Effect of glycogen synthase overexpression on insulin-stimulated glucose uptake and storage

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Fogt, Donovan L., Shujia Pan, Sukho Lee, Zhenping Ding, Angus Scrimgeour, John C. Lawrence Jr., and John L. Ivy. Effect of glycogen synthase overexpression on insulin-stimulated muscle glucose uptake and storage. Am J Physiol Endocrinol Metab 286: E363–E369, 2004. First published October 21, 2003; 10.1152/ajpendo.00115.2003—Insulin-stimulated muscle glucose uptake is inversely associated with the muscle glycogen concentration. To investigate whether this association is a cause and effect relationship, we compared insulin-stimulated muscle glucose uptake in noncontracted and postcontracted muscle of GSL3-transgenic and wild-type mice. GSL3-transgenic mice overexpress a constitutively active form of glycogen synthase, which results in an abundant storage of muscle glycogen. Muscle contraction was elicited by in situ electrical stimulation of the sciatic nerve. Right gastrocnemii from GSL3-transgenic and wild-type mice were subjected to 30 min of electrical stimulation followed by hindlimb perfusion of both hindlimbs. Thirty minutes of contraction significantly reduced muscle glycogen concentration in wild-type (49%) and transgenic (27%) mice, although transgenic mice retained 168.8 ± 20.5 μmol/g glycogen compared with 177.2 ± 2.6 μmol/g glycogen for wild-type mice. Muscle of transgenic and wild-type mice demonstrated similar pre- (3.6 ± 0.3 and 3.9 ± 0.6 μmol·g⁻¹·h⁻¹ for transgenic and wild-type, respectively) and post-contract (7.9 ± 0.4 and 7.0 ± 0.4 μmol·g⁻¹·h⁻¹ for transgenic and wild-type, respectively) insulin-stimulated glucose uptakes. However, the [14C]glucose incorporated into glycogen was greater in noncontracted (151%) and postcontracted (157%) transgenic muscle vs. muscle of corresponding wild-type mice. These results indicate that glycogen synthase activity is not rate limiting for insulin-stimulated muscle glycogen concentration and that the inverse relationship between muscle glycogen concentration and insulin-stimulated glucose uptake is an association, not a cause and effect relationship.

1 Insulin-stimulated glucose uptake during postexercise recovery appears to be inversely related to the muscle glycogen concentration (5, 6, 14, 18, 33). When muscle glycogen is elevated above normal levels, the ability of insulin to activate glucose transport (6, 12, 14, 20, 25, 33) and glycogen synthase (11, 27), the rate-limiting enzyme in glycogen synthesis, is significantly depressed. Several studies have demonstrated an inverse relationship between muscle glycogen concentration and the association of the glucose transporter GLUT4 with the plasma membrane following insulin stimulation (12, 13, 24). This may be a direct result of the glycogen macromolecule (8) or an indirect result of glycogen-mediated inhibition of PKB/Akt (12, 25). PKB/Akt is a key insulin-signaling protein involved in stimulation of GLUT4 translocation to the plasma membrane (7, 17, 26, 38) and inactivation of glycogen synthase kinase-3 (GSK-3) in skeletal muscle (9). GSK-3, in turn, has been implicated in inhibition of glycogen synthase (10). Thus PKB/Akt may play a pivotal role in both insulin-stimulated glucose transport and glycogen synthesis.

Evidence from recent research utilizing the GSL3-transgenic mouse model challenges the normal inverse relationship between muscle glycogen concentration and insulin-stimulated glucose uptake as well as glycogen synthesis. GSL3-transgenic mice overexpress a constitutively active form of glycogen synthase (∼9-fold) in gastrocnemius muscle, which is associated with a markedly elevated (∼4-fold) gastrocnemius muscle glycogen concentration (31). Using this model, Azpiazu et al. (1) demonstrated that overexpression of glycogen synthase is sufficient to promote glycogen accumulation without reducing insulin action on muscle glucose uptake. Because GSL3-transgenic muscle did not demonstrate the classic insulin resistance typically associated with an elevated muscle glycogen concentration, this raised the question of whether or not insulin-stimulated glucose uptake is directly influenced by muscle glycogen. Thus, in an effort to assess the influence of muscle glycogen on insulin-stimulated glucose uptake, we altered the muscle glycogen concentrations of wild-type and GSL3-transgenic mice via in situ sciatic nerve electrical stimulation. Comparisons of GSL3-transgenic and wild-type mice suggest that muscle glycogen does not influence insulin-stimulated glucose uptake in noncontracted or postcontracted muscle.

