Muscle Fiber Type–Specific Defects in Insulin Signal Transduction to Glucose Transport in Diabetic GK Rats

Xiao Mei Song, Yuichi Kawano, Anna Krook, Jeffrey W. Ryder, Suad Efendic, Richard A. Roth, Harriet Wallberg-Henriksson, and Juleen R. Zierath

To determine whether defects in the insulin signal transduction pathway to glucose transport occur in a muscle fiber type–specific manner, post-receptor insulin-signaling events were assessed in oxidative (soleus) and glycolytic (extensor digitorum longus [EDL]) skeletal muscle from Wistar or diabetic GK rats. In soleus muscle from GK rats, maximal insulin-stimulated (120 nmol/l) glucose transport was significantly decreased, compared with that of Wistar rats. In EDL muscle from GK rats, maximal insulin-stimulated glucose transport was normal, while the submaximal response was reduced compared with that of Wistar rats. We next treated diabetic GK rats with phlorizin for 4 weeks to determine whether restoration of glycemia would lead to improved insulin signal transduction. Phlorizin treatment of GK rats resulted in full restoration of insulin-stimulated glucose transport in soleus and EDL muscle. In soleus muscle from GK rats, submaximal and maximal insulin-stimulated insulin receptor substrate (IRS)-1 tyrosine phosphorylation and IRS-1–associated phosphatidylinositol (PI) 3-kinase activity were markedly reduced, compared with that of Wistar rats, but only submaximal insulin-stimulated PI 3-kinase was restored after phlorizin treatment. In EDL muscle, insulin-stimulated IRS-1 tyrosine phosphorylation and IRS-1–associated PI-3 kinase were not altered between GK and Wistar rats. Maximal insulin-stimulated Akt (protein kinase B) kinase activity is decreased in soleus muscle from GK rats and restored upon normalization of glycemia (Krook et al., *Diabetes* 46:2100–2114, 1997). Here, we show that in EDL muscle from GK rats, maximal insulin-stimulated Akt kinase activity is also impaired and restored to Wistar rat levels after phlorizin treatment. In conclusion, functional defects in IRS-1 and PI 3-kinase in skeletal muscle from diabetic GK rats are fiber-type–specific, with alterations observed in oxidative, but not glycolytic, muscle. Furthermore, regardless of muscle fiber type, downstream steps to PI 3-kinase (i.e., Akt and glucose transport) are sensitive to changes in the level of glycemia. *Diabetes* 48:XXX–XXX, 1999

From the Departments of Clinical Physiology (X.M.S., Y.K., A.K., J.W.R., H.W.-H., J.R.Z) and Molecular Medicine (S.E.), Karolinska Hospital, Stockholm, Sweden; and the Department of Molecular Pharmacology (R.A.R), Stanford University School of Medicine, Stanford, California.

Address correspondence and reprint requests to Juleen R. Zierath, PhD, Associate Professor of Physiology, Department of Clinical Physiology, Karolinska Hospital, S-171 76 Stockholm, Sweden. E-mail: jrz@klinfys.ks.se.

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X.M.S. and Y.K. contributed equally to this work.

EDL, extensor digitorum longus; GSK3, glycogen synthase kinase 3; IR, insulin receptor; IRS, insulin receptor substrate; KHB, Krebs-Henseleit buffer; PI, phosphatidylinositol.

In skeletal muscle, early and intermediate steps in the insulin-signaling cascade including the insulin receptor (IR) (1–4), insulin receptor substrate (IRS)-1 (4–7), and phosphatidylinositol (PI) 3-kinase (6–10) are candidates for defects associated with insulin-resistant states. Reduced activity or expression of these insulin-signaling molecules may be genetic and/or acquired (11). However, the extent to which insulin-signaling defects are causative in the development of insulin resistance, or secondary to the altered metabolic state associated with NIDDM, is not clear.

Skeletal muscle is composed of both oxidative (slow-twitch) and glycolytic (fast-twitch) fibers (12). Slow-twitch (type I) muscle fibers have a high oxidative capacity and are characterized by increased fatty acid oxidation, increased triglyceride storage, and low glycolytic capacity, compared with type II fibers (13–15). Fast-twitch fibers can be divided into two subtypes: type IIa (fast-twitch oxidative) and type IIb (fast-twitch glycolytic). Type IIa fibers are characterized by a high oxidative and low glycolytic metabolic capacity, whereas type IIb fibers have a low oxidative and high glycolytic metabolic capacity (16). In view of these different metabolic characteristics, muscle fiber type–specific defects in the insulin-signaling pathway may contribute to the diabetic phenotype. For example, muscle fiber type–specific defects in insulin action on glucose uptake and metabolism have been demonstrated in the high-fat diet-fed rodent (10,17).

