Glycogen loading alters muscle glycogen resynthesis after exercise

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Price, Thomas B., Didier Laurent, Kitt F. Petersen, Douglas L. Rothman, and Gerald I. Shulman. Glycogen loading alters muscle glycogen resynthesis after exercise. J. Appl. Physiol. 88: 698–704, 2000.—This study compared muscle glycogen recovery after depletion of ∼50 mmol/l (∆Gly) from normal (Nor) resting levels (63.2 ± 2.8 mmol/l) with recovery after depletion of ∼50 mmol/l from a glycogen-loaded (GL) state (99.3 ± 4.0 mmol/l) in 12 healthy, untrained subjects (5 men, 7 women). To glycogen load, a 7-day carbohydrate-loading protocol increased muscle glycogen 1.6 ± 0.2-fold (P < 0.01). GL subjects then performed plantar flexion (single-leg toe raises) at 50 ± 3% of maximum voluntary contraction (MVC) to yield ∆Gly = 48.0 ± 1.3 mmol/l. The Nor trial, performed on a separate occasion, yielded ∆Gly = 47.5 ± 4.5 mmol/l. Interleaved natural abundance 13C-31P-NMR spectra were acquired and quantified before exercise and during 5 h of recovery immediately after exercise. During the initial 15 min after exercise, glycogen recovery in the GL trial was rapid (32.9 ± 8.9 mmol·l⁻¹·h⁻¹) compared with the Nor trial (15.9 ± 6.9 mmol·l⁻¹·h⁻¹). During the next 45 min, GL glycogen synthesis was not as rapid as in the Nor trial (0.9 ± 2.5 mmol·l⁻¹·h⁻¹ for GL; 14.7 ± 3.0 mmol·l⁻¹·h⁻¹ for Nor; P < 0.005) despite similar glucose 6-phosphate levels. During extended recovery (60–300 min), reduced GL recovery rates continued (1.3 ± 0.5 mmol·l⁻¹·h⁻¹ for GL; 3.9 ± 0.3 mmol·l⁻¹·h⁻¹ for Nor; P < 0.001). We conclude that glycogen recovery from heavy exercise is controlled primarily by the remaining postexercise glycogen concentration, with only a transient synthesis period when glycogen levels are not severely reduced.

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METHODS

Subjects. Twelve healthy, untrained, nonsmoking subjects (5 men, 7 women, ages 23 ± 2 yr, weight 65 ± 3 kg, height 173 ± 3 cm) participated in this study. Each subject was first assessed by physical examination and medical history and then screened for exercise and dietary habits. Subjects with a family history of diabetes mellitus were excluded from the study, as were those who trained aerobically >5 days/wk (28, 30). Aerobic training was defined as exercise that elevated the heart rate above 120 beats per minute (bpm) over a continuous period of at least 30 min. When accepted into the study, each subject was informed as to the nature and possible consequences of the study and gave informed written consent in accordance with a protocol approved by the Yale University Human Investigation Committee.

