Fiber-specific responses of muscle glycogen repletion in fasted rats physically active during recovery from high intensity physical exertion

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Abbreviated title: Fiber specific muscle glycogen repletion

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ABSTRACT

Mild physical activity performed immediately after a bout of intense exercise in fasting humans results in net glycogen breakdown in their slow oxidative (SO) muscle fibers and glycogen repletion in their fast twitch (FT) fibers. Since several animal species carry a low proportion of SO fibers, it is unclear whether they can also replenish glycogen in their FT fibers under these conditions. Given that most skeletal muscles in rats are poor in SO fibers (<5%), this issue was examined using groups of 24-h fasted Wistar rats (n=10) that swam for 3 min at high intensity with a 10% weight followed by either a 60-min rest (passive recovery, PR) or a 30-min swim with a 0.5% weight (active recovery, AR) preceding a 30-min rest. The 3-min sprint caused 61-79% glycogen fall across the muscles examined, but not in the soleus (SOL). Glycogen repletion during AR without food was similar to PR in the white gastrocnemius (WG), where glycogen increased by 71%, and less than PR in both the red and mixed gastrocnemius (RG, MG). Glycogen fell by 26% during AR in the SOL. Following AR, glycogen increased by 36%, 87%, and 37% in the SOL, RG and MG, respectively, and this was accompanied by the sustained activation of glycogen synthase and inhibition of glycogen phosphorylase in the RG and MG. These results suggest that mammals with a low proportion of SO fibers can also replenish the glycogen stores of their FT fibers under extreme conditions combining physical activity and fasting.

Keywords: glycogen synthesis, regulation, carbohydrate, sprint, and exercise
INTRODUCTION

During a bout of intense physical activity, the stores of muscle glycogen play an important role in supporting muscle energy demands. Unfortunately, a large proportion of these stores is depleted in response to a single intense sprint effort to exhaustion in animals such as fish, amphibians, reptiles and mammals (3,12,20,24,25,26,28,30,41,42). This together with the findings in humans that low muscle glycogen levels can reduce our capacity to engage in a maximal sprint effort (4) suggests that for animals to maintain their capacities to engage in “fight or flight” behaviors, it is important that mechanisms exist for the rapid replenishment of at least part of their muscle glycogen stores, particularly when food is not readily available. In this respect, it is well established that the skeletal muscles of humans and other vertebrates such as fish, amphibians and reptiles display such a capacity when these animals are resting without food during recovery from exercise (21,27,44), a recovery pattern referred to as passive recovery.

Since there are situations where animals recovering from a bout of high intensity physical activity might have to do so while being active rather than at rest, this begs the question of whether mild physical activity performed during recovery from a bout of intense activity, a recovery pattern known as active recovery, is likely to impair muscle glycogen repletion in fasted animals. The effect of active recovery on glycogen repletion after a bout of intense exercise has been examined in humans (5,8,14,15,45) where most studies have reported that active recovery performed at moderate intensity impairs glycogen repletion in skeletal muscles (8,14,15). Interestingly, however, we have recently shown that the pattern of muscle glycogen response to active compared to passive recovery in humans is muscle fiber specific (15). Indeed, active recovery at moderate intensity has no effect on the rates and extent of glycogen synthesis compared to passive recovery in fast twitch (FT) muscle fibers in fasting humans, but not in slow twitch oxidative (SO) fibers where glycogen levels fall during that time (15),
the net effect being the absence of a net increase in total muscle glycogen levels (15). This fall in glycogen concentrations in SO fibers is not surprising given that these are the main fibers recruited during low intensity muscle contraction in humans (51), whereas the rise in glycogen levels in FT muscle fibers is consistent with these fibers being probably near to a rest state during active recovery, since FT fibers are recruited mainly during intense physical activity (51). On the basis of these findings, it has been proposed that the preferential repletion of the glycogen stores in FT fibers under unfavorable conditions combining food absence and active recovery is probably advantageous as it allows these fibers to recover and maintain their capacity to support fight or flight responses.

