Mechanism of muscle glycogen autoregulation in humans

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Laurent, Didier, Ripudaman S. Hundal, Alan Dresner, Thomas B. Price, Suzanne M. Vogel, Kitt Falk Petersen, and Gerald I. Shulman. Mechanism of muscle glycogen autoregulation in humans. Am J Physiol Endocrinol Metab 278: E663–E668, 2000.—To examine the mechanism by which muscle glycogen limits its own synthesis, muscle glycogen and glucose 6-phosphate (G-6-P) concentrations were measured in seven healthy volunteers during a euglycemic (~5.5 mM)-hyperinsulinemic (~450 pM) clamp using 13C/31P nuclear magnetic resonance spectroscopy before and after a muscle glycogen loading protocol. Rates of glycogen synthase (Vsyn) and phosphorylase (Vphos) flux were estimated during a [1-13C]glucose (pulse)-unlabeled glucose (chase) infusion. The muscle glycogen loading protocol resulted in a 65% increase in muscle glycogen content that was associated with a twofold increase in fasting plasma lactate concentrations (P < 0.05 vs. basal) and an ~30% decrease in plasma free fatty acid concentrations (P < 0.001 vs. basal). Muscle glycogen loading resulted in an ~30% decrease in the insulin-stimulated rate of net muscle glycogen synthesis (P < 0.05 vs. basal), which was associated with a twofold increase in intramuscular G-6-P concentration (P < 0.05 vs. basal). Muscle glycogen loading also resulted in an ~30% increase in whole body glucose oxidation rates (P < 0.05 vs. basal), whereas there was no effect on insulin-stimulated rates of whole body glucose uptake (~10.5 mg · kg body wt. · min–1 · kg–1 for both clamps) or glycogen turnover (Vsyn/Vphos was ~23% for both clamps). In conclusion, these data are consistent with the hypothesis that glycogen limits its own synthesis through feedback inhibition of glycogen synthase activity, as reflected by an accumulation of intramuscular G-6-P, which is then shunted into aerobic and anaerobic glycolysis.

nuclear magnetic resonance spectroscopy; glycogen turnover; glycogen synthase

IT IS WELL ESTABLISHED that, despite wide variations in carbohydrate intake, muscle glycogen content remains remarkably stable between 60 and 90 mmol/l muscle (1, 12, 19, 25, 30) and rarely exceeds 130 mmol/l muscle in humans, even under conditions that should promote glycogen synthesis (1, 20, 25). However, the step or process by which glycogen limits its own synthesis is unknown. Glycogen could limit its own synthesis by feedback inhibition of 1) glucose transport, 2) hexoki-nase, or 3) glycogen synthase activity (3, 4, 7, 14, 25, 26, 28, 31). It is also possible that glycogen could limit its own synthesis through promotion of glycogen turnover where an increase in glycogen content induces an increase in phosphorylase activity (19), which in turn results in an increase in glycogen cycling. Muscle glycogen cycling has been observed previously both at rest (4) and during prolonged low-intensity exercise (24). To examine this question, rates of muscle glycogen synthesis and glycogen phosphorylase flux were measured simultaneously using 13C nuclear magnetic resonance (NMR) spectroscopy (4) under euglycemic-hyperinsulinemic conditions before and after a classic muscle glycogen loading protocol (1). This protocol is commonly used by endurance athletes to enhance performance and has been shown to increase muscle glycogen content by ~60% (1). In addition, 31P NMR spectroscopy was used to monitor changes in muscle glucose 6-phosphate (G-6-P) concentration to assess rate-controlling steps in insulin-stimulated muscle glycogen synthesis (28).

METHODS

Subjects

Seven nonsmoking male volunteers (mean age 28 ± 1 yr, mean body wt 73 ± 3 kg, mean body mass index 23.6 ± 0.8 kg/m2) without a family history of diabetes mellitus, hypertension, or any major diseases were studied, and none of the subjects was taking any medications. Subjects were instructed to abstain from strenuous physical activity for at least 3 days before the first clamp study and during the loading protocol. Experimental procedures were all approved by the Yale University Human Investigation Committee. Each subject gave informed consent after the purpose, nature, and potential risks of the study were explained.

