Disruption of microtubules in rat skeletal muscle does not inhibit insulin- or contraction-stimulated glucose transport

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Ai, Hua, Evelyn Ralston, Hans P. M. M. Lauritzen, Henrik Galbo, and Thorkil Ploug. Disruption of microtubules in rat skeletal muscle does not inhibit insulin- or contraction-stimulated glucose transport. Am J Physiol Endocrinol Metab 285: E836–E844, 2003. —Insulin and muscle contractions stimulate glucose transport in skeletal muscle through a translocation of intracellular GLUT4 glucose transporters to the cell surface. Judged by immunofluorescence microscopy, part of the GLUT4 storage sites is associated with the extensive microtubule cytoskeleton found in all muscle fibers. Here, we test whether microtubules are required mediators of the effect of insulin and contractions. In three different incubated rat muscles with distinct fiber type composition, depolymerization of microtubules with colchicine for ≤8 h did not inhibit insulin- or contraction-stimulated 2-deoxyglucose transport or force production. On the contrary, colchicine at least partially prevented the 30% decrease in insulin-stimulated transport that specifically developed during 8 h of incubation in soleus muscle but not in flexor digitorum brevis or epitrochlearis muscles. In contrast, nocodazole, another microtubule-disrupting drug, rapidly and dose dependently blocked insulin- and contraction-stimulated glucose transport. A similar discrepancy between colchicine and nocodazole was also found in their ability to block glucose transport in muscle giant “ghost” vesicles. This suggests that the ability of insulin and contractions to stimulate glucose transport in muscle does not require an intact microtubule network and that nocodazole inhibits glucose transport independently of its microtubule-disrupting effect.

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cellular GLUT4 vesicles (7). However, more recently, disruption of microtubules in adipocytes by nocodazole was found to inhibit insulin-stimulated glucose uptake without significantly affecting GLUT4 translocation, apparently through a direct interaction with the transporter protein itself (14, 29). These apparent differences in the role of microtubules for GLUT4 translocation in adipocytes may be somewhat reconciled by the observation that, although the initial rate of GLUT4 endocytosis was not affected by microtubule depolymerization, the subsequent trafficking to the perinuclear region was blocked (29).

In the present study, we have examined the effect of colchicine and nocodazole on insulin- and contraction-stimulated glucose transport in three different rat muscles with distinct fiber type composition. We conclude that an intact microtubule network is not required for stimulation of glucose transport in skeletal muscle by either insulin nor muscle contractions.

MATERIALS AND METHODS

Materials. Nocodazole, colchicine, 2-deoxy-D-glucose (2-DG), sucrose, glucose, bovine serum albumin fraction V, pyruvic acid, and saponin were from Sigma. 2-Deoxy-D-[^3H]glucose and [14C]sucrose were from DuPont-NEN.

Muscle incubation and stimulation. The experiments were approved by the Animal Experiments Inspectorate of the Danish Ministry of Justice. Fed male Wistar rats (65–75 g body wt), obtained from Charles River Laboratories (Sulzfeld, Germany), were anesthetized by an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt), obtained from Charles River Laboratories (Sulzfeld, Germany), were anesthetized by an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt). The rats were perfused through the left ventricle for 1 min (flow 20 ml/min) with Krebs-Henseleit bicarbonate buffered medium containing 8 mM glucose, 1 mM pyruvic acid, and 0.2% BSA. The epithelium, flexor digitorum brevis (FDB), and soleus muscles, each enriched with a distinct fiber type (11), were gently dissected free with intact tendons at both ends and incubated for 30 min in perfusion medium in test tubes at 29°C. This temperature was maintained throughout the experiments. The medium was continuously gassed with 95% O2-5% CO2. After the 30-min equilibration period, muscles were placed in glucose-free Krebs-Henseleit buffer containing 2 mM pyruvic acid and 0.2% BSA for 8 h with or without colchicine and nocodazole at various concentrations (see legends to figures) before stimulation with either insulin or muscle contractions. To obtain maximum insulin stimulation, muscles were incubated for 30 min with 100,000 μU/ml of insulin (Actrapid, Novo Nordisk), which was also present during transport measurements. The effect of contractions was studied in the absence of any insulin stimulation. Muscles were directly electrically stimulated to contract during the last 11 min of incubation. A small clip was attached to each of the two tendons, and the muscle was vertically suspended in incubation medium, with or without colchicine or nocodazole, with the upper end attached to a force transducer connected to a computer for measurement of total force output (10). Electrodes were placed at both ends of the muscle, and 2 × 5 min of repeated tetanic contractions, separated by a 1-min break, were produced by stimulating with 25 V in 200-ms trains of 100 Hz, each impulse being 0.2 ms, and with a train frequency of 1/6 (17). During the first 10 s of contractions, the length of the muscle was adjusted to yield maximum force; i.e., the muscle was suspended at its resting length. Colchicine and nocodazole were dissolved in dimethyl sulfoxide (DMSO), which was also added to the control groups to a final concentration of 0.05%.

