Postexercise muscle glycogen resynthesis in obese insulin-resistant Zucker rats

CLINTON R. BRUCE, JONG SAM LEE, AND JOHN A. HAWLEY
Exercise Metabolism Group, School of Medical Sciences, Faculty of Life Sciences, RMIT University, Bundoora, Victoria 3083, Australia

Received 2 February 2001; accepted in final form 10 May 2001

Bruce, Clinton R., Jong Sam Lee, and John A. Hawley. Postexercise muscle glycogen resynthesis in obese insulin-resistant Zucker rats. J Appl Physiol 91: 1512–1519, 2001.—We determined the effect of an acute bout of swimming (8 × 30 min) followed by either carbohydrate administration (0.5 mg/g glucose ip and ad libitum access to chow; CHO) or fasting (Fast) on postexercise glycogen resynthesis in soleus muscle and liver from female lean (ZL) and obese insulin-resistant (ZO) Zucker rats. Resting soleus muscle glycogen concentration ([glycogen]) was similar between genotypes and was reduced by 73 (ZL) and 63% (ZO) after exercise (P < 0.05). Liver [glycogen] at rest was greater in ZO than ZL (334 ± 31 vs. 247 ± 16 μmol/g wet wt; P < 0.01) and fell by 44 and 94% after exercise (P < 0.05). The fractional activity of glycogen synthase (active/total) increased immediately after exercise (from 0.22 ± 0.05 and 0.32 ± 0.04 to 0.63 ± 0.08 vs. 0.57 ± 0.05; P < 0.01 for ZL and ZO rats, respectively) and remained elevated above resting values after 30 min of recovery. During this time, muscle [glycogen] in ZO increased 68% with CHO (P < 0.05) but did not change in Fast. Muscle [glycogen] was unchanged in ZL from postexercise values after both treatments. After 6 h recovery, GLUT-4 protein concentration was increased above resting levels by a similar extent for both genotypes in both fasted (~45%) and CHO-supplemented (~115%) rats. Accordingly, during this time CHO refeeding resulted in supercompensation in both genotypes (68% vs. 44% for ZL and ZO). With CHO, liver [glycogen] was restored to resting levels in ZL but remained at postexercise values for ZO after both treatments. We conclude that the increased glucose availability with carbohydrate refeeding after glycogen-depleting exercise resulted in glycogen supercompensation, even in the face of muscle insulin-resistance.

GLUCOSE TRANSPORT and the subsequent activation of glycogen synthase are important steps for controlling the rate of glycogen accumulation in insulin-sensitive tissue such as skeletal muscle (23, 30, 32). At rest or after exercise, the primary fate of glucose entering muscle is conversion to glycogen (9, 16), a reaction catalyzed by glycogen synthase (23). In the postexercise glycogen-depleted state, glycogen resynthesis proceeds in a biphasic manner (11). On cessation of exercise there is an increase in the permeability of muscle to glucose (32) accompanied by a rapid disposal of glucose into muscle (11). These perturbations combined with the increase in the dephosphorylated (active) form of glycogen synthase (37) result in a rapid resynthesis of muscle glycogen lasting up to 60 min.

As the acute, insulin-independent response wears off, there is a gradual increase in the phosphorylated (inactive) state of glycogen synthase, an increase in insulin sensitivity (3, 4, 11, 12), and a 10–30% slower (insulin-dependent) period during which glycogen values return to preexercise resting levels. After exercise there is a prolonged (up to 18 h) and persistent increase in glucose uptake by skeletal muscle (16, 31, 44). Reversal of this increase in muscle insulin sensitivity after exercise occurs simultaneously with muscle glycogen repletion and can be speeded by carbohydrate feeding or slowed by keeping muscle glycogen content low by fasting (44). If adequate carbohydrate is supplied throughout the postexercise recovery period, muscle glycogen stores can be supercompensated to levels that are higher than the fed sedentary state (4).

