GLUT4 Is Reduced in Slow Muscle Fibers of Type 2 Diabetic Patients

Is Insulin Resistance in Type 2 Diabetes a Slow, Type 1 Fiber Disease?

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To gain further insight into the mechanisms underlying muscle insulin resistance, the influence of obesity and type 2 diabetes on GLUT4 immunoreactivity in slow and fast skeletal muscle fibers was studied. Through a newly developed, very sensitive method using immunohistochemistry combined with morphometry, GLUT4 density was found to be significantly higher in slow compared with fast fibers in biopsy specimens from lean and obese subjects. In contrast, in type 2 diabetic subjects, GLUT4 density was significantly lower in slow compared with fast fibers. GLUT4 density in slow fibers from diabetic patients was reduced by 9% compared with the weight-matched obese subjects and by 18% compared with the lean control group. The slow-fiber fraction was reduced to 86% in the obese subjects and to 75% in the diabetic subjects compared with the control group. Estimated GLUT4 contribution from slow fibers was reduced to 77% in the obese subjects and to 61% in type 2 diabetic patients compared with the control subjects. We propose that a reduction in the fraction of slow-twitch fibers, combined with a reduction in GLUT4 expression in slow fibers, may reduce the insulin-sensitive GLUT4 pool in type 2 diabetes and thus contribute to skeletal muscle insulin resistance. Diabetes 50:1324–1329, 2001

It is generally accepted that type 2 diabetes is associated with an impaired insulin-stimulated glucose disposal rate, which has been attributed to insulin resistance in skeletal muscle. A number of studies have focused on the glucose transporter system as part of the underlying mechanisms. Glucose transport across the cell membrane of skeletal muscle is mediated by the glucose transporter proteins GLUT1 and GLUT4 (1). The GLUT1 glucose transporter isoform is believed to support basal glucose transport (2,3), whereas the GLUT4 isoform increases glucose transport in response to insulin and contraction. Insulin and contractions induce translocation of GLUT4 from intracellular storage vesicles to the plasma membrane and to the transverse tubules (4–6). Currently, most studies have failed to demonstrate reduced GLUT4 expression levels in skeletal muscle biopsy specimens from type 2 diabetic patients (7–10). The insulin-stimulated translocation mechanism, however, has been found to be normal in one study (11) and impaired in others (12,13). Therefore, the role of GLUT4 in the pathogenesis of type 2 diabetes has not yet been clearly established.

Human skeletal muscle consists of slow-twitch oxidative (type 1) and fast-twitch nonoxidative (type 2) fibers. Slow-twitch fibers are both more insulin-sensitive and more insulin-responsive compared with fast-twitch fibers (13–16). Fast-twitch fibers are more sensitive to contraction than slow-twitch fibers (17). We have recently developed a sensitive method that allows estimation of GLUT4 density in individually fiber-typed skeletal muscle fibers by a combination of immunohistochemistry and stereology (18). With this technique, a higher GLUT4 expression in slow-twitch fibers compared with fast-twitch muscle fibers was found (18). The fraction of slow muscle fibers has been reported to be inversely related to adiposity (14,16, 19) and significantly lower in patients with type 2 diabetes compared with obese or control subjects (19). Thus, defects specific to type of muscle fiber may contribute to the diabetic phenotype.

To gain further insight into the mechanisms underlying muscle insulin resistance, our aim was to study the influence of obesity and type 2 diabetes on GLUT4 immunoreactivity in slow-twitch and fast-twitch fibers separately, using the combined method of immunohistochemistry and stereology.

RESEARCH DESIGN AND METHODS

Study population. Eight obese type 2 diabetic patients, nine obese control subjects, and nine young lean control subjects participated in the study. Only sedentary men were recruited. The two obese groups were matched for age and BMI, and their obesity was of the android type, as indicated by their high waist-to-hip ratio (Table 1). None of the diabetic patients had received insulin treatment; three patients were treated with diet alone, and five patients were taking oral medication with metformin or sulfonylurea, which was withdrawn 4 days before the study. Simplex retinopathy was the only complication of diabetes noted in these patients. The control subjects had normal fasting glucose and HbA1c, and no family history of diabetes. The protocol for the study was approved by the local ethical committees of Funen and Vejle counties, Denmark, and informed consent was received from all subjects before participation.