Measurement of 2-DG transport in muscles. 2-DG transport was measured as described (9). Briefly, immediately after contractions, muscle clips were removed and the muscles placed in test tubes at 29°C for measurement of glucose transport. Transport was measured as 2-deoxy-D-[3H]glucose ([3H]2-DG) uptake with [14C]sucrose as extracellular marker. Isotopes and unlabeled sugars were added to the incubation medium (Krebs-Henseleit buffer containing 2 mM pyruvic acid and 0.2% BSA with or without colchicine or nocodazole) to yield final concentrations of 0.43 μCi of [3H]2-DG and 0.32 μCi of [14C]sucrose per milliliter and 1 mM of both unlabeled 2-DG and sucrose. After 10 min of exposure to isotopes, muscles were briefly blotted on filter paper and immediately frozen in liquid nitrogen. Muscles were stored at −80°C until analyzed.

Measurement of glucose transport in giant vesicles. Giant vesicles from muscle plasma membrane were produced from pooled rat thigh and calf muscles by collagenase digestion in a KCl buffer as described (22). Transport was measured at room temperature as [3H]2-DG uptake with [14C]sucrose as extracellular marker at a final concentration of 1 mM of both unlabeled 2-DG and sucrose (22). Giant vesicles were incubated for <5 min with colchicine or nocodazole in DMSO, final concentration 0.05%.

Immunocytochemical staining of teased muscle fibers. After incubation of intact muscles with or without colchicine or nocodazole, muscles were pinned down at resting length in petri dishes coated with Sylgard 184 (Dow Corning, La Hulpe, Belgium). Muscles were incubated with Krebs-Henseleit bicarbonate buffer containing procaine hydrochloride (1 g/l) for 3 min followed by 2% freshly depolymerized paraformaldehyde and 0.15% picric acid in 0.1 M Sorensen’s phosphate buffer at room temperature for 45 min. The muscles were left for an additional 4 h in the fixative at 4°C and transferred to PBS. Bundles of one to three fibers were teased from fixed muscles with fine forceps and transferred to 50 mM glycine in PBS. Nonspecific binding was blocked with 50 mM glycine, 0.25% bovine serum albumin, 0.03% saponin, and 0.05% sodium azide in PBS for 30 min. Fibers were then incubated overnight with a mouse monoclonal anti-α-tubulin antibody (Sigma) diluted in blocking buffer. After three washes of 30 min each, they were incubated for 2 h with Alexa 488-conjugated goat anti-mouse antibody (Molecular Probes, Eugene, OR) in blocking buffer, stained for 5 min with Hoechst 33342 (0.5 μg/ml) in blocking buffer, washed three times, and mounted in Vectashield (Vector Laboratories, Burlingame, CA). Confocal immunofluorescence microscopy was performed with a Zeiss LSM 410 system (Carl Zeiss, MicroImaging, Thornwood, NY) or a Leica SP2 confocal system (Leica, Mannheim, Germany).