Given the adaptive value in maintaining adequate glycogen stores in FT muscle fibers, this begs the question of whether mammalian species with a low proportion of SO fibers are also capable of replenishing the glycogen stores of their FT fibers if mildly active and deprived of food after an intense bout of physical activity. This is an important question given that many species of mammals, including rats and mice, carry a very small proportion of SO fibers (<5%; 1,2,37) in comparison to human muscles in whom the proportion of SO fibers across skeletal muscles ranges between 25 and 95% (33). We hypothesized that in mammals with a low proportion of SO fibers, their FOG fibers play a role similar to that of SO muscle fibers in humans, with the glycogen stores in FG fibers being preferentially replenished while being broken down in FOG fibers during active recovery without food. Using the rat as our experimental model, the primary goal of this study was to test this hypothesis.
MATERIALS AND METHODS

Materials

The chemicals were purchased from BDH (British Drug Houses Ltd, Poole, Dorset, UK) and Sigma (St. Louis, MO, U.S.A.). The biochemicals and enzymes were obtained from Boehringer Mannheim (Sydney, NSW, Australia). Dowex 1-X4 resin was purchased from Sigma, and UDP-[U-14C]glucose and [U-14C]glucose 1-phosphate obtained from Amersham International (Little Chalfont, Bucks, U.K.). All chemicals were of analytical grade.

Experimental animals

Sixty Adult male albino Wistar rats (280-320g; n=10 per time point for each treatment) were obtained from the Animal Resource Centre at Murdoch University, Western Australia. Male rats were used in preference to females to avoid the physiological changes associated with the estrous cycle. The rats were kept at approximately 20°C on a 12 h light/12 h dark photoperiod and had unlimited access to water and a standard laboratory chow diet (Glen Forrest Stockfeeders, Glen Forrest, W.A. 6071: 55% digestible carbohydrate, 19% protein, 5% lipid and 21% non-digestible residue by weight). Before experiments, the rats were fasted for 24 h to deplete most of their stores of liver glycogen (11) and to prevent food in the gut from providing carbon precursors for the replenishment of muscle glycogen post-exercise. On the day of the experiment, the animals were exercised and killed between 9.00 and 12.00 h. This study was approved by the Animal Ethics Committee of the University of Western Australia, Western Australia, Australia.

Cannulation

One group of animals was cannulated at least four days before being exercised (n=7). Prior to surgery,
each rat was anaesthetized using a combination of ketamine hydrochloride (90 mg.kg\(^{-1}\) i.p.; Park Davis, Victoria, Australia) and xylazine (6.0 mg.kg\(^{-1}\) i.p.; Bayer, New South Wales, Australia) in conjunction with atropine (0.6 mg.kg\(^{-1}\) i.m.; Astra Pharmaceuticals, New South Wales, Australia). A silastic catheter (Dow Corning, Michigan, U.S.A.) was implanted into the right jugular vein (0.02 mm ID x 0.037 mm OD), and then channeled around the neck and exteriorized through the skin at the vertex of the head. Following surgery, the animals were housed individually and had free access to food and water.

**Exercise protocol**

Rats being natural swimmers, an exercise protocol based on swimming was adopted in this study, the intensity of the exercise being determined by the amount of lead weight attached to the base of the tail (11,18). Previous studies from this laboratory showed that swimming with a lead weight corresponding to 9-10% body mass allows the animal to remain at the water surface while causing rapid and marked reproducible changes in muscle glycogen and lactate levels (11,18,47,48), thus offering a model of choice to investigate the response of muscle glycogen levels to intense physical activity. Another advantage of this exercise protocol over one that uses a treadmill is that a prolonged training period is not required for rats to exercise at near maximal intensity (11,18,47,48). Immediately before swimming, each animal was weighed and a lead weight equivalent to 10% body mass was attached to the base of the tail. Each animal was then placed in a plastic tank (30 cm diameter, 48 cm deep) filled with water at 34°C and forced to swim for 3 min as described previously (18). Upon completion of the 3-min high intensity swim, some animals (n=10) were allowed to rest for either 0 (n=10) or up to 60 min (passive recovery; n=10), whereas other animals were subjected to an active recovery protocol. This protocol was started immediately following the bout of high intensity exercise, and required that each rat swam for another 30 min with a lead weight equivalent to 0.5% of body mass attached to the base of the tail. The period of 30 min for the swim at moderate intensity was chosen on the basis that this corresponds
approximately to the length of time required for muscle and blood lactate to return to near basal levels (18). Following exercise, the rats were either sacrificed (n=10) or allowed to recover in separate grid-bottom cages without access to food for another 30 min (n=10). One group of non-exercised rats served as the pre-exercise group (n=10), and a group of cannulated rats was subjected to the exercise and the recovery protocols described above.

