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Skeletal muscle glycogen synthase subcellular localization: effects of insulin and PPAR-α agonist (K-111) administration in rhesus monkeys

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Skeletal muscle glycogen synthase subcellular localization: effects of insulin and PPAR-α agonist (K-111) administration in rhesus monkeys. Am J Physiol Regul Integr Comp Physiol 288: R1509–R1517, 2005. First published March 10, 2005; doi:10.1152/ajpregu.00692.2004.—Insulin covalently and allosterically regulates glycogen synthase (GS) and may also cause the translocation of GS from glycogen-poor to glycogen-rich locations. We examined the possible role of subcellular localization of GS and glycogen in insulin activation of GS in skeletal muscle of six obese monkeys and determined whether 1) insulin stimulation during a hyperinsulinemic euglycemic clamp and/or peroxisome proliferator-activated receptor (PPAR)-α agonist treatment (K-111, 3 mg·kg−1·day−1; Kowa) induced translocation of GS and 2) translocation of GS was associated with insulin activation of GS. GS and glycogen were present in all fractions obtained by differential centrifugation, except for the cytosolic fraction, under both basal and insulin-stimulated conditions. We found no evidence for translocation of GS by insulin. GS total (GST) activity was strongly associated with glycogen content (r = 0.70, P < 0.001). Six weeks of treatment with K-111 increased GST activity in all fractions, except the cytosolic fraction, and mean GST activity, GS independent activity, and glycogen content were significantly higher in the insulin-stimulated samples compared with basal samples, effects not seen with vehicle. The increase in GST activity was strongly related to the increase in glycogen content during the hyperinsulinemic euglycemic clamp after K-111 administration (r = 0.74, P < 0.001). Neither GS protein expression nor GS gene expression was affected by insulin or by K-111 treatment. We conclude that 1) in vivo insulin does not cause translocation of GS from a glycogen-poor to a glycogen-rich location in primate skeletal muscle and 2) the mechanism of action of K-111 to improve insulin sensitivity includes an increase in GST activity without an increase in GS gene or protein expression.

glycogen metabolism; glycogen synthase gene expression; glycogen synthase protein expression; Macaca mulatta; peroxisome proliferator-activated receptor-α;

GLYCOGEN SYNTHASE (GS) activity and glucose transport are key regulatory components for glycogen synthesis. Under normal circumstances, in vivo insulin increases the fractional activity of GS in skeletal muscle without changing the total activity (measured at a saturating concentration of glucose 6-phosphate) of the enzyme (10, 12, 25, 36). In insulin-resistant subjects, the effect of in vivo insulin to increase the fractional/ independent activity of skeletal muscle GS is reduced compared with insulin-sensitive subjects (10, 20, 36). Studies show that insulin-resistant subjects have lower (15, 20, 36, 41) or normal (10, 19, 20) skeletal muscle GS total activity and lower (18, 23, 42) or normal (19) skeletal muscle GS protein expression compared with insulin-sensitive subjects. At least one study has shown insulin-resistant subjects to have reduced GS total activity and GS mRNA expression with normal GS protein expression (42).

Although the covalent and allosteric regulation of skeletal muscle GS by insulin during a hyperinsulinemic euglycemic clamp has been studied extensively, the role of spatial compartmentalization in insulin regulation of skeletal muscle GS is less clear. A previous study in C2C12 myoblasts reported that GS translocated from the nucleus to the cytosol in the presence of glucose, independent of insulin (13). In another study of rat skeletal muscle, GS translocated from a glycogen-enriched membrane fraction to the cytoskeleton fraction when glycogen levels decreased (29). In 3T3-L1 adipocytes, insulin caused a 75% decrease in GS protein expression in the cytosolic fraction with a concomitant increase of GS protein expression in the plasma membrane (7). The effects of in vivo insulin on the cellular localization of skeletal muscle GS and the possible resulting effects on activation of GS have not been previously reported.