METHODS

Animals and experimental design. GSL3-transgenic and wild-type littermates [(C57BL6 × CBA)F1] weighing 20–35 g were used in these experiments. GSL3-transgenic mice overexpress a constitutively active form of glycogen synthase [GS(2,3a)], a rabbit skeletal muscle glycogen synthase having Ser to Ala mutations at sites 2 and 3a. The generation of the transgenic line is described by Manchester et al. (31). Transgenic expression of the constitutively active glycogen synthase is approximately nine times higher compared with wild-type in gastrocnemius muscle, with approximately five times greater expression in fast-twitch than in slow-twitch muscle (31). Upon receipt of the mice, they were individually housed at The University of Texas Animal Resource Center on a 12:12-h light-dark cycle. Laboratory Chow and water were provided ad libitum, and the animal room temperature was maintained at 21°C. Animals were transferred to the laboratory ≥24 h before the first experimental procedures following

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the same light-dark cycle. The University of Texas Animal Care and Use Committee approved all procedures for this study.

**Muscle contraction procedure.** After a 6-h fast, GSL3-transgenic and wild-type mice were anesthetized with pentobarbital sodium (6.5 mg/100 g body wt). The skin from the right hindlimb was reflected, and a section of the calcaneus, with the Achilles tendon still attached, was clipped from the foot. The triceps surae muscle group was retracted from the tibia and the distal tendon (detached from the bony insertion) of the muscle group was attached to an isometric force transducer (Series 300 B Lever System; Aurora Scientific, Ontario, Canada). However, the distal insertion detached by Johnsson and colleagues (21, 22) and Roy et al. (34) was employed. The gastrocnemius was stimulated for 30 min by using supramaximal stimulation strength at the optimal length for the muscle contracting at 90° to the knee joint.

The goal of this stimulation procedure was to reduce muscle glycogen before the assessment of 2-deoxyglucose uptake. Therefore, a 14-h fasted mouse of the protocol described by Johnsson and colleagues (21, 22) and Roy et al. (34) was employed. The gastrocnemius was stimulated for 30 min by using supramaximal (8 V) trains composed of 1-ms square-wave pulses firing at 100 Hz. Trains of 200 ms were delivered at a rate of 30/min (1/2 s). Tetrican tension was assessed throughout the stimulation period. Gastrocnemius muscle cross-sectional area was estimated by dividing wet weight by the measured optimal muscle length (cm) multiplied by the fiber-to-muscle length ratio for rodent gastrocnemius (0.4) multiplied by mammalian muscle-specific gravity (1.06 g/cm³) (3). Specific force (N/cm²) was then estimated by dividing muscle Po, expressed in newtons (N), by the estimated cross-sectional area (cm²) (3). After the stimulation period, the mice were prepared for in situ hindlimb perfusion to measure insulin-stimulated gastrocnemius 2-deoxy-[3H]glucose uptake or to determine gastrocnemius [14C]glucose incorporation into glycogen by use of a submaximal insulin concentration (0.2 nU/ml).

**Hindlimb perfusion.** The perfusion surgical technique was similar to that described previously (4, 19). Major vessels branching from the abdominal aorta and vena cava, except for the common iliac artery and iliac vein, were ligated. Heparin (100 U) was injected into the inferior vena cava just below the diaphragm. After the heparin injection, the descending aorta and inferior vena cava were cannulated as previously described (19). Catheters were placed in line with the noncirculating hindlimb perfusion apparatus providing a 37°C, continuous gassed (95% O₂-5% CO₂) perfuse flow of 1.8 ml/min. Both hindlimbs were perfused during the equilibrium (10 min) and tracer (20 min) periods. Per fusates consisted of 6% bovine serum albumin in Krebs-Henseleit buffer (pH 7.4) with 0.2 mM pyruvate. Perfusion metabolic tracers varied for assessment of glucose uptake and glucose incorporation into glycogen are described below. Immediately after the perfusion period, gastrocnemii from both hindlimbs were excised, frozen with Wollenberg tongs cooled in liquid N₂, and stored frozen (−80°C) until further analysis.

