Induction of Insulin Resistance in Human Skeletal Muscle Cells by Downregulation of Glycogen Synthase Protein Expression

K.S. Park, T.P. Ciaraldi, L. Carter, S. Mudaliar, S.E. Nikoulina, N.J.G. Webster, and R.R. Henry

Glycogen synthase (GS) is the rate-limiting enzyme controlling nonoxidative glucose disposal in skeletal muscle. A reduction in GS activity and an impaired insulin responsiveness are characteristic features of skeletal muscle in type 2 diabetes. These properties also exist in human skeletal muscle cell cultures from type 2 diabetic subjects. To determine the effect of an isolated reduction in GS on skeletal muscle insulin action, cultures from nondiabetic subjects were treated with antisense oligonucleotides (ODNs) to GS to interfere with expression of the gene. Treatment with antisense ODNs reduced GS protein expression by 70% compared with control (scrambled) ODNs ($P < .01$). GS activity measured at 0.01mmol/L glucose-6-phosphate (G-6-P) was reduced by antisense ODN treatment. The insulin responsiveness of GS was impaired. Insulin also failed to stimulate glucose incorporation into glycogen after antisense ODN treatment. The cellular glycogen content was lower in antisense ODN–treated cells compared with control ODN. The insulin responsiveness of glucose uptake was abolished by antisense ODN treatment. Thus, reductions in GS expression in human skeletal muscle cells lead to impairments in insulin responsiveness and may play an important role in insulin-resistant states.

SKELETAL MUSCLE is the principal glucose-utilizing tissue in the insulin-stimulated state and a major site of insulin resistance in type 2 diabetes.[1,2] Following insulin stimulation, glycogen synthesis is a major pathway of glucose disposal in skeletal muscle and is regulated by the rate-limiting enzyme glycogen synthase (GS).[3,4] In type 2 diabetes, an impaired rate of insulin-stimulated glucose uptake is associated with decreased glycogen formation and impaired GS activity.[4-6] Our group and others have previously shown that both basal and insulin-stimulated muscle GS activity are reduced in type 2 diabetes and defective GS activity has a major role in impaired glycogen formation in diabetes.[4,7] It has also been reported that GS activity is reduced in nondiabetic subjects with a strong family history of type 2 diabetes.[8] These findings led to the hypothesis that certain genetic determinants impair GS activity, although environmental factors such as hyperinsulinemia or hyperglycemia can also exert effects.[9]

Further evidence for a genetic determinant of glycogen synthesis is derived from a report showing that glycogen synthesis is reduced in fibroblasts cultured from type 2 diabetic subjects.[10] Recent studies demonstrated that GS mRNA expression in muscle is decreased by about one third in insulin-resistant type 2 patients compared with matched control subjects, although GS protein expression is normal.[11,12] A possible explanation for this discrepancy may be the existence of compensatory mechanisms in diabetes that ensure an increased translation of GS mRNA or increased stability of GS protein. Using the human skeletal muscle culture system, in which the cells are responsive to insulin and display the morphologic, biochemical, and metabolic characteristics of intact skeletal muscle tissue,[13] we found that muscle cells from type 2 diabetic subjects cultured in physiologic concentrations of glucose and insulin demonstrated reduced GS protein expression in parallel with defects in both glucose uptake and GS activity,[14] reflecting the in vivo behavior of diabetic skeletal muscle. However, the physiologic significance of a reduction in this pivotal protein to the defects in insulin action remains uncertain.

The present study was undertaken to determine the effects of an isolated reduction of GS protein on GS activity, glycogen synthesis, glucose uptake, and insulin responsiveness in human skeletal muscle cultures from nondiabetic subjects. The expression of GS protein was reduced by the use of antisense oligonucleotides directed against GS mRNA.

SUBJECTS AND METHODS

Subjects

Seven healthy nondiabetic subjects without a family history of type 2 diabetes provided muscle tissue for GS gene inhibition studies. Subject characteristics are summarized in Table 1. All subjects underwent a 2-hour 75-g oral glucose tolerance test to confirm normal glucose levels. None of the subjects displayed any evidence of fasting or postprandial hyperinsulinemia. Normal glucose tolerance was defined by American Diabetes Association criteria as fasting glucose less than 126 mg/dL and 2-hour glucose less than 140 mg/dL.[15] Insulin action was determined by a 3-hour hyperinsulinemic (300 mU/m^2/min)-euglycemic (5.0 to 5.5 mmol/L) clamp as described in detail previously.[4] The glucose disposal rates (GDRs) determined during the last 30 minutes of the clamp were normal in these subjects (Table 1). The experimental protocol was approved by the Committee on Human Investigation of the University of California, San Diego. Informed written consent was obtained from all subjects after an explanation of the protocol.

