Effect of streptozotocin-induced diabetes on glycogen resynthesis in fasted rats post-high-intensity exercise

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IN RECENT YEARS, WE AND OTHERS have clearly established that, during recovery from exercise, a wide range of vertebrate species has the capacity to replenish stores of muscle glycogen, even in the absence of food intake (6, 10, 18, 19, 23, 25, 32, 37, 39). Under these conditions, the nature of the carbon sources mobilized for the resynthesis of muscle glycogen stores depends in part on the intensity of exercise. If exercise is of moderate intensity, amino acids and, to a lesser extent, glycerol are the major carbon sources (18, 19), whereas lactate is believed to play a major role during recovery from high-intensity exercise (6, 10, 25, 37, 39). The ability of muscles to replenish their stores of glycogen without food ingestion is clearly advantageous, because it allows the sparing of glycogen for situations eliciting fight-or-flight responses. Despite its physiological importance, the only study on the regulation of this process in vivo was performed only recently on rats (10), which comes as no surprise given that the importance of this process in this animal species was recognized only a few years ago (37). Moreover, an important issue that has never been addressed is the effect of disease states such as diabetes on the capacity of fasted animals, including humans, to resynthesize their muscle glycogen stores after exercise. This is in marked contrast to the impressive literature published on the regulation of muscle glycogen synthesis in postexercised, fed diabetic rats and humans (5, 12, 24, 31, 33, 41, 45).

It is difficult to predict, on the basis of the studies performed on fed animals, whether diabetes impairs glycogen resynthesis in fasted animals recovering from high-intensity exercise. Indeed, several studies have been carried out to determine the effect of diabetes on glycogen resynthesis in humans and rats administered carbohydrate during recovery, but these have led to contradictory findings. For instance, some studies in rats report that, after exercise of moderate intensity, the rate and extent of muscle glycogen resynthesis in response to food intake are not affected by diabetes (5), whereas reduced rates of glycogen deposition are reported in diabetic rats recovering from either exercise (45) or electrical stimulation (12, 24). In these latter studies, the lower rate and extent of glycogen resynthesis in streptozotocin (STZ)-diabetic rats were reversed by the administration of insulin (12, 24). Similarly, in individuals with type 1 diabetes not treated with insulin, the rates of glycogen resynthesis are reduced after exercise (33). Although similar observations have been made in individuals with insulin resistance typical of type 2 diabetes (41), others have reported that insulin resistance is without any effect on glycogen repletion after exercise (31). It is important to stress that, even if there were better agreement among the aforementioned findings, the marked physiological differences between fed and fasted animals would preclude us from predicting whether, in the absence of...
food intake, diabetes affects glycogen resynthesis in fasted animals after exercise. For this reason, the primary aim of the present study was to establish, for the first time, the pattern of response of glycogen metabolism in muscles and liver of fasted STZ-diabetic rats recovering from high-intensity exercise.

**MATERIALS AND METHODS**

**Materials.** Biochemicals and enzymes were obtained from Boehringer Mannheim (Sydney, NSW, Australia). UDP-[U-14C]glucose and streptozotocin were obtained from Amer sham International (Amerham, Bucks, UK) and Sapphire Biochemicals (Alexandria, NSW, Australia), respectively. All other chemicals were of analytical grade. Scintillation cocktail (Emulsifier-Safe) was obtained from Packard Instruments (Sydney, NSW, Australia).