Hyperglycemia is believed to contribute to the development of peripheral insulin resistance associated with both IDDM and NIDDM. In people with NIDDM, a negative correlation between fasting blood glucose level and insulin-stimulated glucose transport and metabolism in skeletal muscle has been reported (18,19). The direct impact of hyperglycemia on insulin action has been demonstrated in several studies in which euglycemia is achieved in diabetic rats by administration of phlorizin, a distal renal tubular inhibitor of glucose reabsorption (20–22). Correction of hyperglycemia by phlorizin treatment restores insulin-stimulated glucose transport in isolated adipocytes and improves whole-body insulin sensitivity in partially pancreatectomized diabetic rats (20,21) and normalizes glucose metabolism in liver and peripheral tissue in streptozotocin-induced diabetic rats (22). The extent to which hyperglycemia contributes to the development of

insulin resistance in glycolytic versus oxidative muscle fibers is presently unknown.

We determined the fiber-type–specific responses of several key components of the insulin signal transduction pathway in oxidative and glycolytic skeletal muscle from Wistar and diabetic GK rats. Soleus (84% type I, 16% type IIa, 0% type IIb) and extensor digitorum longus (EDL) (3% type I, 57% type IIa, 40% type IIb) muscle were chosen because they represent two distinctly different fiber-type populations (12). The GK rat is a spontaneous nonobese model of NIDDM, developed by selective breeding of glucose-intolerant Wistar rats over several generations (23). To evaluate the role of hyperglycemia in the development of insulin resistance, we have treated GK diabetic rats with phlorizin for 4 weeks. Initially, we reported that normalization of blood glucose in GK rats was associated with improved glucose tolerance and full restoration of insulin-stimulated Akt kinase activity in soleus muscle (24). Here, we provide evidence that insulin-signaling defects can occur in a fiber-type–specific manner in skeletal muscle. In diabetic GK rats, tyrosine phosphorylation of IRS-1 and IRS-1–associated PI 3-kinase activity is severely reduced in oxidative (soleus) muscle, with no impairment in glycolytic (EDL) muscle. In contrast, downstream components of the insulin signal transduction pathway, including Akt and glucose transport, are impaired in oxidative (24) and glycolytic muscle from GK rats and fully restored to Wistar levels with improved glycemic control. Thus, downstream rather than upstream steps to PI 3-kinase appear to be sensitive to changes in the glycemic milieu.

RESEARCH DESIGN AND METHODS

Animals. Male GK rats (200–250 g) were obtained from our colony at the Karolinska Institute. Age- and weight-matched male Wistar rats served as controls (B&K Universal, Sollentuna, Sweden). All rats were maintained under a 12-h light/dark cycle and had free access to water and standard rodent chow. Four groups of rats were studied: vehicle-treated Wistar rats (n = 12), phlorizin-treated Wistar rats (n = 5), vehicle-treated GK rats (n = 12), and phlorizin-treated GK rats (n = 11). Phlorizin (0.8 g/kg body wt per day made up as a 40% solution in propylene glycol) or vehicle was administered as a subcutaneous injection in equal doses at 12-h intervals for 4 weeks. Phlorizin was administered 14–18 h before physiological testing of the animals. Levels of plasma insulin and glucose tolerance in treated and untreated Wistar and GK rats have been previously reported (24).

Muscle incubations. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt). Isolated soleus and EDL muscle were used for in vitro incubation. Each soleus and EDL muscle was split into two equal longitudinal portions before incubation. The procedure and suitability of the muscle preparation has been previously described (25,26). All incubation media were prepared from a stock solution of pregassed (95% O_2/5% CO_2) Krebs-Henseleit buffer (KHB), which contained 5 mmol/l Hepes and 0.1% bovine serum albumin (radioimmunoassay grade). The gas phase in the vials was maintained at 95% O_2/5% CO_2 throughout all incubations. Muscles were incubated (30°C) for 15 min in KHB supplemented with 2 mmol/l pyruvate and 38 mmol/l mannitol before addition of insulin. Thereafter, muscles were incubated in the absence or presence of insulin (0.6, 2.4, 120 nmol/l) for 6 min (signaling) or 20 min (glucose transport), as indicated in the figures.

Glucose transport activity. Glucose transport in EDL and soleus muscle was assessed using the glucose analog 3-*O*-methylglucose as described by Wallberg-Henriksson et al. (26). Muscles were incubated at 30°C, in the absence or presence of insulin (0.6, 2.4, 120 nmol/l) for 10 min, in KHB containing 5 mmol/l 3-*O*-methylglucose (2.5 µCi/mmol), and 35 mmol/l [^{14}C]mannitol (26.3 µCi/mmol). Glucose transport activity is expressed as micromoles of glucose analog accumulated per milliliter of intracellular water per hour.

Immunoprecipitation and Western blotting. One half of each soleus or EDL muscle was homogenized in ice-cold buffer and centrifuged at 150,000*g* for 35 min (4°C), as described previously (4). Protein was determined using a kit from Bio-Rad (Richmond, CA). Aliquots of supernatant (500 µg) were immunoprecipitated overnight (4°C) with anti–IRS-1 antibody coupled to protein A-Sepharose (Sigma, St. Louis, MO). The immunoprecipitates were washed (7), resuspended in Laemmli sample buffer with 100 mmol/l dithiothreitol, and heated (95°C) for 6 min. Proteins were separated by SDS-PAGE, transferred to nitrocellulose membranes, and blocked with 5% nonfat milk at room temperature for 1 h. Membranes were washed with TBST buffer (10 mmol Tris, 100 mmol NaCl, 0.02% Tween-20) and incubated with anti-phosphotyrosine antibody (Transduction Laboratories, Lexington, KY) to assess tyrosine phosphorylation of IRS-1. Membranes were washed and incubated with the appropriate secondary antibody as recommended by the supplier (Amersham, Arlington Heights, IL). Proteins were visualized by enhanced chemiluminescence and quantified by densitometry.