Experimental protocol. On a separate day, each subject’s maximum voluntary contraction (MVC) for the gastrocnemius muscle was assessed (29, 30). To determine MVC, subjects were asked to perform a single plantar flex (one leg, knee fully extended) against a resistance and then relax. With the subject relaxed, weight was added and the subject was asked to plantar flex again. This process continued until the resistance was great enough that the subject was unable to plantar flex. This method has been shown to agree with MVC values determined electronically (29). Mean MVC values were 128 ± 7 kg. The study was paired so that on one occasion subjects exercised from a Nor state with glycogen concentrations at ~70 mmol/l (Nor) and in a separate session subjects exercised from a GL state. Before the day of the GL session, each subject performed a 7-day carbohydrate loading protocol. On the day of the study, subjects performed an exercise protocol of single-leg toe raises from an erect stand position (knee fully extended) to isolate the gastrocnemius muscle (28, 30). Exercise intensity (workload) was determined by each subject’s body mass, yielding a mean workload of 51 ± 2% (range of 41–60%) of MVC. To minimize intramuscular lactate accumulation, subjects exercised by alternating 1 min of toe raises with 1 min of rest throughout the period of exercise (28, 30). The cadence, determined by each subject as the rate (number of lifts per minute) that was most comfortable, averaged to ~35 toe raises per minute. The exercise durations were determined by the degree of gastrocnemius glycogen depletion (targeted at ~50 mmol/l). NMR was used to assess glycogen depletion at 15 min and 20 min into each subject’s exercise session. Under the different conditions (Nor vs. GL), the total exercise durations were not significantly different (24 ± 3 min Nor; 22 ± 1 min GL) nor was the total work performed (30.6 ± 3.3 kJ Nor, 29.3 ± 2.1 kJ GL) (1). Mean exercise-induced glycogen depletion rate, calculated from 13C-NMR spectra, was also similar between the two groups (128 ± 19 mmol·1·h⁻¹·Nor, 135 ± 8 mmol·1·h⁻¹·GL).

All studies began at 8 AM following an overnight fast of ~10 h. At least 30 min before any samples were drawn, a Teflon catheter was inserted into an antecubital vein for blood assays. Two interleaved natural abundance 13C-31P-NMR spectroscopy experiments were performed to determine baseline levels of the 13C and 31P metabolites as well as baseline pH. During this same period, baseline blood samples were obtained. Subjects were then asked to perform the exercise protocol (single-leg toe raises). Interleaved 13C,31P-NMR spectra were obtained at 15 min of exercise and as well as at the end of exercise, and blood samples were obtained at the end of exercise (t = 0). The exercise protocol ended when the subject had depleted his or her muscle glycogen concentration by 45–50 mmol/l. When the exercise protocol was completed, recovery was monitored over 5 h with blood sampling and interleaved 13C,31P-NMR spectroscopy. NMR spectra were obtained continuously over the first hour of recovery (5.4 min/spectrum) and on 30-min intervals from hours 2 to 5 (30). Subjects rested quietly outside the spectrometer between scans from hours 2 to 5 of recovery. During the exercise and recovery periods, dietary intake was restricted to water only.

GL protocol. To prepare for the GL portion of the study, each subject performed a carbohydrate-loading (supercompensation) protocol that consisted of a bout of exhaustive exercise followed by 3 days on a low-carbohydrate (LoCHO) diet and a second bout of exhaustive exercise followed by 4 days on a high-carbohydrate (HiCHO) diet. Two identical exercise protocols were employed with the intention of globally depleting glycogen in a major portion of each subject’s musculature. Subjects were asked to run 1 h on a self-propelled treadmill. During the run, subjects worked at 87 ± 5% (171 ± 10 bpm) of their maximum heart rate (196 ± 4 bpm), determined as (220 – age) bpm. At the end of the treadmill run, each subject performed an additional 5 min of single-leg toe raises, alternating legs, to further deplete glycogen specifically in the gastrocnemius of each leg. After the toe raises were completed, subjects were placed immediately (<2 min) in the NMR spectrometer to obtain postexercise spectra. During the 7-day GL protocol, each subject followed two different standardized diets that were prepared by the metabolic kitchen at the General Clinical Research Center at Yale University School of Medicine. After the first bout of exhaustive exercise, subjects consumed a weight-maintenance (45 cal/kg) LoCHO diet containing 20% carbohydrate, 60% fat, and 20% protein over a 3-day period. After the second bout of exhaustive exercise, subjects consumed a weight-maintenance (45 cal/kg) HiCHO diet containing 90% carbohydrate, 8% protein, and 2% fat over a 4-day period. Primary meals were consumed as breakfast (before 9 AM, 25% of total calories), lunch (at 12 PM, 25% of total calories), and dinner (6–8 PM, 25% of total calories). Subjects were allowed to snack between meals and in the evening after 8 PM (25% of total calories).

