The Journal of Experimental Biology 207, 4615-4621
Published by The Company of Biologists 2004
doi:10.1242/jeb.5913

Lactate availability is not the major factor limiting muscle glycogen repletion during recovery from an intense sprint in previously active fasted rats

Ghazala Raja[1], Sally Mills[1], T. Norman Palmer[2] and Paul A. Fournier[1,*]

[1]*School of Human Movement and Exercise Science, University of Western Australia, Crawley, Western Australia, Australia, 6009* and [2]*Department of Biochemistry and Molecular Biology, James Cook University, Townsville, Queensland, Australia, 4811*

*Author for correspondence (e-mail: fournier@cyllene.uwa.edu.au)

Accepted 12 October 2004

Summary

It is not clear whether the amount of accumulated lactate is the main factor limiting muscle glycogen accumulation during recovery from an intense sprint performed by previously active fasted laboratory rats. To address this question, groups of fasted rats swam at moderate intensity for 30 min, each animal with a lead weight equivalent to 0.5% body mass attached to its tail, followed by a 3 min high intensity swim with a 10% lead weight and a recovery period of up to 2 hours afterwards. Moderately intense exercise for 30 min caused a decrease in muscle glycogen levels in the mixed, white and red gastrocnemius and the mixed quadriceps muscles, and a further rapid fall occurred in response to the 3 min sprint effort. During recovery, glycogen increased to comparable or above pre-sprint levels across all muscles, and this occurred to a large extent at the expense of net carbon sources other than lactate, with these carbon sources accounting for at least 36–65% of the glycogen deposited. The sustained dephosphorylation-mediated activation of glycogen synthase, but not the changes in glucose 6-phosphate levels, most probably played an important role in enabling the replenishment of muscle glycogen stores. In conclusion, our findings suggest that the amount of glycogen deposited during recovery from high intensity exercise in fasted animals is not limited by the amount of accumulated lactate.

Key words: carbohydrate, exercise, glycogen, glycogen synthase, muscle, recovery, regulation, rat.

Introduction

Muscle glycogen has been shown nearly four decades ago to play an important role in supporting muscle energy demands during an intense sprint effort in humans (Hultman, 1967). Its importance is such that the depletion of this fuel source can adversely reduce our capacity to engage in high intensity physical activity (Balsom et al., 1999) and as a result impairs one's ability to survive under conditions eliciting 'flight or fight' responses. Fortunately, it is generally the case in humans and across a wide range of animals species (e.g. fish, amphibians, reptiles and other mammal species) that, following an intense sprint effort resulting in a marked depletion of muscle glycogen, skeletal muscles have the capacity to replenish rapidly their glycogen stores even in the absence of food intake (reviewed by Gleeson, 1996; Palmer and Fournier, 1997; Fournier et al., 2002). Under these conditions, lactate (and to a lesser extent the glycolytic intermediates) is an important carbon source for glycogen synthesis, either directly via the intramuscular pathway of lactate glyconeogenesis or indirectly via its hepatic conversion to glucose, with the relative importance of these pathways varying across animal species (Gleeson, 1996; Palmer and Fournier, 1997; Fournier et al., 2002).

Given that, after a sprint, skeletal muscles have the capacity to replenish their glycogen stores even without food intake, this raises the question of what are the factors that limit the extent to which these stores are replenished. Since the lactate accumulated during exercise is a major net carbon source for the synthesis of muscle glycogen, it is generally assumed that the extent of muscle glycogen repletion during recovery depends to some extent on the proportion of accumulated lactate that is converted into glycogen (Gaesser and Brooks, 1984; Gleeson, 1996; Raja et al., 2003). Under conditions where an intense sprint effort is supported primarily by the catabolism of muscle glycogen into lactate, the conversion of all accumulated lactate and glycolytic intermediates into muscle glycogen could result in the complete or near complete replenishment of muscle glycogen stores. This could be the case for the animal species (e.g. fish, amphibians, lizards) known to replenish completely their muscle glycogen stores when recovering from an intense sprint effort (Gratz and Hutchison, 1977; Gleeson, 1982; Milligan and Wood, 1986; Gleeson and Dalessio, 1989; Pagnotta and Milligan, 1991; Fournier and Guderley, 1992; Girard and Milligan, 1992; Scarabello et al., 1992; Gleeson, 1996; Milligan, 1996; Bräu

et al., 1999). In contrast, the partial conversion of lactate into muscle glycogen in other species, such as humans and rats, has been proposed to explain, in part, why their muscle glycogen stores are only partially replenished after a sprint (Hermansen and Vaage, 1977; Astrand et al., 1986; Hatta et al., 1988; Bangsbo et al., 1992; Choi et al., 1994; Nikolovski et al., 1996; Peters et al., 1996; Bangsbo et al., 1997; Ferreira et al., 2001).