A peroxisome proliferator-activated receptor-α (PPAR-α) agonist, K-111, formerly identified as BM 17.0744, has been previously shown in rodents to have antidiabetic and insulin sensitizing activity, including a significant lowering of fasting plasma insulin and triglyceride levels (39). In obese, insulin-resistant monkeys we have observed significant enhancement of whole body insulin-stimulated glucose disposal with K-111 administration (4). Another PPAR-α agonist decreases fasting plasma insulin in obese rhesus monkeys (43). PPAR-γ agonists improve insulin sensitivity in monkeys (21) and in humans (26), and a PPAR-δ agonist has been shown to improve insulin

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sensitivity in monkeys (30). The mechanisms involved in these insulin-sensitizing effects of PPAR agonists may include an increase in skeletal muscle GS activity. We have previously reported that a potent PPAR-γ agonist significantly increased GS total and GS independent activity during the maximal insulin stimulation of a hyperinsulinemic euglycemic clamp in insulin-resistant monkeys (33). In the present study the actions of the PPAR-α agonist K-111 on skeletal muscle glycogen metabolism were examined to identify further the mechanisms of action of this class of agents.

Here we report a series of studies carried out in obese, insulin-resistant rhesus monkeys, including the cloning and sequencing of the rhesus monkey skeletal muscle GS gene, and the subcellular localization of skeletal muscle GS and its relationship to glycogen. We also describe for the first time the effect of maximal insulin stimulation on GS localization, using a hyperinsulinemic euglycemic clamp procedure to hold glucose levels constant. Finally, we determined the effect of a selective PPAR-α agonist on both the subcellular localization of GS and on insulin action to increase GS activity.

MATERIALS AND METHODS

Subjects. Six adult male obese and insulin-resistant rhesus monkeys (Macaca mulatta) were used. Five of the six monkeys were normoglycemic; the sixth monkey had overt type 2 diabetes (fasting plasma insulin values >600 pmol/l and fasting plasma triglyceride values >1.12 mmol/l).

The primates were individually housed, and consistent primate care was provided according to the Guide for the Care and Use of Laboratory Animals (National Research Council-Institute for Laboratory Animal Resources) (28). All protocols were approved by the University of Maryland Institutional Animal Care and Use Committee. The monkeys were provided ad libitum access to standard monkey chow (17% protein, 13% fat, and 70% carbohydrate; PMI Nutrition International, Brentwood, MO) and fresh water continued to be available. Light sedation was accomplished with ketamine hydrochloride (15 mg/kg body wt) with supplemental ketamine as needed at 20- to 30-min intervals during the experiment. For the clamp procedure with muscle biopsies, Buprenex (0.03 mg/kg) was administered intramuscularly 15 min before the initial ketamine dose.

Fasting hormone and substrate concentrations were determined at week 2 and week 4 of vehicle administration and week 2 and week 4 following initiation of dosing with K-111 compound using the protocol described previously (16, 17). The hyperinsulinemic euglycemic clamp technique (11) modified for use in primates (3) was carried out as previously published during week 4 of vehicle and again during week 7 of treatment. Briefly, 2 contralateral peripheral cannulas were placed, one for the administration of glucose (20% concentration) and one for the administration of insulin (given as a priming dose, followed by a continuous infusion of 400 mU/ml), Blood samples for glucose were obtained every 5 min, and the glucose infusion was adjusted to maintain steady-state plasma glucose at ~4.7 mmol/l (85 mg/dl). Whole body glucose disposal rate (M) was calculated as the average of the exogenous glucose infusion rate during steady state at maximal insulin stimulation (steady-state insulin >18,000 pmol/l) and was corrected for metabolically active fat-free mass. Steady state was reached at 51 ± 5 min after the onset of the clamp, and the clamp ended at 101 ± 5 min. Muscle biopsies were obtained after an overnight fast (16 h) and again during the hyperinsulinemic euglycemic clamp (96 ± 5 min), snap frozen ex situ, and stored at −196°C for later assay.

Plasma glucose was assayed using the glucose oxidase method on a Beckman Autoanalyzer II (Beckman Instruments, Fullerton, CA). Plasma insulin was assayed using the standard double-antibody method (Linco Research Laboratories, St. Louis, MO).