**Animals.** Adult male albino Wistar rats (250–300 g) were kept at ~20°C on a 12:12-h light-dark photoperiod and had unlimited access to water and a standard laboratory chow diet (Glen Forrest Stockfeeders, Glen Forrest, WA, Australia; 55% digestible carbohydrate, 19% protein, 5% lipid and 21% nondigestible residue by weight). In one experiment, 90 animals were divided into either control or diabetic groups (45 animals per group). Diabetes was induced by a single intraperitoneal injection of 60 mg STZ/kg body mass (STZ was prepared fresh in 0.1 M citrate buffer, pH 4.5), whereas the control animals were injected with citrate buffer. Three days after injection, blood glucose level in each animal was determined from blood sampled from the tail vein after a 24-h fast, and only rats with glycemia >15 mM were included in the diabetic group (to generate a group of 45 STZ-diabetic rats, 57 animals were injected with STZ, of which 12 were excluded). The experiments were performed 11 days after STZ injection, at which time the rats were fasted for 24 h to deplete most of their stores of liver glycogen (10), because this source of glucose could contribute to muscle glycogen resynthesis postexercise (27). On the day of the experiment, the animals were exercised and then killed between 0800 and 1200. In another experiment, 40 non-diabetic rats had their food removed at 0800 and were exercised and then killed between 1500 and 1700. At that time, each animal was in a postabsorptive state, with preexercise hepatic glycogen levels comparable to those of the 24-h-fasted STZ-diabetic animals (preliminary experiments had been performed to determine the time of day after food withdrawal when hepatic glycogen in non-diabetic animals reached levels similar to those of the diabetic rats used in the experiment described above). This project was approved by the Animal Ethics Committee of the University of Western Australia.

**Exercise protocol.** Because rats are natural swimmers, exercise protocols based on swimming are widely used, the intensity of the exercise being determined by the amount of lead weight attached to the tail (10, 37). Immediately before swimming, each animal was weighed, and a lead weight equivalent to 9% of body mass was attached to the base of the tail to exercise each rat near-maximal intensity. The 9% lead weight consisted of three lead weights of equivalent mass, each suspended by a small hook to a hook holder attached with a rubber band to the animal’s tail. Swimming took place in a 30-cm-diameter plastic tank filled with water (48 cm deep) at 34°C. To exercise the rats to near exhaustion, the size of the weight was progressively reduced on each occasion by one-third, as the animals tired, until two-thirds of the weight was removed (10). Each weight was removed by pulling it off the hook holder while the animal was swimming. Exhaustion was defined as that point at which the animal could not remain at the water surface (the animals swam for ~3 min). One major strength of this exercise protocol is that it results in highly reproducible changes in muscle glycogen and lactate levels (10, 37). After exercise, the rats were either killed or allowed to recover individually in separate cages without access to food for 10, 30, or 120 min. Groups of non-exercised rats served as the non-exercised control group.

**Tissue sampling.** All rats at rest or at time intervals during the postexercise recovery period were anesthetized under halothane, and the following tissues were sampled: individual muscles (soleus and red, white, and mixed gastrocnemius muscles), blood (by cardiac puncture), and liver. Halothane was chosen because it induces anesthesia within only 15–20 s postexercise and does not affect the levels of the metabolites measured in the present study (22). The red, white, and mixed gastrocnemius muscles were selected because 1) they are rich in type Ia, Iib, and Ia,b fibers, respectively, but poor in slow-twitch type I fibers, thus reflecting the composition of the hindlimb musculature as a whole (34, and 2) they are actively recruited during high-intensity exercise (10, 37). By contrast, the soleus muscle, which is rich in type I fibers, was chosen on the basis that its glycogen stores are not recruited during burst exercise (10, 37). After removal, each tissue was immediately freeze-clamped between aluminum plates precooled in liquid nitrogen. The blood samples were obtained by cardiac puncture, as mentioned above, and were transferred into Eppendorf tubes and centrifuged at 720 g for 10 min. After centrifugation, some of the plasma was stored at −70°C, and the remainder was deproteinized in 9 vol of 6% (wt/vol) perchloric acid and centrifuged at 2,000 g for 10 min. After centrifugation, some of the plasma was stored at −70°C, and the remainder was deproteinized in 9 vol of 6% (wt/vol) perchloric acid and centrifuged at 2,000 g for 10 min before being neutralized with 2 M K2CO3. All samples were kept at −70°C until analysis.

**Metabolite assays.** Each muscle was weighed and ground using a mortar and pestle kept in liquid nitrogen. The powdered tissue was mixed with 10 vol of ice-cold 6% (wt/vol) perchloric acid and homogenized. A portion of the homogenate was used for the determination of glycogen. Another portion was centrifuged at 2,000 g for 10 min, the supernatant was removed and kept on ice, and the pellet was reextracted with ice-cold 6% (wt/vol) perchloric acid before recentrifugation (2,000 g for 10 min). The supernatants were combined, neutralized with 2 M K2CO3, and centrifuged before being used for the assays of metabolites. Glycogen, lactate, glucose, and glucose 6-phosphate were assayed as described in Bergmeyer (7).

