Effect of acute exercise on glycogen synthase in muscle from obese and diabetic subjects

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Insulin-resistant subjects have impaired insulin-mediated muscle glucose disposal (14, 44, 46). Some studies also have demonstrated impaired insulin signaling through Akt (also known as PKB) (45) and reduced insulin-stimulated GS activation (12, 45). Exercise plays a fundamental role in the treatment and prevention of type 2 diabetes mellitus (T2DM) (18). Although the molecular mechanisms by which exercise improves glucose homeostasis are not fully understood, it is thought that exercise improves glycemia in part by enhancing glucose transport into the contracting muscles fibers (32), where it is oxidized to generate ATP or stored as glycogen. Similarly to insulin, exercise promotes dephosphorylation of GS, leading to its enzymatic activation for glycogen synthesis (21, 25, 30, 35, 36). The contribution of phosphorylation-dependent regulation on GS kinetic properties in humans is not known.

Insulin-resistant subjects have impaired insulin-mediated muscle glucose disposal (5, 6). Some studies also have suggested that exercise improves glycemia in part by enhancing GS activity (7, 16). It is thought that both mechanisms contribute to the stimulation of glycogen synthesis in response to insulin by increasing GS fractional activity and intramuscular G6P concentration, secondary to increased glucose transport (3, 14, 15, 34). The role of G6P-mediated allosteric activation has been demonstrated in muscles from knockin mice expressing a GS mutant insensitive to G6P. These mice displayed normal insulin-stimulated GS dephosphorylation and activation but impaired insulin-stimulated glycogen synthesis (4). In rat muscle, both insulin and exercise increase phospho-dependent GS activity by enhancing GS sensitivity for G6P and affinity for UDP-glucose (21, 33). The contribution of phosphorylation-dependent regulation on GS kinetic properties in humans is not known.

METHODS

Subjects. Seven obese (BMI = 33.6 ± 1.9 kg/m²) normal glucose tolerant (NGT), six obese (BMI = 31.1 ± 1.7) T2DM, and nine lean (BMI = 24 ± 1.0) NGT subjects participated in this study. No subject proposed to limit glycogen synthesis in insulin-resistant subjects (13, 14).

GS activity is regulated by covalent phosphorylation and allosteric activation by G6P (7, 16). It is thought that both mechanisms contribute to the stimulation of glycogen synthesis in response to insulin by increasing GS fractional activity and intramuscular G6P concentration, secondary to increased glucose transport (3, 14, 15, 34). The role of G6P-mediated allosteric activation has been demonstrated in muscles from knockin mice expressing a GS mutant insensitive to G6P. These mice displayed normal insulin-stimulated GS dephosphorylation and activation but impaired insulin-stimulated glycogen synthesis (4). In rat muscle, both insulin and exercise increase phospho-dependent GS activity by enhancing GS sensitivity for G6P and affinity for UDP-glucose (21, 33). The contribution of phosphorylation-dependent regulation on GS kinetic properties in humans is not known.

Insulin-resistant subjects have impaired insulin-mediated muscle glucose disposal (14, 44, 46). Some studies also have demonstrated impaired insulin signaling through Akt (also known as PKB) (45) and reduced insulin-stimulated GS activation (12, 45). Exercise plays a fundamental role in the treatment and prevention of type 2 diabetes mellitus (T2DM) (18). Although the molecular mechanisms by which exercise improves glucose homeostasis are not fully understood, it is thought that exercise improves glycemia in part by enhancing glucose transport into the contracting muscles fibers (32), where it is oxidized to generate ATP or stored as glycogen. Similarly to insulin, exercise promotes dephosphorylation of GS, leading to its enzymatic activation for glycogen synthesis (21, 25, 30, 35, 36). Notably, it is not entirely clear which phosphorylation sites mediate exercise-induced GS activation in muscle, particularly in humans.

Although exercise can enhance insulin-stimulated glucose disposal (5, 6) in insulin-resistant subjects (5, 6), the effect of exercise on GS phosphorylation and affinity for UDP-glucose has not been studied in these individuals. The goal of this study was to examine the effect of exercise on GS kinetic properties and GS dephosphorylation at Ser7, Ser641, and Ser645,649,653,657 in normal and insulin-resistant (obese nondiabetic and T2DM) subjects.