Experimental Protocol

Muscle carbohydrate (CHO) metabolism was assessed at baseline and again the day after the loading protocol was completed by using 13C/31P NMR spectroscopy under euglycemic-hyperinsulinemic clamp conditions (Fig. 1). The loading protocol consisted of 60 min of exercise, 3 days of a low-CHO/ high-fat diet, a second 60-min bout of exercise, and 4 days of a high-CHO/low-fat diet, as described previously (1). The exercise consisted of running on a treadmill for 55 min (Jogging Machine 2; Tuntur) and performing toe raises with both legs for the last 5 min. The workload for the running session was previously determined to reach ~75% of each subject’s maximal oxygen consumption (heart rate ~160 beats/min). The daily caloric intake for both diets was 210 kJ/kg body wt with a ratio of protein-fat-carbohydrate equal to 33:57:10 for the low-CHO diet and 7:52:5:90 for the high-CHO diet.

Euglycemic-hyperinsulinemia was achieved with the glucose-insulin clamp technique (8). On the evening before each
clamp study, participants were admitted to the General Clinical Research Center of the Yale/New Haven Hospital and were fasted overnight (10–12 h). A Teflon catheter was inserted in an antecubital vein in each arm for blood drawing and for infusions. At time 0, insulin was administered as a prime (100 pmol/kg)-continuous (6 pmol·kg⁻¹·min⁻¹) infusion to raise plasma insulin concentration to ~450 pM and to maintain that concentration for the duration of the study. At the same time, a variable infusion of [1-¹³C]glucose (~20% enriched) was begun to maintain plasma glucose levels at ~5.5 mmol/l. After 120 min, the infusion was switched to unlabeled glucose and was continued for another 100–120 min (chase period). Blood samples for measurements of plasma glucose, insulin, glucagon, lactate, and free fatty acid concentrations and plasma [¹³C]glucose enrichment were obtained at 5- to 30-min intervals until completion of the study.

Indirect Calorimetry

Continuous indirect calorimetry was performed to determine rates of total body glucose and lipid oxidation at baseline and at 100–120 min and 220–240 min into each clamp study, as previously described (29).

In Vivo NMR Spectroscopy

Natural abundance [¹³C/³¹P] NMR spectroscopy was performed in an interleaved fashion at 4.7 T on a Bruker Biospec (Billerica, MA) spectrometer with a 30-cm-diameter magnet bore, as previously described (23). During the measurements, subjects remained supine with the right leg positioned within the homogenous volume of the magnet and with the lower portion of that leg resting on the stage of a radiofrequency (RF) probe. The spectrometer was equipped with a modified RF relay that allowed the hardware to switch the RF power (RF) probe. The spectrometer was equipped with a modified double-tuned circuit to optimize the [³¹P] channel so that the NMR sensitivity would be enhanced to detect G-6-ATP. Shimming, imaging, and ¹H decoupling at 200.4 MHz were performed with a 9 × 9-cm series butterfly coil. Proton water line widths were shimmed to <50 Hz. A microsphere containing [¹³C] and [³¹P] reference standards was fixed at the center of the double-tuned RF coil for calibration of RF pulse widths. Subjects were positioned by an image-guided localization routine that used a T₂-weighted gradient-echo image (repetition time = 82 ms, echo time = 21 ms). The subject’s lower leg was positioned so that the isocenter of the magnetic field was ~1 cm in the medial head of the gastrocnemius muscle. By determining the 180° flip angles at the center of the observation coil from the microsphere standard, RF pulse widths were set so that the 90° pulse was sent to the center of the muscle. This maximized suppression of the lipid signal that arises from the subcutaneous fat layer and optimized signal from the muscle.