Protein expression of the IR, IRS-1, p85α and p110α regulatory and catalytic subunits of PI 3-kinase, GLUT4, and glycogen synthase were determined in muscle lysates. Aliquots (50 µg) of muscle homogenate were resuspended in Laemmli sample buffer with 100 mmol/l DTT. Proteins were subjected to immunoblot analysis as described above using appropriate primary antibodies. The IR antibody was from Dr. Ken Siddle (Cambridge University, Cambridge U.K.), the IRS-1 antibody was from Drs. Morris White and Martin Myers (Joslin Diabetes Center, Boston, MA), p85α and p110α antibodies were from Dr. Jonathan Backer (Albert Einstein College of Medicine, Bronx, NY), and GLUT4 and glycogen synthase antibodies were from Drs. Oluf Pedersen and Sten Lund (Steno Diabetes Center, Copenhagen, Denmark). Membranes were washed and incubated with the appropriate secondary antibodies as recommended by the supplier (Amersham, Arlington Heights, IL). Proteins were visualized by enhanced chemiluminescence and quantified by densitometry.

IRS-1–associated PI 3-kinase activity. A portion of soleus or EDL muscle from Wistar, GK, or phlorizin-treated GK rats was homogenized in 500 µl lysis buffer as described (4), and solubilized by continuous stirring for 1 h at 4°C. After centrifugation (12,000*g* for 4 min at 4°C), the supernatant (500 µg) was immunoprecipitated overnight (4°C) with anti–IRS-1 antibody coupled to protein A-sepharose. PI 3-kinase activity was assessed directly on the protein A-sepharose beads as described (27). The bands corresponding to phosphatidylinositol-3-phosphate were quantified using a PhosphoImager (Bio-Rad).

Akt kinase activity. Akt kinase activity in EDL muscle from Wistar, GK, or phlorizin-treated GK rats was measured against a peptide substrate (GRPRTSSFAEG) based on a motif from glycogen synthase kinase 3 (GSK3) (28). The Akt1 antibody was raised in rabbit, against a fusion protein of the PH domain of human AKT1 and GST. Akt activity in skeletal muscle was determined as previously described by Krook et al. (29).

Statistical analysis. Data are presented as means ± SE. When statistical differences (P < 0.05) were found by two-way or one-way analysis of variance (ANOVA), the location of the significance was determined with the Fisher's least significant differences test.

RESULTS

Animal characteristics. Body weight was similar between GK and Wistar rats (Table 1), as reported in our previously published work (24). After 4 weeks of phlorizin treatment, GK rats weighed significantly less than Wistar rats (P < 0.01). Fasting blood glucose levels were elevated in GK, compared with that of Wistar rats. Phlorizin treatment normalized fasting blood glucose (Table 1) and partially normalized glucose tolerance in GK rats (24).

Glucose transport activity. Phlorizin treatment did not alter basal or insulin-stimulated glucose transport in soleus or EDL muscles from Wistar rats (data not shown). Insulin elicited a concentration-dependent increase in glucose transport in soleus (Fig. 1*A*) and EDL (Fig. 1*B*) muscle from Wistar and GK rats. In soleus muscle, maximal insulin-stimulated glucose transport activity was reduced by 38% (P < 0.05) in GK rats and restored to Wistar levels after treatment with phlorizin. Basal and submaximal insulin-stimulated glucose transport activity in soleus muscle was similar between GK and Wistar rats.

In EDL muscle, basal and maximally insulin-stimulated (120 nmol/l) rates of glucose transport were similar between GK and Wistar rats, whereas submaximal insulin-stimulated (0.6 and 2.4 nmol/l) glucose transport activity was markedly decreased in untreated GK versus Wistar rats (Fig. 1*B*). Submaximal insulin-stimulated 3-*O*-methylglucose transport activity was increased in phlorizin-treated versus untreated GK rats by 64 and 46% (P < 0.05) for 0.6 and 2.4 nmol/l insulin, respectively. Thus, restoration of glycemia in diabetic GK

TABLE 1
Animal characteristics

Treatment	Body weight	Fasted blood glucose (mmol/l)
Wistar	281 ± 3	3.9 ± 0.1
Wistar-phlorizin	278 ± 7	3.2 ± 0.4
GK	274 ± 6	5.2 ± 0.2*
GK-phlorizin	252 ± 6†	3.9 ± 0.2

Data are means ± SE for vehicle-treated Wistar rats (n = 12), phlorizin-treated Wistar rats (n = 5), vehicle-treated GK rats (n = 12), and phlorizin-treated GK rats (n = 11). *P < 0.01, †P < 0.001, significantly different from Wistar rats. Blood glucose was determined using a One Touch II glucose meter (Lifescan, Milpitas, CA).

rats fully normalized insulin responsiveness in oxidative muscle (Fig. 1*A*) and insulin sensitivity in glycolytic muscle (Fig. 1*B*).

