Regulation of glycogen synthase and phosphorylase during recovery from high-intensity exercise in the rat

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The aim of this study was to determine the role of the phosphorylation state of glycogen synthase and glycogen phosphorylase in the regulation of muscle glycogen repletion in fasted animals recovering from high-intensity exercise. Groups of rats were swum to exhaustion and allowed to recover for up to 120 min without access to food. Swimming to exhaustion caused substantial glycogen breakdown and lactate accumulation in the red, white and mixed gastrocnemius muscles, whereas the glycogen content in the soleus muscle remained stable. During the first 40 min of recovery, significant repletion of glycogen occurred in all muscles examined except the soleus muscle. At the onset of recovery, the activity ratios and fractional velocities of glycogen synthase in the red, white and mixed gastrocnemius muscles were higher than basal, but returned to pre-exercise levels within 20 min after exercise. In contrast, after exercise the activity ratios of glycogen phosphorylase in the same muscles were lower than basal, and increased to pre-exercise levels within 20 min. This pattern of changes in glycogen synthase and phosphorylase activities, never reported before, suggests that the integrated regulation of the phosphorylation state of both glycogen synthase and phosphorylase might be involved in the control of glycogen deposition after high-intensity exercise.

INTRODUCTION

During high-intensity exercise the energetic requirements of muscles are met to a major extent by the breakdown of the limited stores of glycogen. Because the depletion of muscle glycogen can adversely affect exercise performance, high carbohydrate intake immediately after exercise is generally acknowledged to promote the rapid replenishment of muscle glycogen and the restoration of exercise performance capacity [1]. In the absence of food intake, there is still repletion of muscle glycogen post-exercise, albeit to a lesser extent. Under these conditions the carbon sources for glycogen resynthesis are endogenous in origin and include glycogen-derived glucose from the liver, amino acids, glycerol and lactate. The relative contribution of these precursors depends on the intensity of the exercise before recovery. For instance, during recovery from prolonged exercise of moderate intensity, which results in only a moderate accumulation of plasma lactate, there is significant glycogen deposition from carbon sources such as amino acids and glycerol [2–6], whereas during recovery from high-intensity exercise associated with massive plasma and muscle lactate accumulation, lactate is the major carbon source [7–11]. The ability of muscles to replenish glycogen from endogenous carbon precursors when food is not readily available is of great adaptive value as this process enables fasted animals to maintain sufficient levels of muscle glycogen for mobilization under conditions eliciting 'fight or flight' responses. Despite the biological importance of this process, little is known about the regulation of glycogen synthesis during recovery from exercise.

Irrespective of whether or not food is ingested after exercise, muscle glycogen metabolism must undergo a transition from a catabolic (glycogen breakdown) to an anabolic (glycogen synthesis) state for net glycogen resynthesis to occur during recovery from high-intensity exercise. Studies on animals recovering from exercise of moderate intensity suggest that the regulation of glycogen synthase might play an important role in this transition [12–18]. Among the mechanisms that modulate the activity of glycogen synthase, covalent regulation via reversible phosphorylation is believed to be of critical importance because the activation of glycogen synthase via a decrease in its phosphorylation state is associated with an increased rate of glycogen synthesis in animals recovering from prolonged exercise of moderate intensity [12–18]. Whether a similar pattern of response occurs in fasted animals recovering from exercise of near-maximal intensity remains to be established.

In theory, for optimal rates of glycogen synthesis to occur, not only must there be an increase in the activity of glycogen synthase but the activity of glycogen phosphorylase would also be expected to decrease. Otherwise marked substrate cycling would take place between glycogen and glucose 1-phosphate. Surprisingly, the role of the phosphorylation state of glycogen phosphorylase in the regulation of glycogen synthesis *in vivo* after exercise is an issue that has not been examined so far. The aim of this study is therefore to determine the role of reversible protein phosphorylation of both glycogen synthase and glycogen phosphorylase in the regulation of muscle glycogen repletion in fasted animals recovering from high-intensity exercise.