SKELETAL MUSCLE IS THE MAIN SITE RESPONSIBLE FOR INSULIN-STIMULATED GLUCOSE DISPOSAL (11). After glucose enters the muscle fibers, it is converted to glucose 6-phosphate (G6P). Upon insulin stimulation during the postprandial period, ~80% of the glucose that is taken up by the muscle is utilized for glycogen synthesis (37). Insulin stimulates glucose transport/phosphorylation and activates glycogen synthase (GS), which catalyzes incorporation of glucose from uridyl diphosphate (UDP)-glucose into glycogen (7). Glucose transport is normally considered the rate-limiting step for glycogen storage (34), although impaired GS activation by insulin also has been

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exercised regularly (0 to 1 exercise sessions/wk). Each subject underwent a medical history, physical examination, and 75-g oral glucose tolerance test (OGTT). Three T2DM subjects took a sulfonylurea, which was stopped 2 days before any experiment to avoid hypoglycemia. Three T2DM subjects were diet treated. In lean and obese subjects normal glucose tolerance was documented with the OGTT (1), and these subjects did not have a family history (first-degree relative) of T2DM. Other than the sulfonylureas, subjects were not taking any medication known to affect glucose or lipid metabolism. The study was approved by the Institutional Review Board of the UTHSCSA, and all subjects gave written voluntary consent.

**OGTT.** After an overnight fast, plasma glucose was measured at baseline and 2 h after the ingestion of 75 g of glucose. Plasma insulin and free fatty acid concentrations were measured at baseline.

V\(_{O_2\text{max}}\) testing. Within 7 days after the OGTT, V\(_{O_2\text{max}}\) was determined using a cycle ergometer and a Metabolic Measurement System (Sensormedics, Savi Park, CA), as described previously (40).

**Measurement of insulin sensitivity.** Within 3–7 days after the V\(_{O_2\text{max}}\) test, subjects underwent a 180-min euglycemic hyperinsulinemic clamp study. Insulin-stimulated glucose metabolism (M) was determined as the mean glucose infusion rate during the last 30 min of the clamp (11).

**Acute exercise protocol.** Within 7–10 days after the insulin clamp, subjects underwent an acute exercise experiment with muscle biopsies. After arriving at the Clinical Research Center, subjects rested in bed for 30 min, and a vastus lateralis muscle biopsy was performed under local anesthesia (1% lidocaine) using a Bergström cannula with suction. The muscle was rapidly (within ~7 s) debrided of adipose and connective tissue and frozen in liquid nitrogen. Subjects then exercised on the cycle ergometer. From the V\(_{O_2\text{max}}\) test, a power output designed to elicit an intensity of 70% of V\(_{O_2\text{max}}\) was calculated for each subject. Subjects cycled at the designated power output for 40 min and were then placed on the bed, and a second muscle biopsy was obtained (at ~35 min, subjects stopped exercising for ~20 s for lidocaine application). The subjects then rested in a bed for 210 min, and a third muscle biopsy was performed under local anesthesia. Each biopsy site was separated by 5 cm. In two lean and two diabetic subjects the 210-min postexercise biopsies were not available due to technical reasons.

**Laboratory analyses.** Plasma insulin was measured by radioimmunoassay (Diagnostic Products, Los Angeles, CA), plasma glucose by the glucose oxidase method (Beckman, Fullerton, CA), and hemoglobin A\(_1c\) (Hb A\(_1c\)) using a DCA2000 Analyzer (Bayer, Tarrytown, NY). Plasma free fatty acid (FFA) concentration was determined using a colorimetric method (Wako, Richmond, VA).