NMR spectroscopy. Interleaved natural abundance 13C-31P-NMR spectroscopy was performed at 4.7 T on a Bruker Biospec spectrometer with a 30-cm-diameter magnet bore. During the measurements, subjects remained supine with one leg positioned within the homogeneous volume of the magnet and with the lower portion of that leg resting on the stage of a surface coil radiofrequency (RF) probe. The spectrometer was equipped with a modified RF relay switch that allowed the hardware to switch the RF power between 13C (50.4 MHz) and 31P (81.1 MHz) channels with a 10-ms switching time (10). A modified pulse sequence allowed switching of the acquisition parameters and preamplifiers between the two channels during the 10-ms switching time. A 5.1-cm-diameter circular 13C,31P double-tuned surface coil RF probe was used for interleaved acquisitions (10). The double-tuned circuit was optimized for the 31P channel so that the NMR sensitivity would be enhanced to detect glucose 6-phosphate (G-6-P). Shimming, imaging, and 1H decoupling at 200.4 MHz were performed with a 9 cm × 9 cm series butterfly coil. Proton linewidths 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 employed a longitudinal relaxation (T1)–weighted gradient-echo image [repetition time (TR) = 82 ms, echo time (TE) = 21 ms]. Subject’s lower legs were typically positioned so that the isocenter of the magnetic field was ~1 cm into 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.

The interleaved 1H decoupled 13C-31P RF pulse sequence was designed so that 72 31P transients were acquired during the same period that 2,736 13C transients were obtained (38 13C scans per 31P relaxation period), and free induction decays were saved separately in two blocks (10). The TR for 31P acquisition was 4.6 s to allow for the long T1 of 31P resonances. Because the acquisition times of both channels had to be identical due to a spectrometer limitation, the optimized acquisition time was 87 ms. 1H continuous wave decoupling could not be turned on during the entire acquisition time because RF power deposition would have been excessive. Therefore, the decoupling time was truncated to 25 ms at the beginning of each 13C acquisition. Power deposition, assessed by magnetic vector potential specific absorption rate (SAR) calculations (9), was <4 W/kg. The total scan time for each interleaved spectrum was 5.5 min.

Intramuscular glycogen concentrations were determined by comparison with an external standard solution (150 mmol/l glycogen + 50 mmol/l KCl) in a cast of the subject’s leg that loaded the RF coil the same as subject legs (29, 39). 13C spectra were processed by methods that have been described in detail in several of our earlier studies (28–30, 39). Briefly, gaussian-broadened spectra (30 Hz) were baseline corrected ±1,000 Hz on either side of the 1-13C glycan resonance of both subject spectra and sample spectra. Areas were then assessed at ±200 Hz about the resonance. The 13C-NMR technique for assessing intramuscular glycogen concentrations has been validated in situ in frozen rabbit muscle (15) and by comparison with biopsied human gastrocnemius muscle tissue samples (39).

Concentrations of Pi and creatine phosphate (PCr) were also calculated by comparison with β-ATP (19). Values of pH were calculated according to the chemical shift difference between the Pi peak and the PCr peak using the equation

