Altered GLUT1 and GLUT3 Gene Expression and Subcellular Redistribution of GLUT4: Protein in Muscle From Patients With Acanthosis Nigricans and Severe Insulin Resistance

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Multiple isoforms of glucose transporters are found in muscle, the tissue that normally accounts for 85% of insulin-stimulated glucose uptake. Glucose uptake into muscle cells in the fasting state is mediated primarily by GLUT1 and GLUT3 glucose transporters, whereas postprandial (insulin-stimulated) and exercise-related increments in muscle glucose uptake are mediated primarily by GLUT4. To determine if glucose transporters are abnormally expressed in muscle from insulin-resistant subjects, muscle samples were obtained from 10 normal subjects and 6 obese, nondiabetic subjects with severe insulin resistance and acanthosis nigricans. Both GLUT4 total protein and mRNA were normal in the insulin-resistant subjects. Muscle GLUT3 protein and mRNA were lower than controls by 62% and 71%, respectively. GLUT1 mRNA was twice normal, whereas GLUT1 protein content was not significantly increased. GLUT4 protein was markedly redistributed to the muscle plasma membrane in subjects with severe insulin resistance compared with normals (92% *v* 40% GLUT4 in plasma membrane-enriched fractions, $P < .001$), whereas the percentage of GLUT1 and GLUT3 protein found in the plasma membrane-enriched fractions was not different from controls. These data document differences in the expression of genes for GLUT1 and GLUT3 in muscle from normal and insulin-resistant subjects. Further, insulin resistance with fasting hyperinsulinemia was associated with a redistribution of GLUT4 to the muscle cell surface with no change in total GLUT4 protein. These data suggest that glucose transporter gene expression and their basal distribution in human muscle are related to insulin resistance and could be determinants of whole body insulin responsiveness.

STIMULATION OF GLUCOSE uptake into muscle by either insulin or exercise results from translocation of intracellular GLUT4 glucose transporters to the muscle plasma membrane,[1] which includes the sarcolemma and the plasma membrane invaginations called t-tubules.[2] Whole body glucose uptake normally is maintained through most of the day at a basal rate that is 20% to 25% of the rate achievable with maximal stimulation.[3,4] In the unstimulated state, GLUT3 and GLUT1 glucose transporters are on the muscle cell surface[1,5] and mediate glucose entry into muscle. Most studies of glucose transporters in normal and insulin-resistant subjects have focused on GLUT4, which has usually been found to be expressed at normal levels in muscle of insulin-resistant human subjects.[6-8] Measurements of GLUT3 expression in muscle have not been previously reported in insulin-resistant subjects. We hypothesized that insulin-resistant subjects lack sufficient GLUT1 and/or GLUT3 to maintain basal glucose transport rates and would manifest, in the basal state, persistent redistribution of insulin-responsive glucose transporters to the cell surface. To test this hypothesis, we quantified GLUT1, GLUT3, and GLUT4 mRNA and protein in muscle tissue and the subcellular distribution of glucose transporter proteins in 6 nondiabetic, insulin-resistant subjects and from 10 control subjects. Each of the insulin-resistant subjects had acanthosis nigricans, a cutaneous manifestation of hyperinsulinemia.[9-11]

MATERIALS AND METHODS

Subjects

Six female subjects with acanthosis nigricans and insulin resistance, but with normal glucose tolerance, were recruited for study. Table 1 shows pertinent characteristics of these subjects and the control group. Control subjects were within 50% of their ideal weight for height[12] and had documented normal glucose tolerance and normal fasting insulin levels. No subjects in either group had diabetes at the time of study. A fasting plasma insulin concentration greater than 350 pmol/mL (50 μU/mL) was considered to indicate severe insulin resistance.[9,13,14] Each subject gave informed written consent before admission to the University of Texas Medical Branch (UTMB) General Clinical Research Center. The UTMB Institutional Review Board approved these studies and the consent document.

Percutaneous biopsies of the midthigh portion of the vastus lateralis muscles were obtained in each subject using a 5-mm Bergstrom-Stille needle (Stille, Stockholm, Sweden) as previously described.[15] All muscle biopsies were performed in the morning after a 12-hour overnight fast and recumbency for at least 2 hours. Muscle specimens (50 to 150 mg) were frozen in liquid nitrogen within 30 seconds.