**Determination of 2-deoxyglucose uptake.** During the perfusion tracer period, the perfusate contained 6 nM 2-deoxy-[1,2-3H]glucose (0.05 μCi/ml; ICN Biochemicals, Costa Mesa, CA), 2 mM [U-14C]mannitol (0.12 μCi/ml; ICN Biochemicals), and 0.2 mM/ml insulin (Humulin R-100; Eli Lilly, Indianapolis, IN). Freeze-clamped gastrocnemius muscles from the perfused wild-type and transgenic hindlimbs were sectioned and weighed frozen. A 60- to 100-mg piece of mixed-fiber muscle was dissolved in 1 ml of 1 N KOH by incubating it for 15 min at 65°C and then mixing and incubating for an additional 5 min at 65°C. An equal volume of 1 N HCl was added to the digested samples and mixed, and aliquots of the neutralized samples were counted for 3H and 14C disintegrations per minute (LS 6000SC; Beckman, Fullerton, CA). Muscle 2-deoxyglucose uptake was calculated from the specific activity of the original perfusate after subtracting out the appropriate volume for extracellular space, as determined from radiolabeled mannitol in the muscle sample. The mixed gastrocnemius was chosen for this study because it exhibits a high transgene expression but contains similar GLUT4 content in wild-type and GSL3-transgenic mice (31).

[14C]glucose incorporation into glycogen. [14C]glucose incorporation into glycogen was assessed by the planar and solvent-front TLC method for a different group of wild-type and transgenic hindlimbs as previously described (41). The perfusion surgery and preparation were essentially the same as described for 2-deoxyglucose uptake during the pretracer perfusion period. During the 20-min tracer perfusion period, both hindlimbs were perfused with 6 mM glucose (0.15 μCi/ml tritiated[U-14C]glucose; ICN Biochemicals) with a submaximal insulin concentration (0.2 nU/ml). After the perfusion period, both gastrocnemii were excised, weighed frozen, and placed in separate screw-top test tubes containing 1 ml of 30% KOH saturated with Na₂SO₄. Samples were digested by incubating the tubes for 30 min at 100°C. After the incubation, the tubes were cooled to room temperature, and the glycogen was precipitated overnight at 4°C after the addition of 1.2 volumes of 95% ethanol. The glycogen pellets were resuspended in 0.2 N H₂O. An aliquot of this resuspension was used for determination of muscle glycogen concentration as per Lo et al. (29). A second aliquot was transferred to a scintillation vial containing 5 ml of scintillation fluid, and the rate of [14C]glucose incorporation into glycogen was determined.

**Measurement of glycogen synthase activity.** Nucleotide inhibition has been shown to increase with the increasing phosphorylation state of glycogen synthase. Therefore, to best approximate the in vivo sensitivity of glycogen synthase to allosteric activation by glucose 6-phosphate (G-6-P), glycogen synthase was determined at 30°C by direct incorporation of [U-14C]UDP-glucose (ICN Biochemicals) into glycogen in the presence of 7 mM ATP and 100 μM UDP-1-glucose with several concentrations of G-6-P (0.01, 0.5, 1.5, and 25 mM) (2, 37). In addition to ATP, G-6-P, and UDP-glucose, the reaction buffer contained 50 mM MOPS buffer, 25 mM KF, 20 mM EDTA, 10 mM KH₂PO₄, and 10 mg/ml glycogen at pH 6.9.