The regulation of exercise-stimulated glycogen synthesis in normal, healthy skeletal muscle has been well documented (for review, see Ref. 17). However, far less is known about the effects of postexercise glycogen accumulation in muscle (and liver) that exhibits chronic insulin resistance. Using nuclear magnetic resonance spectroscopy, Shulman et al. (34) provided the first evidence that muscle glycogen synthesis was impaired in individuals with non-insulin-dependent diabetes mellitus. Subsequently, Price et al. (28) reported that, after glycogen-lowering exercise, the insulin-independent phase of glycogen resynthesis was normal but the insulin-dependent phase of glycogen accumulation was reduced in the muscle of insulin-resistant fasted subjects.

To the best of our knowledge, no study has investigated the impact of carbohydrate refeeding on postexercise glycogen accumulation in insulin-resistant muscle. Although such a treatment would be unlikely to influence the rate of glycogen resynthesis immediately after exercise, carbohydrate administration has been shown to reverse the increase in insulin sensitivity.
that occurs during the slower (insulin-dependent) phase of glycogen accumulation (44). When combined with muscle insulin-resistance, carbohydrate refeeding might be expected to attenuate the normal rate of glycogen accumulation during this phase. However, such a hypothesis remains to be tested.

In the present study we used the genetically obese Zucker rat to determine the impact of insulin resistance on postexercise muscle and liver glycogen resynthesis. Skeletal muscle of the obese Zucker rat exhibits insulin resistance (18) and decreased insulin-stimulated glucose uptake (5, 43) but normal contraction-stimulated glucose uptake (2). In addition, the obese Zucker rat remains hyperinsulinemic and hyperglycemic, even during exercise (38). We hypothesized that, compared with normal muscle, 1) the rate of glycogen resynthesis in skeletal muscle from insulin-resistant rats would be similar during the early, insulin-independent phase of recovery with or without carbohydrate refeeding, 2) the extent of glycogen accumulation in the slower (insulin-dependent) phase after carbohydrate administration would be attenuated in insulin-resistant muscle as a result of reversal of the exercise-induced increase in muscle insulin sensitivity, and 3) glycogen concentrations would be restored to resting levels but not supercompensated in muscle from insulin-resistant rats fed carbohydrate throughout recovery.

METHODS

Animal care and overview of experimental design. Female lean (fa/−; n = 40) and obese (fa/fa; n = 40) Zucker rats aged 10–11 wk and weighing ~177 and ~306 g, respectively, were obtained from Monash University Animal Services, Victoria, Australia. Animals were housed two per cage in an environmentally controlled laboratory (temperature 22 ± 1°C, relative humidity 50 ± 2%) with a 12:12-h light-dark cycle (light 0700–1900). Animals were fed standard rodent chow (67.5% carbohydrate, 11.7% fat, 20.8% protein; Barastoc, Victoria, Australia), given ad libitum access to water, and familiarized to laboratory conditions for 1 wk before experimentation. The Animal Experimentation Ethics Committee of RMIT University approved all experimental procedures.

Animals were assigned to one of six subgroups on the basis of 1) whether they remained sedentary control (Rest) or were exercised, 2) whether they were fasted (Fast) or received carbohydrate (CHO) after exercise, and 3) the time of death after exercise.