**Tissue and blood sampling**

The study examined different muscles selected on the basis of their fiber compositions. The white, red and mixed gastrocnemius muscles were selected because they are actively recruited during high intensity exercise (47,48) and because the white gastrocnemius is rich in FG fibers (~90%) and poor in FOG and SO fibers (2,39), the red gastrocnemius is rich in FOG fibers and poor in FG fibers (~9%), and the mixed gastrocnemius muscle is rich in FG and FOG, but poor in SO fibers (~5%; 2,39) thus reflecting the composition of the hind limb musculature of the rat as a whole (2,37). In contrast, the soleus muscle was chosen on the basis that it is one of the very few muscles rich in SO fibers and poor in FG and FOG in the rat (2,37,39).

Rats at rest or at time intervals during the post-exercise recovery period were anaesthetized under 4% (v/v) halothane (Macclesfield, Cheshire, UK) as described previously (17), and the following tissues were sampled: individual muscles (soleus, white, red and mixed gastrocnemius muscles) and blood. After removal, each tissue was immediately freeze-clamped between aluminum plates pre-cooled in liquid nitrogen, then wrapped in aluminum foil and stored at -80°C. For the experiments performed on the cannulated rats, blood was sampled (80 µl per sample) before the bout of high intensity exercise and at 0, 5, 10, 20, 30 and 60 min afterwards.
**Measurement of metabolites**

Immediately following removal, the blood was transferred into a heparinized Eppendorf microcentrifuge tube and centrifuged at 720g for 5 min. After centrifugation, some of the plasma was deproteinized in 9 volumes of 6% (w/v) perchloric acid and centrifuged at 2000g for 10 min. Following centrifugation, the supernatant was neutralized with 2 M K₂CO₃ and centrifuged at 2000g for 10 min. All samples were kept at -80°C until analyzed.

Each muscle was weighed and ground using a mortar and pestle kept in liquid nitrogen, special care being taken to prevent the tissues from thawing (36). The powdered tissue was homogenized with 10 volumes of ice-cold 6% (w/v) perchloric acid. A portion of the homogenate was used for the enzymatic determination of glycogen as described in Bergmeyer (6), whereas a 700 µl aliquot was centrifuged at 2000g for 10 min, and the resulting supernatant was removed and kept on ice. The pellet was re-extracted with 350 µl of 6% (w/v) perchloric acid before recentrifugation at 2000g for 10 min. Following centrifugation, the two supernatants were combined, neutralized with 2 M K₂CO₃, and centrifuged before being stored at -80°C until analysis of lactate, glucose and glucose 6-phosphate as described in Bergmeyer (6).

**Indirect determination of the phosphorylation state of glycogen synthase and phosphorylase**

Changes in the phosphorylation state of glycogen synthase were estimated indirectly by measuring its fractional velocity, which is defined as the ratio of the enzyme activity in the presence of low and high levels of its activator, glucose-6-phosphate. Although this approach does not allow one to identify which of the many phosphorylation state of glycogen synthase is responsible for the change in its fractional velocity (16,43,49), particularly those without any kinetic effect on this enzyme, a low fractional velocity of glycogen synthase typically reflects a phosphorylation-mediated inactivation of the
enzyme (35,43). The extraction of glycogen synthase and the determination of its fractional velocity were performed using a modification (35) of the filter paper method of Thomas and colleagues (50) as described in our earlier studies (18,48). Changes in the phosphorylation state of glycogen phosphorylase were estimated by measuring its activity ratio, which is defined as the ratio of the enzyme activity in the absence and presence of excess concentrations of its activator, AMP (23). Under these conditions, the higher the phosphorylation state of glycogen phosphorylase, the higher its activity ratio (23). Muscle extraction and assay conditions for the determination of the activity ratios of glycogen phosphorylase were performed as described previously (11,32).