**GS activity.** Muscles were homogenized with a Polytron (Kinetica, Littau-Luzern, Switzerland) in ice-cold buffer (1:400) containing 50 mM Tris·HCl (pH 7.8), 100 mM NaF, and 10 mM EDTA. Homogenates were centrifuged at 3,000 g for 30 min at 4°C. For analysis, 20 μl of supernatant was added to 40 μl of assay buffer containing 25 mM Tris·HCl (pH 7.8), 50 mM NaF, 5 mM EDTA, glycogen (10 mg/ml), different concentrations of UDP-glucose (see below), 0.5 μCi/ml of l-[14C]UDP in the presence of 0.17 mM, and 12 mM G6P. GS %I form was calculated as activity without G6P in percent of activity at 12 mM G6P; the concentration of UDP-glucose was 1.67 mM. GS affinity for UDP-glucose (Km) and V\(_{max}\) were analyzed with 0.17 and 12 mM G6P, and assays were conducted with the following concentrations of UDP-glucose: 1.67, 0.8, 0.4, 0.2, 0.1, 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 V\(_{max}\) as the intercept with the y-axis. Km\(_{12.1}\) and V\(_{max,12.1}\) refer to the analysis performed with 0.17 mM G6P, and Km\(_{12.12}\) and V\(_{max,12.12}\) refer to the analysis performed with 12 mM G6P. Concentrations of UDP-glucose and G6P in stock solutions were determined spectrophotometrically, as described (27). Homogenate protein concentration was measured using a colorimetric assay (DC Protein Assay; Bio-Rad, Hercules, CA).

**Glycogen content.** Glycogen was measured in homogenates prepared for GS analysis. One-hundred fifty microliters of crude homogenate was hydrolyzed with 300 μl of 1.8 M HCl (100°C, 2.5 h) and glycogen determined fluorometrically as glucose units (27).

**Immunoblotting.** Muscles were homogenized (1:25) twice for 15 s in ice-cold buffer containing 50 mM HEPEs, 150 mM NaCl, 10 mM Na\(_2\)PO\(_4\), 30 mM NaF, 1 mM Na\(_2\)VO4, 10 mM EDTA, 2.5 mM benzamidine, and 2 μl/ml protease inhibitor cocktail (P-8340; Sigma), and 1% Triton X-100 was added. Homogenates were rotated for 1 h at 4°C and centrifuged (11,500 g for 10 min at 4°C). Homogenates were diluted to 1.5 μg/ml for immunoblotting, and proteins (~15 μg) were resolved in 10% SDS gels, transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA) and probed with the corresponding primary and secondary antibodies. Antibody binding was detected with enhanced chemiluminescence reagents (Millipore, Billerica, MA) and quantified using FUJI LAS-4000 Mini (FujiFilm, Tokyo, Japan).

**Statistics.** Data are presented as means ± SE. Baseline characteristics between groups were compared with one-way ANOVA. The effect of exercise and recovery was analyzed using one-way repeated measures ANOVA followed by Fisher’s least significant difference test. A P value <0.05 was considered to be significant.

**RESULTS**

**Subject characteristics.** Table 1 shows the subject characteristics. Obese NGT and T2DM had higher BMI than the lean NGT group. T2DM subjects had significantly higher plasma glucose level (baseline and during the OGTT), Hb A\(_1c\), and FFA concentrations than obese and lean NGT subjects. T2DM and obese subjects were more insulin resistant than the lean subjects based on the lower M value during the insulin clamp. Subjects in the T2DM group had lower V\(_{O_2\text{max}}\) than lean subjects.

**Glycogen content.** Glycogen content in muscle was not significantly different in muscle from lean, obese, or diabetic subjects prior to exercise (Fig. 1A). Exercise robustly de-