Materials

Lipofectin and OptiMEM1 were purchased from GIBCO-BRL (Gaithersburg, MD). Phosphorothioate oligodeoxynucleotides were obtained from Quality Controlled Biochemicals (Hopkinton, MA). All radioisotopes were obtained from DuPont-NEN (Boston, MA). Bovine serum albumin (fraction V) was purchased from Boehringer Mannheim (Indianapolis, IN). 2-Deoxyglucose, L-glucose, pepstatin, leupeptin, phenylmethylsulfonyl fluoride, and all other reagents were purchased

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Address reprint requests to R.R. Henry, MD, VA San Diego Healthcare System (V111G), 3350 La Jolla Village Dr, San Diego, CA 92161.

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Table 1. Subject Characteristics (N = 7)

Age (yr)	BMI (kg/m^2)	Fasting Plasma Glucose (mmol/L)	Fasting Plasma Insulin (pmol/L)	2-Hour OGTT Glucose (mmol/L)	2-Hour OGTT Insulin (pmol/L)	GDR (mg/kg/min)
36 ± 2	25.9 ± 1.0	4.9 ± 0.1	30 ± 6	5.5 ± 0.4	192 ± 42	11.2 ± 2.3

Abbreviations: BMI, body mass index; OGTT, 75-g oral glucose tolerance test.[16]

from Sigma Chemical (St Louis, MO). Anti–rabbit and anti–mouse immunoglobulin G conjugated with horseradish peroxidase were purchased from Amersham (Arlington Heights, IL). SuperSubstrate chemiluminescence substrate was from Pierce (Rockford, IL).

Human Skeletal Muscle Cultures

Percutaneous muscle biopsies were obtained from the lateral aspect of the quadriceps femoris (vastus lateralis) using local anesthesia and a 5-mm side-cutting needle as described previously.[4] Muscle tissue (200 to 300 mg) was obtained and immediately processed, and cultures were established from satellite cells of dissociated muscle tissue using modifications of the methods of Blau and Webster[17] and Sarabia et al[18] as described in detail previously.[13]

GS Gene Inhibition With Antisense ODNs; Antisense ODN Design and Synthesis

A region of GS mRNA overlapping the start codon was chosen in the design of antisense ODNs.[19] A Genbank homology search was performed to ensure that the sequences chosen did not share homology with other known gene sequences. The GS antisense phosphorothioate oligodeoxynucleotide overlapping the start codon (5′-TAAAGGCATG-GCTGGCGC-3′) and the control oligodeoxynucleotide (the same base composition as the GS antisense with the sequence scrambled (5′-CGG AGC CAG ACG GTG TAT-3′) were synthesized on an automated solid-phase DNA synthesizer using standard phosphoramidite chemistry with purification through Sep-Pak columns (Applied Biotechnology, Milford, MA). Lyophilized ODNs were dissolved in water, and their concentration was determined by spectrophotometry.

Inhibition of GS Gene Expression

Human myoblasts were grown at 37°C in a 5% CO_2 incubator until 70% to 80% confluence was reached. At this stage, the cells were uniformly fused and differentiated into phenotypically mature myotubes.[13] Fusion and differentiation was achieved by 4-day growth in α-minimum essential medium (α-MEM) with 2% FBS and 1% fungibact and confirmed by visual estimates of multinucleated myotubes. On the first day of fusion, the cells were rinsed with serum-free OptiMEM1 and incubated in the presence of antisense or control ODNs using Lipofectin as the ODN carrier. ODNs (0.5 μmol/L) and Lipofectin (5 μg/mL) were mixed in 1 mL serum-free OptiMEM1 and incubated at room temperature for 15 minutes. The ODNs-Lipofectin mixture was then added to the appropriate culture wells and incubated at 37°C for 4 hours. After transfection, the excess ODNs were washed away and 2 mL α-MEM with 2% FBS was added to each well. The ODNs-Lipofectin mixture was added repeatedly every 2 days for 6 days followed by harvesting of cells for assay of GS activity, glycogen synthesis, total glycogen content, glucose uptake, and GS and glucose transporter protein determination.

Immunoblot Detection of GS

The sample preparation for Western blot analysis and detection of GS protein for human skeletal muscle cell cultures has recently been described in detail.[13] GS protein was blotted using affinity-purified polyclonal antibodies raised in rabbits against an oligopeptide specific for the COOH-terminal sequence (kindly provided by Dr Leif Groop, Malmo, Sweden). Immune complexes were detected using an enhanced chemiluminescence kit and quantified by scanning densitometry (Stratoscan 7000; Stratagene, La Jolla, CA).

