

Overexpression of GLUT5 in Diabetic Muscle Is Reversed by Pioglitazone

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OBJECTIVE — This study was undertaken to quantify the expression of muscle GLUT in type 2 diabetes and to determine if treatment with an insulin-enhancing thiazolidenedione drug, pioglitazone, would alter its expression.

RESEARCH DESIGN AND METHODS — Twelve patients with type 2 diabetes were randomly assigned to treatment with either pioglitazone or placebo in a double-blinded 8-week protocol. Protein and mRNA for *GLUT4* and *GLUT5* were quantified in muscle homogenates from biopsies of vastus lateralis before and after treatment. The five additional GLUT family isoforms expressed in muscle had mRNA quantified in these samples.

RESULTS — Baseline and posttreatment repeat measurements of GLUT4 protein were not different from control measurements. Compared with normal subjects, GLUT5 protein increased 2.5-fold, and *GLUT5* mRNA was 82% higher in the pretreatment samples from the diabetic subjects. Concentrations of mRNA for the six other GLUTs (*GLUT1*, *GLUT3*, *GLUT4*, *GLUT8*, *GLUT11*, and *GLUT12*) were not different from control subjects before or after treatment. The proportion of type I (red) fibers (46%) in diabetic muscle was not affected by pioglitazone treatment. Pioglitazone treatment decreased muscle GLUT5 mRNA and protein by 52 and 40%, respectively, whereas placebo did not alter GLUT5 expression. Both red and white fibers had higher GLUT5 expression in the baseline diabetic muscle samples, and a pioglitazone-related decrease in GLUT5 protein also occurred in both.

CONCLUSIONS — GLUT5 was dramatically increased in diabetic muscle, and pioglitazone treatment reversed this overexpression. The role of this fructose transporter expression in the insulin-enhancing effect of pioglitazone in muscle is unclear.

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Thiazolidenedione drugs facilitate insulin action in patients with type 2 diabetes manifested by improved glycemic control and a decrease in endogenous insulin secretion (1,2). Drugs from this class robustly bind to the nuclear factor peroxisome proliferator-activator receptor- γ and activate multiple gene cassettes (1,3). There are differences in some of the genes activated by members of this class (troglitazone, rosiglitazone, and pioglitazone), but changes in many

genes are similar for these three drugs (1,4,5). The specific genes whose activation or suppression are responsible for enhanced insulin action are unclear. The study we describe here was designed to determine if pioglitazone-induced enhanced insulin action on glucose uptake could be associated with an increase in expression of one or more GLUTs in skeletal muscle. We have previously shown (6) that seven members of the GLUT family of hexose transporters are expressed in

human skeletal muscle, with mRNAs for *GLUT4*, *GLUT5*, and *GLUT12* predominating. Among these facilitative hexose transporters, GLUT5 is unique in that it transports only fructose (7). High dietary fructose (but not glucose) content has been shown to directly increase intestinal expression of *Glut5* in a rat model (8). The current study demonstrated that mRNA concentrations for six of seven GLUTs in diabetic muscle are not different from control subjects. Only *GLUT5* mRNA differed in the muscles from diabetic subjects. Both GLUT5 message and protein were increased in baseline diabetic muscle and decreased substantially with pioglitazone treatment.

RESEARCH DESIGN AND METHODS

Materials

Immunohistochemical studies were examined using a Leica TCS SP2 Laser Scanning Confocal Microscope (Wetzlar, Germany). An iCycler iQ System (Bio-Rad, Hercules, CA) was used for most of the PCR amplifications. The primers and standards for each isoform and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were previously described (6). Reverse transcriptase and oligo-dT primers were purchased from Applied Biosystems (Branchburg, NJ) as part of the GeneAmp RNA PCR Core Kit. Affinity-purified rabbit anti-hGLUT5 (GT52-A) was purchased from Alpha Diagnostics (San Antonio, TX). Mouse monoclonal antibody specific for slow myosin heavy chain (MAB1628) was purchased from Chemicon. AlexaFluor 647 donkey anti-mouse, AlexaFluor 555 donkey anti-rabbit, and AlexaFluor 488 donkey anti-goat were purchased from Molecular Probes (Eugene, OR). NADH was purchased from Sigma (St. Louis, MO). SuperSignal west pico chemoluminescence substrate was purchased from Pierce (Rockford, IL).