<table>
<thead>
<tr>
<th>Table 1. Characteristics of subjects</th>
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<tr>
<td><strong>Lean</strong></td>
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<tr>
<td><strong>Age, yr</strong></td>
</tr>
<tr>
<td>Sex (F/M)</td>
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<tr>
<td>BMI (kg/m(^2))</td>
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<tr>
<td>Weight, kg</td>
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<tr>
<td>BML, kg/m(^2))</td>
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<tr>
<td>V(_{O_2\text{max}}), ml·min(^{-1})·kg(^{-1})</td>
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<tr>
<td>Hb A(_1c), %</td>
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<tr>
<td>Fasting plasma glucose, mmol/l</td>
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<tr>
<td>OGTT 2-h plasma glucose, mmol/l</td>
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<tr>
<td>Fasting plasma insulin, μU/ml</td>
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<tr>
<td>Fasting plasma FFA, μmol/l</td>
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<tr>
<td>M value, mg·kg(^{-1})·min(^{-1})</td>
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<td>Work during exercise, W</td>
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Data are means ± SE. Blood samples were taken after an overnight fast. BMI, body mass index; FFA, free fatty acids; H, Hispanic; C, Caucasian; AA, African-American; F, female; M, male. *P < 0.05 vs. lean; **P < 0.05 vs. obese.
creased muscle glycogen content in lean, obese, and T2DM subjects, and the decrease in glycogen content caused by exercise (delta from baseline to exercise time point) was similar between groups (185 ± 25, 159 ± 32, and 177 ± 39 mmol/kg dry wt in lean, obese, and T2DM, respectively, P < 0.05 in all groups vs. baseline). No significant increase in muscle glycogen content was observed between the preexercise and the 3.5-h postexercise time points.

**GS activity.** Baseline GS fractional activity, measured with a physiological concentration of UDP-glucose (FV0.03), was not significantly different in the lean, obese, and T2DM groups, although there was a tendency for a lower FV0.03 in T2DM compared with lean subjects (Fig. 1B). Exercise increased the FV0.03 in lean, obese, and T2DM subjects by 1.9-, 2.3-, and 3.2-fold, respectively (P < 0.05 vs. baseline in all groups); GS activity measured immediately after 40 min of exercise was similar between groups (Fig. 1C). After 3.5 h of rest, GS activity (FV0.03) remained elevated in the three groups (P < 0.05 vs. baseline in all groups); there were no differences in FV0.03 between groups at the 3.5-h postexercise time point (Fig. 1B). GS fractional activity, measured with a high concentration of UDP-glucose (FV1.67), was increased significantly in lean, obese, and diabetic subjects after 40 min of exercise (P < 0.05 vs. baseline in all groups), and FV1.67 remained elevated during the 3.5-h postexercise period (Fig. 1C). There were no statistically significant differences in FV1.67 between groups after 40 min of exercise or at the 3.5-h postexercise time point (Fig. 1D). Exercise caused a time-dependent increase in GS %I-form that in all of the groups was highest at the 3.5-h postexercise time point (Fig. 1D). There were no differences between groups in maximal GS activity calculated from Eadie-Hofstee plots (Table 2). GS activity measured with 1.67 and 12 mM UDP-glucose (often called total activity) was also similar in all groups (data not shown). Exercise did not affect maximal GS activity (Table 3).

**GS affinity.** GS affinity to UDP-glucose was measured with a physiological (0.17 mM) and a pharmacological concentration (12 mM) of G6P. K_m-0.17 in baseline muscle samples was ~0.5 mM, which is in agreement with our previous data in rat muscle (21, 23). Interestingly, baseline K_m-0.17 was ~47% higher in muscles from diabetic subjects compared with lean individuals (P < 0.05; Fig. 3A). Exercise decreased K_m-0.17 to ~0.2 mM in all groups, and no differences were observed between groups. GS affinity for UDP-glucose remained elevated 3.5 h after exercise in all groups (Fig. 3A). In all three groups, 12 mM G6P increased the affinity of GS to UDP-glucose, as evidenced by a decrease in K_m-0.12 to 0.15 mM (Fig. 3B). Exercise did not significantly affect K_m-0.12 in any of the groups (Fig. 3B).