**Expression of results and statistical analyses**

All metabolite concentrations in tissues and plasma are expressed in µmol·g⁻¹ wet mass and mM, respectively. Glycogen synthase fractional velocities and glycogen phosphorylase activity ratios are expressed as a percentage of maximal activity. Results are expressed as means ± S.E.M. The effects of exercise and post-exercise recovery on the levels of metabolites in muscles and plasma and on enzyme activities were analyzed with a two-way ANOVA followed by a Fisher least significant difference *a posteriori* test with Stat View SE + Graphics version 1.03 (Abacus Concepts, Berkeley, CA, USA, 1988).
RESULTS

**Effects of passive and active recovery from high intensity exercise on muscle glycogen, lactate and glucose 6-phosphate levels.**

High intensity exercise caused marked glycogen breakdown in the mixed, white, and red gastrocnemius muscles, whereas the levels of glycogen remained stable in the soleus muscle (Fig. 1). During the initial 30 min of passive recovery, glycogen levels increased in the mixed, white, and red gastrocnemius muscles, and reached concentrations that did not increase significantly during the subsequent 30-60 min recovery period (Fig. 1). In response to 30 min of active recovery (Fig. 1), the levels of glycogen attained in the white gastrocnemius muscle were not different from those reached after passive recovery, whereas the glycogen levels attained in the mixed and red gastrocnemius muscles were lower in response to active than passive recovery, but higher than those at 0 min of recovery. Following the active recovery period, glycogen levels increased further in the mixed and red gastrocnemius muscles and did not change significantly in the white gastrocnemius muscle. The levels of glycogen attained in all muscles at the end of this 30 min of post-active recovery period were similar to those in the rats subjected only to passive recovery (Fig. 1). In the soleus muscle, glycogen levels decreased only during active recovery and returned to pre-exercise levels within 30 min afterwards (Fig. 1).

High intensity exercise caused a significant increase in the concentrations of lactate in the mixed, white, and red gastrocnemius muscles, as well as in the soleus muscle (Table 1). Lactate in all muscles examined returned to basal levels within 30 min of active recovery and remained low and stable thereafter (Table 1). In plasma, high intensity exercise caused a marked increase in lactate levels, which decreased more rapidly during active than passive recovery over the initial 30-min period of recovery (Fig. 2). Following active recovery, plasma lactate remained at stable basal pre-exercise levels.
In all muscles examined, glucose 6-phosphate concentrations were significantly elevated in response to high intensity exercise, returned to basal pre-exercise levels within 30 min of passive recovery, and remained low and stable afterwards (Fig. 3). During the post-active recovery period, glucose 6-phosphate remained at basal levels in all muscles except for the soleus muscle, and these levels were not significantly different from those after 30 min of passive recovery (Fig. 3). In the soleus muscle, the levels of glucose 6-phosphate measured immediately following active recovery were elevated and similar to those immediately after high intensity exercise, but decreased to pre-exercise levels over the 30-min rest period following active recovery (Fig. 3).

**Effects of passive and active recovery from high intensity exercise on the fractional velocities and activity ratios of glycogen synthase and phosphorylase.**

The onset of recovery from high intensity exercise was accompanied by a significant increase in the fractional velocities of glycogen synthase in the mixed, white, and red gastrocnemius muscles, whereas the fractional velocities of glycogen synthase in the soleus muscle decreased below basal levels (Fig. 4). During passive recovery, the fractional velocities of glycogen synthase in the gastrocnemius muscles decreased to basal levels within 30 min following exercise and did not change significantly afterward. In contrast, throughout the 30-min period after active recovery, the fractional velocities of glycogen synthase in the mixed, white, and red gastrocnemius muscles remained at above basal levels as indicated by the increased fractional velocities at the 30 and 60 min time points (Fig. 4). In the soleus muscle, however, the fractional velocities of glycogen synthase did not change significantly from basal levels during the post-active recovery as indicated by the lack of difference in the fractional velocities between 30 and 60 min of recovery, and the overall pattern of change in glycogen synthase fractional velocity in the soleus muscle was not affected by the protocol of recovery (Fig. 4).
The activity ratios of glycogen phosphorylase decreased significantly in response to high intensity exercise in the mixed, white, and red gastrocnemius muscles (Fig. 5). During passive recovery, the activity ratios of glycogen phosphorylase returned to pre-exercise levels within 30 min in the mixed, white, and red gastrocnemius muscles and did not change significantly afterward (Fig. 5). A different pattern of response was found in the soleus muscle where high intensity exercise and passive recovery were without any effect on the activity ratios of glycogen phosphorylase (Fig. 5). During the post-active recovery period, the activity ratios of glycogen phosphorylase in the soleus and white gastrocnemius muscles did not differ between the 30 and 60 min time points (Fig. 5). In contrast, the activity ratios of glycogen phosphorylase in the mixed and red gastrocnemius muscles were lower than pre-exercise levels at the onset of the post-active recovery period, but increased to pre-exercise levels within 30 min after active recovery (Fig. 5).
DISCUSSION