**Activity ratio of glycogen synthase.** The activity ratio of glycogen synthase (± glucose 6-phosphate) is commonly used as an index of its phosphorylation state (10). Muscles previously weighed and ground were homogenized for 30 s with 10 vol of glycerol buffer [50 mM Tris-HCl, pH 7.8, 100 mM KF, 10 mM EDTA and 60% (vol/vol) glycerol] at −20°C. After the addition of 10 vol of glycerol-free buffer (50 mM Tris-HCl, pH 7.8, at 25°C, 100 mM KF, 10 mM EDTA), the extracts were centrifuged at 2,000 g for 10 min, and the supernatants were further diluted fivefold with glycerol-free buffer before assay to avoid activation of glycogen synthase by endogenous glucose 6-phosphate. Glycogen synthase was assayed in either the absence or presence of 5 mM glucose 6-phosphate (46), as described previously (10).

**Expression of results and statistical analysis.** All metabolite concentrations are expressed as micromoles per gram of wet weight and glycogen synthase activity ratios as a percentage of maximal activity. Results are expressed as means ± SE for nine rats. The effects of exercise and postexercise recovery on the levels of metabolites in muscles and
Effect of high-intensity exercise on levels of lactate in skeletal muscles and liver and diabetic animals decreased markedly in the red, intensity exercise, muscle glycogen levels in control

RESULTS

plasma and enzyme activities were analyzed using a one-factor ANOVA followed by a Fisher protected least significant difference (PLSD) a posteriori test with StatVie SE + Graphics v1.03 (Abacus Concepts, 1988).

RESULTS

Muscle glycogen and metabolite levels. During high-intensity exercise, muscle glycogen levels in control and diabetic animals decreased markedly in the red, white, and mixed gastrocnemius muscles, whereas glycogen concentrations in the soleus muscle were not affected by exercise (Fig. 1). The preexercise levels of glycogen in the mixed, red, and white gastrocnemius muscles in the diabetic animals were significantly higher than in nondiabetic rats. Despite this, muscle glycogen levels attained in response to high-intensity exercise were similar in both experimental groups (Fig. 1). During recovery, significant replenishment of the stores of glycogen occurred in the red, white, and mixed gastrocnemius muscles, whereas glycogen levels in the soleus muscle remained stable. The levels of glycogen attained after 30 and 120 min of recovery in diabetic animals were comparable to those in control rats, with the exception of the red gastrocnemius muscle, where glycogen levels at 120 min were higher in diabetic animals.

In response to high-intensity exercise, the levels of lactate in the red, white, and mixed gastrocnemius muscles increased markedly to concentrations that did not differ significantly between the control and diabetic animals (Table 1). In contrast, lactate in the soleus muscle of control animals reached higher levels than in diabetic animals. During recovery, the levels of muscle lactate returned to preexercise levels within 30 min of the cessation of exercise and remained low and stable thereafter. The patterns of change in lactate levels in all muscles examined (soleus and red, white, and mixed gastrocnemius) in control animals were similar to those in diabetic animals, with minor exceptions.

Blood metabolites. Concomitant with muscle glycogen breakdown, high-intensity exercise caused a marked increase in lactate concentrations in plasma (Table 2), which returned to resting preexercise levels within 30 min of the cessation of exercise. Plasma glucose levels increased after exercise in the diabetic rats and decreased thereafter to preexercise levels during recovery, whereas, in control rats, plasma glucose levels remained stable at preexercise levels (Table 2). Levels of free fatty acids and ketone bodies in control

Table 1. Effect of high-intensity exercise on levels of lactate in skeletal muscles and liver