Experimental protocol. At 1700 on the day before an experiment, ZL animals were restricted to 10 g and ZO animals to 12 g of chow (~80% of the animals’ average daily food intake from the previous 7 days). ZL rats were randomly assigned to one of six experimental groups: sedentary control performing no exercise (ZL-Rest; n = 7), fasted and killed immediately postexercise (ZL-Fast; n = 7), fasted and killed 30 min postexercise (ZL-Fast 30; n = 6), fasted and killed 6 h postexercise (ZL-Fast 360; n = 7), CHO supplemented and killed 30 min postexercise (ZL-CHO 30; n = 7), and CHO supplemented and killed 6 h postexercise (ZL-CHO 360; n = 6). ZO rats were also assigned to sedentary control performing no exercise (ZO-Rest; n = 7), fasted and killed immediately postexercise (ZO-Post; n = 7), fasted and killed 30 min postexercise (ZO-Fast 30; n = 6), fasted and killed 6 h postexercise (ZO-Fast 360; n = 7), CHO supplemented and killed 30 min postexercise (ZO-CHO 30; n = 7), and CHO supplemented and killed 6 h postexercise (ZO-CHO 360; n = 6). ZO animals were given a single 12-g dose of chow before the onset of the recovery period, animals assigned to the CHO-supplemented groups received an intraperitoneal glucose injection (0.5 mg/g BM) and were subsequently allowed free access to chow and water ad libitum. Animals in the fasted groups were allowed only ad libitum access to water. All postexercise food consumption was quantified.

Animal death. Approximately 5 min before the due time of death, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg BM). The then soleus muscle was rapidly excised and clamp frozen with tongs cooled in liquid nitrogen. We deliberately chose to sample the soleus (slow-twitch, oxidative fibers) because previous investigations have reported that GLUT-4 concentration is higher in this muscle than in both the red (fast-twitch oxidative) and white gastrocnemius (fast-twitch, glycolytic), whereas there are no differences in GLUT-4 content between ZL and ZO rats (2). Furthermore, total hexokinase activity is also similar between genotypes (33). In addition to the soleus, the liver was also removed. At this time a blood sample (~2 ml) was obtained from the femoral artery.

Blood biochemistry. Whole blood (~2 ml) was transferred to an EDTA-administered tube and was spun in a centrifuge at 12,000 rpm for 3 min. The plasma was immediately analyzed in duplicate for plasma glucose and plasma lactate concentration using an automated analyzer (Yellow Springs Instruments 2300 stat plus glucose and lactate analyzer, Yellow Springs, OH). The remaining plasma was stored at −80°C and was subsequently analyzed for plasma free fatty acid (FFA) concentration by using an enzymatic colorimetric method (NEFA C test kit, Wako, Richmond, VA) and for plasma insulin concentration by radioimmunoassay using a commercially available kit (Phadeseph, insulin RIA, Pharmacia & Upjohn Diagnostics, Uppsala, Sweden).

Tissue glycogen determination. Tissue glycogen content was assayed as previously described (22). Skeletal muscle and liver samples were placed in 2 N HCl and incubated at 100°C for 2 h. After neutralization with 0.66 N NaOH, the liberated glucose units were assayed fluorometrically, and glycogen content was expressed as micromoles of glucosyl units liberated per gram wet muscle weight (μmol/g wet wt).

Measurement of GLUT-4 protein content. Portions of the soleus muscle (~15 mg) were homogenized for 30 s in 1.0 ml of ice cold buffer (20 mM HEPES, 1 mM EDTA, 250 mM sucrose, pH 7.4) by using a motor-driven homogenizer (Ystral, HD Scientific, NSW, Australia). Homogenates were
heated for 10 min at 90°C, vortexed, and spun at 7,000 g for 10 min at 4°C. Supernatants were removed, and protein content was determined by use of a commercially available kit (Micro BCA protein assay reagent kit, Pierce, Rockford, IL). The supernatant was diluted with Laemmli sample buffer and stored at −80°C until subsequent analysis. Aliquots of muscle homogenates containing 5 μg protein were separated by SDS-PAGE (12.5% resolving gel), transferred to polyvinylidenedifluoride membranes (MSI, 0.45 μm, Osmonics), and blocked for 2 h with 5% nonfat milk. Membranes were incubated overnight at 4°C in a polyclonal antibody specific for GLUT-4. Membranes were washed in 0.05% Tris-buffered saline Tween, incubated for 1 h with secondary antibody conjugated to horseradish peroxidase, and washed again in 0.05% Tris-buffered saline Tween. Proteins were visualized by enhanced chemiluminescence (NEN Life Science Products, Boston, MA) and quantified by densitometry.