Moderate intensity exercise performed during recovery from intense physical activity in fasting humans has been reported not to impair glycogen deposition in FT fibers (15) while net glycogen mobilization is taking place in SO fibers. This study examines the issue of whether mammalian species with a low proportion of SO fibers have also such a capacity to replenish the glycogen stores of their FT muscle fibers if active and without food post-exercise. Here we show that, despite most of rat skeletal muscles having a low proportion of SO fibers, the performance of low intensity physical activity during recovery from a bout of intense exercise is without any significant effect on the extent of glycogen repletion in their muscles rich in FG fibers (white gastrocnemius muscle). However, it reduces the extent of glycogen repletion in muscles rich in FOG fibers (red and mixed gastrocnemius muscles) compared to passive recovery, and causes a fall in glycogen levels in the soleus, one of the few rat muscles rich in SO fibers. Furthermore, during the 30-min period following active recovery, glycogen levels increased significantly in muscles rich in FOG fibers (red and mixed gastrocnemius muscles), with the acute regulation of glycogen synthase and phosphorylase probably playing an important role in this latter pattern of glycogen repletion. Overall, these results suggest for the first time that possessing a musculature rich in SO fibers is not a prerequisite for mammals such as rats to replenish the glycogen stores of their FG fibers even under unfavorable conditions combining food absence and active recovery.

During active recovery, FOG muscle fibers in rats might play a role similar to that of SO muscle fibers in humans. Indeed, the reduced accumulation of glycogen in the FOG-rich red gastrocnemius muscles in rats during active compared to passive recovery is consistent with either a subset of those fibers mobilizing glycogen during that time or an increased proportion of the glucose
and lactate metabolized by these fibers being oxidized to support the energy demands of active recovery while FG fibers are accumulating glycogen. During active recovery in fasting humans, it is the SO fibers that break down their glycogen stores and most probably also oxidize lactate (5) while glycogen levels increase in FT fibers (15). It is important, however, to exert caution when comparing those two species, since the relative intensities of the active recovery protocols were not matched between human and rat studies. This raises the possibility that had the intensity of active recovery been higher in humans, maybe glycogen repletion in some of their FT fibers would have also been partial, whereas had the rats here been subjected to an active recovery of lower intensity, this would have probably led to an increased repletion of the glycogen stores in their FOG fibers. Regardless of these considerations, this study suggests that irrespective of their proportions of SO fibers, mammals have the capacity to replenish the glycogen stores of their FT fibers even if they are fasting and moderately active during recovery from an intense bout of physical activity.

Our findings here in rats and recently in humans (15) that glycogen repletion in FG fibers is unaffected by concurrent physical activity of moderate intensity performed in the absence of food raise the important question of whether this is generally the case across animal species. Unfortunately, the effect of active recovery on glycogen repletion without food in species other than rats and humans has only been examined in the rainbow trout (40). In contrast to humans and rats, active recovery after a bout of intense swimming in rainbow trout increases substantially the rate of muscle glycogen repletion compared to passive recovery (40). This finding has been explained on the grounds that post-exercise inactivity in this species is associated with a cortisol-mediated stress that slows down glycogen repletion (40). This distinct effect of active recovery on the pattern of glycogen repletion in the FG fibers of rainbow trout clearly illustrates the risk of generalizing our results in rats and humans to other animal species and shows that AR in some species is preferable to PR for the rapid repletion of muscle glycogen stores in the absence of food.
The regulation of glycogen synthase and phosphorylase by reversible phosphorylation might explain, at least in part, the fiber-specific response of muscle glycogen synthesis after active recovery. Unfortunately, because of the difficulties in sampling rat muscles during exercise, this study could only examine the role of these enzymes following active recovery and during passive recovery. Our results suggest that both the activation of glycogen synthase and inhibition of glycogen phosphorylase are likely to play an important role in enabling muscles rich in FOG fibers (mixed and red gastrocnemius muscles) to synthesize glycogen without food after active recovery. This is supported by the findings that the fractional velocities of glycogen synthase in these muscles remained elevated throughout the post-active recovery period, while the activity ratios of glycogen phosphorylase were at lower than basal levels immediately after active recovery before increasing to pre-exercise levels thereafter (Fig. 5). The importance of lowering the activation state of glycogen phosphorylase is further supported by the observation that, despite the sustained increase in the fractional velocity of glycogen synthase in the FG rich white gastrocnemius muscle following active recovery, the absence of a transient fall in the activity ratios of glycogen phosphorylase was associated with no net glycogen deposition in this muscle (Fig. 4, 5). It is possible, however, that the lack of difference in glycogen levels in the white gastrocnemius muscle as the post-active recovery period progresses is due to a lack of statistical power as suggested by the 60 min glycogen bars appearing to be higher than the 30 min bars. Overall, these findings are consistent with those of earlier studies from our laboratory on the importance of both enzymes in the regulation of glycogen synthesis without food after an intense bout of exercise (11,18,19,48). Arguably, given the many factors that could play a role in controlling the rate of glycogen synthesis, such as the many phosphorylation sites and kinases affecting the activity and intracellular localization of glycogen synthase (16,46,49), glucose transport activation post-intense exercise (18), and the possible increased blood flow to actively recovering muscle, further work is required to elucidate the relative importance
of these factors in the regulation of glycogen synthesis after active recovery in FOG and FG muscle fibers.