Patients and clinical protocol

This protocol and the consent document were approved by the East Tennessee State University Institutional Review Board. After informed consent was obtained, 12 subjects were interviewed and

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Abbreviations: AMPK, 5'-AMP-dependent protein kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—Study subject characteristics

	Subject	Age (years)	Sex	Diabetes duration (years)	BMI (kg/m ²)	A1C (%)	Fasting insulin (pmol/l)
Control subjects (mean ± SD)		30 ± 4.0			21.8 ± 1.0		52 ± 10.0
	1	24	M	—	21.8	—	29
	2	26	F	—	19.7	—	20
	3	25	M	—	23.4	—	74
	4	51	F	—	21.2	—	64
	5	29	M	—	25.5	—	44
	6	26	F	—	19.0	—	79
Placebo-treated subjects (mean ± SD)		40.0 ± 2.0		2.5 ± 0.3	36.6 ± 1.3	7.0 ± 0.6	206 ± 24.0
	7	41	F	3	31.2	6.9	190
	8	33	F	3	36.7	6.4	155
	9	45	F	1	38.0	7.0	280
	10	37	M	2	40.1	5.9	269
	11	44	M	3	34.5	10.1	140
	12	37	M	3	39.1	5.9	199
Pioglitazone-treated subjects (mean ± SD)		46 ± 5.0		3.7 ± 1.5	32.1 ± 2.2	7.3 ± 1.0	166 ± 34.0
	13	47	M	1	25.5	5.9	207
	14	55	F	6	37.1	8.0	232
	15	53	M	2	34.0	7.2	143
	16	24	F	10	37.7	11.7	266
	17	49	F	2	25.8	5.3	70
	18	45	M	1	32.4	5.6	77

examined. Six normal control subjects were also recruited. Control subjects were nonobese with no diabetes in parents, children, or siblings and were not being treated for an acute or chronic illness. Table 1 displays the characteristics of control and diabetic subjects. In total, 10 of the 12 subjects were obese with a mean ± SE BMI for diabetic subjects of 34.3 ± 1.4 kg/m² (range 25.5–40.1). Subjects who were taking insulin or who had diabetes <1 year were excluded. Three subjects were being treated with sulfonylurea medications as monotherapy. Four subjects were being treated with metformin and a sulfonylurea, and two subjects were treated with pioglitazone, one of whom was also taking a sulfonylurea. Metformin and pioglitazone were discontinued, and these subjects entered a 2-month washout period. The A1C level of the seven subjects who went through the washout period did not change (mean 6.9 ± 0.3% before and 6.9 ± 0.5% at the end). Subjects whose home blood glucose monitoring showed increased average values had sulfonylurea medication added or increased. At the end of the study, 8 of 12 subjects were taking a sulfonylurea agent. The study treatment protocol included twice-daily 15 mg pioglitazone or a placebo pill. This study was double-blinded throughout, and the code was not broken

until the clinical protocol and all laboratory measurements were completed except for the GLUT5 immunoblotting. Western blots to compare GLUT5 protein levels were performed after it was determined that GLUT5 mRNA decreased in the pioglitazone-treated subjects.

Either 1 week after screening or at the end of the washout period, subjects were randomized to pioglitazone treatment or placebo. Each subject underwent a percutaneous biopsy of vastus lateralis before beginning medication and at the end of 8 weeks of treatment. The muscle biopsy was performed after an overnight fast and 2 h of quiet recumbency as previously described (9). Patients were instructed to maintain their prestudy activity and diet programs throughout the 8-week study. Weekly phone contacts were made to assess any changes in home glucose monitoring. There were clinic visits at 4 weeks and again at 8 weeks, during which a second muscle biopsy was performed. At the termination of the study, subjects were returned to their prestudy regimen.

Quantification of mRNA

All measurements of mRNA were performed using a Bio-Rad iCycler Thermal Cycler with the iQ Real-Time PCR Detection System as previously described (6). GLUT quantification was performed at

least two separate times with triplicates of each sample in each experiment. GAPDH mRNA was quantified as an indicator of RNA sample consistency.

Immunoblot technique

Immunoblotting was performed essentially as previously described (10). For most GLUTs to be evaluated, 20 µg protein from muscle homogenate was separated on a 10% polyacrylamide gel using the Laemmli system (11), transferred to a nitrocellulose membrane, subjected to blocking with 2.5% nonfat dry milk in phosphate-buffered saline, incubated with a validated dilution of one of the anti-GLUT antibodies above including 1.25% milk, and developed with enhanced chemiluminescence reagent and X-ray film or a phosphorimager.