MATERIALS AND METHODS

Materials

Biochemicals and enzymes were obtained from Boehringer Mannheim (Sydney, NSW, Australia). Dowex 1-X4 resin was purchased from Sigma (St. Louis, MO, U.S.A.). UDP-[U-^{14}C]glucose and [U-^{14}C]glucose 1-phosphate were obtained from Amersham International (Little Chalfont, Bucks., U.K.). All chemicals were of analytical grade.

Animals

Adult male albino Wistar rats (250–300 g) were kept at approx. 20 °C on a 12 h light/12 h dark photoperiod and had unlimited

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access to water and a standard laboratory chow diet: 55% digestible carbohydrate, 19% protein, 5% lipid and 21% non-digestible residue by weight (Glen Forrest Stockfeeders, Glen Forrest, WA 6071, Australia). Before experiments, the rats were fasted for 24 h to deplete most of their stores of liver glycogen [19], as this source of glucose could contribute to muscle glycogen repletion after exercise [9]. On the day of the experiment the animals were exercised and killed between 08:00 h and 12:00 h.

Exercise protocol

Because rats are natural swimmers, exercise protocols based on swimming are widely used, the intensity of the exercise being determined by the amount of weight attached to the tail [20]. 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. Swimming took place in a 30 cm diameter plastic tank filled with water (48 cm deep) at 34 °C. To exercise the rats to near exhaustion, the weight was progressively decreased on each occasion by one-third of the original weight as the animals tired, until two-thirds of the original weight had been removed. Exhaustion was defined as that point at which the animal could not remain at the water surface. The period of exercise before exhaustion was similar in all groups of rats (approx. 3 min). After exercise, the rats were either killed or allowed to recover individually in separate cages without access to food for either 10, 20, 40, 60 or 120 min. Animals during recovery were wrapped in towels to maintain stable body temperature (38 °C). One group of non-exercised rats served as the control group.

Tissue sampling

Rats at rest or at time intervals during the post-exercise recovery period were anaesthetized under halothane and the following tissues were sampled in sequence: individual muscles (soleus, red gastrocnemius, white gastrocnemius, mixed gastrocnemius), blood (by cardiac puncture), liver and heart. The length of time that elapsed between the end of swimming and the beginning of anaesthesia was less than 5 s. Only 15–20 s was then required to anaesthetize the animal fully with halothane (the first sampling after exercise was therefore initiated at 0.4 min). In marked contrast, close to 2 min was required to anaesthetize the rats sampled at 10, 20, 30, 60 and 120 min. To take this delay into account, these animals were anaesthetized 2 min before sampling time. After the onset of anaesthesia, only 2 min was required to sample the different tissues. Special care was taken to avoid pulling or stretching the muscles, because measurement of glycogen phosphorylase activity ratio is very sensitive to handling and sampling [21–23]. The red, white and mixed gastrocnemius muscles were selected on the basis that: (1) they are rich in type IIa, type IIb and types IIa and IIb fibres respectively, but poor in slow-twitch type I fibres, thus reflecting the composition of the hindlimb musculature as a whole [24]; (2) actively recruited during high-intensity exercise [11]; and (3) have the capacity for glycogen synthesis both via muscle lactate glyconeogenesis and the Cori cycle [11,25–27]. In contrast, the soleus muscle, which is rich in type I fibres, was chosen on the basis that it (1) is not recruited during burst exercise [11] and (2) has little, if any, capacity for glycogen synthesis via muscle lactate glyconeogenesis [25]. After removal, each tissue was immediately freeze-clamped between aluminium plates precooled in liquid nitrogen. Arterial blood samples were collected into heparinized syringes, transferred into heparinized Eppendorf tubes and centrifuged at 720 ***g*** for 10 min. After centrifugation, the plasma was deproteinized in 9 vol. of 6% (w/v) perchloric acid and centrifuged at 2000 ***g*** for 10 min before being neutralized with 2 M K_2CO_3. All samples were kept at −70 °C until analysis.

Metabolite assays

Each muscle was weighed and ground with a mortar and pestle in liquid nitrogen. The powdered tissue was mixed with 10 vol. of ice-cold 6% (w/v) perchloric acid and homogenized. A portion of the homogenate was used for the determination of glycogen. Another portion was centrifuged at 2000 ***g*** for 10 min and the supernatant removed and kept on ice; the pellet was re-extracted with ice-cold 6% (w/v) perchloric acid before re-centrifugation (2000 ***g*** for 10 min). The supernatants were combined, neutralized with 2 M K_2CO_3 and centrifuged before being used for the assays of metabolites. Glycogen, lactate, glucose and glucose 6-phosphate were assayed as described by Bergmeyer [28].

