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Repeated bouts of high-intensity exercise and muscle glycogen sparing in the rat

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Summary

Even in the absence of food intake, several animal species recovering from physical activity of high intensity can replenish completely their muscle glycogen stores. In some species of mammals, such as in rats and humans, glycogen repletion is only partial, thus suggesting that a few consecutive bouts of high-intensity exercise might eventually lead to the sustained depletion of their muscle glycogen. In order to test this prediction, groups of rats with a lead weight of 10% body mass attached to their tails were subjected to either one, two or three bouts of high-intensity swimming, each bout being separated from the next by a 1 h recovery period. Although glycogen repletion after the first bout of exercise was only partial, all the glycogen mobilised in subsequent bouts was completely replenished during the corresponding recovery periods and irrespective of muscle fibre compositions. The impact of repeated bouts of high-intensity exercise on plasma levels of fatty acids, acetoacetate and β-hydroxybutyrate suggests that the metabolic state of the rat prior to the second and third bouts of exercise was different from that before the first bout. In conclusion, rats resemble other vertebrate species in that without food intake there are conditions under which they can replenish completely their muscle glycogen stores from endogenous carbon sources when recovering from high-intensity exercise. It remains to be established, however, whether this capacity is typical of mammals in general.

Key words: exercise, fasting, glycogen, lactate, skeletal muscle, recovery, rat.

Introduction

Over three decades have elapsed since the demonstration that muscle glycogen is one of the major fuels mobilised to support muscle energy demands during physical activity, not only of high but also of moderate intensity (Ivy, 1991). The importance of this fuel is best illustrated by several studies that have reported that the depletion of muscle glycogen stores adversely affects exercise performance of moderate or high intensity (Ivy, 1991; Balsom et al., 1999). Such a fall in an animal's capacity to engage in high-intensity physical activity due to low glycogen stores can have severe consequences as it may impair an animal's ability to survive under conditions eliciting 'fight-or-flight' responses.

Fortunately, even in the absence of food intake, muscles recovering from physical activity have the capacity to replenish some or even all of their glycogen stores (reviewed in Fournier et al., 2002). In particular, during recovery from a maximal sprint effort, muscles replenish at least part of their glycogen stores. Under these conditions, lactate is the most likely carbon source for glycogen synthesis, either directly or indirectly *via* its conversion to glucose (Gleeson, 1996; Palmer and Fournier, 1997; Fournier et al., 2002). This synthesis of muscle glycogen from endogenous carbon sources has been demonstrated not only in humans and rats (Fournier et al., 2002) but also in a variety of other animals including fish (Milligan and Wood, 1986; Pagnotta and Milligan, 1991; Girard and Milligan, 1992; Scarabello et al., 1992; Milligan, 1996), amphibians (Fournier and Guderley, 1992), snakes (Gratz and Hutchison, 1977) and lizards (Gleeson, 1982, 1996; Gleeson and Dalessio, 1989).

Although it is generally the case that animals recovering from high-intensity physical activity can replenish their muscle glycogen stores in the absence of food intake, it is important to note that most lower vertebrates (Gratz and Hutchison, 1977; Gleeson, 1982, 1996; Milligan and Wood, 1986; Gleeson and Dalessio, 1989; Pagnotta and Milligan, 1991; Fournier and Guderley, 1992; Girard and Milligan, 1992; Scarabello et al., 1992; Milligan, 1996) and some mammal species (Bräu et al., 1999) have the capacity to replenish their muscle glycogen stores completely under these conditions whereas the replenishment of muscle glycogen is only partial in other species of mammals, such as humans and rats (Hermansen and Vaage, 1977; Astrand et al., 1986; Bangsbo et al., 1992, 1997; Choi et al., 1994; Nikolovski et al., 1996; Peters et al., 1996; Ferreira et al., 2001). On this basis, one might propose that in those animals where the extent of glycogen repletion is only partial post-exercise, a few consecutive bouts of high-intensity physical activity might

eventually lead to a progressive decrease in the levels of muscle glycogen attained after each consecutive recovery period. Unless mechanisms exist to protect muscle glycogen against sustained depletion, muscle glycogen would be expected to eventually attain levels low enough to impair the ability of these animals to engage in fight-or-flight behaviours (Balsom et al., 1999). Since the rat is one animal species where glycogen repletion post intense exercise is only partial without food intake, the aim of this study was to assess whether, as predicted, repeated consecutive bouts of high-intensity exercise in the rat will eventually result in the sustained depletion of their stores of muscle glycogen or whether mechanisms exist for the sparing of their muscle glycogen stores.