Hexokinase and glycogen synthase activity. Soleus muscle was homogenized (1:50 dilution) in 50% glycerol, 20 mM phosphate buffer (pH 7.4), 5 mM 2-mercaptoethanol, 0.5 mM EDTA, and 0.02% bovine serum albumin. Hexokinase activity was assayed fluorometrically at 25°C by measuring the rate of production of reduced NADP (14). Glycogen synthase activity was assayed by using the same homogenate as for hexokinase activity, as previously described (14). Glycogen synthase activity was measured in the absence (active form) or presence of 10 mM (total activity) glucose-6-phosphate. Glycogen synthase activity is reported as fractional activity of the enzyme (active/total), with total activity expressed as μmol·g wet wt⁻¹·min⁻¹.

Statistical analyses. Analysis of differences between the two treatments (Fast or CHO) within a genotype was performed using a paired t-test. An unpaired t-test was used to assess differences between ZL and ZO animals. All other differences were determined by using a one-way ANOVA with Tukey’s post hoc analysis. Significance was accepted when P < 0.05. All data are presented as means ± SE.

RESULTS

Postexercise food intake. Food consumption was similar between ZL and ZO rats after both 30 (0.7 ± 0.2 vs. 0.5 ± 0.2 g) and 360 min (5.6 ± 0.7 vs. 5.2 ± 0.8 g) of refeeding. Muscle glycogen. Figure 1A displays the muscle glycogen content for ZL and ZO animals at rest and at various time points after exercise for the two treatment interventions. Resting muscle glycogen contents were not significantly different between genotypes, although they tended to be higher in ZO rats (38.2 ± 4.3 vs. 29.4 ± 1.5 μmol·g wet wt). Exercise resulted in a significant reduction in muscle glycogen content in both genotypes (ZL 73% vs. ZO 63%; P < 0.01). In ZL rats, CHO or Fast had little effect on muscle glycogen resynthesis in the 30-min period after exercise (Fig. 1A). Likewise, muscle glycogen content was similar 30 min after exercise in ZO-Fast. However, CHO increased muscle glycogen content 68% (from 14.3 ± 1.2 to 24.1 ± 2.5 μmol·g wet wt; P < 0.05) above postexercise values in ZO rats. At this time, glycogen content in ZO was significantly higher for both Fast and CHO compared with ZL under the same conditions (Fig. 1A; P < 0.01). After 360 min and in the absence of CHO, muscle glycogen content for ZL rats remained 56% below resting values; at the same time point, glycogen content in the ZO rats had been restored to that of Rest. CHO refeeding in ZL rats during 360 min of recovery resulted in muscle glycogen supercompensation such that glycogen content was increased 68% above resting levels (29.4 ± 1.5 vs. 49.5 ± 4.0 μmol·g wet wt; P < 0.01). Under the same conditions, muscle glycogen content was supercompensated by 44% in ZO animals (38.2 ± 4.3 vs. 55.0 ± 2.8 μmol·g wet wt) with no differences in the final glycogen content between genotypes.

Liver glycogen content. Figure 1B displays the liver glycogen content for both genotypes at rest and at various time points after exercise for the two treatment interventions. Liver glycogen content at rest was 35% higher in ZO than ZL rats (334 ± 31 vs. 247 ± 16
µmol/g wet wt; \( P < 0.01 \)). Exercise resulted in a significant reduction in liver glycogen content in both groups (Fig. 1B), although the degree of depletion was more marked in ZL compared with ZO rats (94 vs. 44%; \( P < 0.01 \)). Accordingly, liver glycogen utilization during the exercise bout was greater in ZL than ZO rats (146 ± 44 vs. 233 ± 1 µmol/g wet wt; \( P < 0.01 \)).