Glycogen synthase assay

Muscles previously weighed and ground were homogenized for 30 s with 10 vol. of glycerol buffer [50 mM Tris/HCl (pH 7.8)/100 mM KF/10 mM EDTA/60% (v/v) glycerol] at −20 °C. After the addition of 10 vol. of glycerol-free buffer [50 mM Tris/HCl (pH 7.8 at 25 °C)/100 mM KF/10 mM EDTA], the extracts were re-homogenized for a further 30 s. The homogenates were centrifuged at 2000 ***g*** for 10 min and the supernatants further diluted 5-fold with glycerol-free buffer before assay to avoid the activation of glycogen synthase by endogenous glucose 6-phosphate. Glycogen synthase was assayed in either the absence or presence of 5 mM glucose 6-phosphate [29]. The activity ratio of glycogen synthase (with and without glucose 6-phosphate) is commonly used as an index of its phosphorylation state. The phosphorylation state of glycogen synthase was also estimated by measuring its fractional velocity [16], a process that consists of measuring the activity of the enzyme at a sub-saturating near-physiological level of UDP-glucose (0.03 mM) in the presence of either low (0.1 mM) or high (5.0 mM) glucose 6-phosphate concentrations. 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 amounts of extract used and incubation time. It is important to recognize that both indices provide at the very best only a gross picture of the phosphorylation state because the regulation of glycogen synthase is highly complex and involves multiple phosphorylation sites, the phosphorylation of some of which has no kinetic effect [16,30]. It is also important to note that the site of phosphorylation rather than the amount of phosphate incorporated is more important in determining the kinetic effects of phosphorylation [31].

Glycogen phosphorylase assay

Muscles previously weighed and ground were homogenized for 30 s with 10 vol. of glycerol homogenization buffer [50 mM Mes (pH 7.0 at 25 °C)/50 mM KF/10 mM EDTA/10 mM dithiothreitol/60% (v/v) glycerol] at −20 °C, then combined with 30 vol. of glycerol-free buffer [50 mM Mes (pH 7.0 at 25 °C)/50 mM KF/10 mM EDTA] before re-homogenization. The homogenates were centrifuged at 2000 ***g*** for 10 min and the supernatants subjected to two ion-exchange treatments (with Dowex 1-X4 resin) to remove any endogenous AMP before assay [32]. Glycogen phosphorylase was assayed as described in Gilboe et al. [32] in either the absence or the presence of 5 mM

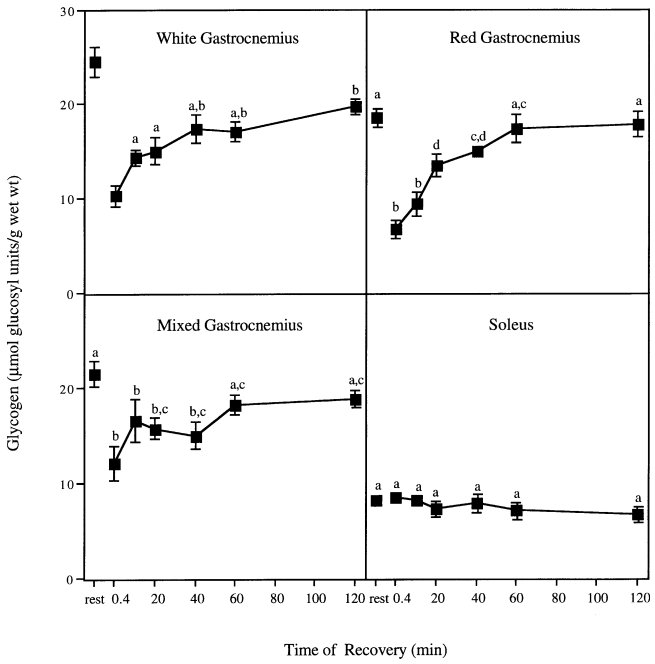

Figure 1 Effect of recovery from high-intensity exercise to exhaustion on levels of glycogen in white, red and mixed gastrocnemius and soleus muscles

Glycogen levels were determined as described in the Materials and methods section. The values are shown as means ± S.E.M. ($n = 8$). Glycogen concentration is expressed in μmol of glycosyl units/g wet wt. Identical superscripts at different time points indicate the absence of significant differences ($P < 0.05$). Points without a superscript differ significantly both between each other and with respect to any point bearing a superscript.