If, as predicted above, the amount of accumulated lactate is the primary factor limiting the extent of muscle glycogen repletion post-intense exercise, one would predict that under conditions where a large proportion of muscle glycogen is oxidised by sustained moderate intensity exercise prior to a sprint, only the glycogen converted to lactate (and to a lesser extent to the glycolytic intermediates) during that sprint would be expected to be replenished, thus leading to the partial replenishment of muscle glycogen. Alternatively, skeletal muscles may possess critical minimal levels of glycogen that are protected against sustained depletion, with the extent of muscle glycogen repletion during recovery from an intense sprint effort in fasted animals being determined primarily by the amount of glycogen required to attain those protected levels, irrespective of lactate availability (Raja et al., 2003). Which of the above mentioned factors limits the extent of glycogen repletion post-intense exercise is an important question that remains to be addressed. For this reason, it is the primary objective of this study to adopt the above mentioned rationale and examine whether the level of accumulated lactate is the major factor determining the extent of glycogen repletion during recovery from an intense sprint effort in fasted rats subjected to moderate intensity exercise prior to the sprint.

Materials and methods

Materials

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

Experimental animals

Adult male albino Wistar rats (280–320 g) 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 oestrous cycle. The rats were kept at approximately 20°C on a 12 h:12 h L:D photoperiod and had unlimited access to water and a standard laboratory chow diet (Glen Forrest Stockfeeders, Glen Forrest, Western Australia 6071: 55% digestible carbohydrate, 19% protein, 5% lipid and 21% non-digestible residue by mass). Before experiments, the rats were fasted for 24 h to deplete most of their stores of liver glycogen (Ferreira et al., 2001) and to prevent food present in the gut from providing carbon precursors for the replenishment of muscle glycogen post-exercise. On the day of each experiment, the animals were exercised and killed by cardiac excision between 9.00 and 12.00 h. The study was approved by the Animal Ethics Committee of the University of Western Australia.

Exercise protocol

In order to test the hypothesis that lactate availability does not limit the extent of muscle glycogen repletion post-intense exercise, groups of rats were subjected to a combination of moderate and high intensity exercise. The rationale for adopting such an exercise protocol to test our hypothesis has already been described in the Introduction. 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 (Ferreira et al., 2001; Raja et al., 2003). The advantage of this exercise protocol over one that uses a treadmill is that a prolonged training period is not required for the animal to exercise at near maximal intensity. Another major strength of this exercise protocol is that it results in highly reproducible changes in muscle glycogen and lactate levels (Nikolovski et al., 1996; Ferreira et al., 2001). Prior to exercise, rats were weighed and a lead weight equivalent to 0.5% body mass was attached to the base of the tail of each animal. Each rat was then placed in a plastic tank (30 cm diameter, 48 cm deep) filled with water at 34°C and forced to swim continuously for 30 min. A period of 30 min was chosen to prevent the complete aerobic depletion of the stores of muscle glycogen during exercise. At the end of the 30 min swim, some rats were sacrificed immediately, others had a lead weight equivalent to 9.5% of body mass added to the base of their tails, and these rats were made to swim for 3 min at high intensity as described previously (Ferreira et al., 2001). Previous studies from this laboratory have shown that swimming with this amount of lead weight results in a rapid and marked changes in muscle glycogen and lactate levels (Nikolovski et al., 1996; Ferreira et al., 2001; Raja et al., 2003). Upon completion of the high intensity swim, the rats were either killed immediately or allowed to recover individually in separate cages without access to food for 30, 60 or 120 min. One group of rats served as the non-exercised control group.

Tissue and blood sampling

Rats at rest or at 0, 30, 60 and 120 min during the post-exercise recovery period were anaesthetised under halothane (Ferreira et al., 1998) and the following tissues were sampled: individual muscles (red gastrocnemius, white gastrocnemius, mixed gastrocnemius and mixed quadriceps muscles), blood by cardiac puncture and liver. The white, red and mixed gastrocnemius muscles and mixed quadriceps muscle were selected because they are rich in fast twitch white, fast twitch red fibres and a combination of both fibre types, respectively (Maltin et al., 1989). These muscles were also selected because their glycogen stores have been shown to be replenished from endogenous carbon sources during recovery from high

intensity exercise in fasted rats (Ferreira et al., 2001). After removal, each muscle tissue was immediately freeze-clamped between aluminium plates pre-cooled in liquid nitrogen, then wrapped in aluminium foil and stored at –80°C. Blood was sampled from anaesthetised rats by cardiac puncture using heparinised syringes and processed as described below.

Extraction of blood and tissue metabolites

Immediately following removal from the heart, blood was transferred into an heparinised Eppendorf microcentrifuge tube and centrifuged at 720 ***g*** for 5 min. After centrifugation, 100 µl of the plasma was deproteinised in 900 µl of 6% (w/v) perchloric acid and centrifuged at 2000 ***g*** for 10 min; the remaining plasma was stored at –80°C. Following centrifugation, the supernatant was neutralised with 2 mmol l^{-1} K_2CO_3 and centrifuged at 2000 ***g*** for 10 min. All samples were kept at –80°C until analysis.