GS Activity

Enzyme activity was measured according to methods described in detail previously for human skeletal muscle cell cultures.[7] At the end of the transfection experiments, GS activity was measured at 0.1 and 10 mmol/L glucose-6-phosphate (G-6-P) in the absence and presence of 33 nmol/L insulin. Results are expressed as nanomoles of UDP glucose (UDPG) incorporated into glycogen per milligram protein per minute. Fractional velocity ([FV] activity at 0.1 mmol/L G-6-P/activity at 10 mmol/L G-6-P) is presented as percent activity.

Glucose Flux Through Glycogen

Glucose incorporation into muscle glycogen was determined at the completion of transfection as previously described,[20] from the incorporation of D-[^{14}C-U]glucose (0.5 μCi/reaction, 5 mmol/L final glucose concentration) in the absence or presence of maximal (33 nmol/L) insulin concentrations for 2 hours. Results are expressed as nanomoles of glucose converted to glycogen per milligram of protein per hour. Total cellular protein was determined by the Bradford method.[21]

Total Glycogen Content

The total glycogen content of the cells was measured by the method of Hassid and Abraham.[22] Results are expressed as milligrams of glycogen per milligram of protein.

2-Deoxyglucose Uptake

The procedure for glucose uptake has been described previously.[23] Briefly, following completion of the transfection, cells were incubated in serum-free media in the absence or presence of 33 nmol/L insulin for 1 hour. Glucose uptake was determined in triplicate after the addition of 10 μL [2-^{3}H]deoxyglucose/L-[^{14}C]glucose (0.1 μCi, final concentration 0.01 mmol/L) to conditions under which cell membrane transport is rate-limiting.[24] Each measurement was performed in triplicate and each value was corrected for trapping and diffusion with L-glucose. Results were normalized to total cellular protein determined by the Bradford method[21] and are expressed as picomoles per milligram of protein per minute.

Membrane Preparation

Cells for membrane preparation were grown in 100-mm dishes and treated as described for uptake assays. Total membranes were prepared by the method developed by Walker et al[25] and described previously.[23] The cells were scraped from the dishes, collected by centrifugation, and homogenized with a Dounce homogenizer. After centrifugation at 750 × *g* for 3 minutes, the pellet was rehomogenized and recentrifuged and the supernatants were combined. Centrifugation of the supernatant at 190,000 × *g* for 60 minutes produced a total membrane pellet. The membranes were resuspended in homogenization buffer and protein content was determined.

Detection of Glucose Transporter Proteins

Membrane preparations were diluted 1:1 in 2× Laemmli buffer without β-mercaptoethanol[26] and heated for 5 minutes at 90°C. Proteins were separated on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels and then transferred to nitrocellulose.[27] GLUT1 was identified using rabbit polyclonal antisera against the rat brain glucose transporter (Chemicon, Temecula, CA). A monoclonal antibody specific for GLUT4 (1F8; Biogenesis, Brentwood, NH)) was also used. The second antibody was anti–rabbit or anti–mouse IgG conjugated

with horseradish peroxidase. Immune complexes were detected using an enhanced chemiluminescence kit. Quantitation was performed with a scanning laser densitometer (ScanAnalysis; BioSoft, Palo Alto, CA).

Data Analysis

Data analysis was performed with the paired or unpaired *t* test as appropriate. Paired comparisons were made to 3 separate sets of controls on cells from the same individual (intraindividual pair): no treatment, Lipofectin alone, and Lipofectin + scrambled (control, GS3) ODN. Differences between GS1 and GS3 would indicate effects of the antisense ODN. The expression of the results as a percent of the control was obtained by comparison to the appropriate paired control for each set of cells. All data calculations and statistical analyses were performed using the Statview II program (Abacus Concepts, Berkeley, CA).

RESULTS

Effect of GS Antisense ODN Treatment on GS Protein Expression

Neither Lipofectin nor the control ODN (GS3) had any effect on GS protein content. However, GS protein expression was decreased significantly following antisense ODN (GS1) treatment (Fig 1). GS protein levels were reduced to 29% ± 6% of the level in control ODN-treated cells ($P < .01$). The content of sarcomeric α-actin protein, a marker of the muscle phenotype, was unaltered by treatment with either ODN or Lipofectin alone (data not shown), suggesting little nonspecific change in protein expression.

