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Effect of impaired glucose uptake on postexercise glycogen repletion in skeletal muscles of insulin-treated streptozotocin-diabetic fasted rats

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Abstract

During recovery from intense exercise performed while fasting, the replenishment of muscle glycogen stores from glucose requires the activation of glucose transport. This study examines if insulin-treated streptozotocin (STZ) diabetes in rats impairs the rate of muscle glucose utilization and glycogen repletion when no food is ingested during recovery from high-intensity exercise. Rats fasted for 24 hours were injected with high doses of STZ (150 mg/kg) to cause severe diabetes, and their glycemia was normalized for 10 days with twice-daily insulin injections. High-intensity exercise in these rats resulted in a marked increase in plasma glucose, which remained higher than preexercise levels thereafter, whereas in control animals, the rise in glycemia was only of a short duration. During recovery, the rates of 2-deoxy-[^{3}H]glucose utilization in muscles rich in fast twitch red fibers (red and mixed gastrocnemius muscles) were much lower in STZ-diabetic than in control rats, but were not affected by diabetes in muscles comprised mainly of fast twitch white fibers (white gastrocnemius muscle). Despite these effects on glucose utilization, STZ diabetes had no inhibitory effect on the rate and extent of glycogen deposition and fractional velocities of glycogen synthase across all muscles. In conclusion, although insulin-treated STZ diabetes in fasted rats inhibits glucose transport rates in fast twitch red muscle fibers post–intense exercise, this has no effect on muscle glycogen repletion either because glucose transport does not control the rate of glycogen synthesis or because of a compensatory increase in the activity of lactate glyconeogenesis in these muscles.

1. Introduction

Glycogen plays an important role in supporting muscle energy demands during exercise of high intensity. Despite its importance, very little glycogen is stored in skeletal muscle and, in theory, a large proportion of it can be depleted within a few minutes of an intense sprint effort. As a result, low muscle glycogen levels can adversely affect ones ability to engage in intense exercise [1,2], thus affecting the ability to elicit flight or fight responses. For this reason, it is not surprising that skeletal muscles across all animal species investigated to date have the capacity to replenish at least part of their glycogen stores even in the absence of food intake [2-4], with the accumulated lactate resulting from this type of exercise being the primary carbon source recruited for glycogen repletion when food is not available.

The postexercise conversion of lactate into muscle glycogen can occur via 2 metabolic pathways, namely muscle lactate glyconeogenesis and the Cori cycle [3,4]. The former pathway takes place exclusively in muscles rich in fast twitch red and white fibers, and has the capacity to convert lactate into glycogen directly [3-5]. The latter pathway involves the participation of gluconeogenic organs such as the liver and kidneys, both of which have the capacity to convert lactate into glucose. This glucose is then released into the blood before being delivered and transported into skeletal muscles where it is stored as glycogen [3,4]. Although muscle lactate glyconeogenesis plays the major role in the postexercise replenishment of muscle glycogen stores in lower vertebrates in the fasted state [3,4], there is compelling evidence that both pathways are involved in mammals, including rats and human beings [3-5], with their involvement in the absence of food intake

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depending to some extent on muscle fiber type and plasma lactate levels [4,5]. Although the relative contributions of these pathways in human beings have been a controversial issue [6-8], recent evidence suggests that glycogen synthesis occurs primarily at the expense of plasma glucose [8].