Prior to assay, muscles and liver were weighed and ground using a mortar and pestle kept in liquid nitrogen, special care being taken to prevent the tissues from thawing (Lehoux and Fournier, 1999). The powdered tissue was homogenised with 10 volumes of ice-cold 6% (w/v) perchloric acid. A portion of the homogenate was used for the determination of glycogen, whereas a 700 µl sample was centrifuged at 2000 ***g*** for 10 min, and the supernatant removed and kept on ice. The pellet was re-extracted with 350 ml of 6% (w/v) perchloric acid before recentrifugation at 2000 ***g*** for 10 min. Following centrifugation, the two supernatants were combined, neutralised with 2 mmol l^{-1} K_2CO_3, and centrifuged before being stored at –80°C until analysis. Glycogen, lactate, glucose and glucose 6-phosphate were assayed as described by Bergmeyer and Gutmann (Bergmeyer, 1974).

Fractional velocity of glycogen synthase

Changes in the phosphorylation state of glycogen synthase can be 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 (Bräu et al., 1997). Since the phosphorylation of glycogen synthase results in its inactivation when measured in the presence of low levels of glucose 6-phosphate, it follows that the higher the phosphorylation state of this enzyme, the lower its fractional velocity. The determination of the fractional velocity of glycogen synthase was performed as described in recent publications from this laboratory (James et al., 1998; Ferreira et al., 2001), using the filter paper method of Thomas et al. (1968). Briefly, muscles previously weighed and ground were homogenised in the presence of 10 volumes of glycerol buffer [50 mmol l^{-1} Tris-HCl (pH 7.8 at 25°C), 100 mmol l^{-1} KF, 10 mmol l^{-1} EDTA, 60% (v/v) glycerol at –20°C]. After the addition of 10 volumes of glycerol-free buffer [50 mmol l^{-1} Tris-HCl (pH 7.8), 100 mmol l^{-1} KF, 10 mmol l^{-1} EDTA], the extracts were re-homogenised for a further 30 s. The homogenates were centrifuged at 2000 ***g*** for 10 min, and the supernatants further diluted fivefold with glycerol-free buffer before assay. The fractional velocity was determined using an assay that consists of measuring the activity of the enzyme at a sub-saturating near-physiological level of UDP-glucose (0.03 mmol l^{-1}) in the presence of either low (0.1 mmol l^{-1}) or high (5.0 mmol l^{-1}) glucose 6-phosphate concentrations (Bräu et al., 1997; James et al., 1998; Ferreira et al., 2001). Under these conditions, the reaction rates of glycogen synthase in the presence of low or high glucose 6-phosphate levels were linear with respect to both the amount of extract used and incubation time.

Expression of results and statistical analyses

All metabolite concentrations in tissues and plasma are expressed in µmol g^{-1} wet mass and mmol l^{-1}, respectively. Glycogen synthase fractional velocities are expressed as a percentage of maximal activity. Results are expressed as means ± S.E.M. for nine rats. The effects of exercise and post-exercise recovery on metabolite levels and enzyme activities were analysed with a one-way ANOVA followed by Fisher LSD test using Stat View SE + Graphics version 1.03 (Abacus Concepts, Berkeley, CA, USA, ’88).

Results

Effect of a combination of moderate intensity exercise and a sprint on muscle glycogen and metabolite levels during and after exercise

Exercise of moderate intensity for 30 min resulted in the breakdown of glycogen in the mixed, white and red gastrocnemius and the mixed quadriceps muscles (Fig. 1). When an intense 3-min sprint was performed immediately afterwards, glycogen levels decreased further in all muscles examined (Fig. 1). After 120 min of recovery, muscle glycogen returned to levels well above those present at the onset of the sprint in the mixed and red gastrocnemius muscles as well as in the mixed quadriceps muscle, whereas the levels attained in the white gastrocnemius muscle were comparable to those prior to the sprint. Despite this marked replenishment of the stores of muscle glycogen, the levels attained were still significantly lower than pre-exercise basal levels in the white and red gastrocnemius and mixed quadriceps muscles, whereas in the mixed gastrocnemius, glycogen returned to pre-exercise levels (Fig. 1). The levels of glycogen attained after 2 h of recovery from the combination of moderate and high intensity exercise across all muscles were also significantly higher than those measured after 30 min. In response to 30 min of moderate intensity exercise, hepatic glycogen levels decreased from 14.2±2.1 to 4.7±1.1 µmol glucosyl units g^{-1} wet mass, and no further changes occurred in response to the subsequent sprint and during the following recovery period.

Prolonged exercise of moderate intensity resulted only in a small rise in muscle lactate levels (Fig. 2). In response to the sprint performed immediately afterwards, lactate levels increased significantly in all muscles examined (Fig. 2). During recovery, lactate returned to pre-exercise levels within 30 min and remained at low and stable levels thereafter (Fig. 2). In response to moderate intensity exercise, plasma