Western blot. Frozen muscles were homogenized with a Polytron PT 3100 (Kinematica, Littau-Luzern, Switzerland) in 1.2 ml of 90°C buffer (4% SDS, 10 mM sodium pyrophosphate, 2 mM sodium orthovanadate, 10 mM EDTA, and 25 mM Tris, pH 6.8), briefly sonicated, and stored in aliquots at −80°C. Protein concentration was determined by the bicinchoninic acid assay (Sigma) using bovine serum albumin as a standard. For Western blot, 25 μg of protein were separated by SDS-PAGE on a 10% Criterion gel (Bio-Rad) and electrophoretically transferred to PVDF membranes for 30 min at 20 V by use of a semidyne system (Bio-Rad). Transfer buffer contained 48 mM Tris, 39 mM glycine, 0.019% SDS, and 5% methanol. Membranes were blocked in 5% defatted milk powder in TS buffer (10 mM Tris (pH 7.4), 150 mM NaCl), incubated for 90 and 60 min with primary and horseradish

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peroxidase-labeled secondary antibodies, respectively, and diluted in blocking solution. Antigen-antibody complexes were visualized by enhanced chemiluminescence and pre-flashed films (Hyperfilm ECL, Amersham Pharmacia) and quantitated with a GS-710 densitometer (Bio-Rad).

Monoclonal antibody F-18 was used for detection of GLUT1 and monoclonal antibody F-27 for detection of GLUT4 (18).

Statistics. Groups were compared by Student’s unpaired t-test or ANOVA as applicable. In the latter case, statistically significant differences were localized by the Student-Newman-Keuls method. \( P < 0.05 \) was considered significantly different in two-tailed testing.

RESULTS

Effect of colchicine on 2-DG uptake in soleus muscles. From Fig. 1 it can be seen that preincubation of soleus muscles with the microtubule depolymerizing agent colchicine at various concentrations (1–25 \( \mu \text{g/ml} \)) for \( \leq 3 \text{ h} \) did not inhibit insulin-stimulated 2-DG uptake at all. In fact, colchicine partially prevented the time-dependent development of insulin resistance in the soleus muscle. Thus, in the absence of colchicine, the maximum insulin-stimulated glucose transport was reduced by \( \sim 35\% \) (\( P < 0.001 \)) after 180-min preincubation. In contrast, during preincubation with either 10 or 25 \( \mu \text{g/ml} \) colchicine, only a \( \sim 20\% \) decrease was seen within this time period (\( P < 0.01 \)).

The lack of an inhibitory effect of colchicine on insulin-stimulated 2-DG uptake in soleus muscles (Fig. 1) could be due to too short an incubation time with the depolymerizing agent. We therefore incubated three different muscles with distinct fiber type composition for \( \leq 8 \text{ h} \) with 10 \( \mu \text{g/ml} \) colchicine. Even 8 h of incubation with colchicine did not inhibit insulin-stimulated, or contraction-stimulated, 2-DG uptake in either soleus, FDB, or epitrochlearis muscles (Fig. 2). Consistent with this, colchicine also did not inhibit the total amount of force produced during 10 min of contractions (Fig. 3). From Fig. 1 it appeared that, in the absence of colchicine, the soleus muscle became increasingly resistant to insulin stimulation, with incubation times \( \leq 3 \text{ h} \). The insulin resistance was not increased any further after 8 h of incubation, when the maximum insulin response was decreased by \( \sim 30\% \) (\( P < 0.05 \)) in the soleus muscle but remained unchanged in the FDB and epitrochlearis muscles (Fig. 2). This was not due to a decrease in the amount of glucose transporters due to long incubation times in vitro. Thus, after 8 h of incubation, GLUT4 protein in soleus muscles, determined by Western blot, was unchanged, whereas the amount of GLUT1 protein had increased by \( \sim 50\% \) (Fig. 4). Interestingly, the development of insulin resistance in the soleus muscle was not accompanied by resistance to the effect of contractions. Furthermore, it was abolished by colchicine.

Morphological effects of colchicine and nocodazole on microtubules in skeletal muscle. The absence of an inhibitory effect of colchicine on insulin- and contraction-stimulated glucose transport could also be due to resistance of the microtubule network to depolymerization. To examine this, teased fibers from muscles that had been preincubated with or without colchicine or nocodazole were incubated with an antibody against tubulin and observed by immunofluorescence microscopy. In the absence of colchicine, dense bundles of microtubules surrounded muscle nuclei and an extensive network of microtubules was also seen between nuclei (Fig. 5). Already, after treatment with colchicine for 2 h, a substantial part of the microtubules had been disrupted, and after 8 h most microtubules between nuclei had disappeared, whereas a substantial number of those surrounding the nuclei was still present (Fig. 5). Treatment of intact muscles for 2 h with a different microtubule-depolymerizing drug, nocodazole, gave results quite similar to those obtained with colchicine, whereas after 8 h most of those microtubules surrounding nuclei had also disappeared (Fig. 5).