Total and phosphorylated PKB/Akt-1α. Three isoforms of PKB/Akt have been identified. The primary insulin-stimulated isoform in rodent muscle is PKB/Akt-1α (39, 40). In addition, postexercise insulin-stimulated PKB/Akt-1α activity was inversely related to skeletal muscle glycogen content according to Derave et al. (12). Therefore, we selected PKB/Akt-1α as the isoform to assess in the present study. Muscle samples (30 mg) were homogenized in ice-cold buffer containing 50 mM HEPES (pH 7.4), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl₂, 1.0 mM EDTA, 10 mM Na₂PO₄, 100 mM NaF, 2.0 mM Na₃VO₄, aprotinin (10 μg/ml), leupeptin (10 μg/ml), pepstatin (0.5 μg/ml), 1% Igepal, and 2 mM phenylmethylsulfonyl fluoride. Homogenates were centrifuged at 200,000 g for 50 min at 4°C, and aliquots of the supernatant were treated with 2× Laemmli sample buffer containing 100 mM dithiothreitol and boiled for 5 min. Samples (80 μg protein) were then subjected to SDS-PAGE (10% resolving gel) and transferred to nitrocellulose membranes. An insulin-stimulated mouse gastrocnemius muscle standard (80 μg protein) was run concurrently on each gel. The membranes were blocked in 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween 10 (TBS-T, pH 7.5) for 1 h. After a rinse in 0.1% Tween 10, the membranes were incubated with sheep anti-phospho-PKB/Akt-1α (Ser473) antibody (1:1,500 vol/vol; Upstate Biotechnology) for 4 h. Membranes were rinsed in 0.1% Tween 10 and incubated with horseradish peroxidase-conjugated goat anti-sheep IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) for 60 min. Antibody-bound protein was visualized by means of an enhanced chemiluminescence (ECL) Western blot detection kit (Amersham) accord-
ing to the manufacturer’s instructions, and arbitrary density units were determined from optical scanning. Phosphorylated PKB/Akt of samples was expressed relative to an insulin-stimulated mouse standard.

Total PKB/Akt-1α protein content was assessed on the nitrocellulose membranes used for phosphorylated PKB/Akt-1α. Membranes were stripped of antibodies by incubation with stripping buffer containing 100 mM 2-mercaptoethanol, 2% sodium dodecyl sulfate, and 62.5 mM Tris-HCl (pH 6.7) for 30 min. Membranes were then washed three times for 15 min in TBS-T buffer. Membranes were blocked overnight, washed three more times for 15 min in TBS-T, and incubated with sheep anti-PKB/Akt-1α (1:1,000 vol/vol) (Upstate Biotechnology) for 4 h with gentle agitation. After three more TBS-T washes, antibody-bound protein was visualized by ECL (Amersham), and arbitrary density units were determined from optical scanning. Total PKB/Akt-1α was expressed relative to an insulin-stimulated mouse standard.

Statistics. One-way analysis of variance was performed on all tissue analyses. Post hoc tests were performed using Fisher’s protected least significant difference test with differences considered statistically significant if P ≤ 0.05.

RESULTS

Muscle contractile performance. Gastrocnemius Pt, optimal muscle length, muscle wet weight, and estimated muscle cross-sectional area were not significantly different between wild-type and GSL3-transgenic mice (Table 1). Transgenic muscle Po was 14% lower than that of wild-type mice (Table 1). This difference was significant at the P < 0.06 level. Specific force was significantly lower in GSL3-transgenic muscle (16.1 ± 0.88 N/cm²) compared with wild-type muscle (21.4 ± 1.5 N/cm²; Table 1). Gastrocnemius tetanic tension production declined to 48% of Po for wild-type mice and 61% of Po in GSL3-transgenic mice by 5 min of stimulation (Fig. 1). Tetanic tension production remained essentially unchanged from 5 min throughout the 30-min stimulation period. Wild-type muscle contractile performance was similar to that found by Johannson et al. (21), using a similar stimulation protocol in the rat.

Muscle glycogen concentration. There was a significant genotype and treatment effect with respect to muscle glycogen concentration. Glycogen concentration of gastrocnemius from transgenic mice was approximately sevenfold greater than that of wild-type mice (Fig. 2). Muscle contraction lowered muscle glycogen significantly in both genotypes. The reduction in muscle glycogen was 49% in wild-type gastrocnemius and 27% in transgenic gastrocnemius. However, in absolute terms, 30 min of contraction reduced glycogen by 63 μmol/g wet wt in transgenic muscle and 17 μmol/g wet wt in wild-type muscle. Quadriceps muscle glycogen concentration was unchanged by stimulation in either genotype (data not shown), demonstrating that the decline in gastrocnemius muscle glycogen was specific to the muscle stimulated by the sciatic nerve.