Materials

The American Type Culture Collection (ATCC) (Rockville, MD) provided cDNAs for human GLUT1 (ATCC no. 59630), human GLUT3 (ATCC no. 61615), and human GLUT4 (ATCC no. 61617). Glyceraldehyde phosphate dehydrogenase (GAPDH) cDNA was purchased from Ambion (Austin, TX). pBS M13 was purchased from Stratagene (La Jolla, CA). Affinity-purified, polyclonal antibodies against human GLUT1, GLUT3, and GLUT4 were purchased from Chemicon International (Temecula, CA). Riboprobe Gemini II Core System kits were purchased from Promega (Madison, WI). α^{32}P-uridine triphosphate (UTP), 800 Ci/mmol, was purchased from DuPont

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Table 1. Subject Characteristics

Subject	Ethnicity	Age/Sex	BMI (kg/m^2)	Weight % Ideal	Insulin (pmol/L)	Glucose (mmol/L)
Insulin resistance (n = 6)						
a	B	18/F	40.9	212	295	4.4
b	H	23/F	50.4	255	505	4.3
c	B	28/F	41.1	204	200	4.1
d	W	26/F	46.0	225	570	5.3
e	W	34/F	52.4	268	265	5.5
f	W	35/F	29.3	152	335	6.7
Mean ± SEM		27 ± 3	43.3 ± 3.4	219 ± 17	360 ± 60	5.1 ± 0.4
Control subjects (n = 10)						
g	W	24/F	26.0	135	57	5.6
h	W	46/F	24.1	122	31	4.3
i	B	32/M	24.4	116	—	4.7
j	B	27/F	23.2	116	56	5.0
k	W	23/M	27.3	125	67	4.8
l	W	21/F	23.1	115	38	4.9
m	B	42/M	20.7	98	42	5.6
n	B	40/M	25.9	122	161	5.9
o	H	25/F	27.5	144	56	5.6
p	B	26/M	23.5	107	42	5.1
Mean ± SEM		31 ± 3	24.6 ± 0.7	120 ± 4	61 ± 13	5.1 ± 0.2

NOTE. Ethnicity: W, white; B, black; H, Hispanic. Insulin and glucose concentrations were measured in plasma after an overnight fast.

NEN (Boston, MA). Vanadyl Ribonucleoside Complex (VRC) was purchased from GIBCO BRL (Grand Island, NY). Ribonuclease T1 was purchased from Sigma (St Louis, MO). Ribonuclease A and DNAse were purchased from Boehringer-Mannheim (Indianapolis, IN). All other chemicals were reagent grade.

Methods

Riboprobes for GLUT3, GLUT4, and GAPDH were prepared as previously described.[16] A 320-bp EcoRI/PstI GLUT1 cDNA fragment was isolated from pSGT for the GLUT1 probe. The fragment was then gel purified and ligated into pBSM13. GLUT1 riboprobes were then generated by the same procedures as the 3 other riboprobes.[16] Probe sizes for GLUT1, GLUT3, GLUT4, and GAPDH were 500 bp, 459 bp, 825 bp, and 376 bp, respectively. Protected band sizes were 330 bp, 256 bp, 650 bp, and 316 bp, respectively.

Ribonuclease protection assays (RPAs) for GLUT1, GLUT3, GLUT4, and GAPDH mRNA were performed using minor modifications of the method of Lowe et al[17] as previously described.[16] Total cellular RNA was isolated from skeletal muscle using RNAzol B (Tel-Test, Friendswood, TX) with 2 mL RNAzol B/100 mg muscle. Twelve consecutive preparations averaged 0.8 μg RNA/mg muscle. Each dried gel was analyzed using images from a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Band intensities were quantified using the ImageQuant software provided with the PhosphorImager. The intensities of protected bands were adjusted by GAPDH band intensity as a measure of total RNA variability. In each RPA, equal amounts of RNA from a reference sample of human muscle were included in triplicate. The GAPDH band intensity in each lane was used to normalize the RNA applied to that lane relative to the mean of the 3 lanes containing equal amounts of RNA from the reference muscle specimen. The GLUT1/GLUT3/GLUT4 RPAs were optimized for linearity over a range of 5 to 30 μg total RNA/lane.