Effect of GS Antisense ODN Treatment on GS Activity

GS activity measured at a low concentration (0.1 mmol/L) of the allosteric activator G-6-P reflects both the amount of enzyme protein and, primarily, the influence of allosteric regulation. Lipofectin treatment alone caused a modest but nonsignificant increase in $GS_{0.1}$ activity in the absence of insulin (118% ± 8% of control; Fig 2A). Treatment with control ODN reduced $GS_{0.1}$ activity (80% ± 8% of untreated control, $P < .05$), but antisense ODN reduced $GS_{0.1}$ even further (to 50% ± 11% of control ODN, $P < .05$). With regard to insulin-stimulated $GS_{0.1}$ activity, Lipofectin treatment had no effect, while the activity was reduced in both control ODN (65% ± 8% of control, $P < .01$) and antisense ODN (50% ± 6%, $P < .0005$) cells. The impairment in insulin-stimulated $GS_{0.1}$ activity was greatest after antisense ODN treatment (to 73% ± 7% of control ODN, $P < .05$). Acute insulin exposure caused a significant stimulation of $GS_{0.1}$ in untreated ($P < .02$ *v* paired basal), Lipofectin-treated ($P < .05$), and control ODN–treated ($P < .05$) cells, but antisense ODN–treated cells did not display a significant response to insulin ($P = .62$ *v* without insulin) and could be considered insulin-resistant for this effect (Fig 2A).

The GS fractional velocity (FV) is indicative of the activation state of the enzyme. The basal GS FV was similarly reduced by control ODN (74% ± 8% of untreated control, $P < .02$) and antisense ODN (80% ± 4%, $P < .01$) treatment (Table 2). There was no significant difference between control and antisense ODN. Insulin stimulated GS FV by 30% to 60% in the 3 control groups; only antisense ODN–treated cells failed to show a significant response to insulin (2% ± 13% increase) and could be considered insulin-resistant for this response.

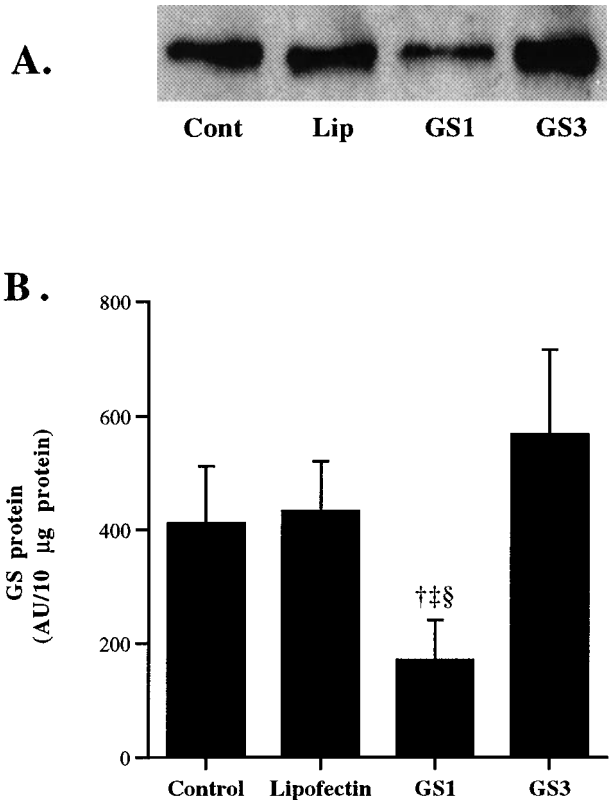

Fig 1. Effect of antisense ODN treatment on GS protein expression in human skeletal muscle cultures from nondiabetic subjects. Cultured muscle cells were pretreated with Lipofectin (5 μg/mL) and 0.5 μmol/L of either a GS antisense ODN (GS1) or GS control ODN (GS3) for 6 days. Control, no addition; Lipofectin, vehicle only; GS1, GS antisense ODN + Lipofectin; GS3, GS control ODN + Lipofectin. (A) Representative autoradiogram of a GS Western blot on muscle cells from a nondiabetic subject. (B) Quantitation of immunoreactive GS protein in cells of 6 nondiabetic subjects. Results are the mean ± SEM. †$P < .01$ *v* untreated control, ‡$P < .05$ *v* Lipofectin, §$P < .05$ *v* GS3.

GS activity measured at a maximal concentration (10 mmol/L) of G-6-P has been considered as an indicator of the total enzyme activity. Lipofectin and control ODN treatment had no appreciable effects on GS activity measured at 10 mmol/L G-6-P in either the absence or the presence of insulin (Fig 2B). However, with antisense ODN treatment, both basal (to 69% ± 10% of control ODN, $P < .05$) and insulin-stimulated (64% ± 8% of control ODN, $P < .02$) GS activity decreased significantly. Thus, the effects of antisense ODN on fully activated enzyme activity (Fig 2B) are in general agreement with those on GS protein expression (Fig 1).