Glycogen synthase activity. Glycogen synthase total activity (25 mM G-6-P), expressed in nanomoles per gram per minute, was significantly greater in noncontracted (1,197%) and contracted (1,761%) transgenic muscle compared with respective wild-type muscle (Fig. 3). In addition, allosteric activation of glycogen synthase was greater at G-6-P concentrations of 0.5 mM (142%) and 1.5 mM (361%) in noncontracted transgenic muscle compared with muscle from wild-type mice. After 30 min of contractions, glycogen synthase allosteric activation was greater (291 and 1,062% for 0.5 and 1.5 mM, respectively) in transgenic muscle compared with muscle from wild-type mice. Contraction of muscle of transgenic mice resulted in greater total glycogen synthase activity (110%) and allosteric activation of

Table 1. In situ contractile performance of the gastrocnemius muscle

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>Transgenic</th>
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<tr>
<td>Pt, N (n = 6)</td>
<td>1.02±0.03</td>
<td>1.11±0.03</td>
</tr>
<tr>
<td>Po, N (n = 7)</td>
<td>3.30±0.10</td>
<td>2.85±0.18*</td>
</tr>
<tr>
<td>Lo, cm</td>
<td>1.68±0.07</td>
<td>1.63±0.09</td>
</tr>
<tr>
<td>Wet weight, g</td>
<td>0.11±0.01</td>
<td>0.12±0.01</td>
</tr>
<tr>
<td>CSA, cm²</td>
<td>0.16±0.01</td>
<td>0.18±0.02</td>
</tr>
<tr>
<td>Specific force, N/cm²</td>
<td>21.4±1.5</td>
<td>16.1±0.88†</td>
</tr>
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Values are means ± SE. Gastrocnemius muscle was stimulated via the sciatic nerve for 30 min using supramaximal (8 V) trains composed of 1-ms square-wave pulses firing at 100 Hz. Trains of 200 ms were delivered at a rate of 30/min (1/2 s). Peak twitch tension (Pt), peak tetanic tension (Po), optimal muscle length (Lo), wet weight, estimated cross-sectional area (CSA), and specific force are presented. *P < 0.05 vs. wild type; †P < 0.05 vs. wild type.
glycogen synthase at G-6-P concentrations of 0.5 mM (62%) and 1.5 mM (131%) compared with noncontracted transgenic muscle. When glycogen synthase was assayed in the presence of 0.01 mM G-6-P, there was no difference in activity among any of the experimental groups.

2-Deoxyglucose uptake. Genotype had no effect on insulin-stimulated glucose uptake of the gastrocnemius muscle. There was, however, a significant treatment effect, as 30 min of contraction resulted in a twofold increase in insulin-stimulated glucose uptake (Fig. 4). This twofold increase occurred in both transgenic and wild-type muscle.

[^14]C glucose incorporation into glycogen. Significant genotype and treatment effects were observed with respect to insulin-stimulated glucose incorporation into glycogen. Glucose incorporation into glycogen in transgenic muscle was significantly higher before (151%) and after (157%) contraction than in the corresponding wild-type muscle (Fig. 5). However, contraction resulted in a 1.5-fold increase in insulin-stimulated incorporation of glucose in muscle of both genotypes.

Total and phosphorylated PKB/Akt-1. Total PKB/Akt-1 protein was similar in wild-type and GSL3-transgenic muscle and was not altered by 30 min of contraction (Fig. 6). Significant genotype, treatment, and interaction effects were evident with respect to insulin-stimulated PKB/Akt-1 Ser473 phosphorylation. Insulin-stimulated PKB/Akt-1 Ser473 phosphorylation was significantly higher in muscle of transgenic mice compared with muscle of wild-type mice (Fig. 7). This difference was significant before (212%) and after (52%) muscle contraction. Contraction significantly increased insulin-stimulated PKB/Akt-1 phosphorylation but only in wild-type muscle (131%).

![Fig. 3](#). Glycogen synthase activity following hindlimb perfusion (0.2 mU/ml insulin) in nonelectrically stimulated and previously in situ electrically stimulated (30 min) gastrocnemii. G-6-P, glucose 6-phosphate. Groups are non-stimulated wild type (n = 6) and transgenic (n = 5). Group means are presented. †P < 0.05, stimulated (STIM) transgenic vs. all groups; ‡P < 0.05, nonstimulated (NON) transgenic vs. all groups.