Immunohistochemistry

Confocal microscopic assessment of specific fluorescent labeling of GLUT protein in normal human muscle sections were performed using methods previously described (6). Muscle fiber type composition was determined by examining sections stained with NADH oxidase as described by Scarpelli et al. (12) or by fluorescent staining using an anti-slow myosin heavy chain monoclonal antibody (6). All sections were coded, photo-

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Table 2—Effect of pioglitazone treatment on skeletal muscle mRNA levels for glucose transporters in patients with type 2 diabetes

mRNA	Control subject†		Diabetic subject‡		P	Pioglitazone treatment*			Placebo treatment*			P	Between-group comparison of change
	subject†	subject‡	subject‡	subject‡		Baseline	8 weeks	Mean change	P	Baseline	8 weeks		
GLUT4	185 ± 63	209 ± 50	0.404	227 ± 64	229 ± 165	2	0.963	192 ± 27	197 ± 140	5	0.625	-3 (-182 to 178)	0.980
GLUT5	56 ± 22	104 ± 44	0.029	105 ± 24	42 ± 22	-64	0.025	102 ± 61	97 ± 54	-5	0.847	-59 (-129 to 12)	0.093
GLUT12	30 ± 9	23 ± 8	0.130	21 ± 7	19 ± 8	-2	0.742	25 ± 10	17 ± 11	-8	0.191	6 (-12 to 24)	0.437
GLUT8	5.3 ± 2.1	7.8 ± 4.2	0.215	9.2 ± 5.7	5.2 ± 2.5	-4.0	0.313	6.3 ± 1.6	6.2 ± 2.6	-0.1	0.950	-3.9 (-12.0 to 4.1)	0.297
GLU11	4.8 ± 0.9	6.1 ± 1.8	0.093	6.1 ± 1.2	5.5 ± 3.0	-0.6	0.656	6.1 ± 2.4	6.8 ± 4.9	0.7	0.622	-1.3 (-5.1 to 2.7)	0.495
GLUT1	0.5 ± 0.2	1.7 ± 3.4	0.871	2.5 ± 4.8	1.2 ± 1.6	-1.3	1.000	0.8 ± 0.9	0.8 ± 0.6	0	0.796	-1.3	1.000
GLUT3	0.27 ± 0.10	0.39 ± 0.19	0.447	0.37 ± 0.16	0.40 ± 0.32	0.03	0.996	0.39 ± 0.25	0.63 ± 0.51	0.24	0.395	-0.21 (-0.86 to 0.39)	0.411

Data are means ± SD. *n = 5; †n = 6; ‡n = 10. All data are expressed as copies per nanogram RNA.

graphed, and then quantified independently by three observers who were unaware of which subject or treatment the photograph represented.

Statistics

All data are displayed as means ± SE except Table 2, which lists SDs. Comparisons between two groups were performed using Student's *t* test for independent groups, and when comparing paired measurements, the paired *t* test was used. ANOVA was used for comparisons of three independent groups.

RESULTS

Impact of the 8-week protocol on glycemic control

This protocol was double-blinded such that neither the subject nor the investigators knew who was receiving active drug. Nine subjects maintained their glucose control during the study, but three subjects in the placebo group had worsening of glycemic control, with A1C increasing by >1%. The mean A1C for the placebo group tended upwards (8.8 ± 0.6 vs. 7.0 ± 0.6%; *P* = 0.07, paired *t* test) but did not change in the pioglitazone group (7.6 ± 1.0 vs. 7.3 ± 1.0%). Weight gain in excess of 1 kg occurred in five of the pioglitazone subjects and one of the placebo subjects. Weight change was 1.5 ± 0.4 kg (*P* = 0.008) for the pioglitazone group and -0.9 ± 1.0 kg (*P* = 0.47) for the placebo group. Mean fasting plasma insulin decreased slightly in both pioglitazone-treated (8%) and placebo-treated (7%) groups, but was not statistically significant (*P* = 0.15, *P* = 0.39). However, plasma C-peptide decreased 21% (*P* = 0.026) after pioglitazone treatment; the placebo-related drop of 6% was not significant (*P* = 0.68). One muscle biopsy was inadequate and one was lost, resulting in one subject from each group not being used in the muscle data analysis.