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f-GLUT4, GLUT4 contribution from fast-twitch fibers; HRP, horseradish-peroxidase; s-GLUT4, GLUT4 contribution from slow-twitch fibers.

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were performed in the sections stained for GLUT4 and fast myosin and vice versa. The area fraction of each fiber type was determined as the number of points associated with a fiber type divided by the total number of points counted. The contribution of GLUT4 from slow fibers (≥GLUT4) in a muscle biopsy specimen was estimated as the mean GLUT4 density in slow fibers multiplied by the slow-fiber area fraction. The contribution of GLUT4 from fast fibers (<GLUT4) was estimated similarly.

**Western blotting.** For muscle biopsy specimens in which >10 mg was available after histomorphometric analyses, total GLUT4 expression was determined by Western blotting. A total of 10–30 μg was homogenized in 50 mmol/l HEPES (pH 7.6), 250 mmol/l sucrose buffer containing molybdate 20 mmol/l, phenylmethylsulfonyl fluoride 1.5 mmol/l, EDTA 10 mmol/l, pepstatin A 1 μmol/l, leupeptin 1 μmol/l, and aprotinin 400 Kallikrein inhibiting U/ml for 2 × 10 s. A crude membrane fraction was isolated from the supernatant, as described previously (7), and its protein concentration was determined by the Bradford method (Biorad, Copenhagen). Western blotting and densitometry were performed as described previously (7).

**Statistical analysis of data.** Data in text, tables, and figures are means ± SE. Statistical analyses were performed with INSTAT 2.01 (GraphPad, San Diego, CA). Nonparametric statistical analyses of data were used: Mann-Whitney U test for unpaired comparisons, Kruskal-Wallis test for unpaired comparisons between more than two groups, and Spearman’s rank correlation coefficient r for analysis of covariance.

**RESULTS**

**Subject characteristics.** Eight middle-aged obese men, eight type 2 diabetic patients matched for age and gender, and nine young healthy control subjects participated in the study. Clinical data are listed in Table 1.

**GLUT4 density and fiber type.** The GLUT4 immunoreactivity in sections of striated muscle from all subjects expressed distinct granular reactions in association with the cell surface. Few grains were found in deeper parts of the sarcoplasm (Fig. 1). Due to its distinct granular appearance, the GLUT4 immunoreactivity could be quantified by counting. GLUT4 density in slow-twitch fibers was significantly higher in slow-twitch fibers compared with fast-twitch fibers in biopsy specimens from both control subjects (2.9 ± 0.1 vs. 2.7 ± 0.1 n/poin, P < 0.04) and obese subjects (2.6 ± 0.1 vs. 2.3 ± 0.0 n/poin, P < 0.02). In contrast, in type 2 diabetic subjects, GLUT4 density was significantly lower in slow-twitch fibers compared with fast-twitch fibers (2.4 ± 0.1 vs. 2.9 ± 0.1 n/poin, P < 0.007) (Fig. 2).

**GLUT4 density and study groups.** GLUT4 density in slow-twitch fibers was reduced by 9% in obese subjects compared with the lean control subjects (P < 0.05) and by 18% in type 2 diabetic patients compared with lean control subjects (P < 0.001) (Fig. 2). GLUT4 density in slow-twitch fibers was significantly lower in type 2 diabetic patients compared with the obese subjects (P < 0.03). BMI and GLUT4 density in slow fibers were inversely correlated (r = −0.48, P < 0.02, n = 25), and fasting glucose and GLUT4 density in slow fibers were inversely correlated (r = −0.7, P < 0.05, n = 25). GLUT4 density in fast-twitch fibers was unchanged in type 2 diabetic patients compared with control subjects; however, in obese subjects, GLUT4 density was reduced by 18% compared with control subjects (P < 0.0002) and by 20% compared with type 2 diabetic patients (P < 0.0001) (Fig. 2).

**Characteristics of muscle fibers.** Needle biopsy specimens of skeletal muscle were analyzed for fiber-type composition. The fraction of slow fibers was 0.51 ± 0.02 in the control subjects and was significantly reduced to 0.44 ± 0.03 in the obese subjects (P < 0.05) and to 0.38 ± 0.01 in the type 2 diabetic patients (P < 0.05). The ratio of fast to slow fibers was significantly increased from the control subjects to the obese subjects and was further increased in type 2 diabetic patients (P < 0.001) (Fig. 2).