### Table 2. Glycogen synthase activity in muscles from lean, obese, and T2DM subjects

<table>
<thead>
<tr>
<th></th>
<th>Maximal Glycogen Synthase Activity, mmol·min⁻¹·kg dry wt⁻¹</th>
<th>Lean</th>
<th>Obese</th>
<th>T2DM</th>
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<tbody>
<tr>
<td>Basal</td>
<td></td>
<td>16.6 ± 1.4</td>
<td>14.7 ± 1.0</td>
<td>13.9 ± 0.8</td>
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<tr>
<td>After exercise</td>
<td></td>
<td>15.2 ± 1.3</td>
<td>14.0 ± 1.1</td>
<td>12.8 ± 1.9</td>
</tr>
<tr>
<td>3 h Postexercise</td>
<td></td>
<td>13.8 ± 1.1</td>
<td>15.3 ± 1.6</td>
<td>16.5 ± 4.1</td>
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Data are means ± SE. T2DM, type 2 diabetes mellitus. Maximal glycogen synthase activity was calculated from Eadie-Hofstee plots with 12 mM glucose 6-phosphate.
**GS protein content and phosphorylation.** At baseline, GS protein content was decreased significantly in T2DM subjects ($P < 0.05$ vs. lean; Fig. 4A) and was accompanied by a trend for a reduction (17%, $P = 0.05$ not significant) in GS total activity (Table 2). At baseline, GS Ser641 phosphorylation (corrected for protein content) was similar in the three groups (Fig. 4B). Exercise significantly decreased GS Ser641 phosphorylation to a similar level in all groups ($P < 0.05$ vs. baseline in the 3 groups), and it remained decreased 3.5 h after exercise (Fig. 4B). Prior to exercise, baseline GS Ser645,649,653,657 phosphorylation (corrected for protein content) was similar in all groups (Fig. 4C). Exercise caused GS Ser645,649,653,657 dephosphorylation to a similar level in the three groups ($P < 0.05$ vs. rest). GS Ser645,649,653,657 phosphorylation remained significantly decreased 3.5 h after exercise in lean and obese subjects ($P < 0.05$ vs. baseline in both groups; Fig. 4C). GS Ser7 phosphorylation was similar between groups after correcting for GS protein content and was not influenced by exercise (Fig. 4D). In line with this finding, there was no difference in baseline GS Ser7 + 10 phosphorylation between groups, and it did not change with exercise (data not shown). Analysis of pooled data from lean, obese, and T2DM subjects before and after exercise revealed that GS Ser641 and Ser645,649,653,657 phosphorylation correlated inversely with GS FV$_{0.03}$ ($r = -0.580$ and $-0.602$, respectively, $P < 0.001$ for both) and directly with $K_m$-0.17 ($r = 0.546$ and 0.524 respectively, $P < 0.001$ for both). Notably, there was a strong curvilinear relationship between GS FV$_{0.03}$ and $K_m$-0.17 (Fig. 5A). GS fractional activity, measured with 0.03 mM (FV$_{0.03}$), correlated closely with GS fractional activity measured with 1.67 mM UDP-glucose (FV$_{1.67}$) in the assay buffer (Fig. 5B).

### Table 3. GSK-3β Ser9 phosphorylation in lean, obese, and T2DM subjects before, immediately after, and 3.5 h after 40 min of exercise

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Obese</th>
<th>T2DM</th>
</tr>
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<tbody>
<tr>
<td>Preexercise</td>
<td>21.1 ± 3.1</td>
<td>26.1 ± 3.1</td>
<td>22.3 ± 1.2</td>
</tr>
<tr>
<td>Postexercise</td>
<td>24.4 ± 4.0</td>
<td>21.7 ± 2.2</td>
<td>20.0 ± 3.5</td>
</tr>
<tr>
<td>3.5 h Postexercise</td>
<td>21.3 ± 2.8</td>
<td>25.7 ± 3.7</td>
<td>25.1 ± 3.3</td>
</tr>
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</table>

Data are means ± SE.

**Fig. 2.** Correlation between glycogen content and GS activity. A: correlation between maximal GS activity with 1.67 mM UDP-glucose (V$_{\text{max}-12}$) and glycogen content at baseline. $r = 0.69$, $P < 0.01$. B: correlation between glycogen content and GS fractional activity (FV$_{0.03}$) measured before, during, and after exercise. Open symbols, before exercise; filled symbols, immediately after exercise; filled symbols with cross, 3.5 h after exercise.