Given that there are several physiological conditions, including recovery from intense exercise, where the rate of muscle glycogen synthesis from glucose is controlled to a large extent by glucose transport [9-11], this raises the possibility that glucose transport might also play an important role in the control of glycogen synthesis when food is not available during recovery from high-intensity exercise, and that any condition that interfere with its activity could affect glycogen synthesis. Unfortunately, on the basis of the studies performed so far, it is difficult to predict whether diabetes is one of those conditions. Indeed, several studies have investigated the effect of diabetes on muscle glycogen repletion during recovery from moderate intensity exercise in streptozotocin (STZ)-diabetic rats or diabetic individuals fed carbohydrates during recovery, and some [12,13], but not all [14-16], have reported that diabetes impairs glycogen repletion in muscle. Moreover, recently, we have examined the effect of diabetes on glycogen repletion without food intake during recovery from high-intensity exercise in non–insulin-treated STZ-diabetic rats and shown that diabetes has no effect on glycogen resynthesis [17]. It is important to stress, however, that glucose uptake activity was not measured and that none of the animals was treated with insulin in that study [17]. It is therefore possible that the marked hyperglycemia in these animals could have opposed, in part, the inhibitory effect of diabetes per se on glycogen synthesis postexercise because of a glucose-mediated stimulation of glucose transport [18] and the favorable mass action effect of elevated glycemia. Thus, it remains to be established whether insulin-treated diabetes impairs glucose transport and glycogen synthesis when no food is ingested during recovery from high-intensity exercise.

Using insulin-treated fasted STZ-diabetic rats with normal resting blood glucose levels as our experimental model, it was our primary goal, firstly, to determine if glucose uptake rate during recovery from high-intensity exercise is impaired under these conditions, and, secondly, to determine whether this is associated with an impaired replenishment of muscle glycogen stores across all fiber types when food intake is not permitted. This is a difficult issue to examine in human beings because their muscles available for biopsies are made out of a combination of all major fiber types. In contrast, it is much easier to explore this question in the rat because of the ease with which it is to sample muscles rich in any given fiber types. It is this feature that has made the rat an experimental model of choice in studies concerned with the effects of muscle fiber composition on glucose and glycogen metabolism in diabetes [17,19,20].

2. Materials and methods

2.1. Materials

Biochemicals and enzymes were obtained from Boehringer Mannheim, Sydney, NSW, Australia. 2-Deoxy-[^{3}H]glucose and streptozotocin (STZ) were purchased from Amersham International, Burks, UK, and Sapphire biochemicals, NSW, Australia, respectively. Insulin radioimmunoassay kit was obtained from Linco, St Charles, Mo. All other chemicals were of Analar grade or equivalent (BDH, Sydney, Australia). Scintillation cocktail (emulsifier safe) was obtained from Packard Instruments BV, Netherlands.

2.2. Animals

Adult male Wistar rats (250-300 g) were kept at approximately 20°C on a 12-hour light/12-hour dark photoperiod and had unlimited access to water and a standard laboratory chow diet (Glen Forrest Stockfeeders, Glen Forrest, WA 6071, Australia). The animals were divided randomly into 2 groups: control and STZ-diabetic group. Severe diabetes was induced by a single subcutaneous injection of 150 mg/kg body wt of STZ prepared fresh in 0.1 mol/L citrate buffer [21] (pH 4.5), whereas the control rats were injected with citrate buffer only. Blood glucose levels in STZ-diabetic rats were kept as close as possible to levels typical of nondiabetic animals with a twice-daily injection of a slow-acting insulin (Humulin-NPH, Eli Lilly, West Ryde, NSW, Australia) for approximately 10 days before the start of the experiments (STZ-diabetic rats received ~2 units of insulin in the morning and ~4.5 units of insulin in the late afternoon, whereas control animals received a physiological saline). In the rats used for the 2-deoxy-[^{3}H]glucose utilization experiments, an indwelling catheter was inserted into the right jugular vein and exteriorized through the back of the neck as described previously [22]. On the day of the experiments, food was withdrawn at the start of the light cycle (06:00 hour), insulin was injected as per normal, and blood glucose levels were measured before the start of every experiment, and only the STZ-diabetic rats with blood glucose levels close to those of nondiabetic animals were used on that day. All animals were exercised as described below and killed between 1:00 PM and 4:00 PM. The Animal Ethics Committee of the University of Western Australia approved this study.

2.3. Exercise protocol

Because rats are natural swimmers, exercise protocols on the basis of swimming are widely used, the intensity of the exercise being determined by the amount of lead weight attached to the tail [17,23]. 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. As described previously [17,23], the animals were swum for 3 minutes in a 30-cm-diameter plastic tank filled with water

(48 cm deep) at 34°C. One major strength of this exercise protocol is that it has been shown to result in highly reproducible changes in muscle glycogen and lactate levels [17,23]. After exercise, the rats were either killed or allowed to recover individually for 60 minutes in separate cages without access to food. Two groups of nonexercised rats (one STZ diabetic and one nondiabetic) served as nonexercised control groups.