Materials and methods

Materials

Chemicals were purchased from BDH (British Drug Houses Ltd, Poole, Dorset, UK) and Sigma (St Louis, MO, USA). Biochemicals and enzymes were obtained from Boehringer Mannheim (Sydney, NSW, Australia). All chemicals were of analytical grade.

Animals

Adult male albino Wistar rats (*Rattus norvegicus* Berkenhout; 280–320 g) were obtained from the Animal Resource Centre at the University of Murdoch, 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 light:dark photoperiod and had unlimited access to water and a standard laboratory chow diet (Glen Forrest Stockfeeders, Glen Forrest, Western Australia: 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) so as to prevent hepatic glycogen and food present in the gut from providing carbon precursors for the replenishment of muscle glycogen post-exercise. All experiments took place between 8.00 h and 13.00 h. This research project was approved by the Animal Ethics Committee of the University of Western Australia.

Exercise protocol

As rats are natural swimmers, an exercise protocol based on swimming was adopted for this study, the intensity of the exercise being similar for each exercise bout and determined by the amount of lead weight attached to the base of the tail (Ferreira et al., 2001). The 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 and this protocol results in reproducible glycogen mobilisation and lactate accumulation (Nikolovski et al., 1996; Ferreira et al., 2001). Immediately before swimming, each rat was weighed and a lead weight equivalent to 10% body mass was attached to the base of its tail. Each rat swam for 3 min in a plastic tank (30 cm diameter, 48 cm depth) filled with water at 34°C as described previously (Ferreira et al., 2001). With the exception of one group of non-exercised rats, which served as the control group, all animals were subjected to either one, two or three bouts of exercise, each bout being separated from the next one by a recovery period of 60 min. The animals were sacrificed either immediately following each bout of exercise or after each of the associated 60 min recovery periods during which each rat recovered alone in a cage without access to food. Other animals were subjected to only one bout of high-intensity exercise and were allowed to recover individually in separated cages for either 0, 10, 20, 40, 60 or 120 min prior to being sacrificed.

Tissue sampling

The study examined different muscles selected on the basis of their fibre compositions. The white, red and mixed gastrocnemius muscles were selected because they are actively recruited during high-intensity swimming and are rich in fast-twitch white, fast-twitch red and a combination of both fibre types, respectively, but are poor in slow-twitch red fibres, thus reflecting the composition of the hindlimb musculature as a whole (Maltin et al., 1989). By contrast, the soleus muscle, which is rich in slow-twitch red fibre, was chosen on the basis that its glycogen stores are not recruited during high-intensity swimming (Nikolovski et al., 1996; Ferreira et al., 2001).

Rats at rest or at time intervals during the post-exercise recovery period were anaesthetised under halothane as described previously (Ferreira et al., 1998), and their tissues, namely individual muscles (soleus muscle and red, white and mixed gastrocnemius muscles), blood and liver, were sampled. After removal, each 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 and processed as described below.

Extraction of blood and tissue metabolites

Immediately following its removal from the heart, blood was transferred into a heparinised Eppendorf microcentrifuge tube and centrifuged immediately at 720 ***g*** for 5 min. Following 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, while the remaining plasma was stored at –80°C. After centrifugation, the supernatant was neutralised with 2 mol l^{-1} K_2CO_3 and centrifuged at 2000 ***g*** for 10 min. All samples were kept at –80°C until analysed.

Muscles were weighed and ground under liquid nitrogen (Lehoux and Fournier, 1999), and the powdered tissue was homogenised with 10 volumes of ice-cold 6% perchloric acid. A portion of the homogenate was used for the determination of glycogen, while a 700 μl aliquot was centrifuged at 2000 ***g*** for 10 min and the supernatant removed and kept on ice. The pellet was re-extracted with 350 μl of 6% perchloric acid

before re-centrifugation at 2000 ***g*** for 10 min. Following centrifugation, the two supernatants were combined, neutralised with 2 mol l^{-1} K_2CO_3 and centrifuged before being stored at –80°C until analysis of metabolites. The levels of glycogen, lactate, glucose, glucose 6-phosphate, glycerol, β-hydroxybutyrate and acetoacetate were assayed as described by Bergmeyer (1974), and fatty acid levels were determined using the Wako NEFA C Kit (Wako Pure Chemicals Industries, Osaka, Japan).