AMP. The activity ratio of glycogen phosphorylase (with and without AMP) is commonly used as an indicator of its phosphorylation state. The reaction rates of glycogen phosphorylase (with and without AMP) were linear with respect to both the amounts of extract and incubation time.

Expression of results and statistical analysis

All metabolite concentrations are expressed in μmol/g wet weight. Glycogen synthase and glycogen phosphorylase activity ratios as well as fractional velocities are expressed as a percentage of maximal activity. Results are expressed as means ± S.E.M. for eight rats. The effects of exercise and post-exercise recovery on the levels of metabolites in muscles and plasma and enzyme activities were analysed with a one-factor ANOVA followed by a Fisher protected least significant difference *a posteriori* test with StatView® SE + Graphics version 1.03 (Abacus Concepts, Berkeley, CA, U.S.A.; 1988).

RESULTS

Muscle glycogen and metabolite levels after exercise

To examine the relation between the phosphorylation states of glycogen synthase and glycogen phosphorylase and glycogen synthesis during recovery from high-intensity exercise, glycogen and other metabolite levels were measured. Burst swimming to exhaustion caused substantial glycogen breakdown in the red, white and mixed gastrocnemius muscles, whereas the glycogen content in the soleus muscle did not change to any significant extent in response to exercise (Figure 1). During the first 40 min of recovery, significant repletion of glycogen occurred in the red, white and mixed gastrocnemius muscles, whereas glycogen levels in the soleus muscle remained stable. During the ensuing 40–120 min recovery period there were no further significant changes in the glycogen contents of all muscles sampled (Figure 1). The pre-exercise level of liver glycogen was 4.91 ± 0.66 μmol of glucosyl units/g wet weight, and no significant change occurred after exercise and during recovery (results not shown).

Concomitant with muscle glycogen breakdown, high-intensity exercise caused a marked increase in lactate concentrations in plasma, soleus, and red, white and mixed gastrocnemius muscles (Table 1). During recovery from high-intensity exercise, the levels of lactate in all muscles and plasma returned to resting pre-exercise levels within 20 min of the cessation of exercise, and remained low and stable for the next 100 min (Table 1). Blood glucose levels remained stable during and after exercise (pre-

Table 1 Effect of recovery from burst exercise to exhaustion on levels of lactate and glucose 6-phosphate (Glc-6-P) in white, red and mixed gastrocnemius and soleus muscles and plasma lactate levels

Metabolite assays were performed as described in the Materials and methods section. The values are shown as means ± S.E.M. ($n = 8$). Metabolite concentrations are expressed as μmol/g wet weight, or mM in plasma. Identical superscripts on different values indicate the absence of significant differences. Values without a superscript differ significantly both between each other and with respect to any value bearing a superscript.