<table>
<thead>
<tr>
<th>Lactate, μmol/g</th>
<th>Rest</th>
<th>0</th>
<th>10</th>
<th>30</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. White</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.89 ± 0.06</td>
<td>12.20 ± 1.50*</td>
<td>8.80 ± 1.80†</td>
<td>1.88 ± 0.22†</td>
<td>0.72 ± 0.04</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.89 ± 0.08</td>
<td>13.00 ± 2.00*</td>
<td>5.70 ± 1.20*</td>
<td>0.80 ± 0.08</td>
<td>0.63 ± 0.03</td>
</tr>
<tr>
<td>G. Red</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.01 ± 0.12</td>
<td>4.93 ± 0.40*</td>
<td>2.65 ± 0.40†</td>
<td>1.13 ± 0.20</td>
<td>0.69 ± 0.05</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.67 ± 0.07</td>
<td>5.27 ± 0.30*</td>
<td>3.54 ± 0.50*</td>
<td>1.19 ± 0.14</td>
<td>0.88 ± 0.12</td>
</tr>
<tr>
<td>G. Mixed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.93 ± 0.50</td>
<td>9.79 ± 1.30*</td>
<td>4.84 ± 0.80*</td>
<td>1.67 ± 0.20†</td>
<td>0.98 ± 0.07</td>
</tr>
<tr>
<td>Diabetic</td>
<td>1.45 ± 0.10</td>
<td>7.93 ± 1.00*</td>
<td>5.39 ± 0.81*</td>
<td>1.09 ± 0.11</td>
<td>0.92 ± 0.09</td>
</tr>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.50 ± 0.09</td>
<td>4.84 ± 0.30†</td>
<td>3.41 ± 0.50*</td>
<td>1.78 ± 0.19</td>
<td>1.62 ± 0.08</td>
</tr>
<tr>
<td>Diabetic</td>
<td>1.15 ± 0.08</td>
<td>5.95 ± 0.30*</td>
<td>4.25 ± 0.50*</td>
<td>1.41 ± 0.12</td>
<td>1.23 ± 0.08</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.84 ± 0.16</td>
<td>7.08 ± 0.80*</td>
<td>4.52 ± 0.47*</td>
<td>3.38 ± 0.34†</td>
<td>2.08 ± 0.33</td>
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<tr>
<td>Diabetic</td>
<td>1.84 ± 0.16</td>
<td>6.31 ± 0.77*</td>
<td>4.53 ± 0.64*</td>
<td>2.08 ± 0.21</td>
<td>1.63 ± 0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9 per time point for each treatment. G., gastrocnemius muscle. *P < 0.05 vs. rest; †P < 0.05 vs. corresponding control animals.
and diabetic rats decreased immediately after exercise but increased during recovery to preexercise or greater than preexercise levels (Table 2).

**Glycogen synthase activity ratio.** Before exercise, there was no difference in the activity ratios of glycogen synthase between diabetic and control rats irrespective of the muscles examined. At the onset of recovery, the activity ratios of glycogen synthase in the red, white, and mixed gastrocnemius muscles in both the control and diabetic rats were higher than those at rest and were not affected by diabetes. During the first 30 min after exercise, the activity ratios of glycogen synthase in the gastrocnemius muscles decreased to basal or near-basal levels (Fig. 2), with some differences between diabetic and control rats after 10 min of recovery in the red gastrocnemius muscle (Fig. 2). A similar pattern of change was also observed for the white gastrocnemius muscle, with the difference that, at 30 min, the activity ratios of glycogen synthase were higher in diabetic animals. A distinct pattern of change was observed in the activity ratios of glycogen synthase in the soleus muscle. In this muscle, exercise resulted in a fall in the activity ratios of glycogen synthase in control rats but resulted in no significant changes in the soleus muscles of diabetic animals (Fig. 2). After 30 min of recovery, the activity ratios of glycogen synthase in the soleus muscle in control rats returned to preexercise levels.

**Liver glycogen.** The pattern of response of hepatic glycogen to high-intensity exercise in diabetic animals was markedly different from that in controls (Fig. 3). Before exercise, the levels of hepatic glycogen in diabetic animals were much higher than those in controls. Although the levels of hepatic glycogen decreased in response to exercise in both experimental groups, the fall in glycogen levels was much more pronounced in diabetic animals. During recovery, glycogen levels remained at low and stable levels in the control rats but returned to preexercise levels in the diabetic rats (Fig. 3). In postabsorptive nondiabetic rats exercised at a time of day chosen so that their preexercise hepatic glycogen levels matched those of the 24-h-fasted STZ-diabetic animals, the stores of hepatic glycogen decreased in response to exercise and remained at stable levels thereafter (Fig. 4).