RT-PCR. For the amplification reaction of the full coding sequence of the rhesus monkey muscle GS, three pairs of primers were designed based on the human muscle GS sequence (GenBank accession number J04501), the primer pairs corresponding to three different overlapping regions of the sequence. The PCR primer sequences were designed against human sequence base pairs 157 to 1021, 837 to 1647, and 1501 to 2401 (region 3). Total skeletal muscle RNA was isolated using Qiagen RNeasy RNA isolation columns and reagents (Qiagen, Valencia, CA). Reverse transcription reaction was performed using Qiagen Omniscript reverse transcriptase and reagents in a volume of 20 μl containing 240 ng total RNA (10 μl), 2.0 μl 10× RT buffer, 2.0 μl dNTP mix (0.5 mM each dNTP), 2.5 μl Omniscript Reverse Transcription, and 4.0 μl water at 37°C for 1 h. Monkey muscle GS was amplified by PCR using 10 μl of the reverse transcription reaction mixture and Cloned Pfu DNA polymerase (Stratagen, La Jolla, CA) in a final volume of 50 μl, containing 5 μl 10× reaction buffer [200 mM Tris-HCl, pH 8.8, 100 mM KCl, 100 mM (NH₄)₂SO₄, 20 mM MgSO₄, 1% Triton X-100, 1 mg/ml nuclease-free bovine serum albumin], 4 μl dNTPs (10 mM; 2.5 mM each of, 5 μl primers (0.5 μM of each), 0.5 μl Pfu DNA polymerase, and 25.5 μl water. Amplification was performed by 36 cycles of denaturation (1 min at 94°C), annealing (30 s at 60°C), and extension (1.5 min at 72°C). Each PCR reaction mixture was then loaded and run on a 1.5% agarose gel. The bands were extracted using Qiagen DNA extraction reagents (Qiagen) and cloned into pPCR-Script Amp SK(+) plasmid vector (Stratagen), and sequenced.

Subcellular localization of skeletal muscle GS in normal and in insulin-resistant monkeys. One hundred milligrams of lysophilized microdissected skeletal muscle (vastus lateralis) were homogenized in 2.5 ml of homogenization buffer (50 mM KCl, 10 mM EDTA, 100 mM KF, 0.5 mM PMSF and 0.1% 2-mercaptoethanol, pH 7.5) and then fractionated by differential centrifugation. The following fractions were collected: pellet 1 (P1) (2,500 g, 5 min), pellet 2 (P2) (post-P1 supernatant centrifuged at 10,000 g, 15 min), pellet 3 (P3) (post-P2 supernatant centrifuged at 20,000 g, 20 min), pellet 4 (P4) (post-P3 supernatant centrifuged at 100,000 g, 40 min), and the remaining supernatant, the cytosolic fraction (CF). These rates and times of centrifugation were chosen to mimic the previous work done in 3T3-L1 adipocytes (7) with the addition of the 20,000 g spin. Similar rates and times of centrifugation have also been used in subcellular fractionation studies of skeletal muscle (2, 40). The pellets were resuspended in 250 μl of homogenization buffer except for P1 which was resuspended in 1 ml. Total protein (6), GS independent activity (0.1 mM G6P), GS total activity (10 mM G6P) (36), and glycogen content (35) were determined in each fraction. GS fractional activity is the ratio of GS independent activity to GS total activity multiplied by 100. An antibody raised in rabbit against an oligopeptide composed of the COOH-terminal 12-amino acid monkey muscle
GS sequence was used to determine GS protein expression in each fraction.

Subcellular localization of adipose tissue GS in normal monkeys. Ten grams of frozen adipose tissue (subcutaneous abdominal) obtained from three monkeys after an overnight fast and during a hyperinsulinemic euglycemic clamp (31, 34) was homogenized in 10 ml of homogenization buffer and treated similarly to skeletal muscle except for the following modification: pellets were resuspended in 100 μl of homogenization buffer except for P1 which was resuspended in 400 μl.

GS mRNA expression. Total RNA was extracted from lyophilized skeletal muscle (20 mg dry weight) by the guanidinium isothiocyanate/phenol/chloroform method developed by Chomczynski and Sacchi (9), using TRizol Reagent (GIBCO-BRL, Gaithersburg, MD), according to the manufacturer’s protocol. Muscle was ground into a fine powder using a mortar and pestle in the presence of liquid nitrogen before homogenization to improve RNA yield. An additional centrifugation at 12,000 × g for 10 min was performed to remove insoluble material. The supernatant was transferred to Phase Lock Gels-Heavy (Brinkmann Instruments, Westbury, NY) to perform phase separation to further improve yield and purity. The entire aqueous RNA phase was recovered without interphase contamination.