The techniques for muscle membrane fractionation have been previously described.[16,18] The method involved sharp blade homogenization in 0.25 mol/L sucrose, DNAse digestion, and differential sedimentation. Beginning with 50 mg muscle thawed from a liquid nitrogen snap-frozen percutaneous biopsy, approximately 1.0 mg plasma membrane-enriched membranes (PM) and 6.4 mg low-density microsomes (LDM) were obtained. The crude PM averaged 5.5-fold enrichment over homogenate in 5′-nucleotidase activity.[19] For comparison, highly purified plasma membranes obtained from 400 to 500 mg muscle using differential sedimentation and sucrose gradients, as described by Garvey et al[20] in his modifications of the methods of Dombrowski et al,[21] had 5′-nucleotidase activity 20-fold enriched over homogenate. This fractionation technique is similar to that used by Mitsumoto and Klip[22] for L6 cells in culture and by Brozinick et al[23] in the evaluation of GLUT4 protein distribution in the muscle of transgenic mice overexpressing GLUT4.

Immunoblots were performed as previously described.[16,18] Each gel lane for Western blots used 5 to 80 μg membrane protein, depending on the fraction and the glucose transporter isoform to be assayed. These membranes fractions were subjected to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)[24] and electrophoretically transferred to nitrocellulose filters. Dried filters were exposed using XAR-5 film (Eastman-Kodak Co, Rochester, NY) for 5 seconds to 3 minutes. Film images were digitized and analyzed using the ImageQuant software provided with the PhosphorImager.

Estimation of the amount of each glucose transporter in the muscle membrane fractions was performed by first quantifying the specific GLUT in an immunoblot using 5 to 80 μg membrane protein. This quantification was on an arbitrary scale defined in each case using a well-characterized human reference muscle specimen. This number was then converted to a total amount for the muscle fraction by extrapolating to the total membrane protein in the specific membrane fraction (PM or LDM). For example, 80 reference units of GLUT1 based on relative band intensity, determined in a lane with 15 μg membrane protein from a total fraction of 1,100 μg, gave an estimate of 5,867 reference units for the entire PM fraction from 50 mg of starting material. The total muscle glucose transporter isoform protein content was estimated by adding the amount calculated for the LDM and the PM fractions.

Data was expressed as mean ± SE, unless otherwise stated. Statistical significance was estimated using Student's *t* test for comparison of

2 independent groups. Significance was achieved with a P value less than .05.

RESULTS

Quantification of GLUT1, GLUT3 and GLUT4 mRNA in Skeletal Muscle RNA

An example of a RPA of GLUT3 and GLUT4 mRNA is shown in Fig 1A. Displayed is a phosphor image from a dried gel containing protected bands specific for GLUT3, GLUT4, and GAPDH. The lanes labeled C1, C2, and C3 are triplicate samples from a single control subject, and the lanes labeled A1, A2, and A3 contain triplicate samples from a single insulin-resistant subject (subject f). No identifiable bands are present for this subject at the apparent size of the protected GLUT3 band from the control subject. Pixel intensity in the region of the expected band was less than 1% of that of the control subject. No additional protected bands of smaller sizes were seen other than those for GAPDH. GLUT4 protected band intensity for the affected subject averaged 78% of that of the control subject in this experiment. GAPDH intensity was 103% of the control lanes.

The results of RPA determinations of GLUT3 and GLUT4 mRNA from muscle biopsies in the control subjects and the 6 severely insulin-resistant subjects are displayed graphically in Fig 2. All of these data were normalized by GAPDH band intensity as described in Methods. GLUT3 mRNA was very low in 5 of the 6 subjects. Subject b had normal GLUT3 and GLUT4 mRNA in spite of insulin resistance that was very similar in severity to that of the other 5 subjects. Figure 1B displays a phosphor image of a typical RPA for GLUT1 mRNA. The 3 lanes labeled a to c contain 15 μg RNA from the reference muscle specimen and lanes h and i 15 μg RNA from control subjects. Lanes d to g contain 10 μg RNA from severely insulin-resistant subjects. Quantifications of GLUT mRNAs were not statistically different between the male and female control subjects (GLUT1, 100 ± 16 *v* 138 ± 16, $P = .22$; GLUT3, 54 ± 7 *v* 51 ± 7, $P = .73$; GLUT4, 164 ± 14 *v* 111 ± 14, $P = .09$, respectively).