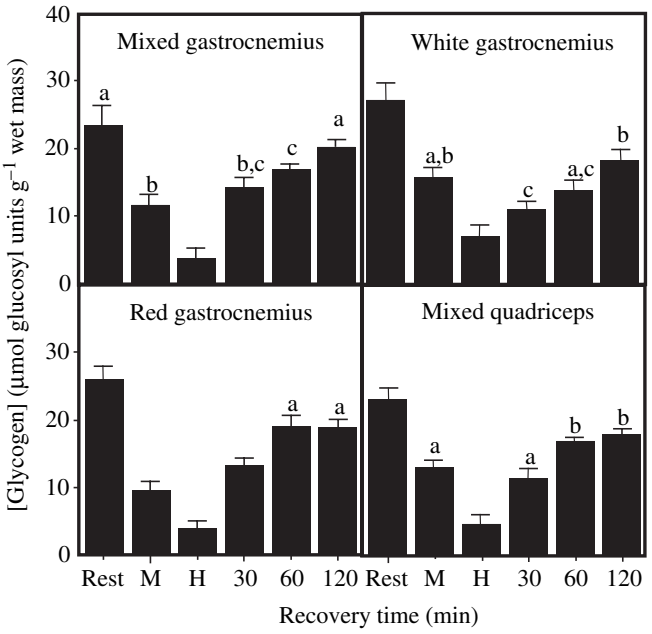

Fig. 1. Effect of a combination of moderate and high intensity exercise on muscle glycogen levels during and after exercise. Glycogen levels were determined as described in the Materials and methods. M and H refer to values found immediately following moderate and high intensity exercise, respectively. The values are shown as means ± S.E.M. (*N*=9). a–c above the bars indicate statistical differences: the same letter indicates no significant differences between the values; different letters indicate that the values differ significantly; no letter indicates that this value differs significantly from all others (ANOVA followed by Fisher PLSD *a posteriori* test; $P<0.05$).

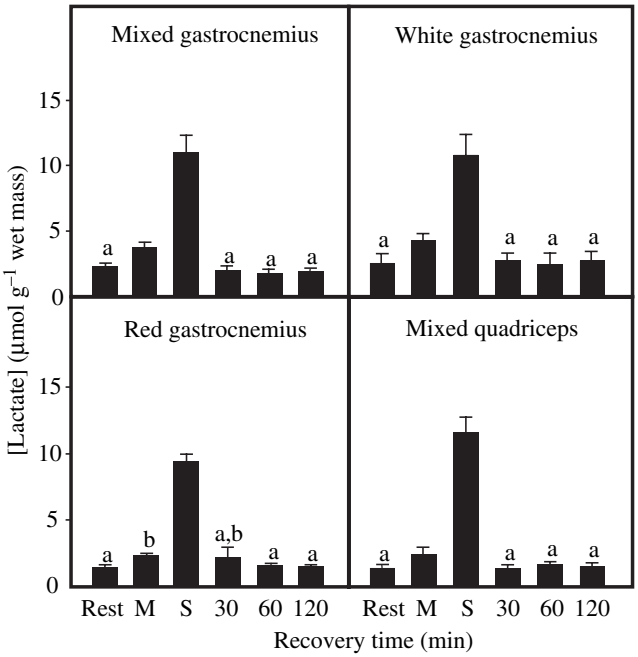

Fig. 2. Effect of a combination of moderate and high intensity exercise on muscle lactate levels during and after exercise. Lactate levels were determined as described in the Materials and methods. M and H refer to values found immediately following moderate and high intensity exercise, respectively. The values are shown as means ± S.E.M. (*N*=9). a,b above the bars indicate statistical differences: the same letter indicates no significant differences between the values; different letters indicate that the values differ significantly; no letter indicates that this value differs significantly from all others (ANOVA followed by Fisher PLSD *a posteriori* test; $P<0.05$).

lactate levels increased from 1.2±0.1 to 2.8±0.7 mmol l^{-1}, and increased further to 16.3±1.8 mmol l^{-1} in response to the subsequent sprint. Within the following 30 min of recovery, plasma lactate levels fell to 1.7±0.3 mmol l^{-1} and remained stable thereafter.

During prolonged exercise of moderate intensity, glucose 6-phosphate remained stable at pre-exercise levels in all muscles (Fig. 3). However, in response to the subsequent intense sprint, glucose 6-phosphate increased above resting levels in all muscles, with the exception of the white gastrocnemius muscle. During recovery, glucose 6-phosphate in all muscles returned to rest levels within 30 min, and remained at low and stable levels thereafter (Fig. 3).

Effects of a combination of moderate and high intensity exercise on the fractional velocity of glycogen synthase during and after exercise

In response to exercise of moderate intensity, the fractional velocities of glycogen synthase increased above rest levels in the mixed, white and red gastrocnemius and the mixed quadriceps muscles (Fig. 4). At the onset of recovery from the subsequent sprint, the fractional velocities of glycogen synthase did not increase further in any of the muscles (Fig. 4). During recovery, the fractional velocities of glycogen synthase remained above basal level in the mixed and white gastrocnemius muscles (Fig. 4). In the red gastrocnemius and mixed quadriceps muscles, the fractional velocities of glycogen synthase returned to basal pre-exercise levels within 60 min of recovery and remained stable throughout the remaining of the recovery period (Fig. 4).