Effect of nocodazole on 2-DG uptake in soleus muscles. Figure 6 shows the dose-dependent effect of 30- or 60-min preincubation with nocodazole (1–25 \( \mu \text{g/ml} \)) on insulin-stimulated 2-DG uptake in soleus muscles. At the lowest concentration tested (1 \( \mu \text{g/ml} \)), insulin-stimulated 2-DG uptake was already inhibited by \( \sim 50\% \) (\( P < 0.05 \) vs. no nocodazole), and it was completely blocked at 25 \( \mu \text{g/ml} \) (\( P > 0.05 \) vs. basal). The inhibitory effect of nocodazole on insulin-stimulated 2-DG uptake was rather fast, as preincubation for 60 min was only slightly more effective than preincubation for 30 min. Nocodazole also effectively inhibited contraction-stimulated 2-DG uptake (Fig. 6). Thus, at 5 \( \mu \text{g/ml} \), contrac-

![Fig. 1. Effect of colchicine on insulin-stimulated glucose transport in soleus muscle. Muscles were preincubated for either 30, 90, or 180 min in the absence or presence of either 1, 10, or 25 \( \mu \text{g/ml} \) colchicine. Muscles were then stimulated with maximum insulin for 30 min or left unstimulated before measurement of 2-deoxyglucose (2-DG) uptake for 10 min. Data are means \( \pm \) SE; \( n = 5 \). *Different from experiments with 10 and 25 \( \mu \text{g/ml} \) colchicine.](http://ajpendo.physiology.org/doi/10.1210/en.285.10.E838)
tion-stimulated 2-DG uptake was inhibited by \(~60\%\) (\(P < 0.05\) vs. no nocodazole), and it was completely blocked at 25 \(\mu\)g/ml (\(P < 0.05\) vs. basal). Finally, basal glucose transport was also progressively inhibited by increasing concentrations of nocodazole (Fig. 6). However, basal transport appeared to be less sensitive to inhibition than insulin- or contraction-stimulated transport. Thus, at the highest concentration tested (25 \(\mu\)g/ml), basal glucose transport was inhibited only \(~50\%\).

**Effect of nocodazole and colchicine on 2-DG uptake in muscle giant vesicles.** The surprising observation that nocodazole, in contrast to colchicine, had a very rapid and complete inhibitory effect on insulin-stimulated glucose transport suggested that nocodazole might have a direct inhibitory action on the glucose transport process itself. This was further supported by the observation that incubation with 25 \(\mu\)g/ml nocodazole for only 2.5 min partially inhibited insulin-stimulated 2-DG uptake in intact soleus muscles (data not shown). Additional evidence was obtained by measuring 2-DG uptake into giant membrane vesicles. Such vesicles consist of a limiting membrane, derived from the muscle fiber plasma membrane, and an interior, filled with mainly cytoplasmic components. The limiting vesicle membrane corresponds to an insulin-stimulated
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DISCUSSION

In the present work, we have shown that the extensive microtubule cytoskeleton in muscle fibers can be disrupted without any deleterious effect on the ability of insulin and muscle contractions to increase glucose transport. Consistent with an unhampered contraction-stimulated glucose transport, the microtubule network also does not appear to play any role for force production during contraction. The tendency of the soleus muscle to become insulin resistant during prolonged incubations is not accompanied by impairment of contraction-induced glucose transport. Surprisingly, it is prevented by disruption of the microtubule cytoskeleton. Finally, nocodazole, in contrast to colchicine, appears to have a marked, direct inhibitory effect on the glucose transport process itself.