On interleaved ¹H decoupled [¹³C/³¹P] RF pulse sequence was designed so that 72 [³¹P] transients were acquired during each 11-min interval averaged from the increment in muscle glycogen concentration (Δ[Gly]) during each 11-min interval was calculated from the increment in C-1 glycogen peak intensity (Δ[Gly]) divided by the intensity of the basal glycogen peak ([Gly₀]). This ratio was multiplied by the basal glycogen concentration ([Gly₀]) and the natural [¹³C] enrichment (1.1%) and was divided by the plasma [¹³C]glucose enrichment [atom percent excess (APE)] measured during the specific 11-min interval as previously described (29)

\[
\Delta[Gly] = \frac{\Delta[Gly]}{[Gly₀] \times 1.1} \times \frac{[Gly₀]}{APE + 1.1}
\]

Rates of glycogen synthesis were then calculated from the slope of the least-squares linear fit to the glycogen concentration curve during the second hour of the clamp period. To obtain an estimate of the rate of glycogen breakdown (ΔVₕₘₙ), the change in [¹³C]glycogen peak intensity during the unlabeled glucose infusion (chase period) was compared with the predicted change in [¹³C]glycogen, with the assumptions of a constant flux through glycogen synthase and no glycogen breakdown. The predicted increase in [¹³C]glycogen concentration (Δ[Glyₚred]) during each 11-min time period (ΔT) of the chase period was calculated by the follow-
where $V_{\text{syn}}$ is the rate of oxygen synthase flux. For each time interval, the observed change in $[1-^{13}\text{C}]$glycogen concentration ($\Delta[1^{13}\text{C}]\text{Gly}_{\text{obs}}$) was estimated to be

$$\Delta[1^{13}\text{C}]\text{Gly}_{\text{obs}} = \Delta\text{Gly} \times [\text{Gly}_0]/\text{Gly}_0$$

The amount of $[1-^{13}\text{C}]$glycogen that is broken down ($\Delta[1^{13}\text{C}]\text{Gly}_{\text{phos}}$) during each time interval then was estimated to equal

$$\Delta[1^{13}\text{C}]\text{Gly}_{\text{phos}} = \Delta[1^{13}\text{C}]\text{Gly}_{\text{pred}} - \Delta[1^{13}\text{C}]\text{Gly}_{\text{obs}}$$

The $\Delta[1^{13}\text{C}]\text{Gly}_{\text{phos}}$ was plotted vs. time, and the best fit of the data to a line was calculated by the method of least squares, the slope of the line representing the average rate of $[1-^{13}\text{C}]$glycogen breakdown. To obtain an estimate of the rate of $V_{\text{phos}}$ for the total amount of glycogen, labeled and unlabeled, this number was divided by the maximum $^{13}\text{C}$ enrichment in C-1 glycogen. The rate of glycogen turnover was then calculated as

$$\text{glycogen turnover (\%)} = V_{\text{phos}} \times 100/V_{\text{syn}}$$

Analytical Procedures

Plasma glucose was measured by the glucose oxidase method using a Beckman glucose analyzer (Fullerton, CA). Plasma immunoreactive insulin and glucagon were measured using commercially available double-antibody RIA kits (insulin [Diagnostic Systems Laboratories, Webster, TX]; glucagon [Linco Research, St. Charles, MO]). Plasma lactate concentrations were measured by the lactate dehydrogenase method. Plasma free fatty acids (FFA) were measured using a microfluorometric assay. $^{13}\text{C}$ atom percent enrichment of plasma glucose was determined by gas chromatography-mass spectrometry, as described previously (29).

Statistics

All values are expressed as means ± SE. A one-way ANOVA with repeated measures was used to analyze time course changes in plasma substrate and hormone concentrations throughout each clamp period. A two-way ANOVA with repeated measures was used to analyze time course differences in plasma substrate/hormone concentrations between both studies. When significant changes were obtained over time, post hoc comparisons were made using a paired t-test. Pairwise comparisons for metabolic flux measurements were made using a paired t-test. All data are expressed as means ± SE.