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, and results are expressed as means ± S.E.M. (*N*=8–12). The effects of exercise and post-exercise recovery on the levels of metabolites in muscles and plasma were analysed with a one-factor analysis of variance (ANOVA) followed by a Fisher protected least significant difference *a posteriori* test using Stat View SE + Graphics version 1.03 (Abacus Concepts, Berkeley, CA, USA).

Results

Effect of repeated bouts of high-intensity exercise and recovery on muscle and liver glycogen levels

Repeated bouts of high-intensity exercise, each separated by a 1 h recovery period, resulted in a significant breakdown of glycogen in the red, white and mixed gastrocnemius muscles (Fig. 1). The extent of glycogen breakdown in response to the first exercise bout was higher than in response to the subsequent bouts in these muscles. Irrespective of the muscle examined, however, glycogen levels reached similar levels in response to each bout of exercise. During the 1 h recovery period following each bout of exercise, significant replenishment of muscle glycogen took place in the red, white and mixed gastrocnemius muscles. During recovery from the first bout of exercise, muscle glycogen returned to lower than pre-exercise levels in these muscles. By contrast, during recovery from the subsequent bouts of exercise, these muscles replenished their stores of glycogen completely and thus the post-recovery glycogen levels attained after the second and third recovery periods were not significantly different from those attained after the first recovery period. A distinct pattern of glycogen metabolism was observed in the soleus muscle, with glycogen levels remaining relatively stable, although a cumulative effect of consecutive exercise/recovery episodes resulted in an eventual fall in glycogen levels (Fig. 1). In response to the first sprint, hepatic glycogen levels decreased from 15.1±2.6 μmol glucosyl units g^{-1} wet mass to 3.2±1.0 μmol glucosyl units g^{-1} wet mass, and no further changes occurred in response to the subsequent bouts of exercise and associated recovery periods.

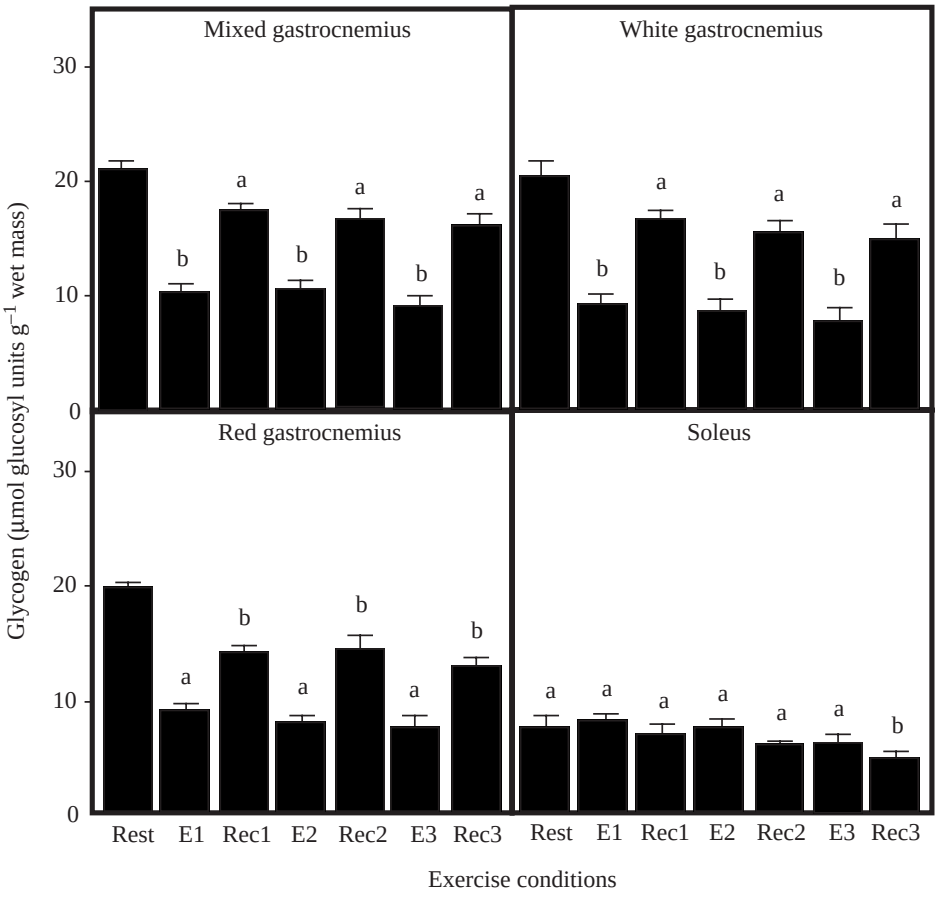

Fig. 1. The effect of repeated high-intensity exercise and recovery on glycogen levels in the soleus and white, red and mixed gastrocnemius muscles. On the *x*-axis, E refers to exercise and Rec to recovery. The values shown represent means ± S.E.M. (*N*=12). Identical superscripts on different columns indicate the absence of significant differences, whereas columns without a superscript differ significantly from any column bearing a superscript (ANOVA followed by Fisher PLSD *a posteriori* test; $P<0.05$).