Insulin-stimulated tyrosine phosphorylation of IRS-1. We next determined whether muscle fiber-type–specific defects in glucose transport were accompanied by reduced phosphorylation of early or intermediate post-receptor components of the insulin signal transduction pathway. Because phlorizin treatment did not alter basal or insulin-stimulated glucose transport in either soleus or EDL muscle from Wistar rats, insulin-signaling assays were not performed in tissue from phlorizin-treated Wistar rats. Insulin-stimulated tyrosine phosphorylation of IRS-1 was determined in soleus and EDL muscle from Wistar, GK, and phlorizin-treated GK rats. In soleus muscle from Wistar rats, IRS-1 tyrosine phosphorylation was increased by 3.1-fold after exposure to 120 nmol/l insulin (P < 0.01). Basal IRS-1 tyrosine phosphorylation was decreased in soleus muscle from GK versus Wistar rats (Fig. 2*A*). Furthermore, IRS-1 tyrosine phosphorylation was decreased in soleus muscle from GK rats by 64% (P < 0.01) after submaximal (2.4 nmol/l) and by 53% (P < 0.01) after maximal (120 nmol/l) insulin stimulation, with no improvement noted after phlorizin treatment. Thus, in soleus muscle from GK rats, restoration of maximal insulin-stimulated glucose transport after phlorizin treatment was not associated with improved insulin-signal transduction at the level of IRS-1.

In EDL muscle from Wistar rats, insulin increased IRS-1 tyrosine phosphorylation in a concentration-dependent manner, with a 2.6-fold increase over basal (P < 0.05) after maximal insulin stimulation (120 nmol/l). In contrast to soleus muscle, where reduced basal and insulin-stimulated IRS-1 tyrosine phosphorylation was noted in GK rats, basal, submaximal, and maximal insulin-stimulated IRS-1 tyrosine phosphorylation in EDL muscle was similar between Wistar, GK, and phlorizin-treated GK rats (Fig. 2*B*).

IRS-1–associated PI 3-kinase activity. We next assessed IRS-1–associated PI 3-kinase activity in soleus and EDL skeletal muscle from Wistar, GK, and phlorizin-treated GK rats. PI 3-kinase activity was measured in anti–IRS-1 immunoprecipitates. A dose-dependent increase in insulin-stimulated IRS-1–associated PI 3-kinase activity was observed in soleus (Fig. 3*A*) and EDL (Fig. 3*B*) muscle from Wistar and GK rats. In soleus muscle from Wistar rats (Fig. 3*A*), maximal insulin stimulation (120 nmol/l) led to a 4.8-fold increase in PI 3-kinase activity (P < 0.01). Submaximal (2.4 nmol/l) and maximal (120 nmol/l) insulin-stimulated PI 3-kinase activity was reduced by 61% (P < 0.01) and 38% (P < 0.01), respectively, in soleus muscle from GK versus Wistar rats. Phlorizin treatment of GK rats restored submaximal, but not maximal insulin-stimulated PI 3-kinase activity to Wistar rat levels. Consistent with our results for IRS-1 phosphorylation, insulin-stimulated IRS-1–associated PI 3-kinase activity was not impaired in EDL muscle from GK rats.

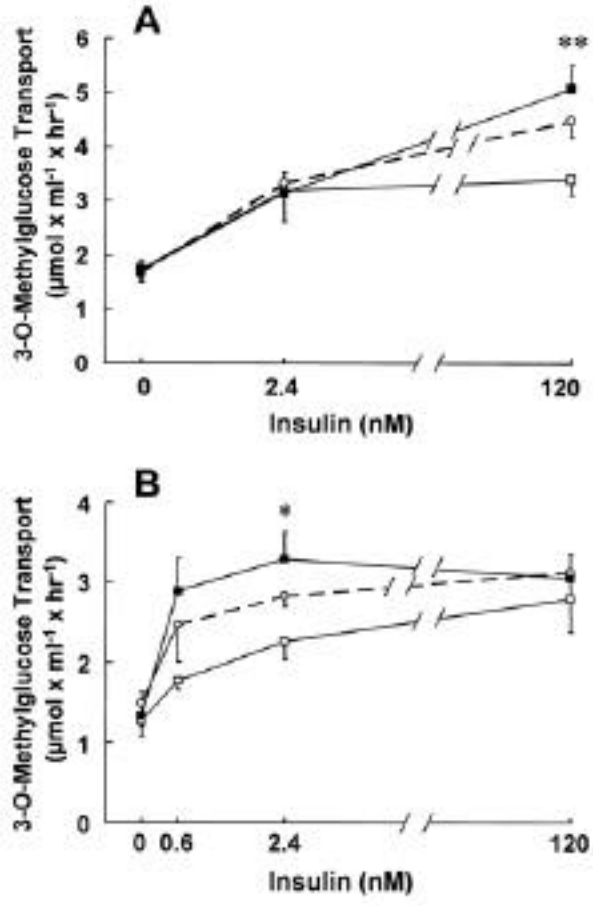

FIG. 1. Insulin-stimulated 3-*O*-methylglucose transport in isolated soleus (*A*) and EDL (*B*) muscle from Wistar, GK, and phlorizin-treated GK rats. Muscles were incubated at 30°C for 30 min in the absence or presence of 0.6, 2.4, or 120 nmol/l insulin. 3-*O*-methylglucose transport was assessed as described in METHODS. Glucose transport is expressed per milliliter of intracellular water. Values are presented as means ± SE for four to eight animals per group. Results are presented for Wistar rats (○), GK rats (□), and GK-treated rats (■). *P < 0.05, **P < 0.01 vs. Wistar rats.