2.4. Tissue sampling and metabolite assay

Rats at rest or at time intervals during the postexercise recovery period were anaesthetized under halothane and the following tissues were sampled: individual muscles (red, white, and mixed gastrocnemius muscles) and blood. The red, white, and mixed gastrocnemius muscles were selected on the basis that (*a*) they contain a high proportion of fast twitch red, fast twitch white, and a mixture of both fiber types, respectively, with the latter reflecting the composition of the hind limb musculature as a whole [24], and (*b*) their glycogen stores are actively recruited during a short bout of high-intensity exercise [17,23].

After removal, each tissue was immediately freeze clamped between aluminum plates precooled in liquid nitrogen and stored at −70°C. Immediately after sampling, the blood was transferred into a heparinized Eppendorf microcentrifuge tube and centrifuged at 720*g* for 5 minutes. After centrifugation, 100 μL of the supernatant was deproteinized in 900 μL of 6% (wt/vol) perchloric acid and centrifuged at 2000*g* for 10 min, whereas the remainder of the plasma was kept for insulin assay and stored at −80°C. After centrifugation, the supernatant was neutralized with 2 mol/L concentration of K_2CO_3 and centrifuged at 2000*g* for 10 minutes. All samples were kept at −80°C until analysis. Metabolites were extracted as described previously [17,23] and stored at −80°C until analysis. Plasma insulin levels were measured using a radioimmunoassay kit (Linco) and glycogen, lactate, and glucose were assayed as described by Bergmeyer [25].

2.5. Glucose utilization rates

In a separate group of rats, experiments were performed to determine the effect of STZ diabetes on the rate of muscle glucose utilization (defined here as the rate at which glucose is delivered, transported, and subsequently phosphorylated by hexokinase). The experimental approach adopted to evaluate average glucose utilization rates by individual muscles was on the basis of an established tracer technique described in more details in an earlier report from this and other laboratories [19,20,22,26]. Briefly, cannulated rats either at rest or immediately after high-intensity swimming were administered an intravenous bolus of 50 μCi 2-deoxy-[^{3}H]glucose (Amersham) in 0.9% NaCl and the line washed with saline. Several blood samples were then taken at time intervals [22] for the next 30 minutes. Immediately after the removal of the last blood sample, the rats were injected via the cannula with sodium pentobarbitone to induce rapid anesthesia, and their red, white, and mixed gastrocnemius muscles were removed, freeze clamped using aluminum tongs precooled in liquid nitrogen, and stored at −80°C until analyzed.

Plasma samples for the determination of 2-deoxy-[^{3}H]glucose radioactivity were deproteinized in $Ba(OH)_2$/$ZnSO_4$ [20] and centrifuged, and an aliquot of the supernatant was counted by liquid scintillation counting. The rate of transport and subsequent phosphorylation of 2-deoxy-[^{3}H]glucose into 2-deoxy-[^{3}H]glucose 6-phosphate was determined as described previously [22,27]. Briefly, frozen muscles were ground under liquid nitrogen, and a portion of the powder was homogenized with 6% $HClO_4$, whereas another portion was homogenized with $Ba(OH)_2$/$ZnSO_4$. After centrifugation, aliquots of the $HClO_4$ and $Ba(OH)_2$/$ZnSO_4$ supernatants were counted by liquid