**TABLE 1**

<table>
<thead>
<tr>
<th>Clinical data</th>
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<tr>
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<tr>
<td>Control</td>
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<tr>
<td>Age (years)</td>
<td>24.6 ± 1.3</td>
<td>50.6 ± 2.3</td>
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<td>Weight (kg)</td>
<td>79.1 ± 3.0</td>
<td>98.9 ± 4.6</td>
<td>98.7 ± 3.0</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>23.4 ± 0.7</td>
<td>31.7 ± 1.0</td>
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<td>Waist-to-hip ratio</td>
<td>0.85 ± 0.01</td>
<td>1.03 ± 0.00</td>
<td>1.02 ± 0.02</td>
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<td>Fasting glucose (mmol/l)</td>
<td>4.5 ± 0.1</td>
<td>4.8 ± 0.1</td>
<td>8.3 ± 0.8</td>
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<td>Fasting Insulin (pmol/l)</td>
<td>37.7 ± 4.4</td>
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<td>HbA1c (%)</td>
<td>4.9 ± 0.1</td>
<td>5.4 ± 0.1</td>
<td>7.3 ± 0.5</td>
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Data are means ± SE. *P < 0.05 vs. control; †P < 0.05 vs. obese.
0.05 in the type 2 diabetic patients \((P < 0.05)\) (Table 2).

The decreased slow-fiber fraction in type 2 diabetic subjects was not significantly different from obese subjects \((P > 0.19)\). The mean fiber diameters were significantly higher in fast fibers compared with slow fibers in all study groups \((P < 0.02)\) (Table 2).

**TABLE 2**  
**Morphometry**  

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Control subjects</th>
<th>Obese subjects</th>
<th>Type 2 diabetic subjects</th>
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<tr>
<td>Fast-fiber fraction (%)</td>
<td>48.7 ± 2.1</td>
<td>56.1 ± 2.7*</td>
<td>62.3 ± 4.9*</td>
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<tr>
<td>Slow-fiber fraction (%)</td>
<td>51.3 ± 2.1</td>
<td>43.9 ± 2.7*</td>
<td>37.7 ± 4.9*</td>
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<td>Fast-fiber diameters (μm)</td>
<td>74.1 ± 1.7</td>
<td>67.3 ± 1.4</td>
<td>71.6 ± 1.1</td>
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<tr>
<td>Slow-fiber diameters (μm)</td>
<td>67.2 ± 1.2†</td>
<td>63.5 ± 1.6†</td>
<td>68.2 ± 0.5†</td>
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Data are means ± SE. *P < 0.05 vs. control; †P < 0.02 the corresponding fast diameter.
**DISCUSSION**

In this study, immunohistochemistry and morphometry were used to study the relationship between GLUT4 expression and muscle fiber type in male obese subjects, type 2 diabetic subjects, and young lean control subjects. This is the first study addressing the GLUT4 expression in individually fiber-typed muscle fibers to gain further insight into the underlying mechanisms of insulin resistance in obesity and type 2 diabetes. Our major findings were a reduction in GLUT4 density determined by Western blotting with data from immunomorphometry, GLUT4 contribution (as defined in **RESEARCH DESIGN AND METHODS**) from slow and fast fibers must be used. Therefore, a reduced GLUT4 contribution (and GLUT4 density) from slow-twitch fibers could be easily blurred by an increased GLUT4 contribution (despite an unchanged GLUT4 density) from fast-twitch fibers, leaving overall GLUT4 unchanged, in parallel with results from studies using the Western blotting technique. Nevertheless, GLUT4 density and contribution from the more insulin-sensitive and insulin-responsive slow-twitch fibers are reduced in type 2 diabetes.