Akt kinase activity in EDL muscle. We have previously reported that improved glucose tolerance restores insulin-stimulated Akt kinase activity in soleus muscle from diabetic GK rats (24). Here, we evaluate whether Akt kinase activity is impaired in glycolytic muscle from GK rats. Consistent with our initial observation in rat soleus muscle (24), insulin increased Akt kinase activity in a concentration-dependent manner in EDL muscle from Wistar rats. Maximal insulin stimulation (120 nmol/l) led to a 3.5-fold increase (P < 0.01 vs. basal) in Akt kinase activity in EDL muscle from Wistar rats. Similar to our previously published observation for soleus muscle, Akt kinase protein expression was not altered in EDL muscle from GK rats (Fig. 4*A*). Stimulation of EDL muscle from GK rats with submaximal or maximal insulin did not

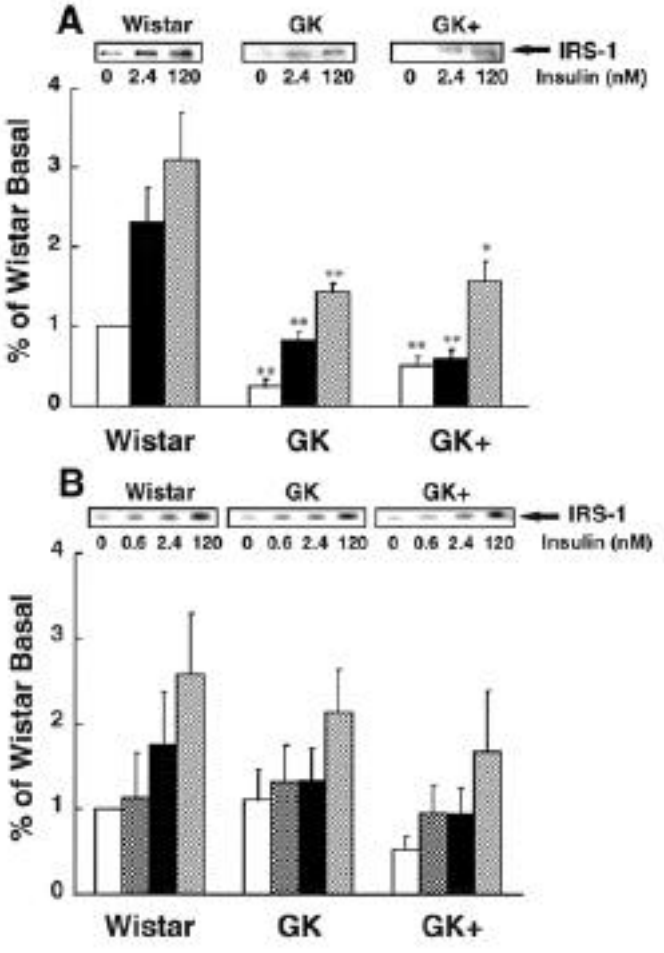

FIG. 2. Insulin-stimulated IRS-1 tyrosine phosphorylation in skeletal muscle from Wistar, GK, and phlorizin-treated GK rats. *A*: Isolated soleus muscles were incubated at 30°C for 6 min in the absence or presence of 2.4 or 120 nmol/l insulin. Muscles were homogenized and equal amounts of protein were immunoprecipitated with anti–IRS-1, followed by SDS-PAGE and immunoblotting with anti-phosphotyrosine antibodies as described in METHODS. The upper panel shows a representative autoradiograph of IRS-1 tyrosine phosphorylation. The lower panel shows data quantification by densitometry. Results are expressed as means ± SE for five to six animals. *B*: Isolated EDL muscles were incubated at 30°C in the absence or presence of 0.6, 2.4, or 120 nmol/l insulin. Samples were prepared and processed as described for soleus. The upper panel shows a representative autoradiograph of IRS-1 tyrosine phosphorylation. The lower panel shows data quantification by densitometry. Results are expressed as means ± SE for four to five animals. *P < 0.05, **P < 0.01 vs. Wistar.