Effect of repeated bouts of high-intensity exercise and recovery on lactate levels in muscle

Repeated bouts of high-intensity exercise, each separated by a 1 h recovery period, resulted in a significant increase in muscle lactate levels (Fig. 2). The extent of lactate accumulation in the soleus muscle and red, white and mixed gastrocnemius muscles was highest in response to the first bout of exercise. During the recovery period following each bout of exercise,

lactate levels returned to pre-exercise levels in all muscles examined.

Effect of repeated bouts of high-intensity exercise on plasma metabolite levels

Each bout of high-intensity exercise resulted in a significant increase in the levels of plasma lactate (Fig. 3). By contrast, the levels of plasma glucose (Fig. 3) and glycerol (Fig. 4) were not significantly affected by the first bout of exercise, but glucose levels decreased in response to both the second and third exercise bouts (Fig. 3). With respect to the levels of plasma fatty acids, acetoacetate and β-hydroxybutyrate, a distinct pattern of changes took place in response to each bout of exercise and ensuing recovery period. Each bout of exercise resulted in a significant decrease in the levels of plasma fatty acids, acetoacetate and β-hydroxybutyrate (Fig. 4). During each of the recovery periods, these metabolites returned to levels comparable or higher than those before exercise, with their concentrations after the third exercise bout being higher than basal pre-exercise levels. In particular, the levels of β-hydroxybutyrate reached after each period of recovery showed a gradual increase to levels that, after the third recovery period, were well above those after the first recovery period (Fig. 4).

In response to a single bout of exercise, glycerol remained at stable levels, whereas the levels of plasma free fatty acids, acetoacetate and β-hydroxybutyrate decreased significantly (Fig. 5). During recovery, the levels of glycerol remained unchanged while those of fatty acids, acetoacetate and β-hydroxybutyrate increased progressively (Fig. 5).

Discussion

It is generally acknowledged that fasted animals recovering from physical activity of near-maximal intensity can replenish their muscle glycogen stores even in the absence of food intake. In some mammal species, such as in rats and humans, the extent of this replenishment is only partial (Hermansen and Vaage, 1977; Astrand et al., 1986; Choi et al., 1994; Nikolovski et al., 1996; Peters et al., 1996; Bangsbo et al., 1997; Ferreira et al., 2001; Fournier et al., 2002), thus suggesting that a few consecutive bouts of high-intensity exercise might eventually lead to the progressive depletion of their muscle glycogen stores. In order to test this prediction, groups of rats were subjected to a series of three bouts of high-intensity swims to exhaustion, each separated from the subsequent one by a recovery period previously shown to be long enough for muscle glycogen and lactate to return to stable levels (Ferreira et al., 2001). This study shows for the first time that repeated bouts of high-intensity exercise do not lead to the eventual sustained depletion of the stores of muscle glycogen in fasted rats, irrespective of whether muscles are rich in fast-twitch red or white fibres (Fig. 1). Indeed, although glycogen repletion after one bout of exhaustive exercise is only partial, all the glycogen mobilised in response to the subsequent exercise bouts is completely replenished during the respective recovery periods, and the levels of muscle glycogen attained after recovery from one sprint do not differ from those attained after recovery from several sprints (Fig. 1).

