Glycogen content and contraction regulate glycogen synthase phosphorylation and affinity for UDP-glucose in rat skeletal muscles

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Lai Y-C, Stuenæs JT, Kuo C-H, Jensen J. Glycogen content and contraction regulate glycogen synthase phosphorylation and affinity for UDP-glucose in rat skeletal muscles. Am J Physiol Endocrinol Metab 293:E1622–E1629, 2007.—Glycogen content and contraction strongly regulate glycogen synthase (GS) activity, and the aim of the present study was to explore their effects and interaction on GS phosphorylation and kinetic properties. Glycogen content in rat epitrochlearis muscles was manipulated in vivo. After manipulation, incubated muscles with normal glycogen [NG; 210.9 ± 7.1 mmol/kg dry weight (dw)], low glycogen (LG; 108.1 ± 4.5 mmol/kg dw), and high glycogen (HG; 482.7 ± 42.1 mmol/kg dw) were contracted or rested before the studies of GS kinetic properties and GS phosphorylation (using phospho-specific antibodies). LG decreased and HG increased GS affinity for UDP-glucose (LG: 0.27 ± 0.02 < NG: 0.71 ± 0.06 < HG: 1.11 ± 0.12 mM; P < 0.001). In addition, GS fractional activity inversely correlated with glycogen content (R = −0.70; P < 0.001; n = 44). Contraction decreased Km for UDP-glucose (LG: 0.14 ± 0.01 = NG: 0.16 ± 0.01 < HG: 0.33 ± 0.03 mM; P < 0.001) and increased GS fractional activity, and these effects were observed independently of glycogen content. In rested muscles, GS Ser641 and Ser7 phosphorylation was decreased in LG and increased in HG compared with NG. GSK-3β Ser9 and AMPKα Thr172 phosphorylation was not modulated by glycogen content in rested muscles. Contraction decreased phosphorylation of GS Ser641 at all glycogen contents. However, contraction increased GS Ser7 phosphorylation even though GS was strongly activated. In conclusion, glycogen content regulates GS affinity for UDP-glucose and low affinity for UDP-glucose in muscles with high glycogen content may reduce glycogen accumulation. Contraction increases GS affinity for UDP-glucose independently of glycogen content and creates a unique phosphorylation pattern.

uridine diphosphate; adenosine monophosphate kinase; glycogen synthase kinase-3; acetyl-coenzyme A carboxylase; enzyme kinetic

GLYCOGEN SYNTHASE (GS) CONTROLS a rate-limiting step for glycogen synthesis with complex regulation by glycogen content, insulin, adrenaline, and contractile activity (7, 8). Furthermore, GS is defectively regulated in type 2 diabetes (4, 11, 16). The regulation of GS includes phosphorylation at no less than nine different sites and allosteric regulation by glucose-6-phosphate (7, 21).

Insulin-mediated GS activation has been studied in detail previously (7, 21). A large number of studies have reported that insulin increases GS fractional activity (8, 11, 14). Increased GS fractional activity reflects increased sensitivity to glucose-6-phosphate (21, 31). GS affinity for UDP-glucose is also regulated, but this regulation has received little attention despite the fact that the Km exceeds physiological concentrations (28, 30). However, it has been reported that insulin increases GS affinity for UDP-glucose (33). Activation of GS during insulin stimulation results from dephosphorylation, particularly of Ser641 and Ser643 (1, 21, 22, 36). These sites are phosphorylated by GSK-3, and inactivation of GSK-3β is required for insulin-mediated GS activation (25).

Glycogen content regulates GS activity. In fact, glycogen content regulates GS fractional activity to a greater extent than insulin (8, 14, 26). However, the effect of glycogen content on GS affinity for UDP-glucose has not been investigated. Furthermore, the role of glycogen content for GS phosphorylation pattern is poorly understood. We reported decreased phosphorylation of Ser641, Ser643, Ser645, and Ser7 in muscles with low glycogen in parallel with increased GS fractional activity (14). Surprisingly, GS Ser645 phosphorylation was also decreased in muscles with high glycogen when GS fractional activity was low (14). However, GS from muscles with high glycogen did not migrate to a lower band during electrophoresis, which suggested that GS was highly phosphorylated. Indeed, GS7 phosphorylation has been reported to be higher in muscles with high glycogen than in muscles with low glycogen (17).