Discussion

It is not clear whether the amount of accumulated lactate is the major factor limiting the extent of muscle glycogen repletion after an intense sprint in fasted laboratory rats. In order to address this question, groups of rats were subjected to 30 min of moderate intensity exercise to mobilize aerobically a large proportion of their muscle glycogen stores. These animals were then subjected to an intense sprint effort, and changes in glycogen levels in the mixed, white and red gastrocnemius and mixed quadriceps muscles were followed throughout recovery. This study shows for the first time that during recovery from a sprint initiated after muscle glycogen stores are already partially depleted by aerobic exercise, the levels of glycogen attained during recovery are not limited by the net amount of lactate available. The extent of glycogen accumulation not only exceeds the amount of glycogen that could be synthesised from the accumulated lactate alone, but

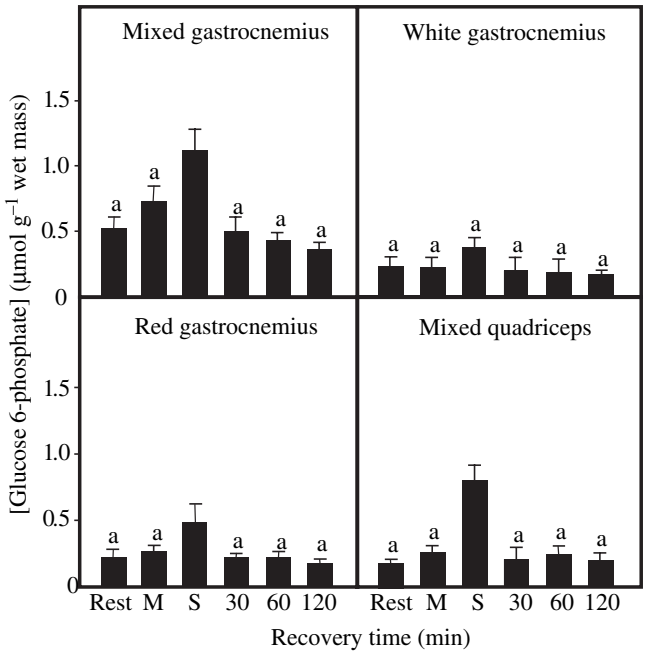

Fig. 3. Effect of a combination of moderate and high intensity exercise on muscle glucose 6-phosphate levels during and after exercise. Glucose 6-phosphate levels were determined as described in Materials and methods. M and H refer to values found immediately following moderate and high intensity exercise, respectively. The values are shown as means ± S.E.M. (*N*=9). The letter a above the bars indicates no significant differences between the values; no letter indicates that this value differs significantly from all others (ANOVA followed by Fisher PLSD *a posteriori* test; $P<0.05$).

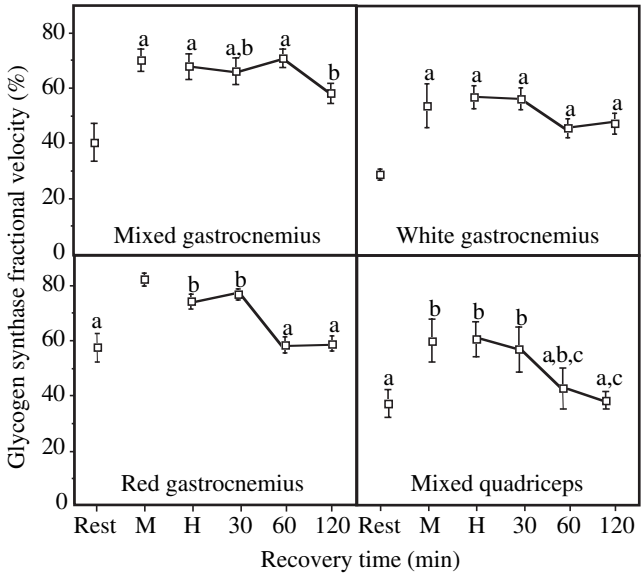

Fig. 4. Effect of a combination of moderate and high intensity exercise on the fractional velocity of glycogen synthase in muscles during and after exercise. Glycogen synthase fractional velocity is the percentage of the enzyme activity in the presence of low and high levels of its activator, glucose-6-phosphate. The fractional velocities of glycogen synthase were determined as described in Materials and methods. M and H refer to values found immediately following moderate and high intensity exercise, respectively. The values are shown as means ± S.E.M. (*N*=9). a–c above the points indicate statistical differences: the same letter indicates no significant differences between the values; different letters indicate that the values differ significantly; no letter indicates that this value differs significantly from all others (ANOVA followed by Fisher PLSD *a posteriori* test; $P<0.05$).

is also consistent with the view that skeletal muscles carry set levels of glycogen that are protected against sustained depletion, with the amount of glycogen required to attain these levels determining the extent of glycogen synthesis during recovery from a sprint. The sustained activation of glycogen synthase probably plays an important role in this process.