RESULTS

Muscle Glycogen Loading

Basal muscle glycogen concentrations before both clamp studies are depicted in Fig. 2. Basal muscle glycogen concentrations were similar before the first clamp study and on day 1 of the loading protocol (73 ± 2 and 72 ± 9 mmol/l muscle, respectively). Performing 1 h of running on the treadmill caused glycogen concentration to decrease by 44 ± 5 mmol/l muscle on day 1 ($P < 0.001$ vs. preexercise). Muscle glycogen content then returned to close to preexercise levels after 3 days of the low-CHO/high-fat diet, although muscle glycogen content still remained slightly lower than baseline (day 4: 62 ± 2 mmol/l muscle, $P < 0.01$ vs. baseline). Performing the second bout of exercise resulted in glycogen depletion to a similar extent as in the first exercise bout (—38 ± 3 mmol/l muscle). High-CHO feeding for the next 4 days resulted in an increase in muscle glycogen to loaded levels (120 ± 5 mmol/l muscle or 165 ± 9% of preexercise glycogen concentrations, $P < 0.001$).

Effects of CHO Loading on Basal Metabolism

Plasma glucose, insulin, and glucagon concentrations were similar before the first clamp study (glucose, 89 ± 2 mg/dl; insulin, 48 ± 6 pM; glucagon, 47 ± 2 pg/ml) and after glycogen loading (glucose, 89 ± 1 mg/dl; insulin, 48 ± 6 pM; glucagon, 45 ± 3 pg/ml). In the basal whole body glucose oxidation rate (first clamp, 0.43 ± 0.14 mM vs. second clamp, 0.81 ± 0.14 mM; $P < 0.05$) and a significant decrease in plasma FFA concentration (first clamp, 551 ± 51 µM vs. second clamp, 380 ± 24 µM, $P < 0.001$) were found on completion of the glycogen-loading protocol. This was paralleled by an increase in the basal whole body glucose oxidation rate (first clamp, 0.41 ± 0.16 mg·kg body wt·$^{-1}·$min$^{-1}$ vs. second clamp, 0.98 ± 0.30 mg·kg body wt·$^{-1}·$min$^{-1}$; $P < 0.005$) and a decrease in the basal lipid oxidation rate (first clamp, 1.81 ± 0.12 mg·kg body wt·$^{-1}·$min$^{-1}$ vs. second clamp, 1.43 ± 0.09 mg·kg body wt·$^{-1}·$min$^{-1}$; $P < 0.005$). Rates of glucose and lipid oxidation were similar in the initial (1–2 h) and late (3–4 h) phase of the clamp studies.

Euglycemic-Hyperinsulinemic Clamps

Plasma glucose, insulin, glucagon, lactate, and FFA concentrations. Plasma glucose concentrations were maintained within a range of 90–110 mg/dl throughout both clamp studies. Plasma insulin concentrations increased rapidly and reached steady-state values within 15 min [time = 30–210 min; first clamp,
Table 1. Effects of glycogen loading on whole body glucose metabolism

<table>
<thead>
<tr>
<th>Glucose Metabolism, mg·kg body wt⁻¹·min⁻¹</th>
<th>Total</th>
<th>Oxidative</th>
<th>Nonoxidative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>10.3±1.1</td>
<td>2.5±0.2</td>
<td>7.8±1.1</td>
</tr>
<tr>
<td>Glycogen loaded</td>
<td>10.9±0.8</td>
<td>3.2±0.3</td>
<td>7.7±0.9</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. NS, not significant.