Pioglitazone-induced changes in skeletal muscle GLUT mRNA

Seven GLUT mRNAs were quantified in muscle homogenates from 6 normal control subjects and from 10 patients with diabetes before and after an 8-week treatment with either 30 mg pioglitazone daily or placebo. Table 2 summarizes those data. Only GLUT5 mRNA was quantitatively different from the control subject data. GLUT5 mRNA was 82% higher in the baseline biopsy, and the treatment with pioglitazone caused the GLUT5

mRNA to decline to within the normal range. The GLUT5 mRNA data are shown graphically in Fig. 1. Normalizing the data to GAPDH mRNA concentrations did not change the findings. The GLUT isoform data we report here are raw data, not adjusted for GAPDH.

Change in GLUT5 protein related to pioglitazone treatment

Immunoblots of muscle homogenates indicated that subjects with type 2 diabetes who were not taking metformin or a thiazolidenedione drug had increased GLUT5 protein. Figure 2A shows a representative immunoblot including control subjects and baseline and posttreatment muscle biopsies from a placebo-treated subject and a pioglitazone-treated subject. Individual data are displayed in Fig. 2B. GLUT5 protein in the control subjects averaged 100 ± 3% of control, the baseline pioglitazone group 251 ± 22%, the baseline placebo 255 ± 31%, the pioglitazone posttreatment 151 ± 16%, and the placebo posttreatment 243 ± 28%. Pioglitazone treatment caused a 40% decrease. Baseline GLUT5 protein did not correlate with age, weight, A1C, or fasting plasma insulin concentration.

Skeletal muscle fiber type composition in patients with diabetes

Because GLUT5 is expressed predominantly in white muscle fibers, we quantified the proportion of fibers that were type I oxidative (red) fibers in our subjects and determined whether that proportion was modified by pioglitazone treatment. Baseline biopsies had 47 ± 3% (*n* = 12) red fibers in diabetic subjects compared with 53 ± 2% in sedentary control subjects (*n* = 6). Even though the proportion of red fibers was 13% lower in the diabetic subjects compared with sedentary control subjects, the proportion of white fibers was not altered by treatment. Postpioglitazone biopsies showed 45 ± 2% (*n* = 5) red fibers compared with 49 ± 3% (*n* = 5) in the placebo-treated subjects. Fiber composition alone could not account for the high amount of GLUT5 mRNA and protein seen in the subjects with diabetes, nor could it explain the dramatic decline that occurred after treatment.