Since, in fasted rats, the extent of muscle glycogen repletion following an intense sprint effort is not limited by the amount of accumulated lactate, this raises the issue of the identity and relative contributions of the different carbon sources recruited for glycogen synthesis. During the first 30 min of recovery, a role for lactate is suggested by the fall in plasma and muscle lactate levels coinciding with glycogen synthesis (Figs 1, 2). However, since during the later stages of recovery (30–120 min), glycogen increases further in all of the muscles examined, despite the absence of any change in muscle and plasma lactate levels (Figs 1, 2), this suggests that the synthesis of muscle glycogen during that stage involves net carbon sources other than lactate, such as glycerol and amino acids derived from triglyceride hydrolysis and protein breakdown, respectively. Hepatic glycogen stores, however, are unlikely to play any role in this process given that they remain at stable levels throughout recovery.

Across the different muscles examined in this study, the carbon sources other than lactate (e.g. amino acids, glycerol) are likely to have contributed at the very least 43, 65, 36 and 49% of the total amount of muscle glycogen replenished during the whole recovery period in the mixed, white, and red gastrocnemius and quadriceps muscles, respectively, with the remainder carbon source being lactate. These are highly conservative estimates calculated simply by determining the proportion of glycogen deposited during the 30–120 min recovery period when there is no change in plasma and muscle lactate levels, relative to the total increase in glycogen levels over the whole duration of the recovery period. The underlying assumption on which these calculations are based are that all the glycogen synthesised while lactate levels are falling during early recovery occurs exclusively at the expense of the pool of accumulated lactate, either directly or indirectly via lactate conversion to glucose. This assumption is most probably unrealistic, since carbon sources other than lactate (glycolytic intermediates, glycerol, amino acids) might contribute to glycogen synthesis during the first 30 min of recovery. For instance, the fall in glucose 6-phosphate levels during that time could have contributed to the synthesis of glycogen (Fig. 1). For these reasons, the actual contributions of carbon sources other than lactate to the synthesis of glycogen are likely to be much higher, and that of lactate much lower, than those calculated here. Although the exact relative contributions of all

carbon sources remain to be established, our findings show that lactate availability does not impose an upper limit on the levels of glycogen attained during recovery from high intensity exercise.

Our findings are consistent with the view that skeletal muscles possess critical levels of glycogen that are protected against sustained depletion, and that the extent of muscle glycogen repletion during recovery from an intense effort in fasted animal is determined primarily by the amount of glycogen required to attain those protected levels, irrespective of lactate availability (Raja et al., 2003). This is supported by our observations that the levels of muscle glycogen attained during recovery from a combination of moderate and high intensity exercise (Fig. 1) are comparable to those attained during recovery from a wide range of different protocols of high intensity exercise in fasted rats, such as a single sprint or multiple short sprints (Ferreira et al., 2001; Raja et al., 2003).

It is noteworthy that the levels at which the stores of muscle glycogen are protected against sustained depletion in fasted rats are high enough to support a little more than one bout of an intense sprint effort to exhaustion. Similarly, following high intensity exercise, most animal species in the fasted state (e.g. fish, amphibians, snakes, lizards and humans) also replenish their muscle glycogen stores to levels such that they can engage in at least one bout of intense sprint to exhaustion without being limited by the size of their glycogen stores (Hermansen and Vaage, 1977; Gratz and Hutchison, 1977; Gleeson, 1982; Astrand et al., 1986; Milligan and Wood, 1986; Peters-Futre, 1987; Gleeson, 1989; Scarabello et al., 1992; Fournier and Guderley, 1992; Girard and Milligan, 1993; Choi et al., 1994; Milligan, 1996; Bangsbo et al., 1997). Such a capacity is important because there are extreme circumstances associated with 'flight or fight' behaviour where an animal might have to engage in a sprint to near exhaustion (Raja et al., 2003). Had accumulated lactate been the major determinant of glycogen accumulation in the present study, the levels of muscle glycogen attained post-exercise would have been not only much lower than those reported here, but also probably low enough to impair the capacity of skeletal muscle to engage in subsequent unimpaired fight or flight responses.

The dephosphorylation-mediated activation of glycogen synthase is likely to play some role in enabling muscles to increase their glycogen to levels well in excess of those that would be attained if lactate were limiting glycogen accumulation. Consistent with this view, glycogen synthase in the red gastrocnemius and mixed quadriceps muscles is activated at the onset of recovery while net glycogen synthesis is taking place, but returns to pre-exercise basal activation state when glycogen reaches stable levels (Figs 1, 4). Moreover, the progressive increase in the glycogen content of the mixed and white gastrocnemius muscles throughout the 120 min recovery period is associated with the maintenance of higher than basal fractional velocities of glycogen synthase during that time (Figs 1, 4). It remains to be shown, however, whether an eventual return of the fractional velocity of glycogen synthase to basal levels in these muscles would also coincide with muscle glycogen returning to stable levels as observed for the mixed quadriceps and white gastrocnemius muscles. That this might be the case is suggested from the observation that, during recovery from a sprint performed by previously rested rats, the fractional velocity of glycogen synthase is at its highest at the onset of recovery when glycogen repletion is taking place, and returns to basal levels within only 30 min of recovery in the mixed, white and red gastrocnemius muscles at a time when the stores of muscle glycogen reach stable levels (Ferreira et al., 2001).