Liver glycogen content in ZL-Fast rats did not increase above Post levels after 30 or 360 min of recovery. However, after 360 min of CHO refeeding, liver glycogen content was restored to resting values in this genotype (Fig. 1B). Liver glycogen content in the ZO rats was unchanged from Post values at all time points after exercise regardless of whether or not CHO was administered. With the exception of the 360-min value with CHO refeeding, liver glycogen content was higher in ZO compared with ZL rats at all time points during recovery from exercise (\( P < 0.01 \); Fig. 1B). Although liver glycogen content after 360 min recovery with CHO was comparable between genotypes, it still remained 38% lower than Rest levels in ZO rats.

**GLUT-4 protein content.** Figure 2 displays the GLUT-4 protein concentration for both genotypes at rest and at various time points after exercise for the two treatment interventions. Resting GLUT-4 protein concentration was similar between ZL and ZO rats. There was little increase in GLUT-4 protein concentration immediately postexercise or after 30 min of recovery for both genotypes regardless of refeeding regimen (Fig. 2). GLUT-4 protein concentration was increased ~45% after 360 min of recovery for both genotypes in fasted rats. However, with CHO refeeding, GLUT-4 protein concentration was increased ~115% above resting values for both genotypes.

**Hexokinase and glycogen synthase activity.** Table 1 shows the activities of hexokinase and glycogen synthase for both genotypes at rest and at various time points after exercise for the two treatment interventions. Hexokinase activity was similar at rest between genotypes. Exercise was associated with a decrease in hexokinase activity in fasted ZL rats, an effect that persisted for the first 30 min of recovery (Table 1). However, hexokinase activity was not different from Rest or Post levels in ZO rats after 30 min of recovery for either Fast or CHO. After 360 min, hexokinase activity was significantly greater than Rest levels in ZO-fasted but not in CHO-fed animals.

The fractional activity of glycogen synthase was similar between genotypes at rest. Immediately after exercise, glycogen synthase activity was significantly increased in both genotypes. Throughout 360 min of recovery, glycogen synthase activity remained elevated for fasted ZL rats (Table 1). However, glycogen synthase activity had returned to Rest values after 360 min of CHO refeeding in ZL animals (Table 1). In the ZO rats, glycogen synthase activity remained elevated for the first 30 min of recovery for both treatments but was back to Rest values after 360 min of either CHO or Fast (Table 1).

**Concentrations of plasma metabolites.** Table 2 shows the concentrations of plasma glucose, plasma lactate, plasma insulin, and plasma FFAs at rest and at various time points after exercise after the two treatment interventions. Resting plasma glucose concentrations were similar between genotypes. However, in ZL rats, exercise was associated with a significant reduction in plasma glucose concentration, an effect that persisted throughout 360 min of recovery (Table 2). On the other hand, after 30 min CHO refeeding, plasma glucose concentration was greater in ZL compared with Fast and was still higher than Post values after 360 min of CHO. Postexercise plasma glucose concentrations in ZO rats were similar to Rest concentrations (Table 2). However, apart from CHO 360, plasma glucose concentration was greater in ZO than ZL animals (Table 2). After 360 min of CHO refeeding, plasma glucose concentration was similar for both genotypes.

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**Table 1. Enzyme activities in soleus muscle**

<table>
<thead>
<tr>
<th>Hexokinase, µmol·g·min⁻¹</th>
<th>Glycogen synthase, active/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean</td>
<td>Obese</td>
</tr>
<tr>
<td>Lean</td>
<td>Obese</td>
</tr>
<tr>
<td><strong>Rest</strong></td>
<td><strong>Post</strong></td>
</tr>
<tr>
<td>4.29 ± 0.32</td>
<td>3.18 ± 0.42</td>
</tr>
<tr>
<td>3.55 ± 0.30*</td>
<td>3.60 ± 0.20</td>
</tr>
<tr>
<td><strong>CHO 30</strong></td>
<td><strong>Fast 360</strong></td>
</tr>
<tr>
<td>3.92 ± 0.27</td>
<td>4.20 ± 0.43*</td>
</tr>
<tr>
<td>0.71 ± 0.05‡</td>
<td>0.41 ± 0.04‡</td>
</tr>
</tbody>
</table>
| *P < 0.01 vs. Post for each genotype. †P < 0.05 vs. Rest for each genotype. ‡P < 0.05 vs. Post for each genotype. §P < 0.05 vs. Fast at same time point for each genotype.
DISCUSSION