The mechanism whereby muscles rich in SO fibers stimulate the replenishment of their glycogen stores after active recovery is probably different from that in muscles rich in FOG fibers. Immediately following an intense sprint, the transient fall in the fractional velocity of glycogen synthase in the soleus muscle suggests that this enzyme experiences a short-duration increase in its phosphorylation state, a pattern of response similar to that reported in previous studies (Fig. 4; 11,18). It has been proposed that this serves to inhibit glycogen synthase in order to favor the channeling of glucose towards the preferential replenishment of the depleted glycogen stores in FT muscle fibers (11), since a bout of high intensity as opposed to moderate intensity exercise has no effect on glycogen levels in the soleus muscle (Fig. 3; 11,18). Given that the fractional velocities of glycogen synthase and activity ratios of phosphorylase did not change significantly after active recovery in the soleus muscle (Fig. 1, 4), the rise in glycogen levels during that time might be the result, at least in part, of the transient increase in glucose 6-phosphate levels in this muscle (Fig. 3). This is not only because glucose 6-phosphate is a potent allosteric activator of glycogen synthase that might also affect its cellular distribution, but also because it is an inhibitor of glycogen phosphorylase (16). There are other physiological conditions where changes in the levels of glucose 6-phosphate have also been reported to play a major role in the acute control of the rates of glycogen synthesis and glucose metabolism in muscle (7,13,34,52). What remains unclear, however, is the extent to which the rise in glucose 6-phosphate levels immediately after active recovery results from prior glycogenolysis during active recovery or stimulation of glucose transport. Finally, it is important to stress that our results do not exclude the possibility that changes in the phosphorylation state of glycogen synthase is involved, since the absence of significant difference in glycogen synthase FV between 30 and 60 min of recovery might be due to a lack of statistical power.
Despite glucose transport being generally involved in the control of muscle glycogen synthesis (7,13,34), its role was not examined here because the potential involvement of muscle lactate glyconeogenesis (MLG) to the repletion of glycogen in FG and FOG fibers makes it difficult to evaluate the importance of glucose transport in vivo during active and passive recovery while lactate levels are elevated. For most physiological situations, such as recovery from moderate intensity exercise or the ingestion of carbohydrates, glycogen repletion involves glucose transport, and not surprisingly glucose transport plays an important role in controlling the rates of glycogen synthesis (7,13,34). In contrast, during recovery from intense exercise resulting in large lactate accumulations, glycogen repletion in FG and FOG fibers is unique in that it can occur independently of glucose transport as a result of the intramuscular conversion of lactate into muscle glycogen via the MLG pathway (27,38). Therefore, under condition of elevated lactate levels, marked changes in glucose transport activity could in theory take place without any effect on the rate of glycogen synthesis. This was illustrated for the first time in one of our recent studies where we showed that insulin-treated streptozotocin-diabetes in fasted rats recovering from a sprint is without any effect on both the activation levels of glycogen synthase and muscle glycogen repletion despite a markedly reduced activation of glucose transport rate (>3-fold lesser activation in some muscles, see ref 19). Although this could be taken as evidence that glucose transport plays little role in controlling the rate of muscle glycogen synthesis under these conditions, glucose transport could still play some role if one assumes that the absence of inhibition of glycogen synthesis in streptozotocin-diabetic rats is due to a compensatory increase in the contribution of MLG (19). Until appropriate methodologies are introduced to measure precisely the contribution of MLG in vivo, any changes in glucose transport activity in FG and FOG fibers recovering from intense contraction while lactate levels are elevated will inform us little about its importance in the regulation of glycogen synthesis. This is in marked contrast to changes in the activity of glycogen synthase, since this enzyme participates in the synthesis of glycogen irrespective of the pathways involved. Nevertheless, the well established observations that muscle contraction activates glucose delivery as
well as glucose transport rate and its sensitivity to insulin in rats (31) are altogether consistent with glucose transport controlling, at least in part, muscle glycogen synthesis during and after active recovery.