Given that there are physiological conditions where both the phosphorylation state and activation state of glycogen synthase are controlled by glucose 6-phosphate levels (Bloch et al., 1994), the question arises of whether a higher than basal level of glucose 6-phosphate contributes to the sustained activation of glycogen synthase and glycogen synthesis during recovery from a combination of moderate and high intensity activity. The elevated levels of glucose 6-phosphate at the onset of recovery in this study might contribute to the initial activation of glycogen synthesis, but the early return of this metabolite to basal pre-exercise levels, despite ongoing glycogen synthesis until late into recovery, suggests that glucose 6-phosphate does not play a major role in the control of glycogen synthesis during late recovery (Fig. 3). This constitutes one of several physiological conditions where changes in glucose 6-phosphate levels do not play a major role in the control of the rate of glycogen synthesis in skeletal muscles (James et al., 1998; Lawrence and Roach, 2000; Fournier et al., 2002).

In conclusion, this study shows that the availability of accumulated lactate is not the major factor limiting glycogen repletion during recovery from an intense sprint effort in fasted rats. Instead, our findings support the view that skeletal muscles possess set levels of glycogen that are protected against sustained depletion, and that the amount of glycogen required to attain these levels determines the extent of glycogen synthesis during recovery from a sprint, irrespective of lactate availability. The sustained dephosphorylation-mediated activation of glycogen synthase, but not glucose 6-phosphate, might play an important role in this process. More research, however, is required to elucidate the nature of the mechanisms that set the levels at which muscle glycogen is protected against sustained depletion and whether these set levels can be altered. Finally, it remains to be established whether it is generally the case across animal species that the amount of accumulated lactate does not limit the level of muscle glycogen attained during recovery from a short sprint effort. The experimental design adopted here might prove helpful in addressing some of these questions.

We would like to thank the Australian Research Council for their financial support to P.A.F. and T.N.P. (ARC Research Grant, AO933 1653).

References

Astrand, O. P., Hultman, E., Dannfelt, A. and Reynolds, G. (1986). Disposal of lactate during and after strenuous exercise in humans. *J. Appl. Physiol.* **61**, 338-343.

Balsom, P. D., Gaitanos, G. C., Soderlund, K. and Ekblom, B. (1999). High-intensity exercise and muscle glycogen availability in humans. *Acta Physiol. Scand.* **165**, 337-345.

Bangsbo, J., Graham, T. E., Johansen, L., Kiens, B. and Saltin, B. (1992). Elevated muscle glycogen and anaerobic energy production during exhaustive exercise in man. *J. Physiol.* **451**, 205-227.

Bangsbo, J., Madsen, K., Kiens, B., and Richter, E. A. (1997). Muscle glycogen synthesis in recovery from intense exercise in humans. *Am. J. Physiol.* **273**, E416-424.

Bergmeyer, H. U. (1974). *Methods of Enzymatic Analysis.* New York: Academic Press.

Bloch, G., Chase, J. R., Meyer, D. B., Avison, M. J., Shulman, G. I. and Shulman, R. G. (1994). In vivo regulation of rat muscle glycogen resynthesis after intense exercise. *Am. J. Physiol.* **266**, E85-E91.

Bräu, L., Ferreira, L. D. M. C. B., Nikolovski, S., Raja, G., Palmer, T. N. and Fournier, P. A. (1997). Regulation of glycogen synthase and phosphorylase during recovery from high-intensity exercise in the rat. *Biochem. J.* **322**, 303-308.

Bräu, L., Nikolovski, S., Palmer, T. N. and Fournier, P. A. (1999). Glycogen repletion following burst activity: A carbohydrate-sparing mechanism in animals adapted to arid environments? *J. Exp. Zool.* **284**, 271-275.

Choi, D., Cole, K. J., Goodpaster, B. H., Fink, W. J. and Costill, D. L. (1994). Effect of passive and active recovery on the resynthesis of muscle glycogen. *Med. Sci. Sports Exerc.* **26**, 992-996.

Ferreira, L. D. M. C. B., Palmer, T. N. and Fournier, P. A. (1998). Prolonged exposure to halothane and associated changes in carbohydrate metabolism in rat muscles in vivo. *J. Appl. Physiol.* **84**, 1470-1474.

Ferreira, L. D. M. C. B., Bräu, L., Nikolovski, S., Raja, G., Palmer, T. N. and Fournier, P. A. (2001). Effect of streptozotocin-induced diabetes on glycogen resynthesis in fasted rats post-high intensity exercise. *Am. J. Physiol.* **280**, E83-E91.

Fournier, P. A. and Guderley, H. (1992). The metabolic fate of lactate after vigorous activity in the leopard frog *Rana pipiens*. *Am. J. Physiol.* **262**, R245-R254.

Fournier, P. A., Bräu, L., Ferreira, L. D. M. C. B., Fairchild, T., Raja, G., James, A. and Palmer, T. N. (2002). Glycogen synthesis in the absence of food ingestion during recovery from moderate or high intensity physical activity: novel insights from rat and human studies (review). *Comp. Biochem. Physiol. A Physiol.* **133**, 755-763.