Treatment of intact soleus muscles with colchicine at various concentrations (1–25 µg/ml) for up to 3 h did not inhibit insulin-stimulated glucose transport at all (Fig. 1). This led us to study muscles during longer incubations with colchicine. Treatment of muscles in vitro with 10 µg/ml (≈25 µM) colchicine for 8 h resulted in depolymerization of the majority of microtubules in the soleus muscle (Fig. 5) as well as in the FDB and epitrochlearis muscles (data not shown). The effect of colchicine was rather rapid, since already after 2 h a substantial part of the microtubules had been disrupted (Fig. 5). A minor fraction of the microtubules surrounding the nuclei was not depolymerized, even after 8 h, possibly because a longer depolymerization time was needed. Colchicine acts by forming complexes with soluble tubulin, a binding reaction that is slow and may take hours. Depending on the concentration, tubulin addition at the microtubule ends is then either reduced or blocked completely (12). However, despite the extensive disruption of the microtubule network by colchicine in all three different muscles, the ability of insulin and muscle contractions to increase glucose transport was unhampered (Fig. 2). This was unexpected considering that, in 3T3-L1 cultured adipocytes, a sizeable decrease in insulin-induced GLUT4 translocation (40–80%) has been reported upon microtubule disruption by colchicine (5) as well as by nocodazole (4, 5, 7, 15). We therefore also studied the effect of nocodazole and found that it agreed with the mentioned fat cell studies. In addition, the effect of contractions on glucose transport was inhibited by nocodazole as well (Fig. 6). Interestingly, basal glucose transport could only be inhibited ~50% by nocodazole in contrast to an ~100% inhibition of insulin- and contraction-stimulated glucose transport (Fig. 6). If we assume that insulin and contraction mediate their effects on glucose transport in muscle exclusively through GLUT4, then the presence of a residual, noninhibitable basal glucose transport may be mediated through a non-GLUT4 transporter (e.g., GLUT1). This suggests that nocodazole preferentially (exclusively?) inhibits GLUT4. The discrepancy between the respective effects of colchicine and nocodazole might reflect actions of nocodazole different from its effects on microtubules. This is supported by the observation that incubation with 25 µg/ml nocodazole for only 2.5 min inhibited insulin-stimulated 2-DG uptake by ~40% in soleus muscles (data not shown). During such a short time, depolymerization of microtubules in intact muscles would probably be negligible. Additional evidence was obtained by measuring glucose uptake into giant ghost vesicles, where uptake was inhibited ~80% by incubation with nocodazole for fewer than 5 min, whereas colchicine did not inhibit glucose transport (Fig. 7). In these ghost vesicles, since the procedure for preparing vesicles probably results in activation of the glucose transport system similar to the activation by insulin but not by contractions (22). In these “ghost” vesicles, uptake of 2-DG was inhibited ~80% by incubation with nocodazole for <5 min (P < 0.001), whereas colchicine did not inhibit glucose transport (P > 0.05; Fig. 7).

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vesicles, the glucose transporters are already located in the vesicle membrane; i.e., no translocation takes place, which indicates that nocodazole acts by direct inhibition of the GLUT4 transport activity. A similar conclusion has recently been obtained from studies of 3T3-L1 adipocytes (14, 29). It was found that, at concentrations below 2–3 μM, nocodazole depolymerized microtubules but did not impair insulin-stimulated glucose transport. At higher concentrations, there was a dose-dependent inhibition of insulin-stimulated transport despite the absence of any significant impairment of GLUT4 translocation. However, in the present study, we did not measure GLUT4 translocation directly, but only glucose transport. In cultured myotubes, insulin stimulation of glucose transport requires at least two steps, i.e., recruitment of transporters to the cell surface from intracellular pools and an increase in the intrinsic activity of the transporters (6). If a similar mechanism is operating in fully differentiated muscle fibers, microtubule disruption could hypothetically lead to an increase in the activation step, which then possibly could compensate for a reduction in GLUT4 translocation. Another pitfall in the interpretation of our findings could result from downregu-

Fig. 5. Confocal imaging of microtubules in single muscle fibers. Single fibers were teased from fixed soleus muscles that had been incubated for 2 h (A, B, C) or 8 h (D, E, F) in the absence (A, D) or presence (B, E) of 10 μg/ml colchicine or 10 μg/ml nocodazole (C, F). Then the fibers were stained with an antibody against α-tubulin and observed with a confocal microscope. In the absence of depolymerizing drugs, nuclei are surrounded by dense bundles of interlacing microtubules in their equatorial plane. Between nuclei an extensive network of microtubules can be seen. Already after 2 h of incubation with either colchicine or nocodazole, a substantial part of microtubules had disappeared (A, B, C). After treatment with colchicine for 8 h (E), most microtubules between nuclei had disappeared, whereas a substantial part of the “perinuclear ring” was still present. However, after treatment with nocodazole for 8 h (F), most of the microtubules surrounding muscle nuclei had also disappeared. Findings are representative of ≥10 fibers from each condition.
lation of GLUT4 during prolonged muscle incubation in vitro. This could hypothetically be masked by a compensatory upregulation of other glucose transporters, which most likely would be constitutively residing in the plasma membrane. However, even after 8 h of incubation, we did not find any significant reduction in GLUT4 protein in muscle (Fig. 4). Interestingly, GLUT1 was upregulated by ~50% after 8 h, despite no increase in basal glucose transport (Fig. 4). This could possibly reflect the newly synthesized transporters not yet having been inserted into the plasma membrane. Taken together, results from adipocytes (14, 29) as well as our present findings in muscle demonstrate that disruption of the microtubule cytoskeleton does not prevent GLUT4 translocation induced by either insulin or contractions.