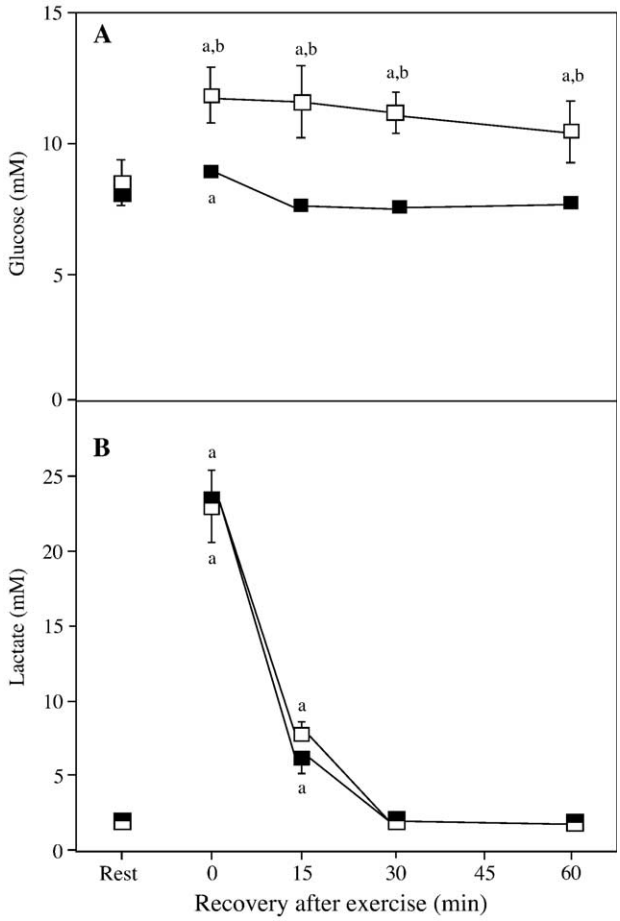

Fig. 1. Effect of high-intensity exercise and subsequent recovery on the levels of plasma glucose (A) and lactate (B) in control (closed squares) and STZ-diabetic rats (open squares). The values shown represent means ± SEM (n = 9). Glucose levels are expressed in mmol/L. [a]Significant difference from rest levels within the same treatment group ($P < .05$). [b]Significant differences ($P < .05$) with the corresponding control animals at the same point (ANOVA followed by Fisher least significant difference [LSD] test).

scintillation counting. As recommended [27], this procedure was chosen instead of separation by anion-exchange chromatography so as to avoid the underestimation of glucose utilization [28]. The radioactivity incorporated as a result of the transport and phosphorylation of 2-deoxy-[^{3}H]glucose was calculated as the difference between the radioactivity in the 6% $HClO_4$,where both 2-deoxy-[^{3}H]glucose and 2-deoxy-[^{3}H]glucose 6-phosphate are found, and $Ba(OH)_2/ZnSO_4$ supernatants, which contain only 2-deoxy-[^{3}H]glucose [26,27].

Both the amount of 2-deoxy-[^{3}H]glucose that had been phosphorylated and the rate of plasma 2-deoxy-[^{3}H]glucose disappearance were used for the calculation of the glucose utilization index (Rg′) for each muscle, which is a measure of glucose transport and subsequent phosphorylation in individual muscles. This index is calculated using the following equation: $Rg' = C_p \cdot C_{m*}/\int^{30} C_{p*}(t)dt$ where C_p is the plasma glucose concentration in millimoles per liter, C_{m*} is the accumulation 2-deoxy-[^{3}H]glucose that has been phosphorylated in muscle, and $C_{p*}(t)$ is the plasma 2-deoxy-[^{3}H]glucose radioactivity. The area under the curve made by the time course of 2-deoxy-[^{3}H]glucose disappearance was used in the calculation of Rg′. The derivation of the equation and the assumptions involved in the calculation have been described previously [19,20,26].

2.6. Glycogen synthase

The fractional velocity of glycogen synthase was determined as described in our recent studies [17,23], using the filter paper method of Thomas et al [29]. Muscles previously weighed and ground were homogenized for 30 seconds with 10 vol of glycerol buffer (Tris-HCl 50 mmol/L, pH 7.8, KF 100 mmol/L, EDTA 10 mmol/L, and 60% [vol/vol] glycerol) at −20°C. After the addition of 10 vol of glycerol-free buffer (Tris-HCl 50 mmol/L, pH 7.8 at 25°C, KF 100 mmol/L, EDTA 10 mmol/L), the extracts were rehomogenized for another 30-second period. The homogenates were centrifuged at 2000*g* for 10 minutes and the supernatants diluted 5-fold with glycerol-free buffer before assay to avoid activation of glycogen synthase by endogenous glucose 6-phosphate. The phosphorylation state of glycogen synthase was estimated by measuring its fractional velocity [17,23], an assay that consists in measuring the activity of the enzyme at a subsaturating near-physiological level of UDP-glucose (0.03 mmol/L) in the presence of either low (0.1 mmol/L) or high (5.0 mmol/L) glucose 6-phosphate concentrations. Under these conditions, the reaction rates of glycogen synthase in the presence of either low or high glucose 6-phosphate levels were linear with respect to both the amount of extract used and incubation time.