Muscle fiber type localization of pioglitazone-induced changes in GLUT5 protein

Muscle biopsies from each subject were subjected to cryosectioning and immuno-

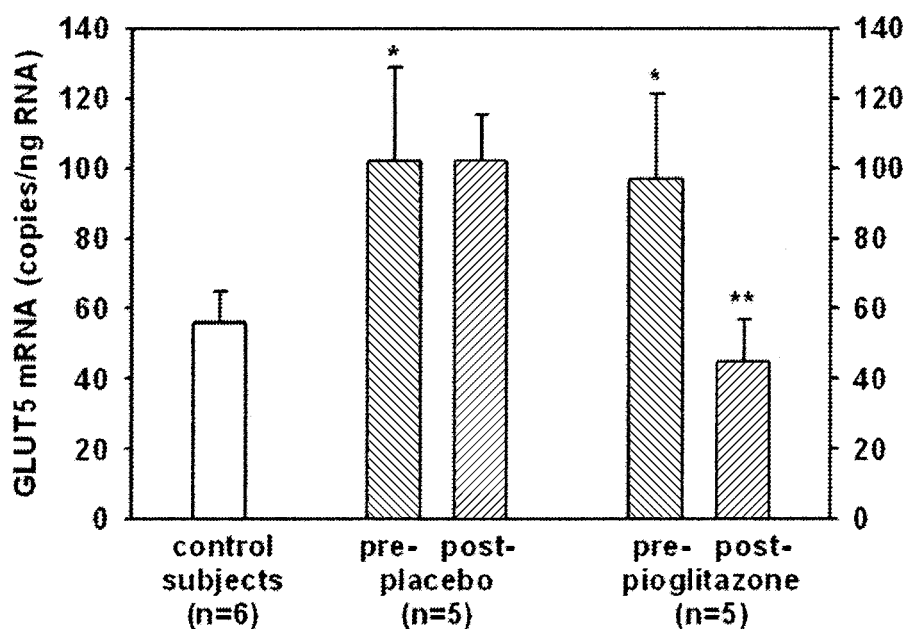


Figure 1—Pioglitazone treatment alteration of GLUT5 mRNA in skeletal muscle from type 2 diabetic subjects. Shown are data from quantitative real-time PCR using a light cycler PCR instrument. Baseline GLUT5 mRNA increased 82% in subjects with diabetes, and pioglitazone treatment specifically decreased GLUT5 mRNA concentrations by 56%. The single asterisk (*) indicates significant difference from control subjects ($P < 0.05$, independent groups), and the double asterisk (**) represents significant difference from the pretreatment values ($P < 0.05$, paired observations).

histochemistry. Figure 3A shows representative sections. Comparing panels 1 and 3 to panel 5 demonstrates that type I and type II fibers of the diabetic subjects express more GLUT5 than the control subject. Treatment with placebo had no effect on the expression of GLUT5 protein, but pioglitazone treatment resulted in a substantial decrease in the GLUT5 protein content of both type I and II fibers. Thereby, panel 4 qualitatively resembles panel 5. This pattern and the change or lack of change in fiber-specific expression of GLUT5 was present in each of the 10 subjects with two evaluable biopsies. Each biopsy of the 10 subjects treated in this protocol was analyzed using image analysis software to quantify the relative intensity of the GLUT5 immunofluorescence. The results of these measurements are displayed in Fig. 3B. These data show changes in fiber-specific GLUT5 protein expression that are similar to the changes demonstrated in Western blots of muscle homogenates.

CONCLUSIONS—Patients with type 2 diabetes had normal expression of GLUT1, GLUT3, GLUT4, GLUT8, GLUT11, and GLUT12 as indicated by mRNA concentrations quantified using reverse transcriptase and real-time PCR.

In contrast, GLUT5 mRNA increased 82%, and GLUT5 protein increased more than twofold compared with control subjects. Pioglitazone treatment of these patients for 8 weeks, while maintaining glycemic control, caused a 52% decline in GLUT5 mRNA and a 40% decline in GLUT5 protein, but had no effect on mRNA concentrations for the remaining GLUTs. Even though A1C level was unchanged, plasma C-peptide data suggested that endogenous insulin secretion was modestly decreased coincident with pioglitazone treatment.

Glucose is by far the predominant carbohydrate fuel used by mammalian tissues, making it no surprise that 12 members of the 14-member GLUT family of membrane proteins transport glucose. Fructose, on the other hand, is found in blood at as much as 500-fold lower concentrations in humans (13). GLUT5, exclusively a fructose transporter with no ability to transport glucose (7), has been demonstrated in intestinal epithelium, kidney, fat, skeletal muscle, testes, and sperm (14). Six other isoforms have been shown to also transport fructose. GLUT2 is a glucose and fructose transporter, which is likely responsible for fructose uptake in the liver (14) and plays a critical role in glucose and fructose transfer at the

basolateral membrane of the intestinal luminal epithelial cell (15). GLUT5, GLUT7, GLUT9, and GLUT11 are all members of class II of the GLUT family, grouped together because of their high degree of amino acid identity (40–60%) and deduced to have fructose transport activity because of their similarity to GLUT5 (16). GLUT7 (17,18) and GLUT9 (19) subsequently have been confirmed to have fructose-inhibitable glucose uptake. In addition, GLUT8 and GLUT12 have been shown to transport both glucose and fructose (20).