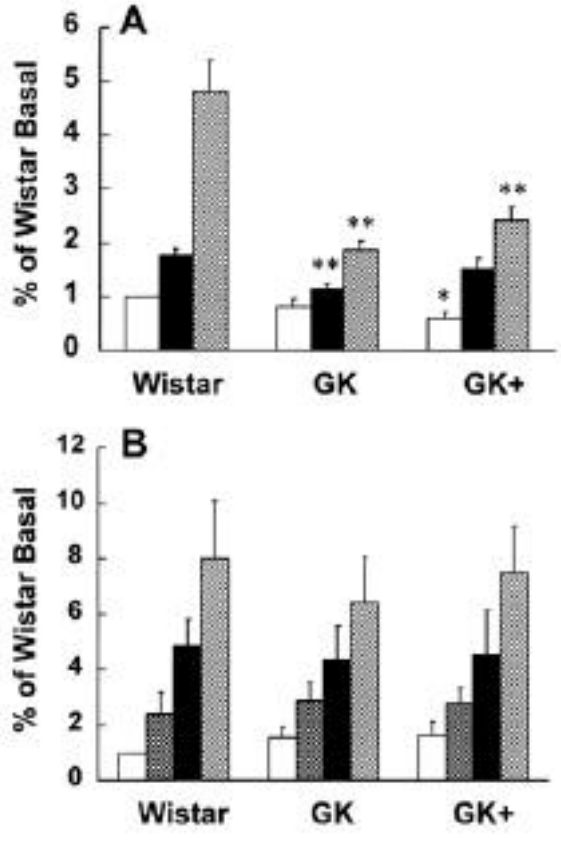

FIG. 3. Insulin-stimulated IRS-1–associated PI 3-kinase activity in skeletal muscle from Wistar, GK, and phlorizin-treated GK rats. *A*: Isolated soleus muscles were incubated as described for Fig. 2*A*. Equal amounts of protein were subjected to immunoprecipitation with IRS-1 antibody followed by PI 3-kinase assay as described in METHODS. PI 3-kinase activity was assessed in IRS-1 immunoprecipitates, as described in METHODS. ^{32}P incorporation into PI 3-phosphate was determined using a PhosphoImager. Results are expressed as means ± SE relative to Wistar basal for five to six animals. *B*: Isolated EDL muscles were incubated in the absence or presence of insulin (0.6, 2.4, or 120 nmol/l) as described for Fig. 2*B*. IRS-1–associated PI 3-kinase was determined as described above for soleus muscle. Results are expressed as means ± SE relative to Wistar basal for five to six animals. *P < 0.05, **P < 0.01 vs. Wistar.

lead to a significant increase in Akt kinase activity (Fig. 4*B*). After phlorizin treatment, maximal insulin (120 nmol/l) stimulation led to a 2.7-fold increase in Akt kinase activity in EDL muscle from phlorizin-treated GK rats (P < 0.01 vs. basal).

Protein expression of key genes involved in insulin signaling. We next determined whether functional defects noted in the insulin signal transduction pathway in skeletal muscle from diabetic GK rats were due to changes in the expression of proteins involved in these pathways (Fig. 5). We first determined IRS-1 protein expression in lysates prepared from soleus or EDL muscle. Although IRS-1 protein expression in soleus muscle was 20 and 25% lower in GK and GK-treated versus Wistar rats, this difference did not reach statistical significance (Fig. 5). Level of IRS-1 expression in EDL muscle was similar between Wistar and GK rats (103 ± 15 and 90 ± 19% of Wistar levels for GK and GK-treated rats, respectively). Because insulin-stimulated PI 3-kinase was impaired in GK soleus muscle, we next determined the protein expression of the p85α regulatory and p110α catalytic subunits of PI 3-kinase in this tissue. Protein expression of the p85α regulatory subunit of PI 3-kinase was similar between GK and Wistar soleus muscle. By contrast, level of p110α was reduced by 33% (P < 0.05) in soleus muscle from GK versus Wistar rats. Furthermore, phlorizin treatment did not restore p110α expression in GK soleus. No significant change was observed in the level of IR, GLUT4, or glycogen synthase expression in soleus muscle from GK versus Wistar rats (data not shown).

DISCUSSION

Skeletal muscle is composed of distinct fiber types with unique contractile and metabolic characteristics. Here, we demonstrate muscle fiber-type–specific defects in the insulin signal transduction pathway in skeletal muscle from diabetic