Effect of GS Antisense ODN Treatment on Glucose Incorporation Into Glycogen and Glycogen Content

GS measurements assess only the activity of the isolated enzyme. Net glycogen synthesis from glucose reflects the balance within the cell of substrate delivery, GS activity, and other pathways for glucose metabolism, as well as the effects of intracellular modulators. The rates of new glycogen synthesis from glucose measured in cells after the various treatments are presented in Fig 3. The flux of glucose through glycogen was

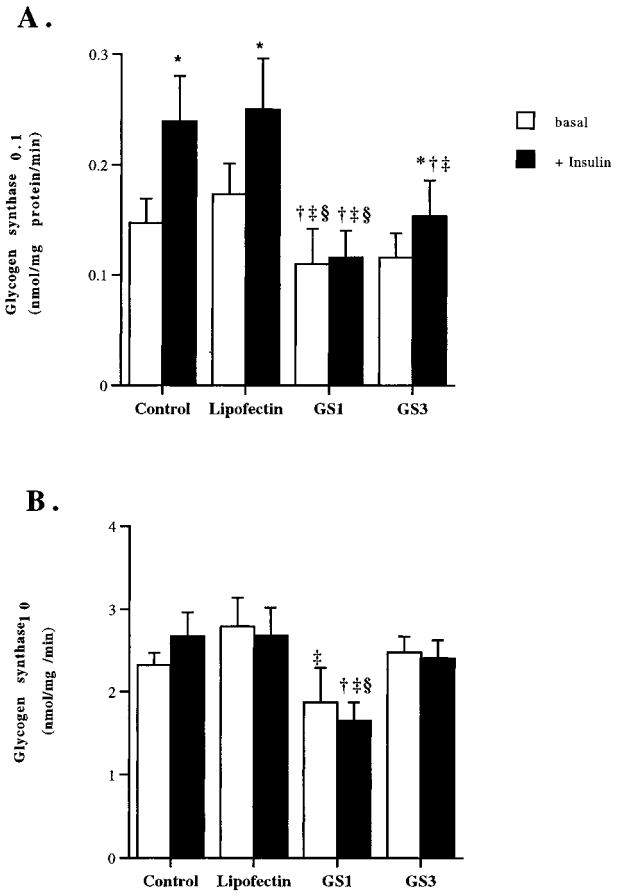

Fig 2. Effect of antisense ODN treatment on GS activity. (A) GS activity at 0.1 mmol/L G-6-P. Basal state, no insulin added to serum-free media; insulin-stimulated state, 33 nmol/L insulin added to serum-free media for 1 hour. (B) GS activity at 10 mmol/L G-6-P. Results are the mean ± SEM (n = 7). *$P < .05$ *v* corresponding basal, †$P < .05$ *v* paired untreated control, ‡$P < .05$ *v* paired Lipofection, §$P < .05$ *v* paired GS3.

not reduced in antisense ODN–treated cells. While insulin caused a 50% to 60% increase in glucose incorporation into glycogen in all control conditions, acute insulin stimulation of glycogen synthesis was impaired in antisense ODN–treated cells. The insulin-induced fold increase was significantly lower in antisense ODN–treated compared with control ODN–treated cells (72% ± 6% of the insulin increment in control ODN–treated cells, $P < .05$).

The total glycogen content, reflecting the long-term balance of metabolic processes, was significantly lower in antisense ODN–treated muscle cells (2.61 ± 0.26 mg glycogen/mg protein) versus control ODN treatment (3.33 ± 0.32, $P < .001$).

Table 2. Effect of Antisense ODN Treatment on GS FV

	Treatment			
	Control	Lipofectin	GS1	GS3
Basal	6.4 ± 1.1	6.4 ± 1.2	5.2 ± 0.9	4.8 ± 1.1
+Insulin	8.6 ± 1.0*	9.2 ± 1.5*	6.8 ± 1.1	6.4 ± 1.5*

NOTE. Values are the mean ± SEM. Basal state, no insulin added to serum-free media; insulin-stimulated state, 33 nmol/L insulin added to serum-free media for 1 hour. GS FV = (activity at 0.1 mmol/L G-6-P/10 mmol/L G-6-P) × 100 and is expressed as a %.

*$P < .05$ *v* corresponding basal value.

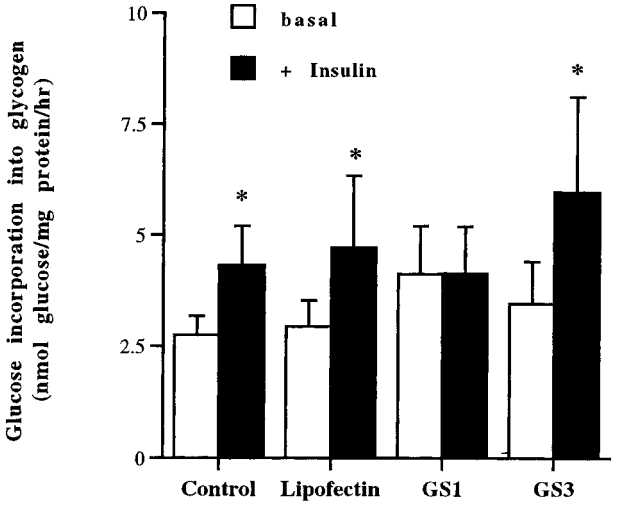

Fig 3. Effect of antisense ODN treatment on rate of glucose incorporation into glycogen in cultured muscle cells. Results are the mean ± SEM (n = 7). *$P < .05$ *v* corresponding basal value.