$$\text{pH} = 6.77 + \log([\Delta \delta - 3.29]/(5.68 - \Delta \delta))$$

where Δδ is the chemical shift difference between Pi and PCr. Corrections were made when exercise resulted in swelling of the muscle that could alter the NMR-sensitive volume and make the NMR peaks appear smaller. In the processing mode of the spectrometer, the postexercise 31P total spectral intensity was corrected to equal the resting spectrum. When the total spectrum area correction factor was applied, there were no significant differences in the β-ATP resonances after exercise. Time domain data were apodized and zero filled using a 6-Hz exponential function. Intramuscular G-6-P was quantified by comparison with the β-ATP resonance as an internal reference standard (8, 28, 32, 33). A constant concentration of 5.5 mmol/l was assumed for resting muscle ATP (17). The quantification of muscle G-6-P differed from the method described by Rothman et al. (33) in that the chemical shift of G-6-P was determined relative to Pi, rather than to PCr. Pan et al. (27) have shown that, over the pH range of 6.60–7.05, exercise-induced changes in the chemical shift of Pi, paralleled those of G-6-P. We measured the chemical shifts of the major constituents of the phosphonooester (PME) region at pH 6.60–7.05 relative to Pi at 0.00 parts per million (ppm) and found them to remain constant [G-6-P, 2.29 ppm; α-glycerol phosphate (α-GP), 2.04 ppm; inosine monophosphate (IMP), 1.61 ppm]. The basal G-6-P concentration was determined by integrating over the chemical shift range of 2.61–2.29 ppm and multiplying the area by 2 to minimize any contribution from upfield (lower ppm) PME resonances (33). When difference spectra were obtained by subtracting resting spectra from spectra obtained after exercise, the increase in G-6-P after exercise was cleanly resolved at 2.29 ppm. Measurement of G-6-P by 31P-NMR has been validated in an animal model by comparison with chemical assay of G-6-P done on rat muscle frozen in situ (7).

Blood samples. Venous blood samples were assayed for glucose, lactate, insulin, free fatty acids (FFA), and glucagon. Glucose and lactate were assayed by enzymatic methods (12, 21). FFA were assayed by a microfluorimetric method (26). Glucagon and insulin were determined by RIA methods (37).

Statistical analysis. Blood data and NMR-determined metabolite concentrations are presented as means ± SE of all exercise/recovery sessions unless otherwise noted. Overall NMR precision was calculated by pooled variance analysis (17, 29). Paired two-tailed t-tests were used for comparison of data within individual subjects. Comparisons of data between groups was performed using ANOVA with Bonferroni correction factor. Glycogen repletion rates for each subject were determined by least-squares linear regression analysis of the increase in glycogen, either over a specified time period or within a specified range of glycogen concentrations.

RESULTS

During the GL protocol, glycogen returned to its initial concentration at the end of the 3-day LoCHO diet (64.5 ± 2.6 mmol/l before the protocol and 66.5 ± 4.0 mmol/l after the LoCHO diet). After the 4-day HiCHO diet, gastrocnemius glycogen levels increased to 1.5 ± 0.2 times the initial resting levels (99.3 ± 4.0 mmol/l GL). Resting glycogen levels at the start of the control protocol (63.2 ± 2.8 mmol/l Nor condition) were 1.6 ± 0.2 times lower (P = 0.01) than at the start of the GL condition (Fig. 1A). According to the design of the study, the exercise-induced decrease in muscle glycogen (ΔGly) was similar after exercise under Nor conditions (ΔGly = −47.5 ± 4.5 mmol/l) and under GL conditions (ΔGly = −48.0 ± 1.3 mmol/l) (Fig. 1A). Glycogen resynthesis rates, compared during three different postexercise recovery periods when subjects were glycogen loaded, were significantly faster during the initial 0–15 min after exercise (32.7 ± 8.9 mmol·l−1·h−1; P = 0.005) (Fig. 2) than during subsequent recovery periods [1.7 ± 2.6 mmol·l−1·h−1 (15–60 min) and 1.2 ± 0.6 mmol·l−1·h−1 (60–300 min)]. After the initial 15 min of recovery under the GL condition, glycogen resynthesis rates did not differ significantly (Fig. 2). After exercise under the Nor condition, glycogen resynthesis rates during the initial 0–15 min of recovery were not significantly faster than during the period 15–60 min after exercise [19.0 ± 4.4 mmol·l−1·h−1 (0–15 min); 14.8 ± 3.0 mmol·l−1·h−1 (15–60 min)] (Fig. 2). During the extended period (60–300 min), the mean glycogen recovery rate under the Nor condition (3.5 ± 0.4 mmol·l−1·h−1) was significantly reduced compared with the earlier periods [0–15 min (P = 0.005) and 15–60 min (P = 0.002)] (Fig. 2). When glycogen recovery rates during each postexercise period were compared under the GL and the Nor conditions, the GL recovery rates were reduced during the 15–60 min period [1.7 ± 2.6 mmol·l−1·h−1 (GL); 14.8 ± 3.0 mmol·l−1·h−1 (Nor) (P = 0.02)] and during
the 60- to 300-min period [1.2 ± 0.6 mmol·l⁻¹·h⁻¹ (GL); 3.5 ± 0.4 mmol·l⁻¹·h⁻¹ (Nor) (P ≤ 0.02)] but not during the initial (0–15 min) period (Fig. 2).