A high-salt precipitation solution (1.2M NaCl, 0.8 M sodium citrate) was added and sensitivity was improved.

GS cDNA sequence. The full length of monkey skeletal muscle GS cDNA sequence is available in GenBank (accession number AF529178).

GS amino acid sequence. The predicted amino acid sequence (737 amino acids) and the comparison to the human skeletal muscle amino acid sequence (8) are shown in Fig. 1. There are six amino acid substitutions in the monkey sequence compared with the human sequence (99.1% identity). The nine in vivo phosphorylation sites (serine) present in human (8) skeletal muscle GS are present in monkey GS.

Total protein content. GS total activity, GS independent disposition, cholesterol, and triglyceride values are shown in Table 1. The means ± SE for the nonobese monkeys (n = 5) are shown separately from the diabetic (DM) monkey. Administration of the PPAR-α agonist K-111 lead to a significant decrease in body weight, food intake, fasting plasma insulin, and in fasting plasma triglyceride in the nonobese monkeys. Fasting plasma high-density lipoprotein-C subclasses 3–5 values significantly increased in the nonobese monkeys. Insulin sensitivity generally improved as indicated by increased mean whole body glucose disposal rate (4 of 5 nonobese monkeys showed an increase).

The effects of K-111 in the present study were similar to those that had been reported in a prior study using a different group of monkeys (4). In the prior dose-escalation study of K-111 compound in obese insulin-resistant, normoglycemic monkeys, significant decreases in fasting plasma insulin and plasma triglyceride, and improved insulin sensitivity were observed (4). Based on these results, the present study was designed to determine the mechanism(s) by which insulin sensitivity was improved.

RESULTS

The effects of K-111 on body weight, food intake, fasting plasma insulin, fasting plasma glucose, whole body glucose.

Table 1. Effect of PPAR-α agonist K-111 on metabolic and clinical characteristics in obese rhesus monkeys

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle Non-DM (n = 5)</th>
<th>DM (n = 1)</th>
<th>K-111 Non-DM</th>
<th>DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>17.1 ± 0.8</td>
<td>14.8</td>
<td>16.2 ± 0.8*</td>
<td>14.4</td>
</tr>
<tr>
<td>Food intake, kcal/day</td>
<td>849 ± 73</td>
<td>936</td>
<td>687 ± 38*</td>
<td>993</td>
</tr>
<tr>
<td>Fasting insulin, pmol/l</td>
<td>1178 ± 251</td>
<td>2904</td>
<td>673 ± 140*</td>
<td>852</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>3.77 ± 0.23</td>
<td>9.27</td>
<td>7.68 ± 0.23</td>
<td>5.43</td>
</tr>
<tr>
<td>Glucose disposal, mg/kg FFM⁻¹·min⁻¹</td>
<td>6.40 ± 0.88</td>
<td>4.87</td>
<td>7.80 ± 1.57</td>
<td>3.98</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>3.98 ± 0.53</td>
<td>7.03</td>
<td>3.55 ± 0.18</td>
<td>6.23</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>3.21 ± 1.03</td>
<td>4.88</td>
<td>1.78 ± 0.48*</td>
<td>6.60</td>
</tr>
<tr>
<td>VLDL-C, mmol/l</td>
<td>4.47 ± 2.27</td>
<td>4.39</td>
<td>2.28 ± 1.10</td>
<td>11.88</td>
</tr>
<tr>
<td>HDL-C, mmol/l</td>
<td>1.64 ± 0.26</td>
<td>1.13</td>
<td>1.97 ± 0.23</td>
<td>1.16</td>
</tr>
<tr>
<td>HDL-C 3, 4, 5, mmol/l</td>
<td>1.35 ± 0.23</td>
<td>1.30</td>
<td>1.82 ± 0.21*</td>
<td>1.02</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. FFM, fat-free mass; VLDL, very low density lipoprotein; HDL, high-density lipoprotein; DM, diabetic monkey; non-DM, nondiabetic monkey; PPAR-α, peroxisome proliferator-activated receptor-α. Triglycerides were log transformed for statistical analysis due to large baseline variation. *P < 0.05 vs. non-DM vehicle.