2.7. Expression of results and statistical analysis

All metabolite concentrations are expressed in micromole per gram wet weight. Results are expressed as means ± SEM. The effect of exercise and postexercise recovery on the levels of metabolites in muscles and plasma were analyzed with analysis of variance (ANOVA) followed by a Fisher

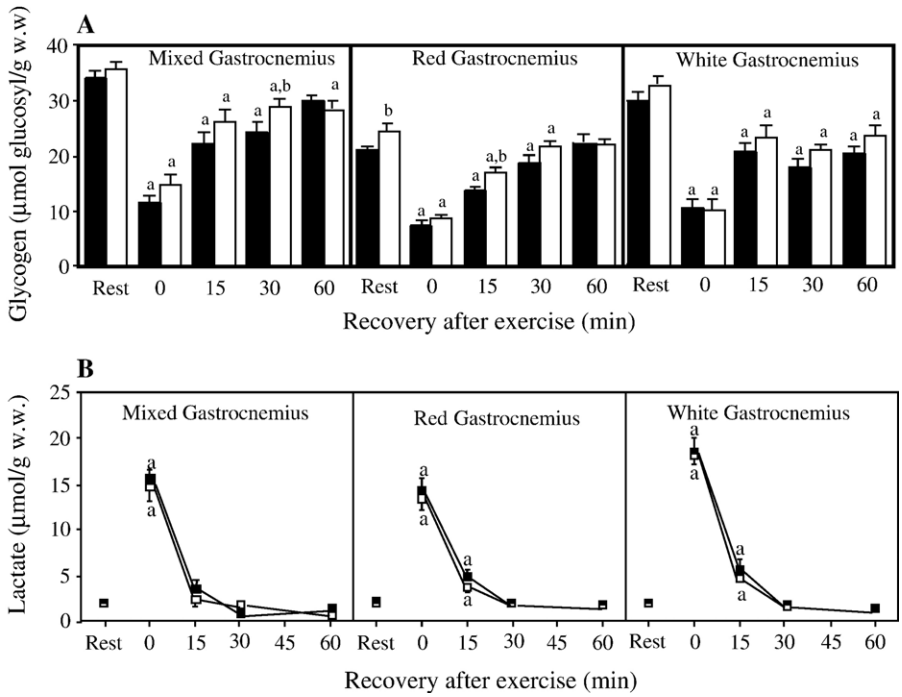

Fig. 2. Effect of high-intensity exercise and subsequent recovery on muscle glycogen (A) and lactate (B) levels in control (closed bars) and STZ-diabetic (open bars) rats. The values shown represent means ± SEM (n = 9). Glycogen contents are expressed as micromole of glucosyl units per gram wet weight and lactate as micromole per gram wet weight. [a]Significant difference from rest levels within the same treatment group ($P < .05$). [b]Significant differences ($P < .05$) with the corresponding control animals at the same point (ANOVA followed by Fisher LSD test).