On the basis of our findings, one must amend the view that rats

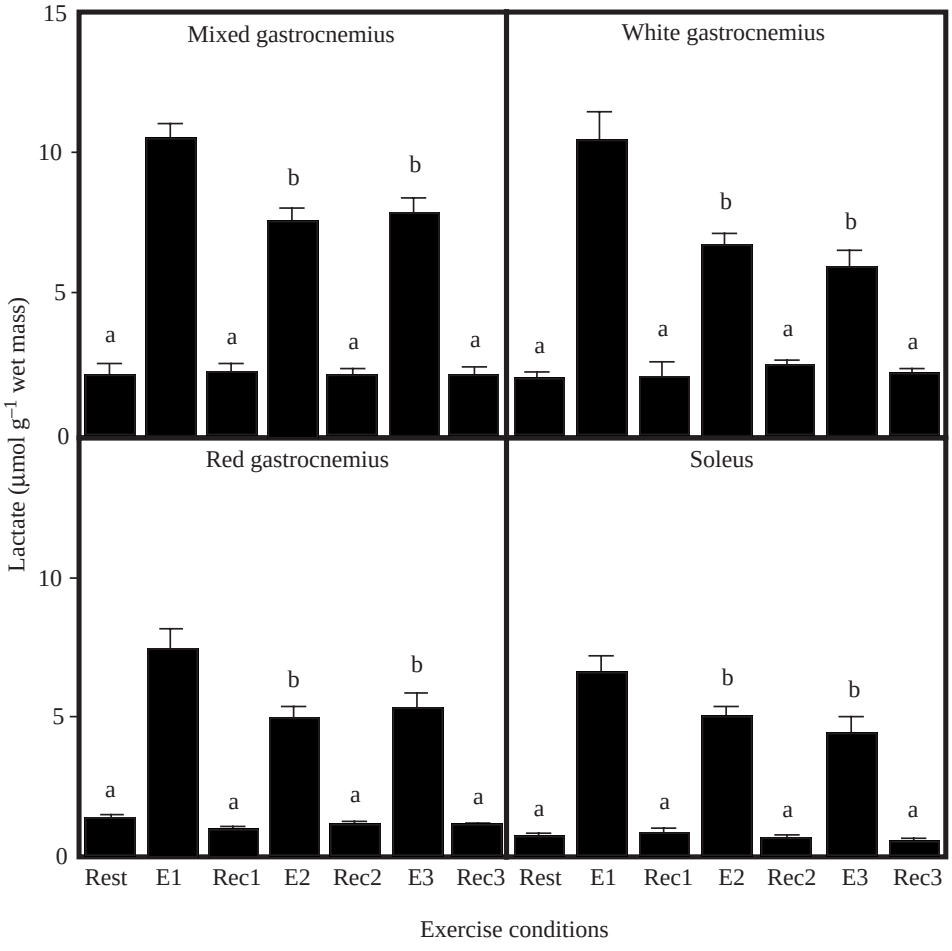

Fig. 2. The effect of repeated high-intensity exercise and recovery on lactate levels in the soleus and white, red and mixed gastrocnemius muscles. On the *x*-axis, E refers to exercise and Rec to recovery. The values shown represent means ± S.E.M. (*N*=12). Identical superscripts on different columns indicate the absence of significant differences, whereas columns without a superscript differ significantly from any column bearing a superscript (ANOVA followed by Fisher PLSD *a posteriori* test; $P<0.05$).

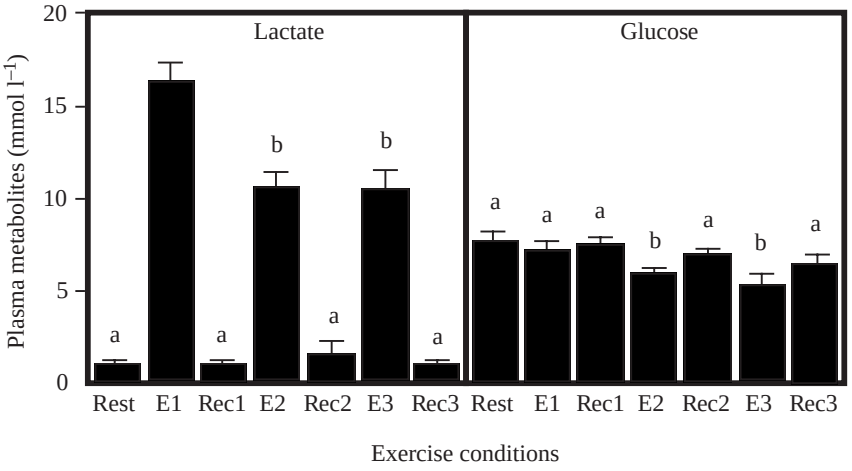

Fig. 3. The effect of repeated high-intensity exercise and recovery on glucose and lactate levels in the plasma. On the *x*-axis, E refers to exercise and Rec to recovery. The values shown represent means ± S.E.M. (*N*=8). Identical superscripts on different columns indicate the absence of significant differences, whereas columns without a superscript differ significantly from any column bearing a superscript (ANOVA followed by Fisher PLSD *a posteriori* test; $P<0.05$).