Total glycogen synthesis is given in Table 1. Under the GL condition, 70% of glycogen resynthesis (8.2 ± 2.2 mmol/l) occurred during the first 0–15 min after cessation of exercise. In contrast, under the Nor glycogen condition, only ~15% of glycogen resynthesis (4.0 ± 1.7 mmol/l) occurred during the first 0–15 min. During the next 15- to 60-min period, a significantly smaller amount of glycogen was synthesized under the GL condition than under the Nor condition [2.8 ± 2.4 mmol/l (GL); 10.7 ± 1.7 mmol/l (Nor)] (P ≤ 0.02). During the final 60- to 300-min period after exercise, the GL glycogen recovery was minimal compared with Nor glycogen recovery [0.4 ± 3.2 mmol/l (GL); 11.4 ± 1.5 mmol/l (Nor)] (P ≤ 0.01) despite similar G-6-P levels (Fig. 1B). In addition, the total glycogen resynthesized was significantly blunted (P ≤ 0.001) following exercise under the GL condition (11.4 ± 2.6 mmol/l) when compared with the Nor condition (26.1 ± 1.6 mmol/l).

Resting gastrocnemius G-6-P concentrations were similar under both conditions (0.11 ± 0.01 mmol/l Nor; 0.13 ± 0.01 mmol/l GL) (Fig. 1B). After exercise, G-6-P rose significantly under both Nor (0.45 ± 0.10 mmol/l, P ≤ 0.01) and GL (0.57 ± 0.13 mmol/l, P ≤ 0.01) conditions, remaining elevated 15 min into the recovery period (0.27 ± 0.06 mmol/l, P ≤ 0.05 Nor, 0.28 ± 0.08 mmol/l, P ≤ 0.05 GL) and returning to approximately basal levels during the remainder of the recovery period (0.14 ± 0.02 mmol/l GL and Nor) (Fig. 1B). Because of the extended acquisition periods (5.5 min) required to obtain 31P-NMR spectra with sufficient signal-to-noise ratios to measure G-6-P, significant
changes in gastrocnemius PCr and Pi were not observed (i.e., time resolution was insufficient to produce meaningful PCr and Pi data). Gastrocnemius pH dropped significantly (7.04 ± 0.02 to 6.82 ± 0.04, P < 0.001 Nor; 7.04 ± 0.01 to 6.90 ± 0.03, P < 0.001 GL) immediately after exercise, quickly returning to basal values.

Venous blood components were measured at rest and throughout exercise and recovery. There were no significant differences between Nor and GL sessions for any of the measured blood constituents. However, although mean venous glucose concentrations did not differ between the two conditions, regression analysis revealed that the time course of plasma glucose was significantly nonzero (P < 0.03) during the Nor recovery period and not during the GL recovery period. The rise in plasma lactate (1.4-fold for Nor and 1.5-fold for GL) immediately after cessation of exercise was not significant. Under both Nor and GL conditions, venous insulin (9.4 ± 1.0 to 6.1 ± 0.6 µmol/l for Nor and 9.8 ± 0.9 to 6.0 ± 0.4 µmol/l for GL) and glucagon (56 ± 6 to 39 ± 4 pg/ml for Nor and 51 ± 3 to 36 ± 4 pg/ml for GL) levels declined significantly (P < 0.05) during the recovery period. During this period, FFA rose steadily [350 ± 46 to 780 ± 176 mmol/l for Nor (P < 0.05) and 377 ± 71 to 737 ± 101 mmol/l for GL (P < 0.05)].