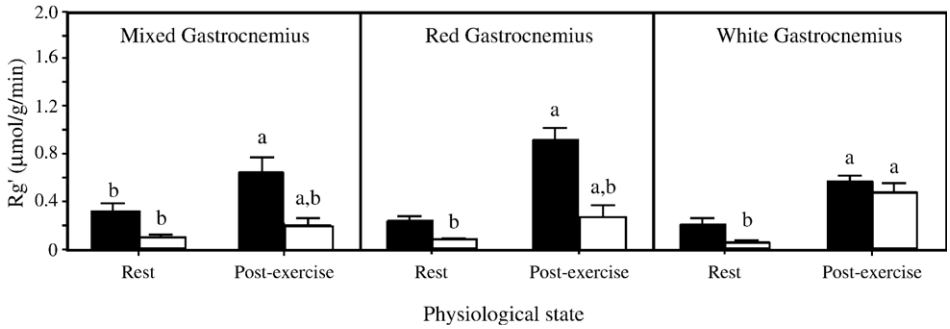

Fig. 3. Effect of recovery from high-intensity exercise on glucose utilization in the mixed, red, and white gastrocnemius muscles in control (closed bars) and STZ-diabetic (open bars) rats. Postexercise refers to muscles sampled at the onset of recovery from exercise. The values shown represent means ± SEM. Glucose utilization is expressed as Rg′ (tissue glucose metabolic index, micromole per gram per minute). [a]Significant difference from rest levels within the same treatment group ($P < .05$). [b]Significant differences ($P < .05$) with the corresponding control animals at the same point (ANOVA followed by Fisher LSD test).

least significant difference (LSD) test using StatView SE + Graphics v1.03 (Abacus Concepts, 1988).

3. Results

3.1. Plasma glucose, insulin, and lactate levels

Before exercise, no difference was observed in plasma glucose levels between control and insulin-treated STZ-diabetic rats (7.9 ± 0.1 vs 8.4 ± 0.9 mmol/L, respectively; Fig. 1A), which was expected because only STZ-diabetic rats with blood glucose levels close to those of nondiabetic rats were used in our experiments to match preexercise glucose levels. Moreover, insulin levels were also similar (241 ± 43 and 282 ± 61 pmol/L for control and diabetic rats, respectively). In response to high-intensity exercise, the increase in plasma glucose levels was much more pronounced in STZ-diabetic as opposed to control animals ($P < .05$; Fig. 1A). During recovery, plasma glucose returned rapidly to preexercise levels in control animals but remained at higher than preexercise levels in the STZ-diabetic rats. High-intensity exercise caused also a marked increase in plasma lactate concentrations (Fig. 1B), which returned to baseline levels during recovery. Plasma lactate response to high-intensity exercise was not affected by diabetes (Fig. 1B).

3.2. Muscle metabolites

Preexercise glycogen levels in the mixed and white gastrocnemius muscles were not significantly affected by STZ diabetes, whereas glycogen levels in the red gastrocnemius muscle were marginally higher in the insulin-treated STZ-diabetic rats ($P < .05$; Fig. 2A). In response to high-intensity exercise, the extent of glycogen breakdown in all muscles examined was not affected by STZ diabetes (Fig. 2A), with substantial glycogen breakdown taking place in these muscles (Fig. 2A). During recovery, a significant replenishment of the stores of glycogen occurred in the red, white, and mixed gastrocnemius muscles, a process that was not affected by STZ diabetes (Fig. 2A). Lactate levels in the red, white, and mixed gastrocnemius muscles increased markedly in response to high-intensity exercise and returned to preexercise levels during the ensuing recovery period (Fig. 2B). The pattern of change in muscle lactate levels was not affected by STZ diabetes.

3.3. Glucose utilization

Before exercise, the rate of 2-deoxy-[^{3}H]glucose utilization in the white gastrocnemius muscle was lower in the STZ-diabetic as opposed to the control rats ($P < .05$; Fig. 3). In response to exercise, the rate of deoxy-[^{3}H]glucose utilization increased in the muscles of diabetic and control