The pattern of glycogen resynthesis in normal muscle after its depletion by exercise is biphasic (29, 37), with an initial rapid (insulin-independent) phase of synthesis, followed by a slower rate of glycogen accumulation that requires the presence of insulin. In this second phase and in the absence of carbohydrate supplementation, glycogen levels are eventually restored to basal concentrations, but if carbohydrate refeeding takes place after exercise and is continued during recovery, muscle glycogen levels are increased above normal, so-called glycogen supercompensation (4).

On the basis of the results of previous investigations performed on insulin-resistant fasting animals (1, 10, 36), it is difficult to predict whether carbohydrate refeeding during recovery from prolonged, strenuous exercise would have an effect on skeletal muscle glycogen resynthesis. However, in light of the finding that contraction-stimulated glucose uptake is normal in insulin-resistant muscle of the obese Zucker rat (2), we originally proposed that the rate of glycogen accumulation would be similar for both obese and lean animals during the insulin-independent phase of recovery with or without carbohydrate refeeding. In agreement with our hypothesis, the first finding of the present study was that insulin resistance had little effect on the immediate (30 min) rate of postexercise glycogen resynthesis in obese fasted animals. Previous investigations have found a normal (1, 10, 36) or a reduced (13, 36) rate of postexercise glycogen resynthesis in diabetic fasted animals. However, these results need to be reconciled with the fact that in those studies (1, 10, 13, 36) diabetes was induced with streptozotocin, an agent that is known to be associated with a fall in GLUT-4 concentrations (7), a reduced insulin-responsiveness (19, 25), and a fall in contraction-stimulated muscle glucose uptake (24, 27, 42).

Perhaps of more significance was our finding of little or no repletion of muscle glycogen after 30 min of carbohydrate refeeding in lean rats. Such an observation is difficult to explain in light of the fact that carbohydrate supplementation had restored blood glucose concentration to resting values, whereas fasting animals were still hypoglycemic at this time. However, in agreement with our results, Terjung et al. (37) also reported no net glycogen accumulation after 30 min in the soleus muscle of rats swum to exhaustion and fed glucose immediately after exercise. These workers reported that the highest rates of glycogen resynthesis after glucose administration were found between 30 and 60 min postexercise (37). In the present study, animals were killed 30 and 360 min after exercise: it may well be that, because of our early and late sampling times, we failed to detect the most rapid phase of glycogen resynthesis. We also considered the possibility that the lack of glycogen resynthesis in lean animals may have been because they consumed less food during the early phase of recovery. Such a theory