Contraction also strongly influences GS fractional activity (10, 19). However, the effect of contraction on GS affinity for UDP-glucose has to the best of our knowledge not been studied. Understanding of the GS phosphorylation pattern after contraction is also limited. It has been reported that contraction reduces GS affinity for UDP-glucose (25, 34, 35), but a systematic investigation of all phosphorylation sites has not been performed. GS activation after contraction does not require GSK-3β inhibition (25). However, contraction does not activate GS in muscles lacking the protein phosphatase-1 glycogen binding regulatory subunit PP1GML/RGL, where dephosphorylation of GS is impaired (2). Furthermore, contraction decreases glycogen content and it has been suggested that contraction activates GS solely by reducing glycogen content (26).

We have previously reported that decreasing glycogen content by fasting increased GS fractional activity, whereas increasing glycogen content by refeeding decreased GS fractional activity (14). In the present study, we used the same protocol to manipulate glycogen content to address questions regarding the regulation of the affinity of GS for UDP-glucose...
and its phosphorylation status. The first aim of the present study was to investigate the effect of glycogen content and contraction on GS affinity for UDP-glucose. Muscle contraction decreases glycogen content, and to determine the effect of contraction on GS activation independently of glycogen content, muscles with different glycogen contents were studied. Finally, we used three phospho-specific antibodies raised against GS (anti-GS Ser7, anti-GS Ser84, and anti-GS Ser645,649,653,657) to assess the phosphorylation status of GS when GS activity was modulated by glycogen and contraction.

METHODS

Chemicals and antibodies. Amyloglucosidase was from Boehringer Mannheim (Indianapolis, IN), and [U-14C]glucose (303 mCi/mmol) and [3-14C]dihydropyridine glucose (200 mCi/mmol) were from Perkin Elmer Life Sciences (Boston, MA). Anti-phospho-GS Ser84 was from Oncogene (San Diego, CA). Anti-phospho-GS Ser7 was a gift from Graham Hardie and has been described previously (17). Anti-GSK-3, anti-mouse HRP-conjugate, and anti-sheep HRP-conjugate antibodies were from Upstate (Lake Placid, NY). Anti-phospho-GS Ser84, anti-phospho-GSK-3α/β Ser21/Ser9, anti-phospho-S-AMP-activated protein kinase-α (AMPKα) Thr172, anti-acetyl-CoA carboxylase (ACC) Ser79 (recognizes also ACCβ Ser218; ACCβ is the major isoforms expressed in skeletal muscles), and anti-rabbit HRP-linked antibodies were from Cell Signaling (Beverly, MA). Anti-GS was a gift from Oluf Pedersen (Copenhagen, Denmark). ECL was from Amersham Pharmacia (Buckinghamshire, UK) and Millipore (Billerica, MA). Other chemicals were standard analytical grades from Merck (Darmstadt, Germany) or Sigma (St. Louis, MO).

Animals. Male Wistar rats were obtained from B & K Universal (Nittedal, Norway) and acclimatized in our laboratory animal facilities for at least 6 days with free access to food and tap water before the experiment. The animal room was maintained at 21°C, humidity ≥ 55%, and a 12:12-h light-dark cycle (lights on from 6:00 AM to 6:00 PM). The experiments were performed between 10:00 AM and 2:00 PM. Muscle glycogen concentration was manipulated to obtain muscles with low (LG), normal (NG), and high glycogen (HG) as described previously (12, 14). In brief, the rats with NG were maintained on their normal rat chow until the experiments. The rats with LG were fasted for 24 h before the experiments. The rats with HG were fasted for 24 h and then fed on normal chow for the 24 h before the experiments. Animals were randomly assigned to treatment groups. On the day of the experiment, the weights of the rats were 120–150 g. Experiments and procedures were approved by the National Animal Research Authority and were performed in accordance with the laws and regulations controlling experiments on live animals in Norway, and the European Convention for the Protection of Vertebrate Animals Used in Experimental and Other Scientific Purposes.

Muscle preparation and incubation. The rats were anesthetized with an intraperitoneal injection of ~10 mg pentobarbital (50 mg/ml). The epimysial and muscles were dissected out, suspended on contraction apparatus at their approximate resting length, and preincubated for 30–50 min in 3.5 ml modified Krebs-Henseleit buffer as described previously (10). Muscles were then kept rested or contracted electrically with 200 ms trains (100 Hz, square wave pulses of 0.2 ms duration, 10 V) delivered every 2 s for 30 min as described previously (3). After stimulation, muscles were incubated for an additional 30 min for analyses of GS activity and Western blot. Glycogen synthesis was measured for 60 min. All incubations were executed at 30°C, and gas (95% O2, 5% CO2) was bubbled continuously through the buffer. After incubations, muscles were removed from the apparatus, blotted on filter paper, frozen in liquid nitrogen, and stored at −70°C until analysis.