recovering from high-intensity exercise differ from most species of lower vertebrates in that, in the absence of food intake, they are incapable of replenishing completely their glycogen stores. Here, we show that there are conditions where the glycogen mobilised in response to a sprint is completely replenished after exercise in fasted rats (Fig. 1). Furthermore, the observation that repeated sprints fail to cause a progressive fall in the levels of muscle glycogen attained after each consecutive recovery period might be taken as evidence that there is a critical amount of muscle glycogen in the rat that is protected against sustained depletion. In order to support further this interpretation, other studies would be required to show that glycogen levels post-exercise are protected irrespective of the type, intensity and duration of exercise and availability of endogenous carbon sources. Fortunately, one such study has been performed in which fasted Wistar rats were forced to engage for nearly 2.5 h in continuous or intermittent aerobic exercise in order to deplete aerobically most of their muscle glycogen stores (Gaesser and Brooks, 1980). This study reported that during recovery, the stores of muscle glycogen increased to levels (17.6 μmol g^{-1}) comparable with those observed in the present study in response to either one or several 3-min sprints, but with the difference that endogenous carbon sources other than lactate were involved since these aerobic exercise protocols resulted only in a marginal increase in blood lactate level (Gaesser and Brooks, 1980). Overall, the observations that in fasted rats muscle glycogen levels return to comparable levels in response to a range of highly different exercise protocols, such as a single 3-min sprint, multiple 3-min sprints, prolonged continuous aerobic exercise or prolonged intermittent aerobic exercise, provide strong support for the existence in skeletal muscles of set glycogen levels that are protected against sustained depletion post-exercise.

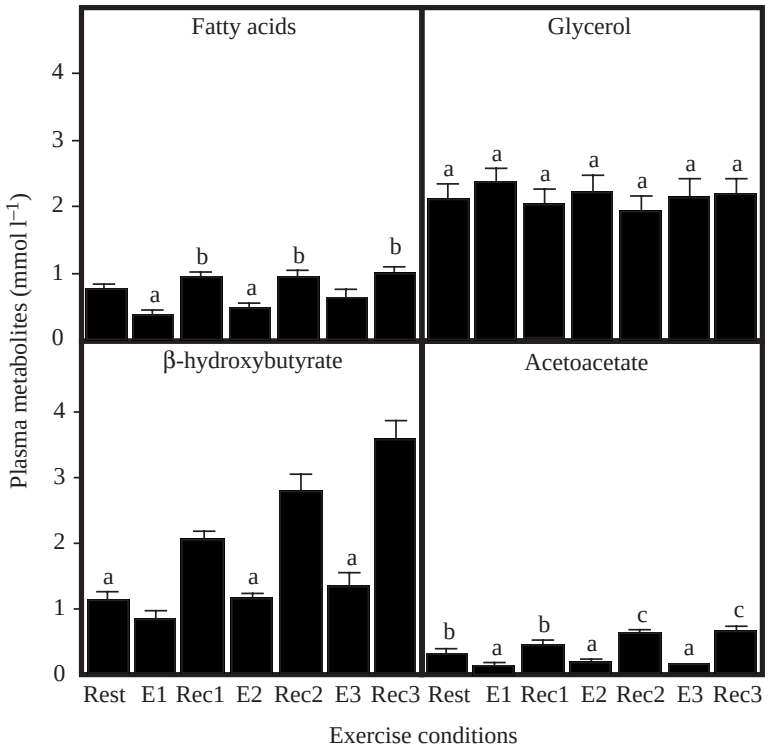

Fig. 4. The effect of repeated high-intensity exercise and recovery on plasma fatty acids, glycerol, β-hydroxybutyrate and acetoacetate levels. On the *x*-axis, E refers to exercise and Rec to recovery. The values shown represent means ± S.E.M. (*N*=8). Identical superscripts on different columns indicate the absence of significant differences, whereas columns without a superscript differ significantly between each other and from any column bearing a superscript (ANOVA followed by Fisher PLSD *a posteriori* test; $P<0.05$).