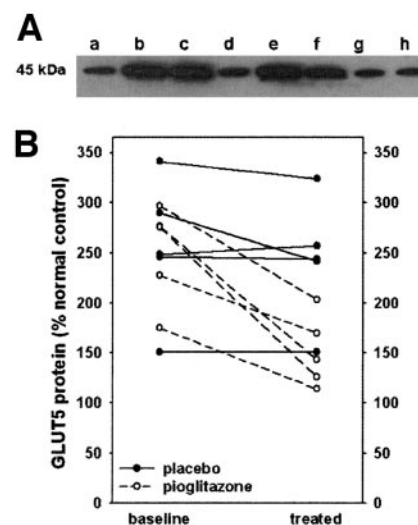


Figure 2—The impact of pioglitazone treatment on muscle GLUT5 expression in five subjects with type 2 diabetes. A shows an immunoblot of GLUT5 in muscle biopsy homogenates from diabetic and control subjects. The GLUT5 protein band migrated with a relative molecular weight of 45 kDa. In this typical immunoblot, lanes a, d, g, and h contained 5 µg protein from homogenized muscle obtained by percutaneous biopsy from four normal control subjects. Lanes b and c contained 5 µg homogenate from subject 4 before and after, respectively, 8 weeks of placebo treatment. Lanes e and f contained 5 µg protein from muscle homogenates obtained from subject 6 before and after 8 weeks of treatment with pioglitazone, 30 mg daily. B shows the individual data points from each of the 10 completed subjects. Each data point represents the mean of determinations from two to five separate experiments. GLUT5 expression was 2.5-fold higher in diabetic subjects compared with control subjects. Pioglitazone treatment resulted in a 40% decline in GLUT5 protein, whereas placebo had no effect. All of the pioglitazone-treated subjects exhibited a decline in skeletal muscle homogenate GLUT5 protein in the second biopsy, whereas only two of the placebo-treated subjects had a decline.