### Table 2. Concentrations of plasma metabolites

<table>
<thead>
<tr>
<th>Glucose, mmol/l</th>
<th>Lactate, mmol/l</th>
<th>Insulin, μU/ml</th>
<th>FFA, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean</td>
<td>Obese</td>
<td>Lean</td>
<td>Obese</td>
</tr>
<tr>
<td>Rest</td>
<td>8.8 ± 0.1</td>
<td>9.3 ± 0.3</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Post</td>
<td>5.3 ± 0.7*</td>
<td>10.3 ± 0.7‡</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td>Fast 30</td>
<td>5.2 ± 0.4*</td>
<td>10.3 ± 0.8§‡</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>CHO 30</td>
<td>7.6 ± 0.7‡</td>
<td>11.9 ± 0.9*‡</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Fast 360</td>
<td>6.3 ± 0.1*‡</td>
<td>9.0 ± 0.6‡</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>CHO 360</td>
<td>7.7 ± 0.3‡§</td>
<td>8.0 ± 0.4</td>
<td>2.4 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. Plasma glucose, lactate, insulin, and free fatty acid (FFA) concentrations were measured in lean and obese Zucker rats as described in METHODS. *P < 0.05 vs. Rest for each genotype. †P < 0.05 vs. Fast for each genotype. §P < 0.05 vs. lean group under identical conditions. $P < 0.05 vs. Fast at same time point for each genotype.
would be consistent with our observations that, compared with obese rats, lean animals tended to be more fatigued and lethargic when placed back in their cages. However, analysis of food records revealed that both lean and obese rats consumed similar amounts of chow during the first 30 min (~0.6 g) and also after 360 min (~5.5 g) of recovery. Because postexercise lactate levels were consistently elevated in obese compared with lean animals, one cannot discount the possibility that lactate provided a major carbon source for muscle glycogen resynthesis during recovery, as has been recently suggested (10). On the other hand, despite higher resting and postexercise concentrations of plasma FFA in obese compared with lean rats (Table 2), there was no evidence of diminished glycogen deposition.

On the basis of impaired rates of insulin-dependent glycogen synthesis in muscle from insulin-resistant fasting humans recovering from exercise (28), along with the observed reversal of the exercise-induced increase in permeability to sugar when fed carbohydrate (44), we hypothesized that the extent of glycogen accumulation in muscle from obese animals would be attenuated in the slower (insulin-dependent) phase after carbohydrate administration. Furthermore, because glycogen supercompensation takes place throughout this slower portion of recovery (11), we also proposed that glycogen concentration would only be restored to the slower (preexercise) levels and not supercompensated in muscle from insulin-resistant rats fed carbohydrate. However, contrary to our expectations, we found that carbohydrate refeeding accelerated glycogen accumulation in muscle of obese compared with lean animals (Fig. 1A) and consequently resulted in supercompensation after 360 min of recovery. These data strongly suggest that, with the increased carbohydrate availability, there was a persistent increase in glucose uptake associated with glycogen-depleting exercise, even in the face of muscle insulin resistance. Although the degree of supercompensation was greater in skeletal muscle from lean than from insulin-resistant rats (68 vs. 44%), there were no differences in the final glycogen content between genotypes because of the slightly higher resting glycogen content in the obese animals. To the best of our knowledge, this is the first study to report glycogen supercompensation in skeletal muscle from untrained insulin-resistant rats. Our findings raise interesting questions regarding the control of muscle glycogen synthesis in insulin-resistant tissue.

Both glucose transport (30, 35) and glycogen synthase activation (23) have been implicated to be major rate-limiting steps for glycogen synthesis in skeletal muscle. Being downstream of GLUT-4 and hexokinase in the metabolic pathway, glycogen synthase is dependent on glucose transport to provide substrate for subsequent glycogen synthesis. Glucose transport would be expected to exert control over the glycogen synthesis pathway if an increase in the transport capacity induced a proportional increase of flux through the pathway. A limitation of the present study was that we did not obtain measures of glucose transport. However, Brozinick et al. (2) previously reported that both basal and the maximum contraction-stimulated glucose uptake is not impaired in insulin-resistant muscle of the obese Zucker rat.

Alternatively, it has been proposed that the level of skeletal muscle GLUT-4 protein serves to set or limit the upper level of glycogen accumulation (20, 21). In this regard, Kuo et al. (21) have recently reported that an acute bout of prolonged swimming induced a 43% increase in GLUT-4 protein concentration above resting control values in fasted rats. They also found that carbohydrate supplementation in the postexercise recovery period further enhanced GLUT-4 protein concentration and reported a significant positive correlation between the level of muscle GLUT-4 protein and subsequent glycogen accumulation after carbohydrate refeeding (21). In close agreement with the results of Kuo et al. (21), we found that exercise alone resulted in a ~45% increase in GLUT-4 protein concentration in fasted animals. Exercise plus carbohydrate supplementation, however, increased soleus GLUT-4 protein concentration ~115% above rest in both genotypes. Accordingly, during this time carbohydrate refeeding resulted in glycogen supercompensation in both lean and obese rats. These data strongly suggest that if glycogen levels are lowered by similar amounts during exercise and sufficient carbohydrate is available, glycogen resynthesis will be stimulated to the same extent in both normal and insulin-resistant muscle.