Gaesser, G. A. and Brooks G. A. (1984). Metabolic bases of excess post-exercise oxygen consumption: a review. *Med. Sci. Sports Exerc.* **16**, 29-43.

Girard, S. S. and Milligan, C. L. (1992). The metabolic fate of blood-born lactate in winter flounder (*Pseudopleuronectes americanus*) during recovery from strenuous exercise. *Physiol. Zool.* **65**, 1114-1134.

Gleeson, T. T. (1982). Lactate and glycogen metabolism during and after exercise in the lizard *Sceloporus occidentalis*. *J. Comp. Physiol.* **147**, 79-84.

Gleeson, T. T. (1996). Post-exercise lactate metabolism: a comparative review of sites, pathways, and regulation. *Ann. Rev. Physiol.* **58**, 565-581.

Gleeson, T. T. and Dalessio, P. M. (1989). Lactate and glycogen metabolism in the lizard *Dipsosaurus dorsalis* following exhaustive exercise. *J. Exp. Biol.* **144**, 377-393.

Gratz, R. K. and Hutchison, V. H. (1977). Energetics for activity in the diamondback water snake, *Natrix rhombifera*. *Physiol. Zool.* **50**, 99-114.

Hatta, M., Atomi, Y., Yamamoto, Y., Shinohara, S. and Yamada, S. (1988). Oxidation of lactate in rats after short-term strenuous exercise. *Int. J. Sports Med.* **9**, 429-432.

Hermansen, L. and Vaage, O. (1977). Lactate disappearance and glycogen synthesis in human muscle after maximal exercise. *Am. J. Physiol.* **233**, E422-E429.

Hultman, E. (1967). Studies on muscle metabolism of glycogen and active phosphate in man with special reference to exercise and diet. *Scand. J. Clin. Lab. Invest.* **19** (suppl. 94), 1-63.

James, A. P., Flynn, C. B., Jones, S. L., Palmer, T. N. and Fournier, P. A. (1998). Re-feeding after starvation involves a temporal shift in the control site of glycogen synthesis in rat muscle. *Biochem. J.* **329**, 341-347.

Lawrence, J. C. and Roach, P. J. (1997). New insights into the role and mechanism of glycogen synthase activation by insulin. *Diabetes* **46**, 541-547.

Lehoux, E. A. and Fournier, P. A. (1999). Liquid N_2 bath for the powdering of tissue. *Anal. Biochem.* **269**, 213-214.

Maltin, C. A., Delday, M. I., Baillie, A. G. S., Grubb, D. A. and Garlick, P. J. (1989). Fiber-type composition of rat muscles. I. Changes during the first year of life. *Am. J. Physiol.* **257**, E823-827.

Milligan, C. L. (1996). Metabolic recovery from exhaustive exercise in fish. *Comp. Biochem. Physiol.* **113B**, 51-60.

Milligan, C. L. and Wood, C. M. (1986). Tissue intracellular acid-base status and the fate of lactate after exhaustive exercise in the rainbow trout. *J. Exp. Biol.* **123**, 123-144.

Nikolovski, S., Faulkner, D. L., Palmer, T. N. and Fournier, P. A. (1996). Muscle glycogen repletion from endogenous carbon sources during recovery from high intensity exercise in the fasted rat. *Acta Physiol. Scand.* **157**, 427-434.

Pagnotta, A. and Milligan, C. L. (1991). The role of blood glucose in the restoration of muscle glycogen during recovery from exhaustive exercise in rainbow trout (*Oncorhynchus mykiss*) and flounder (*Pseudopleuronectes americanus*). *J. Exp. Biol.* **161**, 489-508.

Palmer, T. N. and Fournier, P. A. (1997). Replenishment of muscle glycogen after high-intensity exercise: a role for intramuscular lactate glyconeogenesis? *Biochem. Soc. Trans.* **25**, 25-30.

Peters, T. J., Nikolovski, S., Raja, G. K., Palmer, T. N. and Fournier, P. A. (1996). Ethanol acutely impairs glycogen repletion in skeletal muscle following high intensity short duration exercise in the rat. *Addic. Biol.* **1**, 289-295.

Peters-Futre, E. M., Noakes, T. D., Raine, R. I. and Terblanche, S. E. (1987). Muscle glycogen repletion during active postexercise recovery. *Am. J. Physiol.* **253**, E305-E311.

Raja, G., Bräu, L., Palmer, T. N. and Fournier, P. A. (2003). Repeated bouts of high-intensity exercise and muscle glycogen sparing in the rat. *J. Exp. Biol.* **206**, 2159-2166.

Scarabello, M., Heigenhauser, G. J. F. and Wood, C. M. (1992). Gas exchange, metabolite status and excess post-exercise oxygen consumption after repetitive bouts of exhaustive exercise in juvenile rainbow trout. *J. Exp. Biol.* **167**, 144-169.

Thomas, J. A., Schlender, K. K. and Larner, J. (1968). A rapid filter paper assay for UDP glucose-glycogen glucosyltransferase, including an improved biosynthesis of UDP-^{14}C glucose. *Anal. Biochem.* **25**, 486-499.