Effect of GS Antisense ODN Treatment on Glucose Uptake

Glucose uptake in the absence of insulin was increased significantly ($P < .005$) following Lipofectin treatment (Fig 4). The combination of Lipofectin and ODNs showed additive effects to further increase basal glucose uptake ($P < .005$ *v* untreated control for both antisense and control ODN). Glucose uptake was even higher in cells treated with antisense ODN versus control ODN–treated cells (133% ± 9% of control

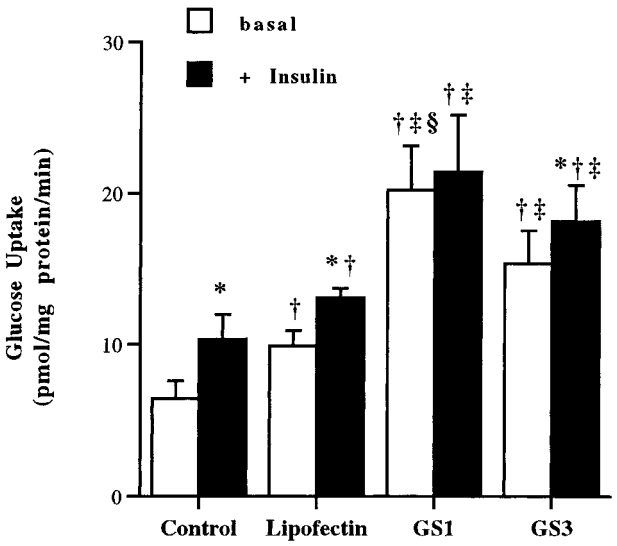

Fig 4. Effect of antisense ODN treatment on glucose uptake in human skeletal muscle culture. Cells were washed and incubated in serum-free α-MEM for 60 minutes in the absence (basal) or presence (33 nmol/L) of insulin. Cells were then washed free of media, and 2-deoxyglucose uptake was measured. Results are the mean ± SEM (n = 7). *$P < .05$ *v* corresponding basal, †$P < .05$ *v* paired control, ‡$P < .05$ *v* paired Lipofectin, §$P < .05$ *v* paired GS3.

ODN, $P < .05$). While Lipofectin (38% ± 10% increase with insulin, $P < .01$ *v* paired basal) and control ODN treatment (20% ± 7% increase, $P < .01$) maintained the insulin responsiveness for uptake, even in the face of elevated basal uptake rates, insulin responsiveness was abolished in antisense ODN–treated cells (5% ± 6% increase over paired basal).

Effect of GS Antisense ODN Treatment on Glucose Transporter Expression

To investigate the possible mechanisms by which the various treatments increased glucose uptake in muscle cells, a total membrane fraction was prepared and glucose transporter expression was determined by Western blotting. None of the treatments had any effect on GLUT4 protein expression. This is a measure of total cellular GLUT4 content and does not reveal whether the treatments had any influence on the subcellular distribution of GLUT4. Each of the treatments resulted in an upregulation of total cellular GLUT1 protein expression, although the increase in Lipofectin-treated cells did not attain statistical significance (Fig 5). The increase in GLUT1 protein generally followed the change in glucose uptake activity (Fig 4), being smallest with Lipofectin treatment and largest after ODN treatment. Interestingly, even though the increase in GLUT1 protein was similar in control and antisense ODN–treated cells, glucose uptake activity was significantly higher with antisense ODN, suggesting some further effect of GS reduction on glucose transporter activity or localization.

DISCUSSION

A number of approaches have established impaired skeletal muscle GS activity and glycogen synthesis as common characteristics of type 2 diabetes.[4-7] Several lines of evidence suggest that defects in GS occur early in the development of diabetes. Skeletal muscle GS activity has been found to be reduced in nondiabetic subjects with a family history of type 2 diabetes.[8] The fact that glycogen synthesis is reduced in cultured fibroblasts from type 2 diabetic subjects,[10] far removed from the in vivo metabolic environment, implies that defects in GS may be an intrinsic property of the diabetic state. GS activity is also reduced in human skeletal muscle cell cultures from type 2 subjects,[14] strengthening this concept in the primary insulin target tissue.