### DISCUSSION

It is now well established that, during recovery from high-intensity exercise, a large proportion of muscle glycogen can be replenished even in the absence of food intake (6, 10, 18, 19, 23, 25, 32, 37, 39). To further our understanding of the regulation of this process in health and in diseases, we undertook to establish its response to mild diabetes. To address this question,
this study examined the response of muscle glycogen metabolism to high-intensity exercise and subsequent recovery in fasted rats rendered diabetic by STZ administration. That the animals were mildly diabetic was evidenced by their high fasting blood glucose levels (18.3 ± 1.1 mM; Table 2) accompanied by ketone body and free fatty acid levels comparable to or marginally different from those found in nondiabetic fasted rats at rest, findings typical of animals treated with moderate doses of STZ (28, 51) (Table 2). This is in marked contrast with the response of plasma ketone body levels in rats administered high doses of STZ, because, under those conditions, these metabolites reach levels severalfold higher than those in nondiabetic animals (28, 51). With the use of this animal model, our results show for the first time that STZ-induced diabetes has little effect on muscle glycogen synthesis after exercise but has a pronounced and unexpected effect on hepatic glycogen metabolism.

In contrast to previous studies, basal glycogen concentrations in muscles from STZ-diabetic rats were slightly higher than those in control animals (5, 12, 13, 24, 45) (Fig. 1). Comparison of our results with those of others must be made with caution, however, because previous studies have measured muscle glycogen concentrations only in fed animals. The presence of higher glycogen levels in muscles of STZ-diabetic rats at rest, findings typical of animals treated with moderate doses of STZ (28, 51) (Table 2). This is in marked contrast with the response of plasma ketone body levels in rats administered high doses of STZ, because, under those conditions, these metabolites reach levels severalfold higher than those in nondiabetic animals (28, 51). With the use of this animal model, our results show for the first time that STZ-induced diabetes has little effect on muscle glycogen synthesis after exercise but has a pronounced and unexpected effect on hepatic glycogen metabolism.

In contrast to the results obtained in several studies performed on fed animals (12, 24, 33, 41), diabetes does not affect the extent to which muscle glycogen is replenished in fasted animals recovering from high-intensity exercise. During recovery, glycogen levels in the white, red, and mixed gastrocnemius muscles from control and diabetic rats increased to near-preexercise levels despite the absence of food intake. The absence of glycogen deposition in the soleus muscle in control and diabetic animals is consistent with the lack of glycogen mobilization in this muscle during exercise. Although previous studies conducted by us and others (6, 10, 25, 37, 39) showed that, even in the absence of food intake, there is marked glycogen deposition during recovery from high-intensity exercise in muscles from nondiabetic humans and rats, the present study is the first one to have examined whether diabetes affects this process.

![Graph](https://example.com/graph1.png)

**Fig. 3.** Effects of high-intensity exercise to exhaustion and subsequent recovery on levels of hepatic glycogen in diabetic and control rats. Values shown represent means ± SE (n = 9/time point). Glycogen content is expressed in μmol of glucosyl units/g wet wt. a, P < 0.05 vs. Rest; b, P < 0.05 vs. corresponding control animals (ANOVA followed by a Fisher PLSD a posteriori F-test).

![Graph](https://example.com/graph2.png)

**Fig. 4.** Effects of high-intensity exercise to exhaustion and subsequent recovery on levels of hepatic glycogen in postabsorptive control rats. Values shown represent means ± SE (n = 9/time point). Glycogen content is expressed in μmol of glucosyl units/g wet wt. a, P < 0.05 vs. Rest (ANOVA followed by a Fisher PLSD a posteriori F-test).
The accumulation of glycogen in muscles despite the absence of food intake indicates that the carbon precursors for glycogen resynthesis must be endogenous in origin. Glucose derived from hepatic glycogen is unlikely to participate in the resynthesis of muscle glycogen stores, since recovery was not accompanied by a fall in hepatic glycogen levels under any of the conditions examined (Fig. 3). The accompanying decrease in muscle and plasma lactate levels during recovery is consistent with a precursor-product relationship between lactate and muscle glycogen, suggesting that lactate is probably an important carbon source for muscle glycogen replenishment. Glycerol derived from triglyceride hydrolysis and the gluconeogenic amino acids derived from protein breakdown may also have contributed to some extent to glycogen resynthesis (18, 19), but their relative contributions and that of lactate remain to be established.