440 ± 13 pM; second clamp, 447 ± 13 pM), whereas plasma glucagon concentrations remained at baseline levels during both clamp studies. During the measurement of glycogen synthase and phosphorylase fluxes (60–120 and 140–210 min, respectively), there was no difference in the mean glucose infusion rate between the two studies (Table 1). During the glucose infusion, plasma lactate concentrations rose significantly (P < 0.0001 vs. baseline) and were significantly higher throughout the glycogen-loaded clamp study (90–210 min, 1.13 ± 0.19 mM, P < 0.05 vs. baseline clamp) compared with the first clamp study (90–210 min, 0.73 ± 0.16 mM). Plasma FFA concentrations decreased to similar levels during each clamp study (first clamp, 124 ± 4 µM; second clamp, 140 ± 13 µM).

Flux measurements. V_syn were linear in both studies (first clamp, r = 0.99 ± 0.01; second clamp, r = 0.98 ± 0.01). V_syn was suppressed by 23% in the glycogen-loaded study compared with the first clamp (0.127 ± 0.031 vs. 0.165 ± 0.029 mmol·l⁻¹·muscle⁻¹·min⁻¹, P < 0.01; Fig. 3). V_phos was slightly but not significantly lower in the second clamp study (0.029 ± 0.005 vs. 0.040 ± 0.010 mmol·l⁻¹·muscle⁻¹·min⁻¹, not significant). Because muscle glycogen turnover (V_phos / V_syn) was similar in the two studies (first clamp, 23 ± 3%; second clamp, 28 ± 3%), the lower rate of net glycogen synthesis (~29%, P < 0.05 vs. baseline clamp study) observed in the glycogen-loaded studies could be attributed to a marked reduction in V_syn. Despite lower rates of insulin-stimulated muscle glycogen synthesis, rates of insulin-stimulated whole body glucose metabolism were unchanged in the muscle glycogen-loaded studies. Assuming that muscle mass is equivalent to ~26% body wt (5), the contribution of muscle glycogen net synthesis accounted for less of nonoxidative glucose metabolism (first clamp, 75 ± 4% vs. second clamp, 51 ± 11%, P < 0.05) in the presence of supercompensated muscle glycogen levels.

Phosphorylated metabolites. Basal intramuscular concentrations of G-6-P were 2.2-fold higher after the glycogen loading protocol (first clamp, 178 ± 21 µM vs. second clamp, 393 ± 66 µM, P < 0.05). Under euglycemic-hyperinsulinemic conditions, intramuscular G-6-P concentrations rose by a similar increment in both clamp studies (first clamp, Δ85 ± 12 µM; second clamp, Δ101 ± 46 µM) but remained higher (P < 0.001 vs. baseline study) in absolute concentrations throughout the glycogen-loaded clamp study (Fig. 4). Finally, although no change in muscle pH was observed during the first clamp study, a slight but significant decrease in pH was noted during the second clamp study (~0.04 pH units vs. first clamp study, P < 0.05). Glycogen loading had no detectable effect on intramuscular concentrations in phosphocreatine (~20 mM) or inorganic phosphate (~3.3 mM).

DISCUSSION

In this study, we found that a 1.6-fold increase in muscle glycogen content to ~130 mmol/l muscle resulted in an ~30% reduction in the rate of insulin-stimulated whole muscle glycogen synthesis. This reduction in insulin-stimulated muscle glycogen synthesis could occur through a reduction in glucose transport, hexokinase, or glycogen synthase activity. To distinguish between these possibilities, we used ³¹P NMR to noninvasively measure intramuscular G-6-P concentrations, which, unlike enzyme activity measurements,
provides unique information regarding rate-controlling steps in muscle glycogen synthesis. Because G-6-P is an intermediate between glucose transport/hexokinase and glycogen synthase, its concentration will reflect the relative activities of these two steps. After glycogen loading, we observed an approximately twofold increase in intracellular G-6-P concentration, suggesting that a decrease in glycogen synthase activity was responsible for this lower rate of muscle glycogen synthesis. These data are consistent with the hypothesis that glycogen limits its own synthesis through inhibition of glycogen synthase activity and are concordant with the in vitro observations that glycogen inhibits the stimulatory effect of insulin on glycogen synthase activity (19) through inactivation of glycogen phosphatase activity (18, 31). It is noteworthy that this rate-controlling step is different from that found in other conditions of decreased insulin-stimulated muscle glycogen synthesis, such as obesity (22), type II diabetes (28), insulin-resistant offspring of type II diabetic parents (27), and poorly controlled type I diabetes (4), where reduced glucose transport/phosphorylation activity has been shown to be responsible for the lower rates of muscle glycogen synthesis. Although it is possible that the preceding high-CHO/exercise protocol and the lower plasma FFA concentrations in the glycogen-loading studies might have contributed to these findings, it is unlikely since all of these factors might be expected to promote muscle glycogen synthesis (1, 2, 21, 23, 26, 32).