In conclusion, our findings and those of others in rainbow trout suggest that irrespective of the proportion of SO fibers in skeletal muscles, animals have the capacity to replenish the glycogen stores of their FT fibers even during active recovery without food. Moreover, muscles rich in FOG fibers can replenish some of their glycogen stores during the post-active recovery period, with the acute regulation of glycogen synthase and phosphorylase probably playing an important role in this process. On the basis of these findings and earlier ones in humans and rainbow trout (15,40), we tentatively propose that the capacity of FT muscle fibers to replenish rapidly their stores of glycogen even under extreme conditions combining fasting and active recovery has the advantage of freeing animals from the constraints of having to rest or ingesting food to rebuild their stores of muscle glycogen to levels sufficient to support the energy demands of their FT fibers for situations eliciting "flight or fight" responses. It remains to be established, however, the extent to which our findings on humans and rats and those of others on rainbow trout extend to other species.

**Perspectives and Significance**

In the literature concerned with the evolution and ecomorphology of muscle structure and function, muscle fiber composition has often been proposed as one of the factors predictive of the trade-off between speed and stamina in animals, with high proportions of FG in high-speed species and higher proportions of oxidative fibers in slower and more aerobic species (e.g. see 9). To the best of our knowledge, our findings raise for the first time the possibility that muscle fiber composition might also be predictive to some extent of the behaviors displayed by animals recovering from fight-or flight responses, with active recovery expected to be preferentially displayed by species with high proportions
of oxidative (SOG, FOG) fibers. It is important to stress, however, that although many species of mammals, birds, reptiles and fish carry a significant proportion of oxidative fibers (1,10,22,29) little is known, with the exception of species such as the rainbow trout or humans, about the extent to which these species not only incorporate active recovery as part of their behavioral repertoire, but also successfully replenish the glycogen stores of their FT fibers under these conditions. With respect to species that carry a low proportion of oxidative fibers, maybe they are contrived to recover passively following fight or flight responses by adopting for instance the use of refuges or crypsis to avoid predation. This raises the intriguing question of whether there is a threshold in the proportion of oxidative fibers for active recovery to be part of an animal’s repertoire of recovery behaviors. Clearly, a better understanding of the interplay between the ecomorphology, physiology, habitat use and behavior of animals differing in their proportion of oxidative fibers is required to explain how those traits might have coevolved together with animals’ patterns of recovery and impacted on their survival and reproductive success.
ACKNOWLEDGMENTS

The support of the Australian Research Council to P.A.F. and T.N.P. is gratefully acknowledged.
References


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**FIGURE LEGENDS**

Fig. 1. Effects of passive and active recovery from high intensity exercise on muscle glycogen levels. Glycogen levels were determined as described in the Materials and Methods section and are expressed in µmol of glucosyl units·g⁻¹ wet mass. Passive recovery (■) consisted of a 60-min rest period post-high intensity exercise, whereas the active recovery (□) protocol involved exercise of moderate intensity for the first 30 min of recovery from high intensity exercise followed by a 30-min rest period. The values are shown as means ± S.E.M. (n = 10). Bars with the same superscript are not significantly different from each other; the lack of a superscript over a bar indicates that this value differs significantly from all others (P < 0.05).