Figure 3 displays the summary data for GLUT1, GLUT3, and GLUT4 mRNA quantification using the RPAs as described above. These data were expressed as arbitrary units defined by use of the our reference muscle sample RNA in each assay. RPAs for GLUT1 mRNA indicated a near doubling (relative to control subjects) of the skeletal muscle expression of GLUT1 in these subjects with severe insulin resistance. GLUT3 mRNA concentrations averaged about one third of the value determined in the control subjects.

Glucose Transporter Protein Content of Muscle Obtained From Insulin-Resistant Subjects

Figure 4 shows a typical immunoblot used to quantify GLUT4 in this case. Image analysis of band intensity generated the glucose transporter isoform protein content in reference units based on the use of fractions from the same reference muscle specimen in each blot. Total muscle membrane content of each glucose transporter, calculated as described in Methods, is displayed in Fig 5. GLUT1, GLUT3, and GLUT4 proteins cannot be directly compared with each other because the scales

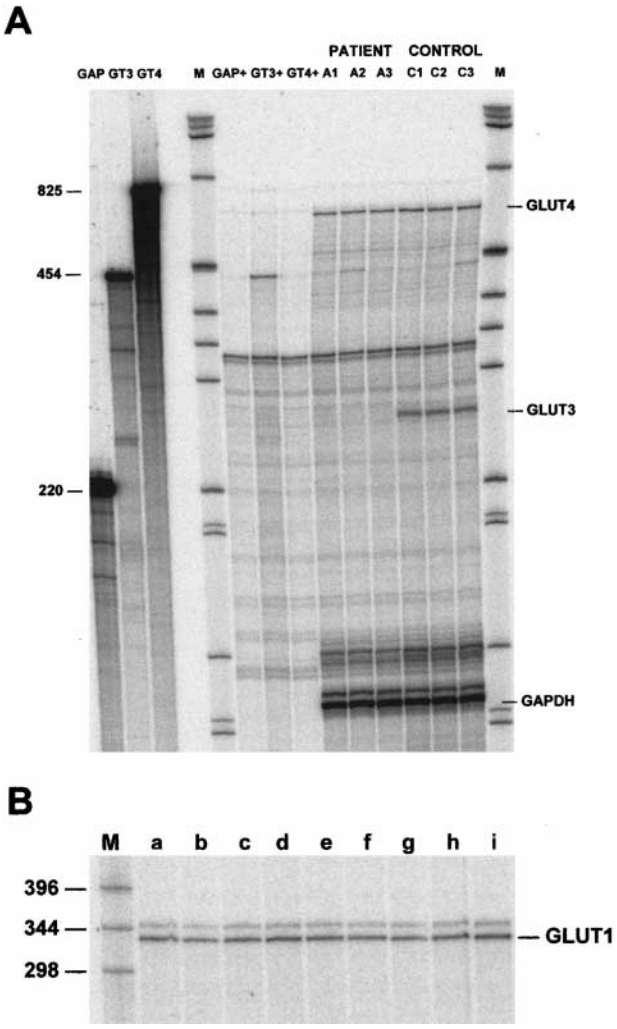

Fig 1. RPAs for GLUT3, GLUT4, and GLUT1. (A) Shows quantification of mRNA for GLUT4 and GLUT3 in RNA from skeletal muscle in a patient with severe insulin resistance. Shown here is a PhosphorImager image of a ribonuclease protection assay performed as described in Methods. The lanes labeled at the top of the figure as GAP, GT3, and GT4 contain the riboprobes only for GAPDH, GLUT3, and GLUT4, respectively. The lanes labeled GAP+, GT3+, and GT4+ contain probe digested with ribonuclease. The lanes labeled "M" contain a molecular weight marker. The lanes labeled A1, A2, and A3 each contain 20 μg total RNA from the patient's muscle specimen, riboprobes GAP, GT3, and GT4, and have been digested with ribonuclease. Lanes C1, C2, and C3 contain 20 μg of skeletal muscle RNA from a control subject, riboprobes, and ribonuclease. The numbers at the left margin are the base pair sizes of the probes. The location of the protected bands for GLUT4, GLUT3, and GAPDH are indicated at the right margin. A doublet seen at about 300 bp apparent size are nonspecific bands seen in each digested probe. (B) Displays a GLUT1 ribonuclease protection assay phosphor image. This phosphor image is typical of the RPAs performed for quantification of GLUT1 mRNA. The protected band was 330 bp. The lighter band at 350 bp mobility is a nonspecific band present in the probe in the absence of sample RNA. Lanes a to c are from the reference muscle specimen, lanes d to g are insulin-resistant subjects, and h and i are control subjects.