Glycogen synthesis. For measurement of glycogen synthesis, 0.2 µCi/ml D-[14C]glucose was added to buffer and the incorporation of radio labeled glucose into glycogen was measured as described previously (10).

Glycogen concentration. In muscles used for analysis of GS activities, glycogen was hydrolyzed with 1 M HCl (2.5 h at 100°C). Glycogen content was determined as glucose units analyzed fluorometrically with appropriate standard curve (27). In muscles where rates of glycogen synthesis were measured, 100 µl of the KOH digest was neutralized and hydrolyzed with amyloglucosidase, and glucose units were analyzed as described previously (15).

Western blot. Muscles were weighed and homogenized (1 mg wet wt: 25 µl) as described previously (5). After determination of protein concentrations (Bio-Rad, DC Protein Assay, Hercules, CA) homogenates were diluted to 2 µg/µl. Muscle homogenates were prepared with Laemmli buffer, and proteins (~25 µg) were separated by electrophoresis in 10% SDS-PAGE. An 8% SDS-PAGE gel was run for detection of GS to obtain clear band-shift reflecting phosphorylation state. Proteins were then transferred from the gel into PVDF membrane (PVDF) and analyzed as described previously (5).

GS activity. GS activity was measured by a modification of the method of Thomas et al. (37). Freeze-dried muscles were homogenized with a Polytron (Kinematica, Littau-Luzern, Switzerland) in 1:400 (dry weight: volume) of buffer containing 50 mM Tris-HCl (pH 7.8), 100 mM NaF, and 10 mM EDTA. The homogenates were centrifuged at 3,000 g for 30 min at 4°C, and the pellet was discarded. For analysis, 20 µl of supernatant was added to 40 µl of assay buffer. Final concentrations in the assay buffer were 25 mM Tris-HCl (pH 7.8), 50 mM NaF, 5 mM EDTA, glycogen (10 mg/ml), 0.5 µCi/ml D-[1-14C]UDP-glucose, 0.17 or 12 mM glucose-6-phosphate, and various concentrations of UDP-glucose. Activity was measured at 37°C during 8 min, and 50 µl of the supernatant/assay buffer were spotted on filter paper (Whatman ET-31, 1 cm × 2.5 cm) and immediately dropped into ice-cold 66% ethanol and washed as described previously (15). Total activity was measured in the presence of 12 mM glucose-6-phosphate. Fractional activity was calculated as activity with 0.17 mM glucose-6-phosphate as a percentage of total activity. Fractional activity was measured in the presence of 0.03 mM UDP-glucose (FV0.03) and 1.67 mM UDP-glucose (FV1.67). Kinetic analysis of GS affinity for UDP-glucose was performed in the presence of 0.17 and 12 mM glucose-6-phosphate. For both concentrations of glucose-6-phosphate, GS activities were measured with the following concentrations of UDP-glucose: 1.67, 0.80, 0.40, 0.20, 0.10, 0.05, and 0.03 mM. Kinetic data were linearized as Eadie-Hofstee plots, and GS Km for UDP-glucose was calculated as the reciprocal to the slope and Vmax as the intercept with the y-axis. Km0.17 and Vmax0.17 refer to measurements performed with 0.17 mM glucose-6-phosphate. Km12 and Vmax12 refer to measurements performed with 12 mM glucose-6-phosphate. Concentrations of UDP-glucose and glucose-6-phosphate in stock solutions were determined spectrophotometrically (27).

Statistics. Data are means ± SE. ANOVA was performed to determine the differences between groups, and least significant difference (LSD) was used as post hoc tests. Pearson’s test was used for correlation analysis. P values <0.05 were considered as significant.