**Fig. 3.** Effect of exercise on GS affinity for UDP-glucose. Muscle biopsies were taken prior to, immediately after, and 3.5 h after 40 min of cycling at 70% V$_{\text{O2max}}$ and GS affinity for UDP-glucose analyzed with a physiological (0.17 mM) concentration of glucose 6-phosphate (A) and with 12 mM glucose 6-phosphate (B). *Significantly different from basal (prior to exercise); †significantly different from lean. Open bars, lean subjects; filled bars, obese subjects; hatched bars, T2DM subjects.

**Fig. 4.** GSK-3β Ser9 phosphorylation. At baseline, GS protein content was decreased significantly in T2DM subjects ($P < 0.05$ vs. lean; Fig. 4A) and was accompanied by a trend for a reduction (17%, $P = 0.05$ not significant) in GS total activity (Table 2). At baseline, GS Ser641 phosphorylation (corrected for protein content) was similar in the three groups (Fig. 4B). Exercise significantly decreased GS Ser641 phosphorylation to a similar level in all groups ($P < 0.05$ vs. baseline in the 3 groups), and it remained decreased 3.5 h after exercise (Fig. 4B). Prior to exercise, baseline GS Ser645,649,653,657 phosphorylation (corrected for protein content) was similar in all groups (Fig. 4C). Exercise caused GS Ser645,649,653,657 dephosphorylation to a similar level in the three groups ($P < 0.05$ vs. rest). GS Ser645,649,653,657 phosphorylation remained significantly decreased 3.5 h after exercise in lean and obese subjects ($P < 0.05$ vs. baseline in both groups; Fig. 4C). GS Ser7 phosphorylation was similar between groups after correcting for GS protein content and was not influenced by exercise (Fig. 4D). In line with this finding, there was no difference in baseline GS Ser7 + 10 phosphorylation between groups, and it did not change with exercise (data not shown). Analysis of pooled data from lean, obese, and T2DM subjects before and after exercise revealed that GS Ser641 and Ser645,649,653,657 phosphorylation correlated inversely with GS FV$_{0.03}$ ($r = -0.580$ and $-0.602$, respectively, $P < 0.001$ for both) and directly with $K_m$-0.17 ($r = 0.546$ and 0.524 respectively, $P < 0.001$ for both). Notably, there was a strong curvilinear relationship between GS FV$_{0.03}$ and $K_m$-0.17 (Fig. 5A). GS fractional activity, measured with 0.03 mM (FV$_{0.03}$), correlated closely with GS fractional activity measured with 1.67 mM UDP-glucose (FV$_{1.67}$) in the assay buffer (Fig. 5B).
GSK-3 phosphorylation. GSK3β Ser9 phosphorylation was similar in lean, obese, and diabetic subjects (Table 3). Exercise did not influence GSK-3β Ser9 phosphorylation in any group of subjects.

DISCUSSION

GS activity is regulated by covalent phosphorylation and the allosteric activator G6P (7, 16). Recent studies performed in rodents suggest that allosteric activation by G6P plays a more prominent role than phosphorylation in the regulation of muscle GS activity and glycogen synthesis in response to insulin (3, 4). Nonetheless, the phosphorylation state of GS influences its enzymatic activity by altering its affinity for G6P and the $K_m$ for its substrate UDP-glucose (16). In addition, G6P increases GS affinity for UDP-glucose (21, 23, 24, 33). These interactions allow for a marked increase in the rate of glycogen synthesis in response to relatively small changes in the level of the allosteric activator G6P and the substrate UDP-glucose when GS phosphorylation is reduced.

The originality of this study lies in that we demonstrate that exercise promotes an increase in GS affinity for UDP-glucose in human skeletal muscle and that exercise increases GS affinity for UDP-glucose with similar efficiency in muscles from lean, obese, and T2DM subjects. Previous studies in rodents have shown that strenuous contractions induced via electrical stimulation increase GS affinity for UDP-glucose (21, 22). The present study is novel in that it demonstrates that a more physiological mode of exercise (40 min of moderate-intensity cycling) is able to reduce significantly the $K_m$ of GS for UDP-glucose in human subjects that are both insulin sensitive and insulin resistant (obese and type 2 diabetic). The GS $K_m$ of $0.2 \text{ mM}$ UDP-glucose after exercise at a physiological concentration of G6P (0.17 mM) is far above the physiological concentration of UDP-glucose (0.03 mM) in resting muscles (28, 31), and the increased affinity for UDP-glucose would be expected to increase the rate of glycogen synthesis after exercise. Our data suggest that increased GS affinity for UDP-glucose represents an important physiological mechanism by which contraction promotes glycogen synthesis.