The lack of any effect of diabetes on glycogen resynthesis needs to be reconciled with the fact that STZ-induced diabetes in rats is known to be accompanied by a fall in muscle glucose transport capacity due to decreased GLUT-4 levels (17, 26). Assuming that lactate is the main carbon source for muscle glycogen resynthesis, two pathways may be considered as possible routes for the conversion of lactate into muscle glycogen, namely, hepatic gluconeogenesis and muscle lactate glycogenogenesis (39). Hepatic gluconeogenesis has the capacity to convert lactate into glucose, which, after its release into the blood, may then be stored as muscle glycogen. The second pathway, muscle lactate glycogenogenesis, operates exclusively in muscles rich in either fast-twitch red or fast-twitch white fibers (39) and provides an intramuscular route for the conversion of lactate into glycogen. Although hepatic gluconeogenesis plays only a minor role in the postexercise replenishment of muscle glycogen in lower vertebrates, there is still some debate as to the relative contributions of hepatic gluconeogenesis and muscle lactate glycogenogenesis to the postexercise muscle glycogen resynthesis in humans and rats (39). In contrast to processes such as glucose transport and glycogen synthesis in muscle and hepatic gluconeogenesis, the rate of lactate glycogenogenesis in muscle is not affected by insulin (9, 36). Thus this pathway is likely not to be affected by diabetes, and were lactate glycogenogenesis to play the major role in the conversion of lactate into muscle glycogen, as suggested recently in rats (37, 39), this would explain why the low plasma insulin-to-glucagon ratios typical of STZ-diabetic rats (1) appear not to affect glycogen synthesis during recovery from high-intensity exercise (Fig. 1). Alternatively, one may argue that, despite the fall in GLUT-4 levels normally associated with STZ-diabetes (17, 26), the exercise-mediated increase in the translocation of GLUT-4 to the sarcolemna, together with the mass action effect of the hyperglycemia associated with diabetes, may be sufficient to support optimal rates of glycogen resynthesis from endogenously derived glucose of hepatic/renal origin. One interesting feature with the former interpretation, however, is that it may explain why glycogen synthesis has been reported to be reduced in fed STZ-diabetic rats recovering from prolonged exercise of moderate intensity (12, 24, 45). Indeed, during recovery from exercise of moderate intensity, muscle lactate glycogenogenesis is known to play a role of marginal importance in the replenishment of the stores of muscle glycogen (23) as opposed to glucose. Because the rate of glucose conversion into muscle glycogen depends in part on plasma insulin levels, the low plasma insulin-to-glucagon ratio typical of STZ-diabetes would thus be expected to inhibit glucose uptake and glycogen synthesis. In summary, the question of which mechanism is responsible for the lack of effect of diabetes on muscle glycogen synthesis in rats recovering from a short bout of high-intensity exercise is one that remains to be answered. A definitive answer to this question awaits the development of experimental methodologies to evaluate the relative contributions of the Cori cycle and lactate glycogenogenesis to the resynthesis of muscle glycogen in the rat (39).