RESULTS

Glycogen content was reduced by ~50% after 24 h fasting (Fig. 1A). When 24-h fasted rats were given access to normal chow for another 24 h, glycogen content increased to more than double of that observed in rats maintained on normal diet (Fig. 1A). Glycogen content remained ~100 mmol/kg dry weight higher in contracted HG than in rested NG (P < 0.001). Glycogen content in contracted NG was reduced to the content of rested LG muscles (Fig. 1A). After contraction, rates of glycogen synthesis were increased in all groups (Fig. 1B).
Glycogen synthesis post contraction was higher in LG than in NG and higher in NG than in HG.

GS fractional activity was measured with physiological UDP-glucose concentration (FV0.03) and a high (1.67 mM) UDP-glucose concentration (FV1.67). GS FV0.03 and FV1.67 in rested muscles decreased gradually as the glycogen content increased (Fig. 2). Pearson’s correlation between FV0.03 and glycogen content showed: \( R = -0.70 \) (\( P < 0.001; n = 44 \)) and between FV1.67 and glycogen content: \( R = -0.88 \) (\( P < 0.001; n = 24 \)). After contraction, GS fractional activities measured with a physiological concentration of UDP-glucose were elevated at all glycogen contents (Fig. 2A). GS FV1.67 was elevated in NG and HG (Fig. 2C). Of particular note, the observed effects of contraction on GS fractional activity depended on the UDP-glucose concentration in the assay buffer. At a physiological concentration of UDP-glucose, GS FV0.03 was higher in contracted HG than in rested NG, although glycogen content was higher in contracted HG than in rested NG (Figs. 1 and 2). In contrast, GS FV1.67 was lower in contracted HG than in rested NG (Fig. 2C). Furthermore, plotting GS fractional activity against glycogen content showed that GS FV0.03 in contracted muscles clustered above rested muscles, showing that contraction increases fractional activity independently of glycogen content (Fig. 2B). On the other hand, GS FV1.67 did not increase independently of glycogen content in contracted muscles with the relationship between glycogen content and GS FV1.67 following the same regression line in rested and contracted muscles (Fig. 2D). Moreover, higher GS fractional activities were observed when the UDP-glucose concentration was increased in the assay buffer.

Analysis of GS kinetic properties showed that a high glycogen content strongly decreases GS affinity for UDP-glucose when measured with a physiological glucose-6-phosphate concentration (\( K_{m,0.17} \), Table 1). A high glucose-6-phosphate concentration decreased GS \( K_m \) for UDP-glucose (\( K_{m,12} \)) to a low level in all groups, and a less pronounced effect of glycogen content was observed. Importantly, GS \( K_{m,0.17} \) for UDP-glucose was reduced to low levels after contraction in all groups when measured at a physiological glucose-6-phosphate concentration (Table 1) although not significantly in LG (\( P = 0.12 \)). However, \( K_{m,0.17} \) remained higher in HG than in NG and LG after contraction (Table 1). \( K_{m,12} \) was reduced in LG and HG after contraction (Table 1). Interestingly, when GS affinity for UDP-glucose was measured with a physiological concentration of glucose-6-phosphate (\( K_{m,0.17} \)), contraction increased GS affinity for UDP-glucose independently of glycogen content (Fig. 3). However, when GS affinity for UDP-glucose was measured with 12 mM glucose-6-phosphate (\( K_{m,12} \)), such an increase was not seen (Fig. 3). The maximal GS activity obtained with a physiological glucose-6-phosphate concentration (\( V_{max,0.17} \)) was higher in LG than in NG and lower in HG than in NG. In contrast, maximal GS activity at a high glucose-6-phosphate concentration (\( V_{max,12} \)) was similar in all groups. Glycogen content in the muscles used for GS measurements was similar to the muscles used for measurement of glycogen synthesis (Table 1; Fig. 1A).

Effects of contraction and glycogen on GS phosphorylation are shown in Fig. 4. GS Ser645,649,653,657, Ser641, and Ser7 phosphorylation was lower in rested LG than in NG. In HG, GS Ser641 and Ser7 phosphorylation was higher than in NG. GS Ser645,649,653,657 phosphorylation was slightly lower in HG than in NG as we have previously reported (14). GS Ser641 phosphorylation was reduced in all groups after contraction (Fig. 4), whereas GS Ser645,649,653,657 phosphorylation was reduced only in NG and LG. After contraction, GS Ser7 phosphorylation was increased in all groups despite the increase in fractional activity and affinity. Dephosphorylation of GS increases electrophoretic mobility, and GS migrated as low bands in contracted NG and LG. In HG, no band shift occurred after contraction, supporting the finding that GS remained highly phosphorylated (Fig. 4A). Data from Western blot showed similar expression of GS in muscles with different glycogen contents (arbitrary units: 107.6 ± 4.7, 100.0 ± 4.7, and 101.2 ± 9.7 in LG, NG, and HG, respectively).