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inal subcutaneous adipose tissue from three young healthy rhesus monkeys before and during a hyperinsulinemic euglycemic clamp (Fig. 2). Neither GS protein nor GS total activity were detected in CF in basal or insulin-stimulated skeletal muscle or adipose tissue in these young healthy monkeys, thus confirming our findings in the obese monkeys of no GS activity in CF.

GS total (GST) activity before and during maximal insulin stimulation (hyperinsulinemic euglycemic clamp) was determined under two conditions, vehicle and PPAR-α agonist treatment, in the various subcellular fractions (Fig. 3). After vehicle administration, the average GST activity was not different in basal vs. insulin-stimulated samples (basal vs. insulin: 37 ± 8 vs. 34 ± 6 nmol·min⁻¹·mg protein⁻¹). After PPAR-α agonist administration, the average GST activity was significantly higher in the insulin-stimulated vs. basal samples (basal vs. insulin: 33 ± 7 vs. 50 ± 11 nmol·min⁻¹·mg protein⁻¹, P < 0.05), indicating enhanced insulin action on GST as a possible insulin-sensitizing mechanism. Neither GS protein expression nor GS mRNA expression were affected by insulin or by PPAR-α agonist treatment (Fig. 3).

Glycogen content. Glycogen was located in P1 through P4 under basal and insulin-stimulated conditions. Glycogen was not detected in the CF in any of the monkeys under any condition, including the young healthy monkeys. Glycogen content before and during insulin stimulation under vehicle and

![Fig. 1. Comparison of the amino acid sequences from rhesus monkey and human skeletal muscle glycogen synthase (GS). Amino acids that are identical to the monkey sequence are represented by dashed lines.](http://ajpregu.physiology.org/)
PPAR-α agonist administration in the various subcellular fractions is shown in Fig. 4. After vehicle administration, the average glycogen content was not different in basal vs. insulin-stimulated samples (basal vs. insulin: 0.89 ± 0.25 vs. 0.65 ± 0.15 μmol/mg protein). After PPAR-α agonist administration, the average glycogen content was significantly higher in insulin-stimulated vs. basal samples (basal vs. insulin: 0.51 ± 0.12 vs. 0.81 ± 0.20 μmol/mg protein, \( P < 0.05 \)) providing additional evidence of an enhancement in glycogen synthesis.

GS independent activity. GS independent (GSI) activity before and during insulin stimulation under vehicle and PPAR-α agonist administration in the various subcellular fractions is shown in Fig. 5. After vehicle administration, the average GSI activity was not significantly different in basal vs. insulin-stimulated samples (basal vs. insulin: 13.6 ± 4 vs. 16.8 ± 3.3 nmol⋅min\(^{-1}\)⋅mg protein\(^{-1}\)). After PPAR-α agonist administration, the average GSI activity was significantly higher in insulin-stimulated vs. basal samples (basal vs. insulin: 9.7 ± 2.1 vs. 20.2 ± 4.2 nmol⋅min\(^{-1}\)⋅mg protein\(^{-1}\), \( P = 0.002 \)).

The effect of insulin to increase GSI activity was positively associated with the effect of insulin to increase GST activity following PPAR-α agonist administration (\( r = 0.55, P < 0.005, n = 24 \)).

GS fractional activity. GS fractional activity before and during insulin stimulation under vehicle and PPAR-α agonist administration in the various subcellular fractions is shown in Fig. 6. The average GS fractional activity increased significantly after insulin stimulation after both vehicle (basal vs. insulin: 45 ± 4 vs. 57 ± 4%, \( P < 0.05 \)) and PPAR-α agonist...
administration (basal vs. insulin: 43 ± 5 vs. 54 ± 4%, P < 0.05), with no difference between the vehicle and PPAR-α agonist treated conditions.

GS activity vs. glycogen content. The significant positive relationship between GST activity and glycogen content in all of the samples (6 monkeys, P1 through P4, basal and insulin, vehicle and clamp, n = 96 fractions) is shown in Fig. 7. The relationship between these two variables was also significant under each condition separately: basal vehicle, n = 24, r = 0.75, P < 0.001; insulin vehicle, n = 24, r = 0.58, P < 0.005; basal PPAR-α agonist, n = 24, r = 0.78, P < 0.001; insulin PPAR-α agonist, n = 24, r = 0.75, P < 0.001. Following PPAR-α agonist administration, the effect of insulin to increase GST activity (r = 0.74, P < 0.001, n = 24; Fig. 8) and GSI activity (r = 0.42, P < 0.05, n = 24) was positively

Fig. 4. Glycogen content under basal (open bar) and insulin-stimulated (hatched bar) conditions after vehicle (top) and PPAR-α agonist treatment (bottom) in pellet fractions 1 through 4. No glycogen was detected in the CF. Mean glycogen content was significantly increased during the clamp after PPAR-α agonist treatment (*P < 0.05). Neither basal nor insulin-stimulated values were significantly different between vehicle and PPAR-α agonist treatment.