![Fig. 4](#). Insulin-stimulated 2-deoxyglucose uptake in nonelectrically stimulated and previously in situ electrically stimulated (30 min) gastrocnemii during hindlimb perfusion. Groups are nonstimulated wild type (n = 6) and transgenic (n = 6) and stimulated wild type (n = 6) and transgenic (n = 6). Group means ± SE are presented. *P < 0.05 vs. respective nonstimulated groups.

![Fig. 5](#). Insulin-stimulated (0.2 mU/ml) [^14]C glucose incorporation into glycogen in nonelectrically stimulated and previously in situ electrically stimulated (30 min) gastrocnemii during hindlimb perfusion. Groups are nonstimulated wild type (n = 9) and transgenic (n = 5) and stimulated wild type (n = 9) and transgenic (n = 5). Group means ± SE are presented. †P < 0.05 vs. respective wild-type treatment; *P < 0.05 vs. respective nonstimulated treatment.

![Fig. 6](#). Total PKB/Akt-1 protein as assessed following hindlimb perfusion with 0.2 mU/ml insulin in nonelectrically stimulated and previously in situ electrically stimulated (30 min) gastrocnemii. Values are expressed as percent insulin-stimulated mouse standard run on all gels. Groups are nonstimulated wild type (n = 6) and transgenic (n = 6) and stimulated wild type (n = 6) and transgenic (n = 6). Group means ± SE are presented.
Insulin-stimulated glucose uptake during postexercise recovery appears to be inversely related to the muscle glycogen concentration (5, 6, 14, 18, 33). However, evidence from recent research utilizing the GSL3-transgenic mouse model does not support a cause and effect relationship (1). In an effort to assess the influence of muscle glycogen on insulin action following muscle contraction, we electrically stimulated muscle of GSL3-transgenic and wild-type mice in situ via the sciatic nerve. With this process, we were able to significantly reduce the muscle glycogen concentrations of both wild-type and transgenic mice, thereby demonstrating that the muscle glycogen in GSL3-transgenic mice is metabolically active. Moreover, we found that insulin-stimulated muscle glucose uptake was similar in transgenic and wild-type mice before and after muscle contraction. However, this occurred despite a markedly greater transgenic muscle glycogen concentration, suggesting that the normal inverse relationship between elevated muscle glycogen levels and insulin action on glucose uptake is absent in GSL3-transgenic mice.

When muscle glycogen is elevated above normal levels, the ability of insulin to activate glucose transport and uptake (6, 12, 14, 20, 25, 33) is normally depressed. With use of the 2-N-4-(1-azi-2,2,2-trifluoroethyl)-benzoyl-1,3-bis-(o-mannos-4-ylxy)-2-propylamine (ATB-BMPA) cell surface GLUT4-labeling technique, muscle glucose transport has been directly related to the number of active glucose transporters associated with the plasma membrane (13, 30). Recent studies have demonstrated an inverse relationship between muscle glycogen concentration and GLUT4 protein association with the plasma membrane following insulin stimulation (12, 13, 24). Inhibition of GLUT4 translocation by elevated muscle glycogen has been suggested to occur directly via the glycogen macromolecule (8). However, evidence has also been reported that the development of insulin resistance and elevation in muscle glycogen occur concurrently but are not necessarily in a cause and effect relationship.

Kawanaka et al. (23) reported that, under certain conditions, an elevation in muscle glycogen did not result in an increase in insulin resistance. Using an isolated muscle preparation, Kawanaka et al. induced insulin resistance by incubating muscle for 5 h with 2 mU/ml insulin and 36 mM glucose. However, when protein or mRNA synthesis was inhibited during incubation with cycloheximide and actinomycin D, respectively, muscle did not become insulin resistant despite the muscle glycogen concentration increasing to levels in excess of the level that occurred in the absence of the inhibitors. Kawanaka et al. concluded that muscle insulin resistance is mediated by a rapid influx of glucose and not the accumulation of muscle glycogen. In this regard, it should be noted that despite a more rapid rate of muscle glycogen storage in the transgenic mice, insulin-stimulated glucose uptakes of transgenic and wild-type mice were similar. Thus our results support the findings of Kawanaka et al. and provide additional evidence that an overabundance of muscle glycogen is not responsible for the development of muscle insulin resistance.