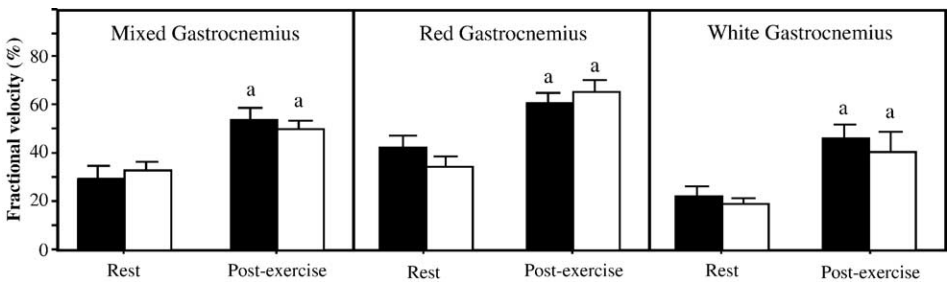

Fig. 4. Effect of recovery from high-intensity exercise on the fractional velocity of glycogen synthase in the mixed, red, and white gastrocnemius muscles in control (closed bars) and STZ-diabetic (open bars) rats. Postexercise refers to muscles sampled at the onset of recovery from exercise. The values shown represent means ± SEM. Fractional velocity is expressed as percentage. [a]Significant difference from rest levels within the same treatment group ($P < .05$). [b]Significant differences ($P < .05$) with the corresponding control animals at the same point (ANOVA followed by Fisher LSD test).

animals. In the red and mixed gastrocnemius muscles, the rates the of 2-deoxy-[^{3}H]glucose utilization attained post-exercise, as well as the magnitude of their increases were much lower in STZ-diabetic animals ($P < .05$), whereas the rates of the rate of 2-deoxy-[^{3}H]glucose utilization were not affected by STZ diabetes in the white gastrocnemius muscle (Fig. 3).

3.4. Glycogen synthase fractional velocity

The fractional velocities of glycogen synthase in the red, white, and mixed gastrocnemius muscles were higher at the onset of recovery than before high-intensity exercise (Fig. 4). Streptozotocin diabetes did not affect the fractional velocities of glycogen synthase in these muscles both at rest and during recovery from high-intensity exercise (Fig. 4).

4. Discussion

During recovery from high-intensity exercise, skeletal muscles have the capacity to replenish their glycogen stores even in the absence of food intake. It is unclear whether insulin-treated diabetes has the capacity to impair the rate of glucose transport and of glycogen synthesis in skeletal muscle when no food is ingested during recovery from high-intensity exercise, and whether the activation of glucose transport is essential for unimpaired glycogen synthesis from glucose to occur. To examine these issues, insulin-treated STZ-diabetic rats with close to normal preexercise plasma glucose levels were exercised at intensities comparable to those of a control group of nondiabetic rats. That the exercise was of comparable intensity in both groups is indicated by the absence of any effect of diabetes on the magnitude of the fall in muscle glycogen levels (Fig. 2A) and rise in plasma and muscle lactate concentrations (Figs. 1B and 2B). Here we show that glycogen repletion and the activation state of glycogen synthase postexercise were not affected by STZ diabetes across all muscles investigated (Figs. 2A and 4) despite the marked impaired stimulation of glucose utilization rates in muscles rich in fast twitch red fibers (Fig. 3). Moreover, as observed in human beings with type I diabetes [30-32], high-intensity exercise in diabetic rats caused a sustained increase in blood glucose levels (Fig. 1A) due, in part, to an impaired exercise-mediated activation of glucose utilization.

The absence of any fall in the postexercise glycogen repletion rates in muscles rich in fast twitch red fibers in STZ-diabetic animals (Fig. 2A) must be reconciled with the finding that the exercise-mediated activation of glucose utilization rates is markedly depressed in these muscles (Fig. 3). Lower rates and extent of glycogen synthesis were expected under these conditions because there are several physiological conditions, such as recovery from high-intensity exercise, where the rate of glucose transport plays a more important role than glycogen synthase in controlling the rate of muscle glycogen synthesis from glucose [9-11]. This raises the question of the mechanisms whereby the rates of glycogen accumulation are unaffected by such low glucose transport activities. Because plasma lactate levels are well above those required for lactate glyconeogenesis to occur at high rates (4 mmol/L) [33], this pathway could be the primary one responsible for the replenishment of muscle glycogen during recovery from high-intensity exercise in rats, and if so, this could explain our findings because insulin is without any effect on muscle lactate glyconeogenesis [33,34]. For this reason, this pathway would be expected to be unaffected by diabetes in marked contrast with the Cori cycle where several of its components are sensitive to insulin (eg, hepatic gluconeogenesis and muscle glucose transport).