Although microtubules are not involved in acute GLUT4 translocation, they may well be of importance for the positioning of GLUT4 within the cell, because disruption of microtubules in adipocytes prevents the return of endocytosed GLUT4 transporters to the perinuclear region (29). Furthermore, Emoto et al. (4) have shown that microtubule disruption in 3T3-L1 adipocytes disperses the perinuclear GLUT4-containing vesicles. This observation can be explained by the well-known dispersal of the Golgi complex and, thereby, the large GLUT4 storage sites in myotubes or muscle fibers. Still, all of the large and some of the small GLUT4 storage sites in skeletal muscle are found along microtubules (19, 24, 26). This suggests that, rather than being functionally implicated in the acute effects of insulin and muscle contractions on GLUT4 translocation, the microtubules may be important for the fiber type-specific subcellular distribution of the large GLUT4 elements (24). Experiments with chronic stimulation of denervated muscle are in accordance with this view. Slow fibers stimulated with the pattern of a fast motor neuron acquire fast-fiber properties and vice versa (3, 16, 28). Such experiments in originally slow, type I fibers have, within 2 wk, elicited a transition in the distribution of both the large GLUT4 elements and the microtubule system toward a type IIB phenotype and vice versa in originally fast, type IIB fibers (19, 26). The driving force behind the plasticity of the distribution of the large GLUT4 elements is probably to be found in the plasticity of the microtubule cytoskeleton, which also affects the localization of the endoplasmic reticulum exit sites (26). How different muscular activity patterns are translated into determining the subcellular organization of the microtubules is not known and may affect several mechanisms (nucleation, stability, and dynamics).

![Fig. 6. Effect of nocodazole concentration on basal and insulin- and contraction-stimulated glucose transport in soleus muscle.](image)

![Fig. 7. Effect of nocodazole and colchicine on glucose transport in muscle giant vesicles.](image)

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Contraction-induced glucose transport varies directly with the force output of the contracting muscle (10, 11). This is consistent with the present finding that colchicine-induced disruption of the microtubule network affected neither contraction-stimulated glucose transport (Fig. 2) nor total force output (Fig. 3). The latter observation is similar to findings in normal cardiac myocytes, where the majority of evidence shows that disruption of the microtubule cytoskeleton by colchicine does not significantly modulate cardiac contractility or the intracellular Ca$^{2+}$ concentration transient (2).

Interestingly, the soleus muscle, but not the FDB or epitrachelis muscles, became increasingly insulin resistant during incubation for up to 8 h. This could be almost completely prevented by incubation with colchicine (Figs. 1 and 2). The mechanism for this effect cannot be explained at present but likely requires disruption of microtubules, which were already substantially depolymerized after 2 h of incubation with either colchicine or nocodazole (Fig. 5). However, it is interesting to note that, in cultured myotubes, depolymerization of the microtubule network results in an apparent fusion of the intracellular GLUT4 storage sites with the endosomal system (25). In the present study, the decrease with time in insulin-stimulated glucose transport in soleus muscles was not accompanied by a decrease in the ability of contractions to increase glucose transport. The lack of parallel changes in the effect of insulin and contractions on glucose transport has been noted previously during other conditions (18, 20) and further adds to the view that these stimuli act by different mechanisms.

In conclusion, the present findings suggest that the ability of insulin and muscle contractions to stimulate glucose transport in muscle does not require an intact microtubule cytoskeleton. Furthermore, nocodazole inhibits glucose transport independently of its microtubule-disrupting effect.

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DISCLOSURES

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