are related to the reference specimen and are different for each glucose transporter. These 5 severely insulin-resistant subjects (a, c, d, e, f) and 5 controls (h, j, n, o, q) are a subset of those

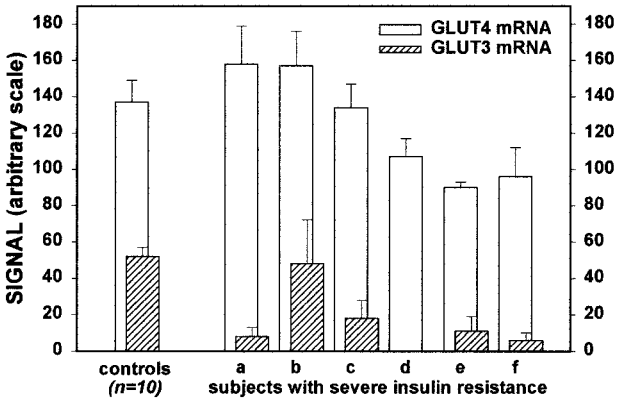

Fig 2. RPA quantification of mRNA for GLUT3 and GLUT4 in muscle RNA obtained from 10 control subjects and 6 normoglycemic subjects with severe insulin resistance. Each bar represents the mean and SE of 2 to 5 separate measurements of RNA from each subject (indicated by the same letters used in Table 1). The arbitrary scale was defined in each RPA autoradiogram by triplicate determinations of GLUT3 and GLUT4 mRNA concentrations in RNA from a reference muscle specimen. The open bars represent GLUT4, and the shaded bars represent GLUT3 mRNA quantification.

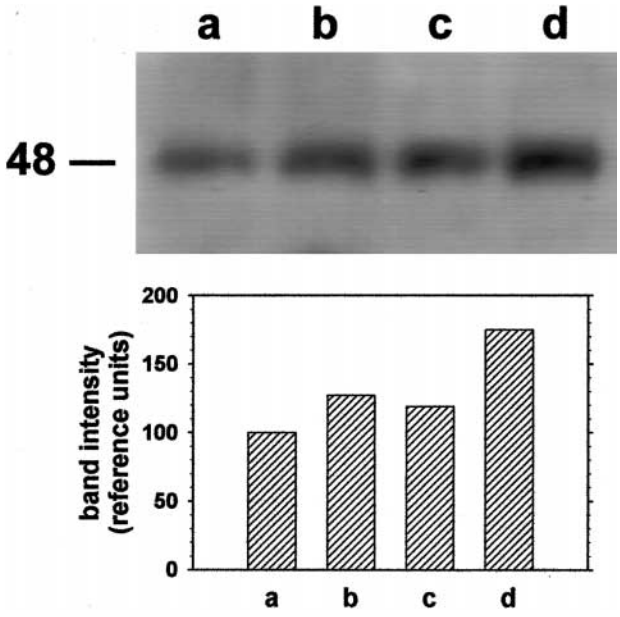

Fig 4. Muscle PM fraction GLUT4 protein immunoblot. In the upper portion is shown a typical GLUT4 immunoblot. The lower graph shows analysis of this blot using the ImageQuant software described in Methods. Lane a contains 20 μg of the PM fraction from the reference muscle specimen, lanes b and c contain 20 μg from biopsies of subjects with severe insulin resistance, and lane d contains 20 μg of PM fractions from a normal subject muscle biopsy.

subjects who had the quantification of mRNA. The subjects not represented in these data were those whose muscle specimen was not sufficient to prepare the membrane fractions or the sample was lost in preparation (1 subject).