In agreement with previous studies, exercise increased GS fractional activity similarly in normal, obese, and diabetic subjects (8, 30), indicating elevated sensitivity for G6P. Our study also demonstrated a much greater increase in GS fractional activity after exercise with a physiological UDP-glucose concentration in the assay buffer ($=30 \mu\text{M}$) compared with a high UDP-glucose concentration (16, 19). Glycogen content is
a powerful regulator of GS fractional activity (9, 15, 21). In this study, 40 min of exercise at 70% $V_{\text{O}}_{2}\text{max}$ decreased glycogen content similarly in lean, obese, and T2DM subjects and caused a comparable increase in GS fractional activity. These findings support the concept that glycogen breakdown (i.e., decreased muscle glycogen concentration) contributes to the activation of GS (17), and the regulation of GS activity by glycogen is preserved in insulin-resistant subjects.

In this study, we performed a detailed kinetic analysis of GS activity at rest, during exercise, and after exercise in muscle from lean insulin-sensitive and insulin-resistant (obese nondiabetic and T2DM) subjects. At rest, GS affinity for UDP-glucose was lower (higher $K_{m}$) in obese and T2DM subjects compared with lean individuals, whereas GS fractional activity was comparable between the three groups. This suggests that GS affinity for UDP-glucose is a more sensitive parameter of changes in enzymatic activity than measurements of GS fractional activity. It should be noted that the function of GS may also be affected by other mechanisms aside from covalent phosphorylation and allosteric modification, including subcellular compartmentalization (30) and posttranslational modification (42). Although one study found that the cellular localization of glycogen particles was similar between lean and T2DM subjects at baseline and after 10 wk of endurance training (26), in the future it will be important to evaluate the effect that acute exercise has on GS localization and posttranslational modification in muscle from healthy and T2DM individuals.

Another goal of this study was to examine whether exercise stimulated dephosphorylation of GS in insulin-resistant subjects to the same degree as in lean individuals. Interestingly, exercise decreased GS Ser641 and Ser645,649,653,657 phosphorylation with similar efficiency in lean, obese, and T2DM subjects. These sites, which have been shown to regulate GS activity (39), are dephosphorylated by exercise in normal (insulin-sensitive) muscle (21, 25, 30). GSK-3 phosphorylates the COOH-terminal sites of GS (Ser641, Ser645, Ser649, and Ser653) (7). Nonetheless, we did not observe changes in GSK-3$\beta$ phosphorylation during exercise despite the decreases in GS Ser641 and Ser645,649,653,657 phosphorylation. These findings indicate that, in human skeletal muscle, exercise-mediated GS dephosphorylation and activation occur independently of changes in GSK-3 inactivation. This is consistent with a prior study performed in mice that showed that insulin, but not exercise, requires GSK-3 inactivation to promote dephosphorylation and activation of GS (3, 25). Experiments in knockout mice demonstrated that exercise, unlike insulin, requires the presence of protein phosphatase-1 (PP1) regulatory subunit RGL/GM for the activation of GS (2). In this study, we did not have sufficient muscle tissue to evaluate the role of PP1 RGL/GM on the regulation of GS activity during exercise, but this would be an important topic for investigation in the future.

