous occurrence of GLUT4 in mice (41). We find highest expression of the transgene in the brown adipocytes relative to white adipocytes, heart, or skeletal muscle. This finding is consistent with a previously published study using the same promoter driving the expression of a CAT (chloramphenicol acetyltransferase) reporter gene (41). However, expression of the GLUT4myc transgene is much lower than that of the reporter gene. Transgenic mice overexpressing GLUT4 (greater than twofold the endogenous levels) were shown to be more glucose tolerant and insulin sensitive (28–30). In contrast, glucose and insulin tolerance tests in the GLUT4myc-expressing mice do not differ significantly from littermate controls, probably due to the low amount of transgene expression.

Previous studies reported that the insulin-induced GLUT4 translocation in rodent brown adipocytes, measured by subcellular fractionation or ATB-BMPA photolabeling, did not correlate quantitatively with the magnitude of insulin-stimulated glucose uptake (9,10,42). Surface GLUT4 increased 1.5- to 3-fold when subcellular fractionation was used and 3- to 7.4-fold with ATB-BMPA photolabelling. In contrast, using immunogold electron microscopy to detect GLUT4 in ultrathin slices of brown adipocytes, the surface signal was calculated to increase by 40-fold (43). The large variation in translocation reported by these studies likely reflects the particularities of the approach. In none of these approaches was the cell surface intact at the time of analysis (isolated membranes, immunoprecipitates, and ultrathin slices are analyzed in each case). Hence, a method to directly measure surface GLUT4 in intact cells was wanting. The immunological procedures used here detect only the surface-exposed myc epitope, giving a measure of GLUT4 fully incorporated into the plasma membrane. Our results provide an alternative approach to measure surface GLUT4 through an epitope away from the glucose binding site and reveal that GLUT4 translocation (twofold) in brown adipocytes is markedly lower than the gain in glucose uptake (sixfold) in parallel cell preparations.

Given our above-mentioned observations, it became important to determine the possible contribution to glucose uptake of GLUT1, the other isoform expressed in these cells. In brown adipocytes from the rat, insulin stimulation did not alter the content of GLUT1 at the cell surface (10). In the present study, we show that the HIV protease inhibitor indinavir, which was previously shown to interact selectively with GLUT4 but not with GLUT1 or GLUT3 (33), inhibited basal and insulin-stimulated glucose uptake into mouse brown adipocytes by 50 and 90%, respectively (Fig. 3C). Collectively, these results strongly suggest that insulin-induced increase in glucose uptake in brown adipocytes is entirely dependent on GLUT4.

We hypothesize that the observed difference between the magnitude of GLUT4 translocation and glucose transport is due to an insulin-induced increase in the intrinsic activity of GLUT4. In addition, insulin-dependent GLUT4 translocation was dissociated from glucose uptake using the pyridinylimidazole SB203580, which inhibited insulin-dependent glucose uptake by 55% without preventing GLUT4 translocation. These results suggest that GLUT4 translocation is not sufficient to cause maximum stimulation of glucose uptake. An additional activation of GLUT4 appears to be necessary. This reduction in insulin-stimulated glucose uptake is unlikely due to a direct nonspecific interaction of SB203580 with GLUT4 because the inclusion of the pyridinylimidazole during the 2-deoxyglucose-uptake assay alone did not have an inhibitory effect, as demonstrated in the current study for brown adipocytes and recently reported for L6 myotubes (18). Our findings suggest that SB203580 interferes with an insulin-derived signal that leads to activation of GLUT4 rather than directly binding to GLUT4.

SB203580 is a selective inhibitor of two (α and β) of the four known p38 MAPK isoforms (44,45). Initially, p38 MAPK was identified as a “stress kinase” mounting a substantial activation in response to hyperosmolarity, hypoxia, or ultraviolet light in different tissues (46,47). However, it is now clear that p38 MAPK responds to insulin in muscle cells in culture as well as in rat skeletal muscle (18,35,48). In skeletal muscle and L6 myotubes, insulin increased the activity of both the α- and β-isoforms of this enzyme, each by about twofold (18,35). p38 MAPK is expressed in brown adipocytes (49), and, when activated via β-adrenergic signals, p38 MAPK induces an increase in uncoupling protein-1 expression (50). Interestingly, in the present study, we found that the β- but not the α-isoform of p38 MAPK is activated by insulin in isolated brown adipocytes. In contrast, both isoforms are stimulated to a similar extent by osmotic stress induced by 600 mmol/l sorbitol. Our findings may suggest that insulin’s proposed stimulatory effect on GLUT4 activity is mediated by the β-isoform of p38 MAPK. Several studies have confirmed a reduction in insulin stimulation of glucose uptake when preincubating L6 muscle cells and 3T3-L1 adipocytes with p38 MAPK inhibitors (51,52). Expression of a dominant-negative mutant of the p38 MAPKα-isoform in 3T3-L1 adipocytes, however, had no effect (52). These observations highlight the need to identify the p38 MAPK isoform specifically involved in regulating glucose uptake.

In conclusion, the GLUT4myc mouse is a useful model for measuring GLUT4 translocation in intact cells and tissues. In isolated brown adipocytes, insulin-stimulated
glucose uptake involves GLUT4 translocation and activation, the latter possibly via a p38 MAPK–dependent pathway. Future quantitative analysis of GLUT4 translocation and glucose transport in muscle and fat tissues from GLUT4myc mice with induced insulin resistance or diabetess will contribute to a better understanding of the underlying defects in insulin action.

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