It is interesting to note that the levels at which muscle glycogen levels are protected against sustained depletion in the rat are high enough to support a little more than one bout of an intense sprint effort to exhaustion. Normally, during a sprint to exhaustion, it is the accumulation of H^+ and inorganic phosphate ions rather than the depletion of muscle glycogen that causes fatigue (Fitts and Metzger, 1993). However, if muscle glycogen stores were to be protected at much lower levels, the limited supply of glycogen would become the main factor

limiting an animal's capacity to engage in an intense sprint to exhaustion by causing premature fatigue (Balsom et al., 1999). Although, under most conditions, a sprint would be expected to last only a few seconds, there are extreme conditions associated with fight-or-flight behaviour where an animal might have to engage in a sprint to near exhaustion. For these animals, it would be highly advantageous to maintain their muscle glycogen stores at levels high enough so that the build-up of H^+ and inorganic phosphate ions, rather than the size of their glycogen stores, limits their capacity to engage in an intense sprint effort to exhaustion. In this regard, it is noteworthy that after a sprint most animal species in the fasted state generally 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; Gleeson and Dalessio, 1989; Astrand et al., 1986; Milligan and Wood, 1986; Scarabello et al., 1992; Fournier and Guderley, 1993; Girard and Milligan, 1992; Choi et al., 1994; Milligan 1996; Bangsbo et al., 1997).

In light of the above discussion, the observation that glycogen repletion in the rat is only partial after a single bout of exercise (Fig. 1) may be explained on the basis that muscle glycogen levels in 24 h-fasted rats are higher than the minimal critical levels normally protected in this species. Along this line of reasoning, the observation that in several vertebrate species the stores of muscle glycogen are completely replenished after a single bout of intense exercise (Gratz and Hutchison, 1977; Gleeson, 1982, 1996; Milligan and Wood, 1986; Gleeson and Dalessio, 1989; Pagnotta and Milligan, 1991; Fournier and Guderley, 1992; Girard and Milligan, 1992; Scarabello et al., 1992; Milligan, 1996; Bräu et al., 1999) might be explained on the basis that their muscle glycogen stores are kept at their species-specific protected levels. This raises the novel question of whether the muscle glycogen stores in these animal species would also be only partially replenished post high-intensity exercise if one were to manipulate their glycogen stores so that more than protected glycogen levels were to be stored in their muscles prior to exercise. This is an issue that remains to be investigated.

The capacity of fasted rats to replenish their muscle glycogen stores between each consecutive bout of exercise raises the question of the identity of the carbon sources mobilised for the synthesis of glycogen. Since, in the present study, the animals were in a fasted state, the synthesis of muscle glycogen had to depend solely on endogenous substrates. The lactate built up in response to exercise is a likely candidate (Fig. 3), as it is generally acknowledged as one of the major carbon sources for the synthesis of muscle glycogen in many vertebrate species (Gleeson, 1996; Palmer and Fournier, 1997; Fournier et al., 2002). That lactate is also likely to be a major carbon source for the replenishment of muscle glycogen during recovery from each bout of exercise in rats is suggested indirectly by the observation that the fall in plasma and muscle lactate levels is temporally linked with glycogen synthesis. In this respect, the changes in lactate levels in the soleus muscles may seem at first surprising, considering the absence of net glycogen breakdown and synthesis in this muscle, but, as argued before (Bräu et al., 1997; Ferreira et al., 2001), it is more than likely that the exercise-mediated rise and subsequent fall in lactate levels in this muscle result from an exchange of lactate between the soleus muscle and the blood. The stores of hepatic glycogen are unlikely to provide a net source of glucose for the resynthesis of muscle glycogen stores, since liver glycogen remains at stable levels in response to several sprints and recovery periods. It is important to stress that amino acids and glycerol *via* their conversion to glucose by the liver could