Mutagenesis analysis of overexpressed muscle GS in COS cells (38) as well as analysis of muscle biopsies from human subjects have suggested that GS Ser7 and/or GS Ser7$^{+}$10 phosphorylation are important regulatory sites in controlling GS activity and glycogen synthesis. Previously, we found that contraction increases GS Ser7 phosphorylation in rat muscles, but phosphorylation of this site was not associated with GS FV (21, 23). In the present study, we did not observe increases in GS Ser7 or GS Ser7$^{+}$10 phosphorylation in rested and exercised muscles in any group. These data agree with results from Prats et al. (30), who reported that acute exercise did not increase GS Ser7 or GS Ser7$^{+}$10 phosphorylation in muscle from young healthy humans, although we cannot rule out transient phosphorylation of these sites at earlier time points. Overall, the present data suggest that exercise promotes GS activity via dephosphorylation of GS Ser641 and Ser645,649,653,657, and this is supported further by the observed direct correlation between phosphorylation of these sites and GS activity (increased affinity for UDP-glucose and G6P). These findings agree with those reported in previous studies (20, 23, 30) and suggest that exercise regulates GS via dephosphorylation of Ser641 and Ser645,649,653,657 in muscles from both insulin-sensitive and insulin-resistant subjects.

Although both GS fractional activity and affinity for UDP-glucose correlated with GS Ser641 and Ser645,649,653,657 phosphorylation, there was no linear relationship between GS FV$_{0.03}$ and $K_{m}$ (26). Rather, there was a curvilinear relationship, as seen in rat muscles when GS activity was manipulated by altering glycogen content and by exercise (16). Also, we found that GS FV$_{0.03}$ declined below 20% before a substantial increase in $K_{m}$ was noted. Although we do not know the reason for this early decline in GS FV$_{0.03}$, this finding could explain why GS affinity for UDP-glucose may be a more
sensitive parameter for changes in enzymatic activity than measurements of fractional activity. It would be important to investigate in future studies whether the higher $K_{m}$ of 0.17 observed in T2DM occurs in conjunction with impaired insulin-induced GS activation.

Insulin-mediated GS activation and dephosphorylation are impaired in insulin-resistant subjects (14, 29, 43). It is well documented that exercise increases nonoxidative glucose disposal (8, 10). Christ-Roberts et al. (6) showed that nonoxidative glucose storage was enhanced immediately after exercise in the diabetic subjects, and glucose disposal has been reported to correlate with GS fractional activity (5). Therefore, our finding that exercise normally dephosphorylates and activates GS may help to explain why exercise stimulates glycogen synthesis and enhances insulin sensitivity in insulin-resistant subjects.

This study evaluated the GS response to a single bout of aerobic exercise. However, it is possible that differences in the GS response could be identified in insulin-resistant subjects by testing the effect of a different exercise challenge. For example, St-Onge et al. (41) demonstrated that GS protein content in muscle increases in response to 6 wk of electrical stimulation in subjects that do not carry the XbaI GS polymorphism, whereas muscle contraction does not increase GS content in carriers of this mutation. In the future, it will be interesting to investigate whether abnormalities in GS function can be detected by testing the response to other exercise/contraction protocols.

In summary, exercise increased GS activity during exercise, and its activity remained elevated in the postexercise period. The sustained effect of exercise on GS activity may contribute to the increases in insulin-mediated glucose disposal after exercise in human skeletal muscle. Overall, we did not observe major differences in exercise-stimulated GS dephosphorylation and activation between lean, obese, and T2DM individuals, suggesting that the molecular mechanism by which exercise promotes glycogen synthesis in muscle is preserved in insulin-resistant subjects. Future studies will be important to further examine how exercise works to activate GS in muscle from healthy and insulin-resistant individuals.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.J., P.T., J.T.S., M.M.-C., and N.M. performed the experiments; J.J., P.T., J.T.S., M.M.-C., R.A.D., and N.M. analyzed the data; J.J., P.T., J.T.S., M.M.-C., R.A.D., K.S., and N.M. interpreted the results of the experiments; J.J., R.A.D., and N.M. prepared the figures; J.J., K.S., and N.M. drafted the manuscript; J.J., P.T., M.M.-C., R.A.D., K.S., and N.M. edited and revised the manuscript; J.J., P.T., J.T.S., M.M.-C., R.A.D., and K.S., and N.M. approved the final version of the manuscript; N.M. did the conception and design of the research.

REFERENCES