Glycogen content did not influence GSK-3β Ser9 phosphorylation in rested muscles. GSK-3β Ser9 phosphorylation measured 30 min after contraction was increased to a similar level in all groups (Fig. 5A). Phosphorylation of AMPK was not observed under basal conditions in any of the groups. Thirty minutes after contraction, AMPKα Thr172 phosphorylation was increased in LG and NG and more in LG than in NG (Fig. 5B). AMPKα Thr172 phosphorylation was not significantly increased in HG 30 min after contraction. ACCβ Ser218 phosphorylation was elevated 30 min after contraction in all groups but more in NG than in HG (Fig. 5C).

DISCUSSION

Glycogen content and contraction are strong regulators of GS, and the present study reports several novel findings about contraction- and glycogen-mediated regulation of GS kinetic properties.
properties and phosphorylation. The most important new findings are as follows: 1) high glycogen content reduced GS affinity for UDP-glucose, 2) high glycogen content increased GS Ser\(^{641}\) phosphorylation, 3) GS affinity for UDP-glucose was increased after contraction, and 4) GS Ser\(^{3}\) phosphorylation was increased after contraction in conjunction with increased GS activation. Moreover, our data show that contraction causes a unique phosphorylation pattern and activates GS independently of glycogen content.

A significant new finding is that high glycogen content in skeletal muscles decreased GS affinity for UDP-glucose. Importantly, high muscle glycogen content increased \(K_m\) nearly twofold similar to alloxan-induced diabetes (20) and far above the physiological UDP-glucose concentration (28, 30). Therefore, an increase in GS \(K_m\) may have a limiting role for glycogen accumulation. On the other hand, low glycogen content reduced GS \(K_m,0.17\), which may be a physiological mechanism for the stimulation of glycogen synthesis when glycogen content is low. As reported in other experimental settings (1, 32, 33), a high glucose-6-phosphate concentration increased GS affinity for UDP-glucose, but \(K_m,12\) was still regulated by glycogen content.

At all glycogen contents, the rate of glycogen synthesis was increased after contraction but the rate of synthesis was 50% lower in HG than in LG. Only a few studies have addressed this question, but the rate of glycogen synthesis has been reported to be reduced similarly in super-compensated human muscles after exercise (29). Interestingly, in contracted muscles, GS \(K_m,0.17\) was reduced to low values at all glycogen contents. Although \(K_m,12\) remained slightly higher in HG than in NG after contraction, the reduction of \(K_m,0.17\) from 1.11 to 0.33 mM in HG after contraction was remarkable. This is the first study to report data on GS affinity after contraction, and we hypothesize that increased GS affinity for UDP-glucose is an important physiological mechanism for the stimulation of glycogen synthesis after contraction.

GS fractional activity, which sheds light on affinity for glucose-6-phosphate, is normally used to evaluate GS activa-
In agreement with previous studies, we also found that GS fractional activity inversely correlates with glycogen content (8, 13, 14, 26). GS fractional activity was increased after contraction at all glycogen contents in agreement with other studies (8, 10, 26). Contraction decreases glycogen content, and reduced glycogen content increases GS activation (8, 14, 26). To determine the effects of contraction on GS activation and phosphorylation independently of glycogen content, we contracted muscles with different glycogen contents. Importantly, the present study shows that contraction regulates GS with increased FV0.03, K_m, and phosphorylation independently of glycogen content. In contracted HG, glycogen content remained higher than in noncontracted NG, whereas FV0.03 was higher and K_m was lower. However, our data highlight that the GS assay condition influences the physiological interpretation of the data. At a high concentration of glucose-6-phosphate, contraction did not decrease K_m independently of glycogen content (Fig. 3B). Furthermore, as reported by Nielsen et al. (26), contraction did not increase GS fractional activity independently of glycogen content when activity was measured with a high concentration of UDP-glucose (FV1.67; Fig. 2D). Our data clearly show that the GS affinity for UDP-glucose has to be considered together with the affinity for glucose-6-phosphate when GS activation is evaluated under physiological conditions.