Glucose Transporter Protein Subcellular Localization in Muscle in Hyperinsulinemic Patients

Figure 6 displays the immunoblot quantification for PM and golgi-enriched fractions from muscle obtained from 5 subjects (subjects a, c, d, e, f, Table 1) with severe insulin resistance and 5 control subjects (subjects h, j, n, o, q, Table 1). Each immunoblot quantification was performed 2 to 4 times, and then the means of the results for each subject were used to determine group averages. The data displayed here are expressed as percent of the total that was present in the PM fractions. This calculation was accomplished after determining by immunoblot the immunoreactivity in arbitrary units (reference sample de-

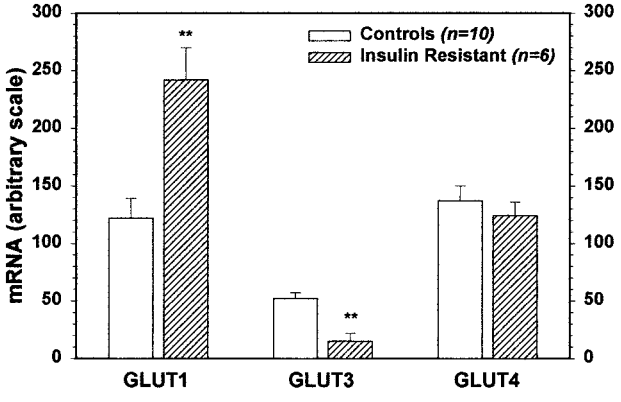

Fig 3. Quantification of mRNA concentrations for GLUT1, GLUT3, and GLUT4 in skeletal muscle obtained from normal subjects and subjects with severe insulin resistance. The data shown here represent the mean and SEM of RPA-determined concentrations for each of the 3 major glucose transporters expressed in muscle. The primary data were determined in arbitrary units defined by a reference muscle RNA sample and adjusted for GAPDH band intensity as described in Methods. The numbers in parentheses are the number of subjects included in the group. Significant difference from the determination in control subjects is indicated by asterisks. Double asterisks indicate $P < .001$.

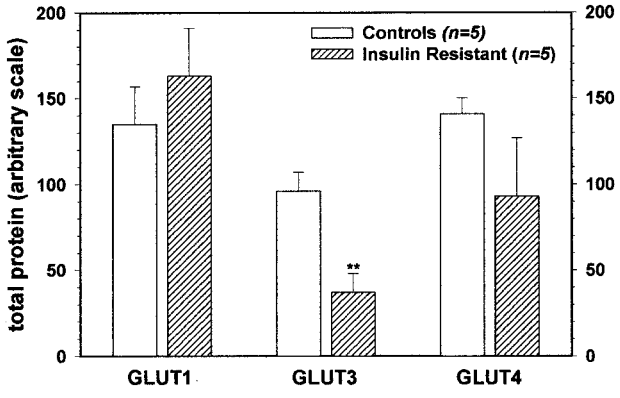

Fig 5. Muscle glucose transporter protein content in insulin-resistant subjects. The data displayed here represent the sum of the protein content for GLUT1, GLUT3, and GLUT4 in the PM fractions and the LDM obtained from 50 mg skeletal muscle obtained from vastus lateralis needle biopsies. The amount is quantified in relation to the content measured in our human muscle reference specimen for each immunoblot. Only the lower amount of GLUT3 protein achieved statistical significance with a $P < .001$.