For net glycogen deposition to occur after exercise, glycogen metabolism must undergo a transition from net glycogen breakdown to net glycogen synthesis. At the onset of recovery, the elevated activity ratios of glycogen synthase in the red, white, and mixed gastrocnemius muscles in both the diabetic and control rats (Fig. 2) suggest that an increased proportion of the enzyme is in its dephosphorylated active form. Throughout recovery, the progressive decrease in the activity ratios of glycogen synthase suggests that the phosphorylation state of the enzyme increases gradually until it stabilizes at the point where little or no further glycogen is being actively deposited. This pattern of change is similar to that observed in muscles recovering from tetanic contraction in situ (8, 15, 40) or high-intensity exercise (10) but is different from that in animals recovering from exercise of moderate intensity. In the latter condition, a delay of $\pm$ 2–4 h is required before the activity ratio of glycogen synthase returns to preexercise levels (14, 29, 52). The similarity in the pattern of change in the phosphorylation state of glycogen synthase in the red, white, and mixed gastrocnemius muscles of diabetic and control rats (Fig. 2) is consistent with the absence of marked differences in their respective rates of glycogen synthesis postexercise. In the soleus muscles of the control rats, the transient inactivation of glycogen synthase during recovery is a finding that has already been reported (10). One may argue that, because there is no net glycogen mobilization in the soleus muscle during high-intensity exercise, the inactivation of glycogen synthase at the onset of recovery may be favorable to the preferential channeling of the glucose carbons away from the soleus muscle and toward muscles in need of glucose for glycogen resynthesis. This explanation, however, is difficult to reconcile with the observation that, during recovery, the absence of change in the activity ratios of glycogen synthase in the soleus muscles of diabetic rats (Fig. 2) is not associated with any net glycogen deposition in this muscle (Fig. 1).
One unexpected finding of the present study is that hepatic glycogen levels at rest and in response to high-intensity exercise are markedly affected by diabetes in fasted rats (Fig. 3). Although it has been reported that liver glycogen levels in fed animals are lower in diabetes (20, 37), the present study indicates that hepatic glycogen levels in diabetic rats after a 24-h fast are much higher than in controls (Fig. 3), a finding consistent with those of others (21, 38). These results suggest, therefore, that fasting in diabetic rats results in a much smaller fall in hepatic glycogen levels, despite the reported lower plasma insulin-to-glucagon ratios in STZ-diabetic rats (1), a hormonal environment normally favorable to hepatic glycogenolysis. As discussed in more detail below, one may argue that the hyperglycemia resulting from low plasma insulin-to-glucagon ratios may in turn impair hepatic glycogen mobilization due in part to glucose-mediated stimulation of glycogen synthase and inhibition of glycogen phosphorylase (42–44).

Another major difference between diabetic and nondiabetic animals is that, in response to exercise, hepatic glycogen levels in control animals decreased only marginally and remained stable at low levels during recovery, a finding attributable in part to the low preexercise glycogen concentrations in fasted control rats. In contrast, high-intensity exercise in diabetic rats was accompanied by a marked fall in hepatic glycogen levels, which returned to preexercise levels during recovery (Fig. 3). This resynthesis of hepatic glycogen is all the more surprising, considering that the animals did not ingest any food during recovery. Moreover, these findings differ also not only from those obtained in postabsorptive nondiabetic rats, where hepatic glycogen levels remained at stable levels during recovery (Fig. 4), but also from those in fed diabetic rats, where it has been reported that there is no hepatic glycogen repletion for the initial 2 h after moderate-intensity exercise (30).

The identity of the carbon sources mobilized for the resynthesis of the stores of hepatic glycogen poses an interesting problem, because most of the resynthesis of hepatic glycogen occurred between 30 and 120 min after exercise, a period during which lactate levels had already returned to basal. Clearly, carbon sources other than lactate must be mobilized for the de novo synthesis of hepatic glycogen. Extracellular glucose is a possible candidate, because the fall in plasma glucose levels between 30 and 120 min (Table 2) coincides with the rise in hepatic glycogen levels (Fig. 3). Glycerol derived from triglyceride hydrolysis and gluconeogenic amino acids derived from protein breakdown may also contribute to some extent to glycogen resynthesis after their conversion to glucose via hepatic/renal gluconeogenesis, but their relative contributions remain to be established. It is important to stress that the rate of hepatic gluconeogenesis required to replenish liver glycogen stores (~26 μmol·g wet wt⁻¹·h⁻¹) is well within the liver’s reported gluconeogenic capacity (50).