Danforth (8) described strong regulation of GS by glycogen more than 40 years ago, but the GS phosphorylation pattern has not been clarified. In the present study, all three antibodies demonstrated reduced GS phosphorylation in muscles with LG where GS fractional activity was increased. This agrees with the common view that dephosphorylation activates GS (7, 21). We are the first to report that GS Ser641 phosphorylation is regulated by glycogen content, and phosphorylation of this site strongly regulates GS activity (36). Jørgensen et al. (17) have previously reported that GS Ser7 phosphorylation was higher in HG than in LG, and we now show that NG phosphorylation is intermediate. The phospho-antibody raised against Ser645,649,653,657 showed higher phosphorylation in HG compared with both LG and HG, which agrees with our previous report (14). Furthermore, similar GS Ser641,645 phosphorylation has been reported in HG and LG in rat muscles (17) and in cultured human primary muscle cells (24). A limitation in the interpretation of data from studies with antibodies raised against peptides with two or more close phos-
phorylation sites is the lack of sufficient characterization of antibody binding when these sites are phosphorylated simultaneously, and the data must be interpreted with caution. However, electrophoretic mobility of GS shows that the protein was highly phosphorylated in HG.

In the present study, phosphorylation of GSK-3 and AMPK in rested muscles was not modulated by glycogen content in agreement with previous findings (9, 14, 18), suggesting that protein phosphatase activity regulates glycogen accumulation under these conditions. In line with this interpretation, high glycogen content has been reported to decrease protein phosphatase-1 (PP-1) activity (39) and overexpression of PP-1 glycogen binding regulatory subunit Gm/RGL, or PTG in cultured human primary muscle cells increases glycogen content (23), whereas deletion of Gm/RGL decreases glycogen content in mouse muscles (2, 38). Genetic approaches are required to determine the phosphorylation sites of GS, which regulate glycogen accumulation.

We show here for the first time that GS Ser7 phosphorylation was increased after contraction. Moreover, GS Ser7 phosphorylation was increased at all glycogen contents after contraction. GS activity was also increased after contraction, and the phosphorylation of GS Ser7 was surprising because AMPK phosphorylates purified GS at Ser7, which decreases its activity (6). Furthermore, Jørgensen et al. (17) have reported that inactivation of GS is associated with GS Ser7 phosphorylation during 5-aminomidazole-4-carboxamide-1-β-d-ribofuranside stimulation and that deletion of AMPK-α2 abolishes both inactivation and phosphorylation. However, we now report that GS Ser7 phosphorylation does not prevent GS activation after contraction.

The present study was not designed to determine the kinase that phosphorylates GS Ser7 after contraction. However, phosphorylation of this site was highest in HG where AMPKα Thr172 phosphorylation was not detectable. Nevertheless, ACCβ Ser218 phosphorylation was elevated in HG after contraction, suggesting that AMPK-mediated phosphorylation had occurred. Other protein kinases also phosphorylate GS Ser7 (7), and it is possible that GS Ser7 phosphorylation after contraction was mediated by another protein kinase.

In the present study, phosphorylation of GS Ser641 was reduced after contraction at all levels of glycogen content, whereas dephosphorylation of GS Ser645,649,653,657 phosphorylation was only seen in NG and LG. Dephosphorylation at these sites after contraction has previously been reported in NG and LG (25, 34, 35). Interestingly, knockin studies have shown that contraction leads to normal GS dephosphorylation and activation even when GSK-3 cannot be inactivated (25), which may explain the discrepancy we observed between GSK-3 Ser9 phosphorylation and GS phosphorylation in contracted muscles. On the other hand, GS dephosphorylation and activation are impaired in muscles from mice lacking Gm/RGL (2).

In the present study, GS phosphorylation after contraction...
remained higher in HG than in NG and we conclude that high glycogen content diminishes dephosphorylation and activation of GS after contraction.

In summary, glycogen content strongly regulates GS by decreasing the affinity for UDP-glucose and in this way may limit glycogen accumulation. In rested muscles with different glycogen contents, GS Ser7 and Ser641 phosphorylation was inversely related to GS affinity for UDP-glucose and fractional activity. After contraction, GS affinity for UDP-glucose was increased at all levels of glycogen content, which was associated with decreased Ser641 phosphorylation but increased Ser7 phosphorylation. Furthermore, our data show that contraction activates GS through mechanisms other than a reduction in glycogen content. However, a physiological concentration of UDP-glucose is required to observe increased GS fractional activity independent of glycogen content.

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