**DISCUSSION**

Gastrocnemius glycogen recovery patterns were different under the two conditions tested in this paired study. When subjects depleted 48.0 ± 1.3 mmol/l of glycogen from an elevated starting concentration of 99.3 ± 4.0 mmol/l (GL condition), there was an initial burst of glycogen resynthesis during the first 15 min of the recovery period that was followed by significantly blunted synthesis over the remaining 285 min of recovery (Figs. 1–3). This initial burst accounted for ~70% of the total glycogen resynthesized (8.2 ± 2.2 mmol/l) under the GL condition. In contrast, when subjects on a separate occasion depleted a similar amount of gastrocnemius glycogen (47.5 ± 4.5 mmol/l) from a Nor concentration of 63.2 ± 2.8 mmol/l (Nor condition), the glycogen recovery pattern was biphasic, as previously reported (24, 28, 30). Under Nor conditions, the first 15 min of recovery accounted for only ~15% of the total glycogen resynthesized (4.0 ± 1.7 mmol/l). In addition, the total glycogen resynthesized was greater under the Nor condition [26.1 ± 1.6 mmol/l for Nor and 11.4 ± 2.7 mmol/l for GL (P < 0.001)]. These different muscle glycogen recovery patterns (GL vs. Nor) were observed despite similar G-6-P patterns that increased significantly (P < 0.05) immediately after exercise and returned to basal levels within 30 min (28). The present study is unique in that it is the first to directly compare glycogen resynthesis after heavy depletion (ΔGly = 50 mmol/l) down to very low glycogen levels (~25 mmol/l for Nor) with the same amount of heavy depletion down to moderate glycogen levels (~50 mmol/l for GL), demonstrating different glycogen resynthesis patterns under the two conditions.

The finding that under Nor conditions rapid postexercise glycogen resynthesis continued for 45–60 min, whereas in GL conditions the rapid phase ceased after 15 min, supports an important role of glycogen concentration in regulating the rate of glycogen synthesis after exercise. According to the design of the study, both the change in muscle glycogen and the exercise duration and intensity were similar under the GL and Nor conditions, so that the concentration of glycogen remaining after exercise was the sole variable to be tested. In the Nor condition, after partial recovery to ~35 mmol/l, glycogen resynthesis continued at a reduced rate throughout the remainder of the 5-h recovery period.

**Table 1.** Total gastrocnemius glycogen synthesized during three periods of recovery from glycogen-depleting exercise

<table>
<thead>
<tr>
<th>Recovery Period, min</th>
<th>Glycogen Synthesized, mmol/l</th>
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<tbody>
<tr>
<td></td>
<td>Nor condition</td>
</tr>
<tr>
<td>0–15</td>
<td>4.0 ± 1.7</td>
</tr>
<tr>
<td>15–60</td>
<td>10.7 ± 1.7</td>
</tr>
<tr>
<td>60–300</td>
<td>11.4 ± 1.5</td>
</tr>
<tr>
<td>Total resynthesis</td>
<td>26.1 ± 1.6 mmol/l</td>
</tr>
</tbody>
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Glycogen concentrations are given as means ± SE. GL, glycogen-loaded condition; Nor, normal glycogen condition. *P < 0.02. †P < 0.01.
This finding is in agreement with earlier studies that have demonstrated an increased rate of glycogen resynthesis at greatly reduced concentrations (35, 41, 43).