Fig. 5. GS independent activity (GSI) under basal (open bar) and insulin-stimulated (hatched bar) conditions after vehicle (top) and PPAR-α agonist treatment (bottom) in pellet fractions 1 through 4. GSI in P2 was significantly increased during the clamp after PPAR-α agonist treatment (**P < 0.05). Mean GSI was significantly increased during the clamp after PPAR-α agonist treatment (***P = 0.002). Neither basal nor insulin-stimulated values were significantly different between vehicle and PPAR-α agonist treatment.

Fig. 6. GS fractional activity (GSfv) under basal (open bar) and insulin-stimulated (hatched bar) conditions after vehicle (top) and PPAR-α agonist treatment (bottom) in pellet fractions 1 through 4. GSfv in P3 was significantly increased during the clamp after vehicle (†P < 0.05). Mean GSfv was significantly increased during the clamp after vehicle and PPAR-α agonist treatment (*P < 0.05). Neither basal nor insulin-stimulated values were significantly different between vehicle and PPAR-α agonist treatment.

Fig. 7. Glycogen content is significantly related to glycogen synthase total activity (r = 0.70, P < 0.001, n = 96). Vehicle basal, square; vehicle insulin, diamond; PPAR-α agonist basal, circle; PPAR-α agonist insulin, triangle.
associated with the effect of insulin to increase glycogen content.

**DISCUSSION**

Insulin activation of muscle GS is strongly correlated to whole body insulin sensitivity. Furthermore, reduced insulin activation of skeletal muscle GS during a hyperinsulinemic euglycemic clamp is associated with insulin resistance and type 2 diabetes (5, 20, 36). To address the mechanisms underlying these associations we have examined the effects of in vivo insulin stimulation and of a PPAR-α agonist, K-111, to alter GS localization and activity and the relationship between GS activity/localization and glycogen content in adult obese rhesus monkeys.

Many studies have reported the effects of in vivo insulin on fractional activity and/or independent activity of GS during a hyperinsulinemic euglycemic clamp, while fewer studies have reported effects on the apparent affinity of GS for the allosteric activator, G6P, during in vivo insulin administration (1, 32). We present here the first report of subcellular localization of skeletal muscle GS before and during in vivo insulin administration.

Our results showed that neither GS activity nor GS protein were present in the cytosolic fraction of subfractionated skeletal muscle obtained after an overnight fast or after 90 min of insulin infusion during a hyperinsulinemic euglycemic clamp in healthy or in insulin-resistant monkeys. GS activity, GS protein, and glycogen were present in all other fractions (2,500 g, 10,000 g, 20,000 g, and 100,000 g pellets). These results are in agreement with Nielsen et al. (29), who found GS activity and GS protein in the cytoskeleton and membrane fractions but not in the cytosolic fraction of subfractionated rat gastrocnemius muscle. Cytosolic GS has been reported to be present in 3T3-L1 adipocytes (7); the difference between our findings and those of the 3T3-L1 study could be related to a difference between fat and muscle. In the present study, however, using subfractionated subcutaneous abdominal adipose tissue from several healthy rhesus monkeys, we were unable to detect GS activity or GS protein in the cytosolic fraction of basal or in vivo insulin-stimulated adipose tissue. It should be noted that we used centrifugation speeds and times (with the addition of the 20,000 g spin) to collect the subcellular fractions that were similar to those used in the 3T3-L1 study (7).