We also examined whether increased glycogen cycling might have contributed to decreased insulin-stimulated muscle glycogen synthesis in the presence of elevated muscle glycogen concentration. We found that glycogen turnover (V_{syn} / V_{phos}) was similar under both normal and glycogen-loaded conditions, suggesting that increased muscle glycogen turnover does not play a major role in limiting net synthesis of muscle glycogen under glycogen-loaded conditions. Although muscle (14, 19) and liver (13) phosphorylase activity have both been shown to be stimulated by an increase in glycogen content, it is possible that the increased intramuscular G-6-P concentration observed in the glycogen-loaded subjects inhibited phosphorylase activity under these conditions (15).

Despite a lower rate of muscle glycogen synthesis in the glycogen-loaded state, rates of insulin-stimulated whole body glucose disposal were similar in the two protocols. This was somewhat surprising, because muscle glycogen synthesis typically accounts for the majority of insulin-stimulated glucose disposal (29). These data imply that, under glycogen-loaded conditions, a significant portion of the infused glucose was being shunted into alternative pathways. Consistent with this hypothesis were the higher rates of whole body glucose oxidation (+30%) and plasma lactate concentrations (+90%) observed in the subjects after the glycogen-loading protocol. These data suggest that, under glycogen-loaded conditions, glucose is diverted from muscle glycogen synthesis into aerobic and anaerobic glycolysis, with some of the glycolytically derived lactate taken up by the liver for gluconeogenesis and/or lipogenesis and other tissues for oxidation (Fig. 5). Although it is also possible that decreased lactate clearance contributed to this increase in plasma lactate concentration, the decrease in intramuscular pH observed during the glycogen-loaded clamp studies would support the former possibility. These observations are consistent with previous in vitro rat studies that have demonstrated that muscles with high glycogen content converted less of the glucose entering the cell into glycogen and more into lactate (32). However, they are in contrast to the results of other in vitro rat studies that have demonstrated a negative correlation between muscle glycogen content and insulin-stimulated glucose uptake (9). One possible explanation for this latter finding is that these studies examined glucose uptake when muscle glycogen was kept low by CHO restriction, resulting in a relatively high proportion of fat in

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**Fig. 5.** Glycogen-induced changes in insulin-stimulated muscle CHO metabolism. Intracellular G-6-P is diverted from glycogen synthesis into aerobic and anaerobic glycolysis in the glycogen-loaded state. [G-6-P], G-6-P concentration; TCA, tricarboxylic acid; GT/HK, glucose transport/hexokinase. Arrow thickness reflects relative flux rates.
the diet that is well known to cause insulin resistance (16).

In summary, these are the first in vivo studies in humans to demonstrate that an elevated concentration of muscle glycogen is associated with a reduction in the rate of insulin-stimulated net muscle glycogen synthesis. This reduced rate of muscle glycogen synthesis could be attributed to a reduction in glycogen synthase activity as opposed to a reduction in glucose transport/hexokinase activity or an increase in the rate of muscle glycogen cycling. This mechanism likely plays an important role in limiting muscle glycogen synthesis in humans under fed conditions.

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