Because hexokinase expression and activity (39) and glycogen synthase expression (40) have been reported to be decreased in patients with type II diabetes, we wished to quantify the activities of both hexokinase and glycogen synthase in an attempt to determine their role in subsequent glycogen resynthesis. In contrast to the finding of a reduced hexokinase activity in skeletal muscle from humans with type II diabetes (39), we found that hexokinase activity was similar between normal and insulin-resistant muscle and also between fed and fasted animals (Table 1). On the basis of this finding, one might reasonably predict the same flux through the glucose transporter-hexokinase step (35) that would result in comparable activation of glycogen synthase (26). Indeed, the activity of glycogen synthase was remarkably similar between genotypes before and after exercise (Table 1). A dissociation between glycogen synthase activity and the subsequent rate of glycogen resynthesis has been previously demonstrated by Price et al. (29). These workers reported a 10-fold drop in the rate of glycogen synthesis between the early (first 30 min) and late (>1 h) period of recovery after exercise with only minor (<twofold) changes in the fractional activity of glycogen synthase.

In agreement with previous reports (9), we found that muscle contraction and glycogen depletion resulted in an increase in the fractional activity of glycogen synthase. Accompanying postexercise glycogen resynthesis was a concomitant inactivation of glycogen synthase: it is well known that glycogen plays an important role in regulating its own synthesis, with glycogen synthase activity varying inversely with glycogen concentration (6) and glucose transport being...
significantly higher in muscles in which glycogen content is kept low (9, 15).

Although the major focus of the present study was to determine the pattern of glycogen accumulation in insulin-resistant skeletal muscle, we also determined the time-course changes in liver glycogen repletion after exercise with and without carbohydrate refeeding. In agreement with other studies (10), the level of hepatic glycogen before exercise was substantially higher in the insulin-resistant compared with the lean animals, but whereas exercise only resulted in a mild (~50%) loss of liver glycogen in insulin-resistant animals, there was an almost complete depletion in lean animals. This latter observation concurs with the results of others (1, 8), but the finding of only a mild exercise-induced decrease in hepatic glycogen stores in insulin-resistant rats contrasts with the recent findings of Ferreira et al. (10), who reported that streptozotocin-induced diabetic animals had a more marked fall in glycogen concentration than control animals after exercise. The most likely reason for such a discrepancy is the animals in the study of Ferreira et al. (10) were fasted for 24 h before their high-intensity short-term (~3 min) exercise protocol.

The pattern of postexercise liver glycogen accumulation deviated markedly between genotypes. Hepatic glycogen concentration in lean animals remained suppressed throughout recovery and only returned to resting levels after 360 min of CHO refeeding. On the other hand, liver glycogen stores in obese insulin-resistant rats showed little fluctuation throughout the recovery period. In agreement with the results of a previous study (8), there was preferential resynthesis of muscle compared with liver glycogen in lean rats after exhausting exercise.

In conclusion, this is the first study to show that the increased glucose availability with carbohydrate refeeding after an acute bout of glycogen-depleting exercise resulted in muscle (but not liver) glycogen supercompensation in both untrained lean and obese insulin-resistant Zucker rats. For this reason, frequent exercise bouts of sufficient intensity and/or duration to markedly deplete muscle glycogen stores may benefit those individuals with a genetic predisposition for the development of insulin resistance.

We are grateful to S. Potocnik for providing the GLUT-4 antibodies used in this study. This study was supported by an RMIT Faculty of Life Sciences Research Grant to J. A. Hawley.

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