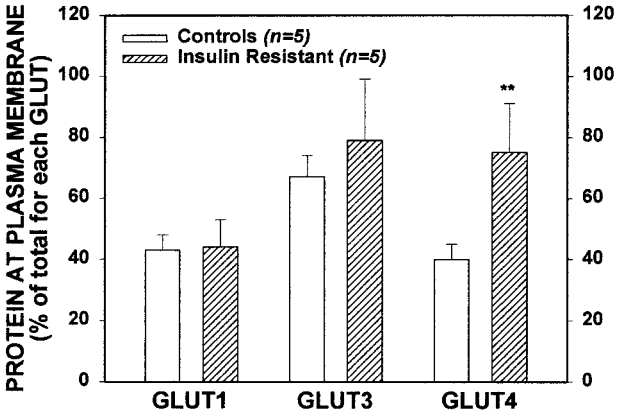

Fig 6. Glucose transporter protein in muscle PM fractions from insulin-resistant subjects. Five insulin-resistant subjects and 5 control subjects had subcellular fractions prepared from muscle needle biopsies. The data displayed represents the percent of total muscle cell-specific protein that was present at the plasma membrane for GLUT1, GLUT3, and GLUT4. GLUT1 was predominantly intracellular in both the controls and the insulin-resistant subjects. GLUT3 was predominantly at the cell surface for both groups of subjects, but GLUT4 was distinctly different. GLUT4 was mostly intracellular in the controls, but was determined to be largely at the plasma membrane in the basal state in the subjects with severe insulin resistance ($P < .001$).

fined 100 units)/μg membrane protein and multiplying that number by the total protein yield in the PM or LDM fractions. The sum of these 2 was considered to be the total tissue immunoreactivity.

DISCUSSION

Subjects in this study with severe insulin resistance and acanthosis nigricans had a lower amount of GLUT3 mRNA and protein in skeletal muscle compared with control subjects. GLUT1 mRNA was increased, but total muscle GLUT1 protein was not different from control values. Muscle GLUT4 mRNA and protein content were normal, but the GLUT4 protein was redistributed from its normal intracellular location to predominantly the cell surface.

Previous studies have found a link between glucose transporter expression and insulin responsiveness in some circumstances.[25] For example, animal and human studies have shown that GLUT4 expression in muscle and insulin responsiveness can be modulated by exercise training,[26-28] and increased insulin sensitivity is associated with increased muscle GLUT4 protein.[28-30] Transgenic mice that overexpress either GLUT1 or GLUT4 have an associated enhancement of insulin responsiveness.[31-33] In type 2 diabetes patients with insulin resistance, however, muscle GLUT4 protein concentrations are not decreased.[6,7,34,35] Further, no abnormalities of the GLUT4 structural gene have been found to be associated with diabetes.[25,36,37]

GLUT4 is normally sequestered in intracellular tubulo-vesicular elements, which have similar biochemical and morphologic characteristics in fat and muscle cells.[38] Subcellular redistribution of GLUT4 protein in hyperinsulinemic conditions has been noted previously in at least 3 human studies and in 1 rat model of insulin resistance. Garvey et al[39] isolated abdominal fat cells from term subjects with gestational diabetes. They found that the plasma membrane contained 2-fold more GLUT4 than did plasma membranes from control subjects, and very little additional GLUT4 moved to the cell surface when the cells were incubated with insulin.[39] Garvey et al[20] recently reported GLUT4 redistribution within the muscle of insulin-resistant subjects and patients with type 2 diabetes. They determined that this redistribution to more dense membrane vesicles was associated with a substantial decrease in the insulin-mediated translocation of GLUT4 to vesicles normally found in higher density sucrose fractions.[20] While Garvey et al[20] speculated that the insulin-resistant subjects might be sequestering GLUT4-containing vesicles into a separate intracellular compartment where they would be unavailable to insulin stimulation,[20] their data do not eliminate the possibility that this "higher density vesicle" compartment might be the muscle t-tubule. Zierath et al[40] isolated skeletal muscle PM fractions from 7 patients with type 2 diabetes using methods similar to ours. They found that their diabetic subjects, in the basal state, had 35% more GLUT4 protein in the plasma membrane fractions than did the fractions from the control subjects. After 40 minutes of in vivo insulin infusion, no further increase in plasma membrane GLUT4 was seen in the diabetic subjects, whereas it increased 1.6-fold in the control subjects.[40]

The Otsuka Long-Evans Tokushima Fatty (OLETF) rat is a hyperinsulinemic laboratory animal with skeletal muscle insulin resistance and visceral obesity.[41] Sato et al[42] have recently evaluated the plasma membrane content of GLUT4 protein in muscles of these rats in the basal state and found increased levels of GLUT4 compared with control rats. Hyperinsulinemic clamps failed to further increase the plasma membrane content of GLUT4 in these animals.[42]

The impact of chronic hyperinsulinemia alone on glucose transporter expression in muscle is not known. Cell culture studies suggest that insulin maintains or increases glucose transporter protein levels. Wilson et al[43] have evaluated the role of insulin in regulation of cell surface GLUT1, GLUT3, and GLUT4 in differentiated L6 cells in culture. The L6 cell line is a transformed rat skeletal muscle-derived line that differentiates on contact inhibition in culture dishes and exhibits many biochemical characteristics of skeletal muscle including contraction.[44,45] The studies of Wilson et al[43] in L6 cells showed that insulin and insulin-like growth factor (IGF)-1 both stimulate GLUT3 gene expression rather than suppressing it.