Metabolites	Rest	Time of recovery (min) ... 0.4	10	20	40	60	120
White gastrocnemius							
Lactate (μmol/g)	1.52 ± 0.41[a]	10.73 ± 0.89	3.64 ± 0.75	1.66 ± 0.34[a]	0.91 ± 0.08[a]	1.02 ± 0.14[a]	0.90 ± 0.24[a]
Glc-6-P (μmol/g)	0.27 ± 0.05[a]	0.84 ± 0.31	0.31 ± 0.08[a]	0.38 ± 0.12[a]	0.21 ± 0.03[a]	0.34 ± 0.04[a]	0.35 ± 0.08[a]
Red gastrocnemius							
Lactate (μmol/g)	1.12 ± 0.22[a]	7.39 ± 0.75	2.29 ± 0.34	1.03 ± 0.37[a]	0.91 ± 0.13[a]	1.15 ± 0.18[a]	1.69 ± 0.28[a]
Glc-6-P (μmol/g)	0.17 ± 0.05[a]	0.29 ± 0.05[b]	0.18 ± 0.03[a]	0.16 ± 0.02[a]	0.16 ± 0.02[a]	0.24 ± 0.02[b]	0.17 ± 0.02[a,b]
Mixed gastrocnemius							
Lactate (μmol/g)	2.32 ± 0.43[a]	8.62 ± 1.24	5.47 ± 1.4	12.05 ± 0.26[a]	1.79 ± 0.25[a]	1.51 ± 0.14[a]	2.32 ± 0.35[a]
Glc-6-P (μmol/g)	0.47 ± 0.09[a,b]	0.35 ± 0.09[a,b]	0.53 ± 0.13[b]	0.28 ± 0.05[a,b]	0.43 ± 0.09[a,b]	0.42 ± 0.09[a,b]	0.22 ± 0.07[a]
Soleus							
Lactate (μmol/g)	1.15 ± 0.10[a]	7.78 ± 0.85	2.97 ± 0.34	1.80 ± 0.30[a]	1.17 ± 0.16[a]	1.10 ± 0.09[a]	0.97 ± 0.14[a]
Glc-6-P (μmol/g)	0.12 ± 0.02[a]	0.36 ± 0.05	0.11 ± 0.02[a]	0.09 ± 0.01[a]	0.11 ± 0.02[a]	0.08 ± 0.20[a]	0.09 ± 0.02[a]
Plasma							
Lactate (mM)	0.95 ± 0.11[a]	10.52 ± 1.3	85.88 ± 1.06	1.98 ± 0.57[a]	1.49 ± 0.22[a]	1.76 ± 0.41[a]	2.09 ± 0.77[a]

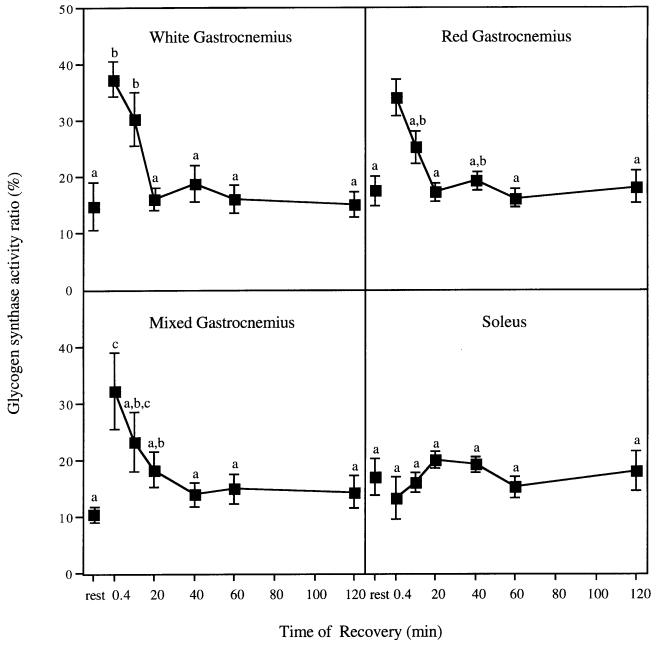

Figure 2 Effect of recovery from high-intensity exercise to exhaustion on glycogen synthase activity ratio in white, red and mixed gastrocnemius and soleus muscles

The activity ratios of glycogen synthase were determined as described in the Materials and methods section. The values are shown as means ± S.E.M. ($n = 8$). Glycogen synthase activity ratio is expressed as a percentage. For the interpretation of statistical significance see the legend to Figure 1.