The question has been raised as to whether a putative reduction in GLUT4 would be reflected in glucose-uptake rates. In a recent study, a mouse model of type 2 diabetes was generated by genetic disruption in one allele of GLUT4 in muscle and adipose tissue (32). The mice exhibited reduced whole-body glucose utilization as well as reduced glucose uptake in skeletal muscle, hyperglycemia, and hyperinsulinemia. Insulin sensitivity and glucose uptake rates could be normalized by introduction of transgenic GLUT4 expression. These results emphasize the decisive role of GLUT4-mediated skeletal muscle glucose uptake in insulin-stimulated whole-body glucose utilization. Here, we found a 20% reduction in the GLUT4 contributed by the insulin-sensitive slow fibers in the obese subjects and a further decrease to 60% of control subject values in the type 2 diabetic patients, a decrease of the magnitude corresponding to that obtained by Tsao et al. (32). This progression of a decline in GLUT4 from control through obese to obese type 2 diabetic patients parallels the classic progression of insulin resistance. Therefore, our finding of a 40% reduction in GLUT4 from slow-twitch fibers is proposed to be part of the underlying mechanism of insulin resistance in type 2 diabetes. However, our results emphasize that a reduction in the fraction of slow-twitch fibers should be accompanied by a reduc-

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**GLUT4 by Western blotting.** No significant differences in GLUT4 expression levels determined by Western blotting of muscle biopsy specimens were found among the control subjects (9.1 ± 2.1, n = 3), obese subjects (5.9 ± 1.3, n = 4), and diabetic patients (7.7 ± 2.7, n = 4) (optical density/mg tissue, P > 0.29).

**FIG. 3.** Estimated f-GLUT4 and s-GLUT4: the contribution of GLUT4 from fast and slow fibers in muscles from control subjects, obese subjects, and patients with diabetes were determined as described in **RESEARCH DESIGN AND METHODS.** The contribution of GLUT4 from fast fibers (A) and slow fibers (B) are shown as mean ± SE, n = 9 in the control subjects and n = 8 in the type 2 diabetic subjects and the obese subjects. *P < 0.001, f-GLUT4 versus f-GLUT4 in diabetic subjects; †P < 0.001, s-GLUT4 versus s-GLUT4 in control subjects.
tion in slow-twitch fiber GLUT4 expression before type 2 diabetes might develop.

Physical training of type 2 diabetic men nearly normalizes insulin-stimulated glucose uptake rates (33). This is associated with an elevated GLUT4 expression in muscle biopsy specimens. In accordance with the results from Tsao et al. (32), it could be speculated that this increase in GLUT4 expression could compensate for the insulin resistance that may partly be the result of a reduced GLUT4 expression in slow fibers from type 2 diabetic patients. This notion is, however, not supported by our finding of diabetes despite an elevated GLUT4 contribution from fast fibers, as compared with the age-matched control group. In our previous study, GLUT4 was reduced in fast fibers from elderly compared with young control subjects (18), a finding that is reproduced here. The background for the lack of a decrease in the diabetic group is not clear. It is suggested that a higher motivation for exercise was present in the diabetic group due to the diagnosis of diabetes. GLUT4 expression was unaffected by age in type 1 fibers from lean healthy subjects (18). Therefore, the reduced GLUT4 in slow-twitch fibers from obese, elderly subjects compared with young, lean control subjects reported in the present study is likely to be secondary to obesity and not a result of age difference among the study groups.

Hyperglycemia is believed to contribute to the development of peripheral insulin resistance. In this study, hyperglycemia was inversely related to GLUT4 contribution from slow fibers and directly related to GLUT4 contribution from fast fibers. Normalization of blood glucose after weight loss (34,35) or diabetes treatment (36,37) enhances insulin sensitivity in skeletal muscle in type 2 diabetic patients. Incubation at normoglycemia of fresh muscle fiber preparations from type 2 diabetic patients completely restores 3-O-methyl-glucose transport after 2 h (38). Skeletal muscle insulin resistance in the diabetic GK rat is associated with slow fiber–specific defects in the insulin-signal transduction pathway to glucose transport (39). Therefore, it could be speculated that hyperglycemia affects slow muscle fibers more severely than fast fibers and selectively reduces GLUT4 expression in slow fibers in type 2 diabetes.

In conclusion, in this study, immunohistochemistry and morphometry were used to study the relationship between GLUT4 expression and skeletal muscle fiber type. We demonstrated a reduced GLUT4 expression in slow-twitch type 1 fibers from type 2 diabetic patients compared with age- and weight-matched obese subjects and from obese subjects compared with the lean control group. The estimated GLUT4 contribution from slow fibers was decreased in obese subjects and further decreased in type 2 diabetic patients. We propose that the reduced GLUT4 contribution from the more insulin-sensitive slow-twitch fibers may contribute to the reduced insulin-stimulated glucose uptake in skeletal muscle in type 2 diabetes.

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