It is not established which of the glucose transporters that are found in muscle play an important role in muscle basal glucose uptake. It has been suggested that GLUT3 in muscle samples might be due entirely to the contamination of the specimens with nerve tissue.[46] However, our immunofluorescence studies suggested that only about 10% of the GLUT3 protein was associated with nerves, and the remainder was distributed throughout the muscle fibers.[47] In situ hybridization techniques confirmed diffuse expression of GLUT3 mRNA throughout the normal muscle specimens.[47]

The subcellular localization of each of the 3 major glucose transporters in muscle has not been well established, but the distributions of GLUT1, GLUT3, and GLUT4 are apparently quite different from each other. Even though GLUT3 mRNA

from muscle homogenate is only about half that of GLUT1 mRNA when quantified using a quantitative RPA, as we recently described,[18] GLUT3 protein is almost entirely on the cell surface, whereas our data suggest that GLUT1 protein is about 60% intracellular. Handberg et al,[48] using immunofluorescence techniques similar to ours, found that more than half of the GLUT1 protein signal in rat muscle was associated with contaminating perineural cells. More recently, this group has suggested that the remainder of muscle GLUT1 protein is actually from red blood cell membrane contamination, where capillaries are in contact with muscle fibers.[49] If their conclusions are correct, then it would appear that an even higher proportion of the basal muscle cell surface glucose transporter pool is made up of GLUT3. Our data showing a predominance of GLUT1 protein in LDM rather than in PM were unexpected and unexplained, but could be due to lower densities of membranes from red blood cells or capillary endothelial cells that may bear the majority of GLUT1 found in the muscle biopsy specimens.

The data presented in this report show that hyperinsulinemia is associated with decreased total GLUT3 protein (Fig 5), which is still predominantly at the cell surface (Fig 6), and redistribution of GLUT4 protein to the cell surface without a significant change in total GLUT4 mRNA or protein. In contrast, GLUT1 mRNA production appears to be increased by hyperinsulinemia, but this does not result in increased GLUT1 protein concentrations. How the difference in mRNA concentrations occurs is unclear, as we have not directly measured mRNA production and degradation rates. Modifications of either or both could explain the changes in concentrations that we found.

These data cannot determine whether fasting hyperinsulinemia results from the decrease in skeletal muscle GLUT3 gene expression. The possibility exists that the decreased GLUT3 expression is caused by hyperinsulinemia rather than being the primary event. The data from 1 normal control subject speak against this, in that he had moderate hyperinsulinemia (fasting 23 μU/mL or 161 pmol/L), but also expressed levels of GLUT3 protein and message about twice that of the mean of the group (data not shown). Moreover, the in vitro studies of Wilson et al[43] in cultured muscle cells suggest that insulin does not suppress GLUT3 mRNA. Further studies are needed to better understand the complex regulation of these 3 glucose transporters in human muscle.

We conclude that fasting hyperinsulinemia in subjects with acanthosis nigricans and severe insulin resistance was associated with lower expression of GLUT3 mRNA and protein in skeletal muscle. GLUT1 mRNA was increased without a corresponding increase in GLUT1 protein. GLUT4 mRNA and total protein were normal in our insulin-resistant subjects, but hyperinsulinemia in these subjects with severe insulin resistance is associated with a basal redistribution of GLUT4 from its normal intracellular sequestered compartment to the plasma membrane. These data are consistent with the hypothesis that a primary defect in the normal muscle basal glucose uptake mechanism can cause a chronic, hyperinsulinemia-associated redistribution of GLUT4, which in turn, blunts the glucose uptake response to further increments in circulating insulin.

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