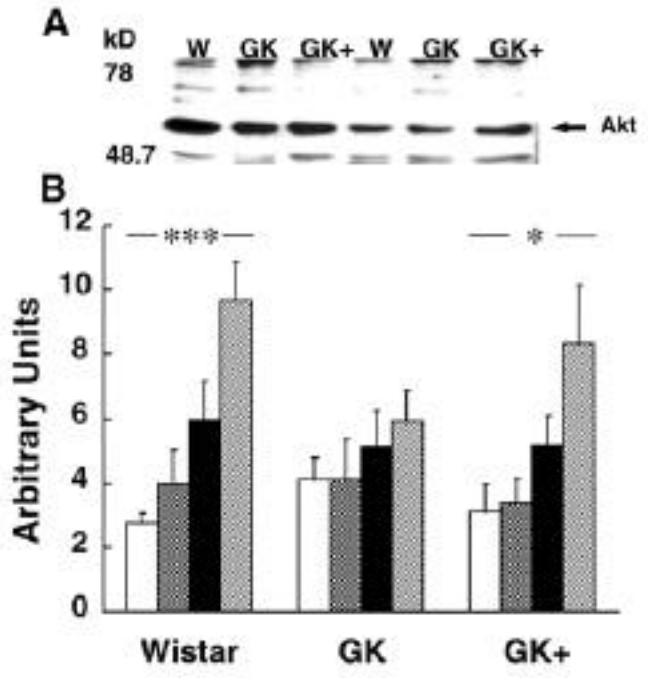

FIG. 4. Insulin-stimulated Akt kinase protein expression and activity in EDL muscle from Wistar, GK, and phlorizin-treated GK rats. *A*: Akt protein expression was determined by immunoblot analysis of total lysate using anti-Akt$_1$ antibody. A representative autoradiograph for Akt$_1$ expression in EDL muscle from Wistar (W), GK, or phlorizin-treated GK (GK+) rats. *B*: EDL muscle was incubated in the absence (□) or presence of 0.6 (▨), 2.4 (■), or 120 (▨) nmol/l insulin as described in Fig. 2*B*. Equal amounts of muscle protein were immunoprecipitated with anti-Akt$_1$, and Akt kinase activity was assessed as described in METHODS. Results are expressed as means ± SE arbitrary PhosphoImager units for five to six animals.

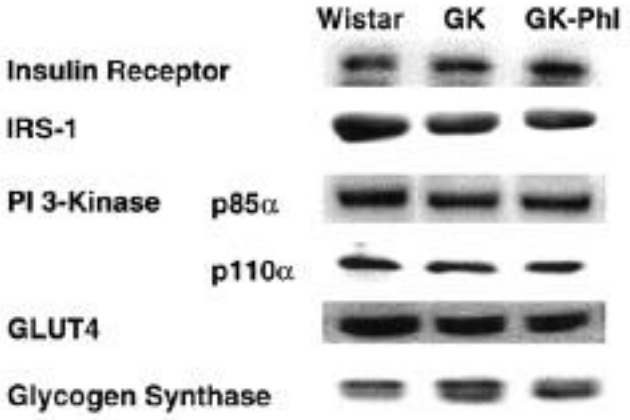

FIG 5. Protein expression of IR, IRS-1, p85α regulatory and p110α catalytic subunits of PI3 kinase, GLUT4, and glycogen synthase in soleus muscle from Wistar, GK, and phlorizin-treated GK rats. Equal amounts of protein were separated by SDS-PAGE and immunoblotted with specific antibodies as indicated in METHODS. Representative immunoblots are presented for Wistar (W; *n* = 6–8), GK (*n* = 6–8), and phlorizin-treated GK (GK+; *n* = 6–8) animals.

GK rats. In slow-twitch oxidative soleus muscle, submaximal and maximal insulin-stimulated IRS-1 phosphorylation and IRS-1–associated PI3-kinase activity were markedly impaired in GK rats, compared with that of Wistar rats. By contrast, in fast-twitch glycolytic EDL muscle, insulin signaling through IRS-1 was intact. However, regardless of muscle fiber-type, downstream steps from PI 3-kinase, including Akt kinase and glucose transport, were impaired in GK rats and restored to Wistar rat levels with improved glycemic control. To our knowledge, this is the first report describing fiber-type–specific defects in early post-receptor components of the insulin-signaling pathway in skeletal muscle from diabetic animals.

Our results suggest that insulin resistance is more pronounced in skeletal muscle composed predominantly of oxidative fibers, such as the soleus muscle. These defects may be related to changes in functional activity and/or protein expression of the insulin-signaling proteins. Multiple molecular isoforms of IRS have now been cloned (30–34). IRS-2 shares many of the features of IRS-1 (31) and serves as an alternative insulin receptor substrate, which allows for significant residual insulin and IGF-1 signaling in IRS-1 knockout mice (35). However, in insulin-resistant skeletal muscle, the decrease in total phosphotyrosine-associated PI 3-kinase activity more closely parallels the reduction in IRS-1 associated PI 3-kinase activity (35–37). In skeletal muscle from *ob/ob* mice, functional defects in IRS-1 rather than IRS-2 are responsible for impaired insulin signaling (37). Thus, we focused on the muscle fiber-type–specific role of IRS-1, rather than IRS-2. Despite functional defects at the level of IRS-1 and PI 3-kinase in soleus muscle from GK rats, protein expression of IRS-1 was not significantly reduced. Furthermore, the expression of the β-subunit of the insulin receptor, of p85α regulatory subunit of PI 3-kinase, of GLUT4, and of glycogen synthase was similar between Wistar and GK rats. In contrast, the p110α subunit of PI 3-kinase was decreased by 33% in soleus muscle from GK and phlorizin-treated GK rats, as compared to that of Wistar rats. This decrease paralleled the reduction in PI 3-kinase activity. Nevertheless, restoration of maximal insulin-stimulated glucose transport in soleus muscle from phlorizin-treated GK rats occurred despite any profound functional improvement at the level of IRS-1. The disassociation between maximal insulin-stimulated IRS-1–associated PI 3-kinase activity and glucose transport activity in soleus muscle from GK rats suggests that full activation of glucose transport activity may not entirely be dependent on full activation of IRS-1–associated PI 3-kinase activity. Support for this comes from the finding that submaximal insulin-stimulated IRS-1–associated PI 3-kinase activity in soleus muscle from GK rats was normalized by phlorizin treatment.