The ability of livers from STZ-diabetic rats to replenish their stores of glycogen even in the absence of food intake, in contrast to what is observed in 24-h-fasted and postabsorptive rats, raises the intriguing question of the mechanism responsible for the activation of glycogen synthesis, particularly considering that the low plasma insulin-to-glucagon ratios typical of STZ-induced diabetes in rats would be expected to stimulate hepatic glycogenolysis. On the basis of the work of Stalmans and colleagues (42–44), one may propose that the high plasma glycogen levels postexercise in diabetic rats may result in an increase in glucose binding to glycogen phosphorylase, a process that would be predicted to increase its susceptibility to dephosphorylation by protein phosphatase (44). This fall in the proportion of phosphorylated glycogen phosphorylase, an inhibitor of protein phosphatase, could allow protein phosphatase to dephosphorylate and activate glycogen synthase (42, 43) and thus result in net glycogen accumulation (35). Moreover, the glucose-mediated increase in glucose 6-phosphate levels would also be expected to promote the activation of glycogen synthase by protein phosphatase (11, 35). It is also possible that the translocation of glucokinase and glycogen synthase plays a major role also in the glucose-mediated stimulation of glycogen synthesis. Recent studies have indicated that glucokinase is translocated from a bound to a free state in response to glucose (2, 4) and that glucose incorporation rates into glycogen are positively correlated with levels of unbound glucokinase (3). Similarly, high blood glucose levels result in the translocation of glycogen synthase to the plasma membrane (20, 49). On the basis of these observations, one may propose that the glucose-mediated inhibition of glycogen phosphorylase and activation of glycogen synthase after high-intensity exercise in diabetes might counteract to some extent the stimulatory effects of low insulin-to-glucagon ratios on glycogenolysis and facilitate higher net carbon flux between glucose and glycogen by decreasing substrate cycling between glycogen and glucose 1-phosphate (16, 35). This, together with the expected activation of hepatic gluconeogenesis by low plasma insulin-to-glucagon ratios, might also facilitate the channeling of carbons derived from hepatic gluconeogenesis toward glycogen synthesis. Clearly, further investigation is required to identify the mechanism(s) responsible for the activation of hepatic glycogen synthesis in fasted diabetic animals recovering from high-intensity exercise.

In summary, this study has investigated for the first time the issue of whether mild diabetes in fasted animals affects the synthesis of glycogen when food intake is precluded during recovery from high-intensity exercise. Our findings show that STZ-diabetes does not affect the extent of muscle glycogen repletion from endogenous carbon sources after high-intensity exercise in fasted animals, as opposed to what has been found by many studies on fed diabetic animals (12, 24, 33, 41). Consistent with these findings, the patterns of change in the phosphorylation state of glycogen synthase in muscles are only marginally affected by diabetes. In marked contrast, the response of hepatic glycogen to high-intensity exercise is markedly af-
fected by diabetes, with a marked fall in liver glycogen levels being observed in diabetic rats. Moreover, de-
spite the absence of food intake, hepatic glycogen returns to preexercis levels only in diabetic animals,
while remaining at basal postexercise levels in both the 24-h-fasted rats and the postabsorptive nondiabetic
animals normalized for preexercis liver glycogen con-
tent. Although lactate has the potential to be a major carbon source for the resynthesis of muscle glycogen,
other precursors are most probably involved in the resynthesis of hepatic glycogen.

In conclusion, there is a need to elucidate the mechanism(s) responsible not only for the absence of any
effect of diabetes on muscle glycogen resynthesis after exercise in fasted animals but also, and more impor-
tantly, for the unexpected return of hepatic glycogen to preexercis levels in animals precluded from eating
food during recovery. Moreover, it remains to be seen whether similar patterns of response of muscle and
hepatic glycogen are also shared by severe non-insulin-
treated STZ-diabetic fasted rats as well as by fasted humans with type 1 and 2 diabetes mellitus. It is
noteworthy that the questions addressed by and arising from this study have been overlooked for so long.
This is most probably because, until recently, it has been the generally held view that food intake is essen-
tial for the resynthesis of muscle and liver glycogen posteexercis and that for this reason, there has been no justifica-
tion for investigating the effect of diabetes on glycogen metabolism posteexercis in fasted animals.
The present study not only refutes these views but also opens a whole new line of research that will contribute
further to our understanding of the regulation of car-
bohydrate homeostasis in health and disease.

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