One major weakness, however, with the above explanation is that glucose transport most probably plays an important role in the replenishment of muscle glycogen postexercise as suggested by the large increase in muscle glucose utilization rates in control rats recovering from exercise (Fig. 3). For this reason, other mechanisms must be proposed to explain the absence of a fall in the rate of glycogen accumulation despite the reduced stimulation of glucose usage rate. One possibility is that the rate of glucose transport during recovery from a sprint in fasted rats does not control and limit muscle glycogen synthesis from glucose. If this were to be the case, our findings would differ from those of others who have reported that glucose transport plays an important role in the control of glycogen synthesis during recovery from high-intensity exercise [11]. Another possibility is that a compensatory increase in the relative contribution of lactate glyconeogenesis to the replenishment of muscle glycogen in STZ-diabetic animals might explain the absence of any effect of diabetes on muscle glycogen synthesis. In this respect, it is important to stress that lactate glyconeogenic capacity in muscle is high enough to support the high rates of glycogen accumulation reported here [33]. Moreover, because the conversion of glucose into glycogen is known to inhibit muscle lactate glyconeogenesis in vitro [33,34], it is possible that the lower rates of glucose usage in STZ-diabetic rats might result in a lesser inhibition of glycogen synthesis from lactate and thus cause a proportional compensatory increased activity of this pathway in diabetic rats.

To test this latter hypothesis, one would have to compare the rates of lactate glyconeogenesis between control and STZ-diabetic rat, a difficult task to perform in small mammals [6-8]. Indeed, the approach of choice for measuring lactate glyconeogenesis in vivo consists in determining changes in muscle glycogen and lactate levels together with the calculation of the arterial-venous balance of lactate and glucose across the muscle vascular bed [6-8]. Unfortunately, these measurements are much more difficult to perform in rats than in human beings because of their small size, and for this technical reason, a precise estimate of the activity of lactate glyconeogenesis in vivo using this methodology has been performed only in human beings [6-8].

Consistent with the postexercise rate of glycogen deposition being similar between STZ-diabetic and control rats,

the exercise-mediated increase in the fractional velocity of glycogen synthase is also not affected by diabetes, irrespective of muscle fiber composition (Fig. 4). A similar lack of effect of STZ diabetes on both the pattern of glycogen repletion and of glycogen synthase activation post–high-intensity exercise has been reported recently in non–insulin-treated mild STZ-diabetic fasted rats [17]. Our findings thus support the view that factors such as glycogen concentration might play a more important role in determining the phosphorylation state of glycogen synthase and rates of glycogen repletion than the rate of glucose transport. It must be stressed, however, that in STZ-diabetic rats fed carbohydrate during recovery from a bout of moderate intensity exercise causing a fall in muscle glycogen levels similar to that observed here [12], there is a lower rate and extent of glycogen synthesis than in control animals [12]. This is probably because the replenishment of muscle glycogen stores from dietary glucose is insulin-sensitive, and muscle lactate glyconeogenesis is not involved with this type of exercise [4,33].

In conclusion, this study shows for the first time that despite its inhibitory effect on glucose usage in fast twitch red muscle fibers, insulin-treated STZ diabetes is without any effect on the rate and extent of glycogen deposition and the phosphorylation state of glycogen synthase in muscle when no food is ingested during recovery from high-intensity exercise. This suggests that either glucose utilization does not control the rate of glycogen synthesis from glucose or that there might be an increased contribution of lactate glyconeogenesis to the replenishment of muscle glycogen under these conditions in STZ diabetes. Arguably, these findings raise several novel questions and challenges, an important one being the need to introduce appropriate methodologies to measure precisely the rate of lactate glyconeogenesis in vivo in the rat. Moreover, there is the obvious question of whether the responses of glucose transport, glycogen synthase, and glycogen synthesis to high-intensity exercise in normal and insulin-treated diabetic human beings are similar to those described here in the rat. Our general understanding of the regulation of carbohydrate metabolism in diabetes would greatly benefit from exploring further the above novel questions.

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