Insulin-stimulated tyrosine phosphorylation of IRS-1 leads to the formation of the IRS-1/PI 3-kinase complex, which transmits the insulin signal to further downstream metabolic events, including activation of glucose transport (11). To date, the molecular link between PI 3-kinase and glucose transport is unknown. The serine/threonine kinase Akt is a downstream target of PI 3-kinase (38–41) and has been implicated as a component of the insulin-signaling pathway to glucose transport. Expression of a constitutively active membrane-bound form of Akt in 3T3-L1 adipocytes (38,39,42) or L6 myotubes (40,41) directly promotes glucose transport and GLUT4 translocation, glycogen synthase, and protein synthesis. However, a recent study (42), whereby a dominant-negative mutant of Akt was transiently expressed in 3T3-L1 adipocytes, provides evidence that Akt may not be necessary

for glucose transport. Whether Akt is directly coupled to glucose transport remains a topic of debate.

Here we show that in phlorizin-treated diabetic GK rats, improved glucose transport occurs in parallel with improved insulin action at the level of Akt kinase in both EDL and soleus muscle. Interestingly, in both soleus and EDL muscle from GK rats, activation of Akt kinase and glucose transport was disassociated from IRS-1/PI-3 kinase under maximal insulin-stimulated conditions. In soleus muscle from GK rats, restoration of insulin-stimulated activity of Akt kinase and glucose transport was observed after phlorizin treatment, despite a persistent impairment at the level of IRS-1/PI-3 kinase. However, in EDL muscle from GK rats, where PI 3-kinase activity was normal, the impaired insulin-stimulated Akt kinase and glucose transport activity was restored after phlorizin treatment. Thus, hyperglycemia appears to impair insulin signaling at the level of Akt, rather than at the level of PI 3-kinase, because there is a disparity between these parameters in both soleus and EDL muscle. Recently, Summers et al. (43) reported that incubation of 3T3-L1 adipocytes with the sphingomyelin-derivative ceramide inhibits activation of Akt, GLUT4 translocation, and glucose transport independently of any effect on IRS-1. Collectively, these findings show that under some situations, activation of Akt kinase can be disassociated from upstream signaling via the IRS-1/PI-3 kinase.

Chronic hyperglycemia is a characteristic feature of NIDDM, and may be one factor leading to the development of insulin resistance in skeletal muscle. Restoration of euglycemia in NIDDM people after weight loss (44,45), sulfonylurea treatment (46,47), or insulin therapy (48,49) enhances insulin sensitivity in adipocytes and skeletal muscle. Previously, we have reported that incubation of skeletal muscle from people with NIDDM, in the presence of 4 mmol/l glucose for 2 h, led to a complete restoration of insulin-stimulated 3-*O*-methylglucose transport activity (50). The molecular mechanism for this improvement may involve improved insulin signal transduction at the level of IRS-1, PI 3-kinase, Akt kinase, and/or GLUT4 translocation, because functional defects have been reported at each of these steps in skeletal muscle from diabetic rodents (4,5,8–10,24) and humans (6,7,19,29). Normalization of glycemia in diabetic GK rats by phlorizin treatment leads to improved glucose tolerance and full restoration of insulin-stimulated activity of Akt kinase and glucose transport in soleus muscle (24), despite impaired insulin signaling at the level of IRS-1/PI-3 kinase. In glycolytic EDL muscle from diabetic GK rats, insulin signaling at the level of IRS-1/PI 3-kinase is not altered, whereas insulin-stimulated activity of AKT kinase and glucose transport is impaired, and fully restored after phlorizin treatment. Thus in both slow-twitch and fast-twitch skeletal muscle, normalization of glycemia ameliorates defects in the insulin signal transduction pathway to Akt kinase and glucose transport. Although phlorizin treatment did not restore maximal insulin stimulation of either IRS-1 tyrosine phosphorylation or IRS-1–associated PI 3-kinase activity in soleus muscle, signal transduction through this pathway was improved under submaximal insulin-stimulated conditions. Thus, downstream steps to PI 3-kinase, including Akt and glucose transport, appear to be sensitive to normalization of glycemia.

In summary, skeletal muscle insulin resistance in the diabetic GK rat is associated with fiber-type–specific defects in the insulin signal transduction pathway to glucose transport. Specifically, reduced IRS-1 tyrosine phosphorylation and IRS-1–associated PI 3-kinase are observed in oxidative soleus muscle, whereas insulin signaling at the level of IRS-1/PI 3-kinase is intact in glycolytic EDL muscle. Furthermore, Akt kinase and glucose transport activity is impaired in skeletal muscle regardless of fiber type composition. Improvement of glycemia by phlorizin treatment restores insulin-stimulated Akt kinase activity and glucose transport in EDL and soleus muscle. Importantly, in soleus muscle, the restoration of maximal insulin-stimulated Akt kinase activity and glucose transport occur despite a profound impairment at the level of IRS-1 and PI 3-kinase. Thus, steps in the insulin-signaling cascade downstream from PI 3-kinase appear to be more sensitive to changes in the glycemic milieu.

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Q1: Correct to say “changes in”?
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Q3: Please spell out TBST.
Q3a: LSD expanded correctly as least significant differences?
Q4: Please identify the “*” P value in Table 1.
Q5: Which figure is this indicated in?

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