In the present study, before and during the hyperinsulinemic euglycemic clamp following vehicle, there were no significant differences in GS total activity between basal and insulin-stimulated fractions. In addition, GS total activity was significantly positively related to glycogen content under all conditions studied. These results suggest that skeletal muscle GS is associated with glycogen under fasting conditions and during insulin administration and that in vivo insulin does not induce translocation of GS from one site to another. These results are in contrast to the study in 3T3-L1 adipocytes in which there was a decrease in GS protein in the cytosolic fraction with a concomitant increase of GS protein in the plasma membrane fraction after treatment of the cells with insulin (7). In the present study, in vivo insulin appears to increase GS protein expression in the 10,000 g, 20,000 g, and 100,000 g pellets of subcutaneous adipose tissue from normal monkeys. The effect of insulin to increase GS total activity in adipose tissue from monkeys (34) and in adipocytes from humans (24) has been reported.

There were significant increases in GS total activity, GS independent activity, and in glycogen content during the hyperinsulinemic euglycemic clamp following PPAR-α agonist treatment. The increases in GS total activity and in GS independent activity were associated with the increase in glycogen content during the hyperinsulinemic euglycemic clamp following PPAR-α agonist treatment. A correlation between the change in skeletal muscle glycogen content and the change in GS independent activity following a hyperinsulinemic euglycemic clamp in humans has been reported (5). An increase in GS total activity may contribute to the improvement in insulin sensitivity after PPAR-α agonist treatment (4, 43) as we have previously demonstrated a positive relationship between whole body glucose disposal and skeletal muscle GS total activity in monkeys (36). In addition, in rhesus monkeys, PPAR-α protein expression is highest in skeletal muscle (skeletal muscle > heart > liver > brown adipose tissue) (43) making this tissue a likely target for PPAR-α agonist action. The increase in GS total activity during the euglycemic clamp appears to be unique to PPAR agonist action; i.e., in vivo insulin during a hyperinsulinemic euglycemic clamp has not been previously shown to increase skeletal muscle GST (36) or GS maximal activity (Vmax) (32) in healthy or in insulin-resistant monkeys.

The mechanism by which the PPAR-α agonist K-111 increases GS total activity was neither via an increase in GS gene expression nor by an increase in GS protein expression. PPAR-α agonists have been shown to reduce muscle lipids in rodent models of obesity and insulin resistance (22, 45). Skeletal muscle triglyceride has been shown to be inversely related to insulin activation of GS in non-diabetic humans (38), and rat liver GS activity (total) was shown to be reduced by palmitoyl-CoA, a long-chain fatty acyl-CoA (44). K-111 has been shown to significantly reduce skeletal muscle triglyceride content during a euglycemic hyperinsulinemic clamp in obese monkeys (37). Therefore, we suggest that the mechanism by which skeletal muscle GST is increased by PPAR-α agonist during the clamp is by a decrease in triglyceride content, secondary to an increase in fatty acid oxidation (14). Although K-111 increases the expression of enzymes involved in fatty acid oxidation in rodent liver (27), the effect of K-111 on

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Fig. 8. After chronic PPAR-α treatment, the effect of insulin stimulation (insulin-stimulated minus basal) on glycogen synthase total activity (nmol/min mg protein) is shown. P1, square; P2, diamond; P3, circle; P4, triangle.
primate skeletal muscle fatty acid oxidation has not yet been reported.

Insulin administration during the hyperinsulinemic euglycemic clamp caused an identical increase (significant) in GS fractional activity following vehicle and following PPAR-α agonist treatment. The effect of insulin to increase GS fractional activity was similar in each of the four cellular fractions. Because GS total activity did not increase in one or more fractions with a concomitant decrease in another fraction during in vivo insulin administration, we suggest that the mechanism of in vivo insulin activation (as determined by an increase in fractional activity) of skeletal muscle GS does not involve changes in cellular localization of GS, contrary to what has been reported in 3T3-L1 adipocytes (7).

In summary, under basal fasting and in vivo insulin-stimulated conditions, skeletal muscle GS was strongly associated with glycogen and both GS and glycogen were located in all cellular fractions except for the cytosolic fraction. In vivo insulin did not change the localization of GS but significantly increased GS total activity, GS independent activity, and glyco- gen content following PPAR-α agonist treatment. GS fractional activity was similarly increased during the hyperinsulinemic euglycemic clamp after both vehicle and PPAR-α agonist treatment. We conclude that in vivo insulin regulation of skeletal muscle GS activity does not involve changes in the subcellular localization of GS and that one mechanism by which PPAR-α agonist treatment improves whole body insulin sensitivity is by increasing skeletal muscle GS total activity.

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