Fig. 2. Effects of passive and active recovery from high intensity exercise on plasma lactate levels. Lactate levels were determined as described in the Materials and Methods section and are expressed in mM. Passive recovery (●) consisted of a 60-min rest period post-high intensity exercise, whereas the active recovery (○) protocol involved exercise of moderate intensity for the first 30 min of recovery from high intensity exercise followed by a 30-min rest period. The values are shown as means ± S.E.M. (n = 7). The superscript ‘a’ indicates the presence of significant differences between active and passive recovery treatments (P < 0.05); the superscript ‘b’ indicates the absence of significant differences between the 30 and 60 min time points (P < 0.05).

Fig. 3. Effects of passive and active recovery from high intensity exercise on muscle glucose 6-phosphate levels. Glucose 6-phosphate levels were determined as described in the Materials and Methods section and are expressed in µmol·g⁻¹ wet mass. Passive recovery (■) consisted of a 60-min rest period post-high intensity exercise, whereas the active recovery (□) protocol involved exercise of moderate intensity for the first 30 min of recovery from high intensity exercise followed by a 30-min rest period. The values are shown
as means ± S.E.M. (n = 10). Bars with the same superscript are not significantly different from each other; different superscripts indicate that the values differ significantly; the lack of a superscript over a bar indicates that this value differs significantly from all others (P < 0.05).

Fig. 4. Effects of passive and active recovery from high intensity exercise on the fractional velocity of glycogen synthase. The fractional velocities of glycogen synthase were determined as described in the Materials and Methods section and are expressed as a percentage of maximal activity. Passive recovery (■) consisted of a 60-min rest period post high intensity exercise, whereas the active recovery (□) protocol involved exercise of moderate intensity for the first 30 min of recovery from high intensity exercise followed by a 30-min rest period. The dotted line joining each 0 and 30 min time points highlights the fact that no measurement of the fractional velocity of glycogen synthase was performed during active recovery and that our graphic interpolation might not be valid. The values are shown as means ± S.E.M. (n = 10). Bars with the same superscript are not significantly different from each other; different superscripts indicate that the values differ significantly; the lack of a superscript over a bar indicates that this value differs significantly from all others (P < 0.05).

Fig. 5. Effects of passive and active recovery from high intensity exercise on the activity ratio of glycogen phosphorylase. The activity ratios of glycogen phosphorylase were determined as described in the Materials and Methods section and are expressed as a percentage of maximal activity. Passive recovery (■) consisted of a 60-min rest period post high intensity exercise, whereas the active recovery (□) protocol involved exercise of moderate intensity for the first 30 min of recovery from high intensity exercise followed by a 30-min rest period. The dotted line joining each 0 and 30 min time points highlights the fact that no measurement of the activity ratio of glycogen phosphorylase was performed during active recovery and that our graphic interpolation might not be valid. The values are shown as means ± S.E.M. (n = 10). Bars with the same superscript are not significantly different from each other; different superscripts indicate that the values
differ significantly; the lack of a superscript over a bar indicates that this value differs significantly from all others (P < 0.05).
Mixed Gastrocnemius

White Gastrocnemius

Red Gastrocnemius

Soleus

Glycogen phosphorylase activity ratio (%)

Post-active recovery

Post-active recovery

Post-active recovery

Post-active recovery

Recovery time (min)

Rest 0 30 60 Rest 0 30 60
TABLE 1. Effect of passive and active recovery from high intensity exercise on the levels of lactate in the white, mixed and red gastrocnemius and soleus muscles.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Rest</th>
<th>Post-intense Exercise</th>
<th>Passive</th>
<th>Recovery (min)</th>
<th>Post-active</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>White gastrocnemius</td>
<td>2.2</td>
<td>17.3</td>
<td>3.5</td>
<td>1.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Mixed gastrocnemius</td>
<td>1.9</td>
<td>11.9</td>
<td>1.9</td>
<td>1.7</td>
<td>2.2</td>
</tr>
<tr>
<td>Red gastrocnemius</td>
<td>1.1</td>
<td>7.6</td>
<td>2.0</td>
<td>1.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Soleus</td>
<td>1.0</td>
<td>7.8</td>
<td>1.7</td>
<td>0.9</td>
<td>1.2</td>
</tr>
</tbody>
</table>

The values are shown as means ± S.E.M. (n = 10). Lactate concentrations are expressed as µmol/g wet weight. Identical superscripts on different values for a given muscle indicate the absence of significant differences. Values with the same superscript (a or b) are not significantly different from each other; different superscripts indicate that the values differ significantly; the lack of a superscript indicates that this value differs significantly from all others (P < 0.05).