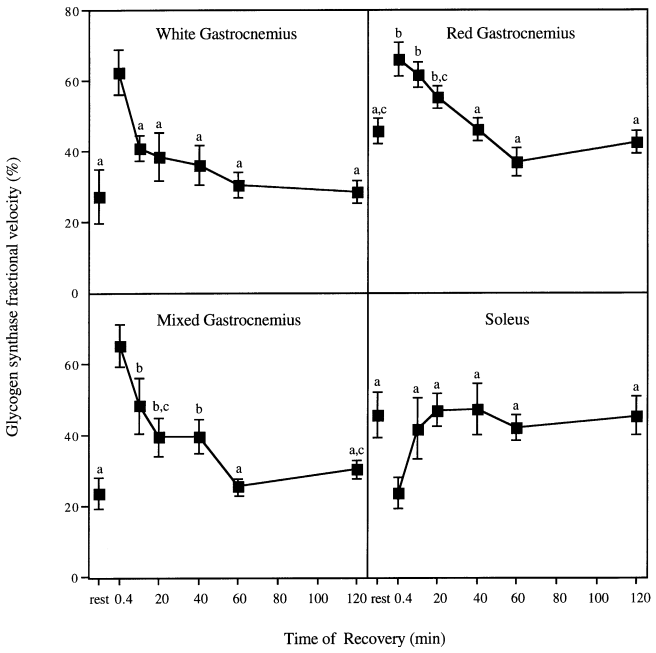

Figure 3 Effect of recovery from high-intensity exercise to exhaustion on glycogen synthase fractional velocity in white, red and mixed gastrocnemius and soleus muscles

The fractional velocities of glycogen synthase were determined as described in the Materials and methods section. The values are shown as means ± S.E.M. ($n = 8$). Glycogen synthase fractional velocity is expressed as a percentage. For the interpretation of statistical significance see the legend to Figure 1.

exercise, 6.53 ± 0.27 mM; immediately after exercise, 6.60 ± 0.50 mM; at 120 min after exercise, 7.30 ± 0.88 mM). Glucose 6-phosphate levels in the soleus and red and white gastrocnemius muscles increased in response to burst swimming (Table 1), but returned to pre-exercise levels within 10 min of recovery in all muscles examined, and remained stable for the remainder of the recovery period (Table 1).

Glycogen synthase and phosphorylase activities after exercise

At the onset of recovery, the activity ratios of glycogen synthase in the red, white and mixed gastrocnemius muscles were higher than those at rest. During the first 20 min after exercise, the activity ratios in these muscles decreased to basal, pre-exercise levels and remained low and stable thereafter (Figure 2). In contrast, exercise had no significant effect on the activity ratio of glycogen synthase in the soleus muscle (Figure 2), a finding consistent with the lack of glycogen mobilization and deposition in this muscle during and after exercise respectively (Figure 1). The pattern of changes in the fractional velocities of glycogen synthase in the red, white and mixed gastrocnemius muscles was similar to that of the activity ratios of glycogen synthase (Figure 3). However, a different pattern was found in the soleus muscle, where the fractional velocities of glycogen synthase were significantly lower than basal immediately after exercise and returned to pre-exercise levels within 10 min of recovery (Figure 3). These findings are in agreement with the view that fractional velocity is a more sensitive tool for the detection of small changes in the phosphorylation state of glycogen synthase [16].

The activity ratios of glycogen phosphorylase in the red, white and mixed gastrocnemius muscles measured immediately after exercise were lower than pre-exercise basal levels (Figure 4).

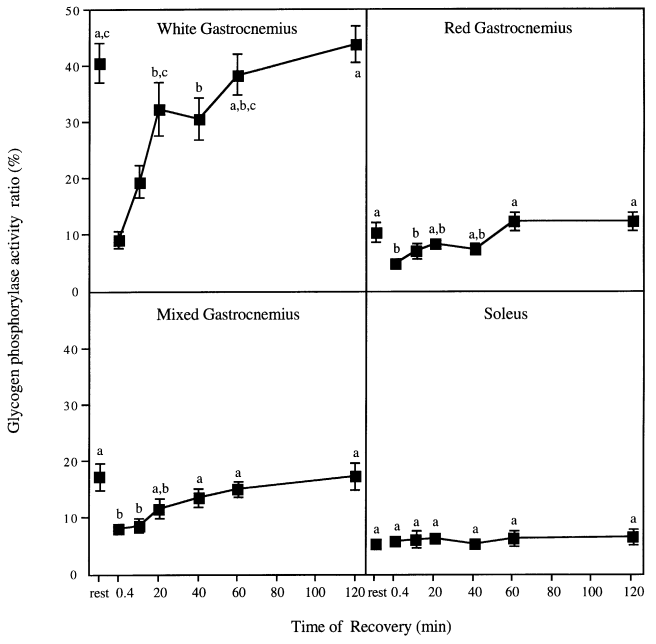

Figure 4 Effect of recovery from high-intensity exercise to exhaustion on glycogen phosphorylase activity ratio in white, red and mixed gastrocnemius and soleus muscles

The activity ratios of glycogen phosphorylase were determined as described in the Materials and methods section. The values are shown as means ± S.E.M. ($n = 8$). Glycogen phosphorylase activity ratio is expressed as a percentage. For the interpretation of statistical significance see the legend to Figure 1.