The finding of a dependency of glycogen synthesis on glycogen concentration in vivo is consistent with studies that have found that the activity of the G-6-P-independent form of GSase is increased at low glycogen levels (6, 43). It has been suggested that the size of the glycogen molecule, which has a reduced number of terminal glucosyl units as size increases, may be a determining factor (41). It is also possible that the rate of formation of new glycogen molecules increases when a highly activated form of glycogen is uncovered (1). It is well known that, as the glycogen molecule decreases in size, GSase and GSase phosphatase, both normally bound to glycogen, are released and become available for activation (40). As glycogen approaches resting levels, the activity of the G-6-P-independent form of GSase returns to the level that is normally seen in muscles at rest (13).

A potential explanation for the rapid glycogen resynthesis during the first 15 min after exercise under the GL condition is that the initial burst of postexercise glycogen resynthesis resulted from elevated G-6-P levels that allosterically activated GSase and increased synthesis in a “feed forward” manner. Once G-6-P concentration returned to near baseline levels, the high-glycogen concentrations that remained after exercise (>50 mmol/l) were unable to activate the G-6-P-independent form of GSase. The ability to allosterically activate GSase through a selective increase in glucose transporter activity has been demonstrated recently in transgenic mice overexpressing glucose transporters. In this study, it was shown that muscle glycogen increases dramatically above resting levels despite a significant reduction in the activity of GSase (31), indicating the ability of increased glucose transport and phosphorylation to drive glycogen synthesis in the absence of high-G-6-P-independent GSase activity (31).

Under both Nor and GL conditions, rapid initial glycogen synthesis rates occurred because, in addition to increased glycogen synthase activity, the activities of the enzymes responsible for glucose transport and phosphorylation, as well as the exercise-induced glucose transporter pool, were also increased. It has been shown that immediately after cessation of exercise there is an increase in glucose transport into exercised muscles (42) that may also be seen as a transient rise in muscle G-6-P (5, 28). In the present study, the initial high concentration of G-6-P directly indicates that glucose transport and phosphorylation activity were increased during the early recovery period under both GL and Nor conditions (Fig. 1B). Although the G-6-P concentration rapidly returned to basal level, the continued high rate of glycogen synthesis under the Nor condition is evidence for a continued high level of glucose transport and phosphorylation activity. The reduced glycogen synthesis in the GL condition, despite similar G-6-P concentration, suggests that the activity of glucose transport and phosphorylation declined rapidly under the GL condition relative to the Nor condition. This conclusion is supported by the regression analysis of the change in plasma glucose concentrations over time, which demonstrates a significantly nonzero negative slope (P < 0.03) under the Nor condition and not under the GL condition (i.e., glucose concentrations are declining under the Nor condition). This is most likely due to greater muscle glucose uptake throughout the Nor recovery period. The possibility that under the Nor condition glucose transport and phosphorylation are bypassed in favor of glycogen synthesis directly from plasma lactate seems unlikely based on the high rate of glycogen synthesis. Whereas high plasma lactate can provide a substrate for limited glycogen synthesis (5, 38), lactate is not considered a major substrate for glycogen resynthesis after exercise (3, 5, 38). In addition, plasma lactate levels were similar under both conditions, in agreement with recent work (16).

The similar initial high concentration of G-6-P and glycogen synthesis rate immediately after exercise under both the GL and Nor conditions is consistent with the duration and intensity of exercise being a factor in the exercise-induced activation of glucose transport activity. However, under the Nor condition the continued high rate of glycogen synthesis after the initial 15-min period suggests that the low concentration of glycogen may have the dominant role in the maintenance of high glucose transport and phosphorylation activity. At present there is no known molecular mechanism by which the concentration of glycogen may influence the activity of glucose transporters or hexokinase.

In summary, glycogen-depleting exercise results in an early increase in glucose transport and phosphorylation and glycogen synthesis in both groups, as reflected by early increases in both G-6-P and glycogen concentrations. However, under the GL condition, the high glycogen concentration remaining after exercise acts to inhibit glycogen synthase activity in a feedback-inhibition manner. These findings strongly support a key role for glycogen concentration in regulating the rate of glycogen resynthesis in the first few hours after heavy exercise.

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REFERENCES