The impaired GS activity in diabetic muscle has several characteristics. While GS mRNA has been shown to be reduced,[11] protein expression is essentially normal. Fully activated (10 mmol/L G-6-P) enzyme activity, a general indicator of enzyme protein, is also normal in diabetic muscle.[11] The greatest reductions are found for activity measured at low levels of the regulator G-6-P ($GS_{0.1}$) and the FV,[7] suggesting that the major defects involve allosteric and covalent regulation of enzyme activity. Many of these characteristic defects of GS are retained in skeletal muscle cultures. Both $GS_{0.1}$ and FV are reduced in diabetic cultures compared with nondiabetic cells.[14] The total activity does not differ significantly from that in normal human muscle cells but GS protein is lower, possibly reflecting the removal of compensatory mechanisms such as hyperinsulinemia that are present in vivo. Beyond the impairments of GS present in diabetic muscle in vivo, as well as in muscle cells, other characteristics of type 2 diabetes include reduced glucose uptake and impaired insulin responsiveness for uptake and glycogen synthesis. The current studies have addressed 2 questions: What impact will reducing GS activity in nondiabetic human skeletal muscle cells have on insulin action and glucose metabolism? In addition, will reducing GS activity to levels present in diabetic cells cause normal cells to develop other characteristics of diabetic muscle with regard to glucose metabolism?

A gene-knockout approach was used to influence GS expression. In mammalian culture systems, numerous studies have demonstrated effects of antisense ODNs and have used this technology to understand the normal function of a particular gene.[28-31] We used phosphorothioate ODNs, which are resistant to intracellular degradation, and cationic lipids to enhance the uptake and activity of ODNs.[32] With GS antisense ODN treatment, we were able to decrease GS protein expression by up to 70%, which is similar to the level we observed previously in diabetic muscle cells (50% reduction[14]). This effect is specific to antisense ODN treatment, since GS protein expression did not change with the scrambled (nonsense) sequence control ODN.

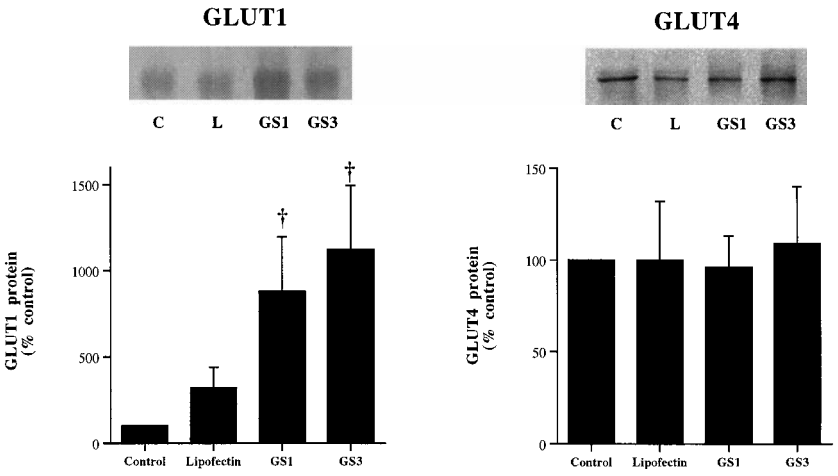

Fig 5. Effect of ODN treatment on glucose transporter protein expression. (A) GLUT 1 protein expression: top, representative Western blot for GLUT1 in cells from 1 individual; bottom, quantitation of autoradiographs. Results are normalized against the untreated control for each subject. (B) GLUT4 protein expression—representative Western blot and quantitation. Mean ± SEM (n = 6). †$P < .05$ *v* paired control.

The behavior of fully active GS was qualitatively reflective of that of GS protein, with changes only in the antisense ODN–treated cells. This result differs somewhat from the diabetic cells, where activity at 10 mmol/L G-6-P is either normal or slightly reduced.[14] There may be several reasons that GS activity at 10 mmol/L G-6-P after treatment with antisense ODN (35% decrease) is not reduced to the same extent as GS protein (70% decrease). GS activity was measured at a single nonsaturating substrate concentration (0.3 mmol/L UDPG). It is possible that after antisense ODN treatment, the affinity for substrate is altered so that enzyme activity would be higher at 0.3 mmol/L UDPG, underestimating the change in enzyme activity. Most importantly, GS activity at 0.1 mmol/L G-6-P in antisense ODN–treated cells was reduced to 50% of the control value, the same relative decrease reported in diabetic muscle cells.[13,14] The GS FV did not differ in antisense ODN–treated cells versus control ODN–treated cells (Table 2). In diabetic muscle cells, GS activity at 0.1 mmol/L G-6-P decreased more markedly than total GS activity, and FV was also significantly lower.[14] Thus, in antisense ODN–treated cells, GS is maintained in a normal activation state and the absolute activity is lower due to the decrease in GS protein, whereas in diabetic muscle cells, it is both the expression and the activation state of GS that are impaired.