During the recovery period, the activity ratios of glycogen phosphorylase returned to basal values within 10 min in the red gastrocnemius and 20 min in the white and mixed gastrocnemius muscle (Figure 4), whereas the ratios remained stable in the soleus muscle (Figure 4).

DISCUSSION

Rats resemble other animal species in that, even in the absence of food intake, their stores of muscle glycogen are substantially repleted during the recovery period after high-intensity exercise (Figure 1) [7–11]. For glycogen repletion to occur, the co-ordinated regulation of glycogen synthase and phosphorylase seems to play an important role. The elevated activity ratios and fractional velocities of glycogen synthase in the red, white and mixed gastrocnemius but not in the soleus muscle, where there is no glycogen mobilization, suggest that the phosphorylation state of glycogen synthase at the onset of recovery (Figure 2) is lower than basal and that an increased proportion of the enzyme is in its active dephosphorylated form. During recovery the progressive decrease in the activity ratios and fractional velocities of glycogen synthase suggests that the phosphorylation state of the enzyme increases progressively until it stabilizes at the point when no further glycogen is being deposited (Figure 2). This pattern of change in the phosphorylation state of glycogen synthase is similar to that observed in muscles recovering from tetanic contraction *in situ* [33,34], but different from that of animals recovering from prolonged exercise of moderate intensity, under which conditions a prolonged delay of at least 2–4 h is required for the activity ratio and fractional velocity of glycogen synthase to return to basal levels [14,16,17].

Glucose 6-phosphate, an activator of glycogen synthase and an inhibitor of glycogen phosphorylase, has been shown to be important in controlling the rate of glycogen synthesis after exercise [12]. The elevated levels of this metabolite immediately after exercise should in theory favour glycogen synthesis. However, the observation that the rate of glycogen synthesis changes markedly after glucose 6-phosphate levels return to basal indicates that other mechanisms, including reversible phosphorylation, also play a major role. The relative importance of each of these mechanisms in the regulation of glycogen synthesis remains to be established.

For optimal glycogen deposition to occur our findings support the proposal that it is not only necessary to activate glycogen synthase but also that the proportion of glycogen phosphorylase in the active phosphorylated form must decrease. The changes in the activity state of glycogen phosphorylase suggest that an increased proportion of the enzyme is in the less active dephosphorylated form at the onset of recovery and that the state of phosphorylation increases to basal pre-exercise levels as muscle glycogen is deposited. In contrast, the phosphorylation state of glycogen phosphorylase in the soleus muscle remains low and stable at its pre-exercise level (Figure 4), a finding consistent with the lack of glycogen deposition in this muscle after exercise. Our findings support those of others [22] that the lowest activity ratios at rest are found in muscle rich in type I fibres (soleus), whereas the highest ratios are in muscle rich in type IIb fibres (white gastrocnemius). Whether these differences correspond to different rates of glycogen turnover has not been examined yet. However, it can be proposed that muscles rich in type IIb fibres, which are recruited mainly during high-intensity exercise, maintain an elevated proportion of glycogen phosphorylase in the active phosphorylated state at rest so as to permit the rapid activation of glycogenolysis in response to exercise. At the onset of exercise, the rapid increase in the levels of muscle inorganic phosphate, one of the substrates of glycogen phosphorylase, would be predicted to result in a marked activation of glycogenolysis [35].