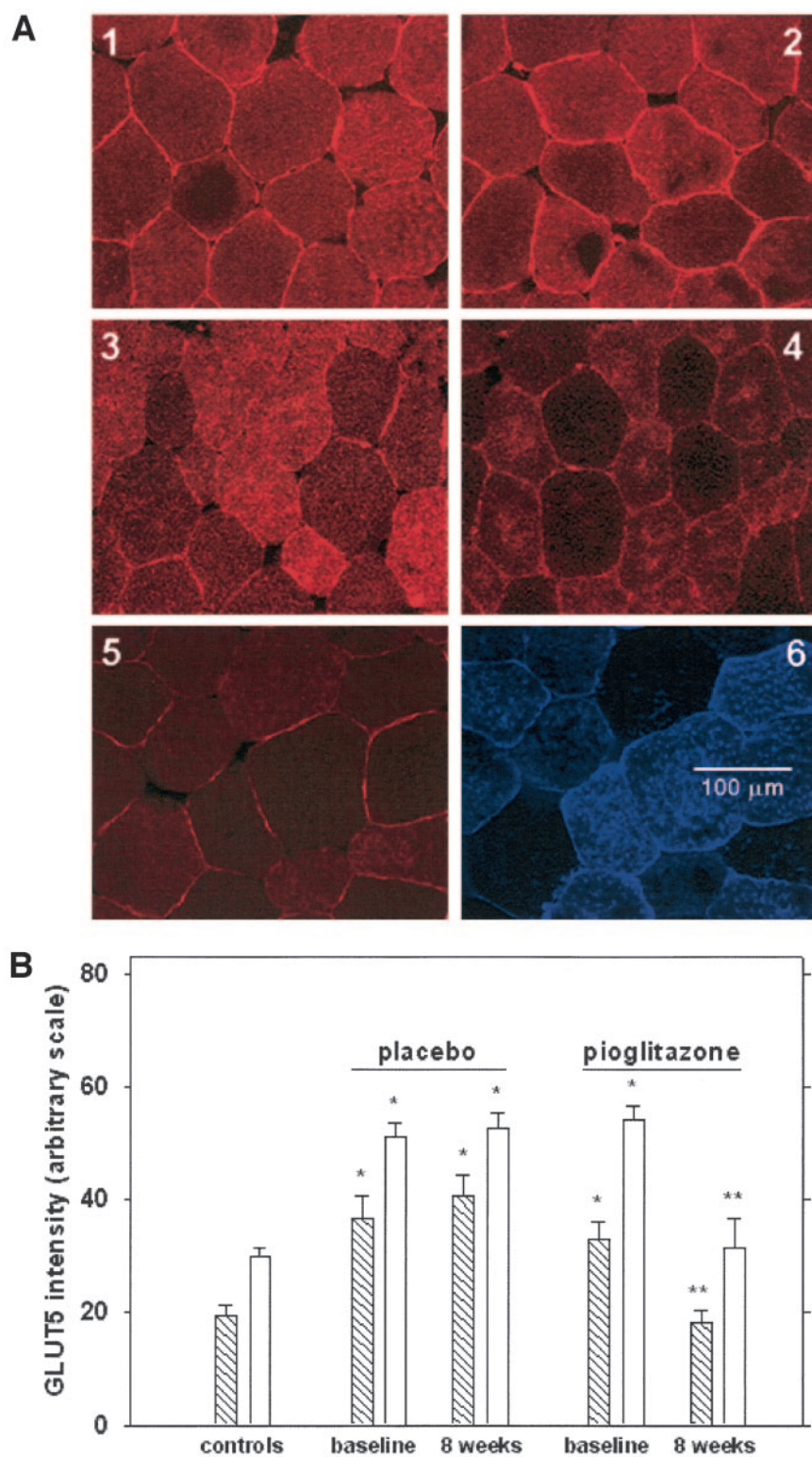


Figure 3—Impact of pioglitazone treatment on the fiber type distribution of GLUT5 in muscle from patients with type 2 diabetes. Immunohistochemical methods were used to evaluate potential change in fiber type distribution of GLUT5 in muscle biopsy material from patients treated with pioglitazone. A displays representative immunohistochemical images. Panels 1 and 2 are from a placebo-treated subject, and panels 3 and 4 are from a pioglitazone-treated subject. Panels 1 and 3 were from baseline biopsies, and panels 2 and 4 were from posttreatment biopsies. Panels 5 and 6 are from biopsies of a normal subject. Cryosections from the two biopsies from each study subject were placed on the same glass slide so that they were simultaneously incubated with the same antibody solution. Each of panels 1–5 were probed with affinity-purified rabbit anti-hGLUT5

Substrate availability has been shown to affect hexose transporter expression in intestinal epithelial cells but has not been directly evaluated in muscle. Hyperglycemia directly increases intestinal glucose absorption severalfold (21). Dyer et al. (21) demonstrated threefold increased transport of D-glucose into brush-border membrane vesicles isolated from duodenal biopsies of patients with type 2 diabetes. They found that mRNAs for SGLT1, GLUT2, and GLUT5 were increased threefold in duodenal biopsies from these diabetic subjects. Protein levels for SGLT1 and GLUT5 were increased more than fourfold compared with control subjects (21). Dyer's group hospitalized several patients with diabetes and intensively managed their blood glucose levels. They found that the overexpression of GLUT5 and SGLT1 in the intestinal epithelium returned to normal (21), suggesting that hyperglycemia had played a role in the overexpression of the transporters in the gut of the diabetic subjects.

Experimental insulin-deficient diabetes is also associated with alteration in the expression of GLUTs. Streptozotocin-induced diabetes in rats results in dramatic increases in both mRNA and

antibodies, as described in RESEARCH DESIGN AND METHODS. Panel 6 is the same as panel 5 except that it is probed with anti-slow myosin heavy-chain antibody to demonstrate which fibers are type I (red) fibers. B shows the change in GLUT5 expression induced by pioglitazone treatment in type I and II muscle fibers. Image analysis software was used to quantify the intensity of the red image due to anti-hGLUT5 primary antibody in sections from 6 control subjects and the 10 diabetic subjects before and after the 8-week treatment protocol. Images similar to those of Fig. 3 were used for each subject. The image analysis was performed using before and after transverse muscle sections mounted on the same slide treated with the same antibody dilutions and imaged on the Leica confocal microscope with identical settings for all sections. The operator was not aware of which subjects received placebo or pioglitazone. Ten type I and 10 type II fibers were identified on each image, and the intensity of the GLUT5 signal was quantified using the Quantity One software from BioRad. The single asterisk (*) indicates significant difference ($P < 0.01$) from the corresponding fiber type of the control subjects. The double asterisk (**) indicates a significant treatment-induced difference ($P < 0.01$, repeated measures) from the corresponding fiber baseline data. Red fibers (▨) indicate type I, and white fibers indicate type II, as indicated by slow myosin heavy-chain antibody fluorescence.

protein for SGLT1, GLUT2, and GLUT5 in jejunum and ileum (22). Insulin treatment of streptozotocin-induced diabetic rats decreases the overexpression of these GLUTs in intestinal epithelial cells (22). These data are consistent with an effect of glucose concentration on GLUT expression in the intestine.