To control for any nonspecific influence of the experimental manipulations, paired comparisons were made to Lipofectin and scrambled ODN–treated cells. These conditions were neutral, with no change versus the untreated control for total GS protein and activity expression, as well as GS FV. While $GS_{0.1}$ appeared lower than the control value in scrambled ODN–treated cells (Fig 2), this difference was significant only for insulin-stimulated activity. In addition, there was a significantly greater decrement in $GS_{0.1}$ resulting from antisense ODN treatment. Thus, some portion of the reduction in $GS_{0.1}$ activity is a specific result of the decreasing GS protein.

Having attained the goal of diabetic-like 50% reductions in GS protein and $GS_{0.1}$ activity, albeit by a different mechanism, how does glucose metabolism in antisense ODN–treated cells compare with that in type 2 diabetic cells? As already mentioned, GS FV in the absence of insulin is normal in antisense ODN cells, confirming that the reduced GS activity is due to the change in protein expression, while in diabetic cells, it is primarily due to changes in enzyme activation. The absolute insulin-stimulated increase in GS FV is reduced to 50% of control or nondiabetic values in both antisense ODN and type 2 diabetic[14] cells. Thus, both instances of reducing GS activity lead to impaired insulin responsiveness for GS stimulation.

Total glycogen content reflects the long-term consequence of glycogen synthesis and breakdown. Glycogen content in type 2 diabetic muscle is usually normal,[33] but this observation may be influenced by hyperglycemia through a mass-action mechanism. Glycogen content was significantly lower in antisense ODN–treated cells versus control ODN cells, which might be an expected consequence of lower GS activity in the presence of matched glucose levels attained in culture.

Insulin-stimulated skeletal muscle glycogen synthesis is impaired in type 2 diabetics[3,7] and their offspring.[33] Unexpectedly, the antisense ODN reduction of GS protein did not significantly alter the rate of new glycogen synthesis in the absence of added insulin (P = NS). However, antisense ODN–treated cells failed to display normal insulin stimulation of glucose incorporation into glycogen (P = NS *v* paired control; Fig 3).

A surprising result is the finding that glucose uptake was upregulated following treatment (Fig 4). This change could be accounted for by increases in GLUT1 protein expression (Fig 5). While a portion of these changes are induced by the control manipulations, through unknown mechanisms, there is a further increase in uptake that can be attributed to a specific effect of antisense ODN treatment, as the uptake is significantly higher compared with control ODN–treated cells. The increase in uptake could represent an attempt by the muscle cell to compensate for impaired glucose storage in glycogen through an increase in substrate delivery, by increasing the ''push'' of glucose to overcome the lessened ''pull'' through GS. Increased glucose entry into the cell could help explain why basal glucose incorporation into glycogen was not lower than the control value in antisense ODN–treated cells (Fig 3) even when GS protein and activity was reduced. Yet glucose flux through glycogen in antisense ODN–treated cells could be considered inappropriately low for the amount of glucose uptake, suggesting that the rate-determining step for glycogen synthesis now resides at GS rather than glucose uptake. The fate of the additional glucose entering antisense ODN cells, since it is not being stored in glycogen, is currently unknown and a question worthy of further study. It is interesting to note that both glucose uptake and GLUT1 protein expression are lower in diabetic compared with nondiabetic muscle cells.[23] This suggests that diabetic muscle cells lack the ability to upregulate glucose uptake to compensate for defects in GS. Thus, one characteristic of diabetic muscle may be an inability to adapt appropriately to changes in the metabolic environment.

A common consequence of the decrease in GS expression was the failure of insulin to significantly stimulate GS FV (Table 2), glucose incorporation into glycogen (Fig 3), or glucose uptake (Fig 4) in antisense ODN–treated cells. Thus, a targeted reduction in GS can not only perturb glucose metabolism but also generate relative insulin resistance. The specific mechanisms of this insulin resistance, if it involves insulin signal transduction or the final effector systems, remain to be determined.

Although the identity of the primary defect(s) of glucose metabolism or insulin action leading to type 2 diabetes is a controversial topic,[8-10,33,34] our results suggest that decreased skeletal muscle GS protein expression and/or reduced GS activity can explain impairments in GS activity. This perturbation also leads to impaired insulin responsiveness for multiple aspects of glucose metabolism, mirroring the insulin resistance characteristic of type 2 diabetes. However, normal to elevated basal glycogen synthesis following a decrease of GS protein is not in agreement with the activities found in type 2 diabetes. This discrepancy can be accounted for by the elevated glucose uptake that occurs in GS-knockout cells but not in diabetic muscle. Impairments in GS activity can contribute to some but not all of the characteristic features of glucose metabolism in diabetic skeletal muscle. More likely, as already proposed previously, type 2 diabetes results from the convergence of an array of metabolic defects with synergistic effects on metabolism and insulin action.

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