PKB/Akt is an insulin-signaling protein that has been implicated in the development of insulin resistance associated with an overabundance of muscle glycogen storage (12, 25). Derave et al. (12) and Kawanaka et al. (25) found that an elevated muscle glycogen concentration (~60–130 μmol/g) in the rat was associated with attenuation in insulin-stimulated phosphorylation of PKB/Akt. Furthermore, we (Fogt DL, unpublished observation) have demonstrated this attenuation in wild-type mice with a muscle glycogen concentration of 103.8 ± 5.8 μmol/g. In the present study, insulin-stimulated glucose uptake of noncontracted transgenic muscle was similar, whereas PKB/Akt phosphorylation was significantly higher, compared with wild-type muscle. After muscle contraction, glucose uptake was increased in both transgenic and wild-type muscle. This increase was also accompanied by an increase in PKB/Akt phosphorylation in wild-type and transgenic muscle, although the increase in PKB/Akt phosphorylation was not significant in the transgenic muscle. The state of PKB/Akt phosphorylation in transgenic muscle, however, remained higher than that of wild-type muscle. These results therefore suggest that the normal suppression of insulin signaling associated with a high muscle glycogen concentration is disrupted in GSL3-transgenic mice, which may account for their normal insulin-stimulated glucose uptake.

Our results also provide important new information regarding the regulation of PKB/Akt phosphorylation. First, they indicate that phosphorylation of PKB/Akt by insulin is increased following contraction. Whether this is due to contraction per se, as recently demonstrated (32, 35), or is due to an increase in sensitivity to insulin stimulation cannot be determined from this study. However, it has been reported that activation of PKB/Akt can occur via increases in intracellular cAMP (15) and Ca²⁺ (42) in the absence of phosphatidylinositol 3-kinase activation. Second, these results indicate that there is an association between the activation states of glycogen synthase and PKB/Akt. How the activities of these enzymes are coordinated and the significance of this coordination remain to be determined.

It was of interest to find that an overexpression of glycogen synthase had a significant effect on intracellular glucose disposal but had no effect on insulin-stimulated glucose uptake. Normally, glucose transport, or the penetration of glucose through the plasma membrane, is rate limiting for glucose uptake. However, under certain conditions the rate-limiting...
reaction can shift from transport to intracellular glucose disposal (14). For example, impairment in glycogen storage, a major pathway for glucose disposal, is characteristic of insulin-resistant muscle (28, 36). In the present study, transgenic mice were able to convert ~45% of glucose uptake to glycogen in both noncontracted and postcontracted muscle, whereas the glucose uptake converted to glycogen was only 16% in muscle of wild-type mice. This increased efficiency appeared to be due to an increased responsiveness of glycogen synthase to allosteric activation by G-6-P. However, despite a significant difference in glycogen storage efficiency between transgenic and wild-type mice, there was no difference in insulin-stimulated glucose uptake. Therefore, our results suggest that the normal glucose disposal pathways of muscle are sufficient to compensate for an initial rapid influx of glucose, even in the presence of a high physiological insulin concentration, and that overexpression of glycogen synthase does not improve insulin-stimulated glucose uptake in non-insulin-resistant muscle.

Finally, differences in peak tetanic tension and specific force in GSL3-transgenic muscle compared with wild-type muscle could not be explained by muscle weight or estimated cross-sectional area. Differences in peak tetanic tension and specific force may result, at least in part, from the sevenfold greater glycogen content in the GSL3-transgenic muscle. Considering the water content and space associated with glycogen storage (16), the extensive glycogen macromolecule in GSL3-transgenic muscle may have disrupted myofibrillar mechanics.

In summary, we have demonstrated that overexpression of a constitutively active form of glycogen synthase facilitates a repartitioning of intracellular glucose intermediates toward glycogen synthesis before and after muscle contraction. This allows for an abundant storage of muscle glycogen without excessive glucose transport and the development of insulin resistance. Furthermore, we have demonstrated that glycogen is not responsible for the inhibition of insulin-stimulated PKB/Akt phosphorylation and that PKB/Akt phosphorylation can be increased with muscle contraction. Thus we conclude that the normal inverse relationships between muscle glycogen concentration and activation of glucose transport and PKB/Akt phosphorylation by insulin are associations and not cause and effect relationships.

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