In addition to endogenous hyperglycemia increasing intestinal glucose uptake, dietary content of sugars directly affects GLUT expression. Miyamoto et al. (8) fed rats a high-glucose diet and found that intestinal uptake of glucose was increased coincident with increased jejunal SGLT1 and GLUT2 mRNAs. They found that D-glucose, D-galactose, and D-fructose feeding stimulated expression of GLUT2, but only D-fructose could increase jejunal GLUT5 mRNA. Burant et al. (23) showed that the D-fructose diet effect was due to direct contact of the sugar with the intestinal epithelial cells and the effect was rapidly reversible. These studies concentrated on intestinal glucose transport and did not evaluate the impact of glucose or fructose on skeletal muscle expression of GLUTs. However, Darakhshan et al. (24) did not find an effect of fructose feeding on fat or muscle expression of GLUT5.

Kawasaki et al. (13) demonstrated that blood fructose concentrations were elevated 50% and urine content was more than threefold increased in patients with diabetes. These investigators found that improving glycemic control resulted in normalization of the serum fructose concentrations and a marked decrease in the urine excretion of fructose (13).

Our GLUT5 data from skeletal muscle did not correlate with glycemic control. The dramatic decrease in GLUT5 protein and message in the pioglitazone-treated subjects was in spite of no significant change in A1C.

The connection between GLUT5 and insulin action is unclear. GLUT5 is not acutely regulated by insulin (25). The increased GLUT5 expression in both red and white muscle fibers in type 2 diabetes that decreases toward control subject levels after treatment with pioglitazone suggests an inverse relationship between muscle GLUT5 and insulin sensitivity. The decrease in GLUT5 could be an indirect or secondary effect of heightened insulin responsiveness, or it could be somehow directly involved in the insulin-enhancing action of thiazolidinedione drugs.

Alternatively, pioglitazone may exert its effects on GLUT5 expression via the

same system that modulates the oxidative enzyme content of muscle. Elite athletes may have as high as 75% type I (red and oxidative) muscle fibers (26), and subjects with type 2 diabetes have been shown to have as low as 35% type I with 65% type II (white and glycolytic) muscle fibers (27). Type I fibers have higher levels of mitochondria and oxidative enzymes and much higher levels of GLUT4 and GLUT12 (6) in contrast to type II fibers, which express low numbers of mitochondria but much higher levels of GLUT5 (6).

Troglitazone, rosiglitazone, and pioglitazone have each been shown to activate 5'-AMP-dependent protein kinase (AMPK) through increasing the ratio of 5'-AMP to ATP in muscle cells (28,29). Activation of AMPK increases the expression of peroxisome proliferator-activated receptor- γ coactivator-1 α , the major regulator of mitochondrial biogenesis (30). This action of thiazolidinedione drugs shows important parallels to the effects of muscle contraction and exercise training on AMPK and peroxisome proliferator-activated receptor- γ coactivator-1 α (31,32). These effects are pro-oxidative in that they increase mitochondrial oxidative enzymes and thereby increase β -oxidation of fatty acids (33,34). Chronic stimulation of AMPK in rats has also been shown to increase GLUT4 expression in muscle (31). Taken together, these effects on mitochondria and GLUT4 expression would move diabetic muscle away from predominantly white, fast-twitch characteristics toward red, slow-twitch biochemistry. A drug treatment that acts through gene expression to shift muscle biochemical characteristics toward type I fibers may result in a reciprocal repression of GLUT5 gene expression.

We believe that the high level of GLUT5 expression in type 2 diabetic muscle is directly tied to the predominance of type II muscle fibers within which mitochondrial oxidative enzyme expression is suppressed and GLUT5 expression is increased. Pioglitazone treatment then reverses the gene regulation cascades to shift muscle to a pattern of gene expression that is more oxidative (red fiber-like), with increased mitochondria and decreased GLUT5.

Our data show that pioglitazone-induced enhanced insulin action in diabetic subjects is accompanied by decreasing the high muscle expression of GLUT5 without any change in muscle fiber type composition or in concentrations

of mRNA for GLUT4 or GLUT12. We speculate that the hyperglycemia of our patients is accompanied by higher blood fructose levels that may directly stimulate GLUT5 expression in muscle by mechanisms that parallel the direct fructose effects on the intestinal epithelial cell. Our data show that enhanced insulin action induced by pioglitazone is associated with a major decline in the diabetes-related increase in GLUT5 expression in muscle. The mechanism by which pioglitazone treatment decreases GLUT5 expression may be directly tied to its stimulatory effects on mitochondrial oxidative enzymes.

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