The pattern of changes in the phosphorylation state of glycogen phosphorylase in response to exercise has never been reported previously *in vivo* under physiological conditions. In fact, all studies concerned with the regulation of glycogen deposition after exercise *in vivo* have neglected glycogen phosphorylase [12,14–18]. Most of the studies have been concerned with the activation of glycogen phosphorylase during contraction. Earlier works performed either *in situ* [34] or with the perfused hindquarter preparation [21] indicated that continuous tetanic contraction causes a rapid and transient rise in the active phosphorylated form of glycogen phosphorylase, followed by a sharp decline in its phosphorylation state to lower than basal levels despite continuous contraction [21,34]. During recovery from stimulated tetanic contraction, the activity ratio of phosphorylase remains low and stable [34], whereas our results show that during recovery from high-intensity exercise the phosphorylation state of the enzyme rises rapidly after the onset of recovery (Figure 4).

The lower than basal phosphorylation state of glycogen phosphorylase at the onset of recovery from exercise might have important physiological implications. The absence of net glycogen mobilization in muscles under basal resting conditions, despite the presence of a significant proportion of glycogen phosphorylase in its active phosphorylated form, results most probably from the presence of low levels of the activators AMP, IMP and P_i [35,36]. Nevertheless the occurrence of glycogenolysis under these conditions is indicated by the presence of an active substrate cycle between glycogen and glucose 1-phosphate in resting muscles [37]. Because the onset of recovery from high-intensity exercise is typically characterized by elevated levels of inorganic phosphate and IMP, the increased levels of these metabolites would be expected to promote increased glycogenolysis. However, the lower than basal phosphorylation state of glycogen phosphorylase after exercise might be one mechanism that prevents (together with low post-exercise intramuscular pH) marked glycogenolysis and substrate cycling between glycogen and glucose 1-phosphate under conditions of elevated AMP, IMP and P_i levels, the end result being an optimal rate of glycogen deposition.

The lower than basal phosphorylation state of glycogen synthase and phosphorylase at the onset of recovery, as well as the progressive rise in their degree of phosphorylation thereafter, might be explained in part by the pattern of changes in the levels of glycogen, glucose 6-phosphate and H^+ in response to exercise. At the onset of recovery, the low levels of muscle glycogen are expected to (1) result in a decrease in the glycogen-mediated inhibition of both glycogen synthase phosphatase and phosphorylase phosphatase activities [38–40] (however, this proposal should be taken with caution as earlier studies reported that high levels of glycogen activate [41,42] rather than inhibit [40] glycogen phosphorylase phosphatase) and (2) cause a decrease in the glycogen-mediated activation of phosphorylase kinase [43–45], an enzyme that can phosphorylate not only glycogen phosphorylase but also glycogen synthase [46]. The increased levels of glucose 6-phosphate after exercise (Table 1) might also favour a decrease in the state of phosphorylation of glycogen synthase [38,47] and phosphorylase [42,48] because binding of this metabolite to glycogen synthase and phosphorylase enhances their susceptibility to dephosphorylation [38,42,47,48], whereas binding of glucose 6-phosphate to phosphorylase decreases its susceptibility to phosphorylation by phosphorylase kinase [49,50]. Finally, low post-exercise intramuscular pH is also likely to be

conducive to a fall in the state of phosphorylation of glycogen synthase and phosphorylase because phosphorylase kinase is inhibited by elevated H^+ levels [45,51]. Overall, these metabolic changes would be expected to over-ride the increases in the phosphorylation state of glycogen synthase and phosphorylase that would normally be expected to occur as a result of the catecholamine-mediated increase in cAMP and cAMP-dependent protein kinase activity in response to exercise [52].

After the onset of recovery, the progressive rise in muscle glycogen levels and fall in the levels of glucose 6-phosphate and H^+ would be expected to favour a progressive increase in the phosphorylation state of glycogen synthase and phosphorylase via reversal of some or all of the mechanisms described above. Changes in the phosphorylation state of the glycogen-binding G-protein [53], translocation of protein phosphatase I between the cytosol and the protein–glycogen complex [54], as well as a change in the phosphorylation state of inhibitor I, which when phosphorylated is a potent inhibitor of protein phosphatase I activity [55], are other mechanisms that might be involved in the regulation of the phosphorylation of glycogen synthase and phosphorylase. Which of these mechanisms plays a significant role in the regulation of these enzymes in response to physical activity and how these regulatory processes are integrated are important biochemical questions that are yet to be addressed.

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