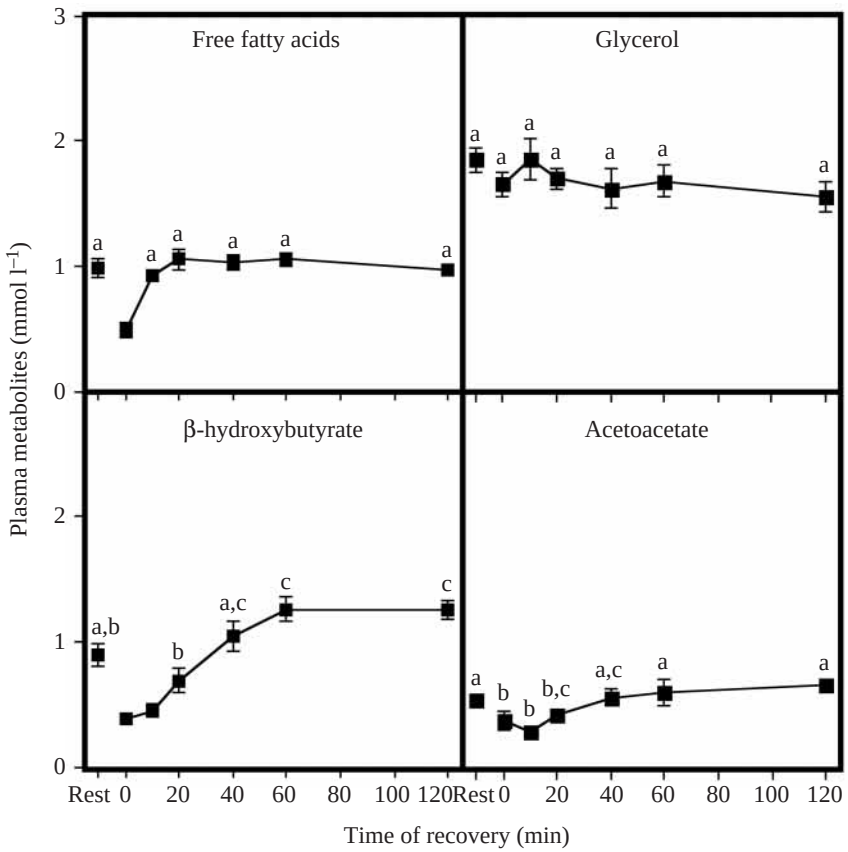

Fig. 5. The effect of high-intensity exercise and recovery on plasma fatty acids, glycerol, β-hydroxybutyrate and acetoacetate levels. The values shown represent means ± S.E.M. (*N*=8). Identical superscripts on different data points indicate the absence of significant differences, whereas data points without a superscript differ significantly between each other and from any data point bearing a superscript (ANOVA followed by Fisher PLSD *a posteriori* test; $P<0.05$).

contribute to the replenishment of muscle glycogen (Gaesser and Brooks, 1980; Fournier et al., 2002), but their relative contributions, as well as that of lactate, remain to be established.

The mechanisms by which the proportion of muscle glycogen replenished post-exercise differs between the first and subsequent bouts of exercise remain to be elucidated. Any attempt at explaining this finding must take into consideration the observation that the absolute amount of glycogen deposited during recovery from the first exercise bout is not different from that after each subsequent bout (Fig. 1). It is because more glycogen is mobilised during the first exercise bout than during the following bouts, maybe because of a higher work output, that the proportion of muscle glycogen replenished after the first exercise bout is lower than after the other two exercise bouts. It is interesting to note that such a lower extent of glycogen mobilisation and lactate accumulation in response to the second and third bouts of high-intensity exercise is not shared by all animals species, since, in the rainbow trout (*Oncorhynchus mykiss*), for instance, the extent of glycogen mobilisation and lactate accumulation does not differ between consecutive exercise bouts (Scarabello et al., 1992). Considering that in the rat more lactate is eliminated during recovery from the first exercise bout than during subsequent bouts (Figs 2, 3), with equivalent amounts of muscle glycogen being replenished, a lower proportion of lactate is likely to be converted into glycogen during recovery from the first bout of exercise. This interpretation holds as long as lactate is the main carbon source for glycogen synthesis, and this might partly explain the partial replenishment of muscle glycogen stores in response to a single bout of exercise.

The differences in the pattern of glycogen repletion between the first and subsequent bouts of exercise raise the question of whether other aspects of fuel metabolism differ among these bouts of exercise. We have addressed this question indirectly by examining the effects of a single bout as well as that of repeated bouts of high-intensity exercise on plasma levels of glycerol, free fatty acids, β-hydroxybutyrate and acetoacetate. As reported previously by others in humans and rats (Drury et al., 1941; Balasse et al., 1978; Romijn et al., 1993), the levels of plasma fatty acids and ketone bodies decreased significantly from rest levels in response to a single bout of high-intensity exercise and increased throughout recovery to attain levels comparable with or higher than those measured before exercise (Figs 4, 5). The impact of repeated bouts of high-intensity exercise on the plasma levels of fatty acids, acetoacetate and β-hydroxybutyrate suggests that the metabolic state of the rat prior to the second and third bouts of exercise was different from that before the first bout (Fig. 4). Indeed, the levels of plasma fatty acids and ketone bodies attained after each of the recovery periods were higher than those prior to the first exercise bout (Fig. 4). Since the rates of utilisation of fatty acids and ketone bodies by muscle are partly determined by their concentrations (Newsholme and Leech, 1983), the higher levels of fatty acids and ketone bodies prior to the second and third bouts of high-intensity exercise, as well as during recovery from these bouts, would be expected to enhance their oxidation by muscles. As a result, this would be predicted to provide muscles and other organs with an increased supply of fuels other than glucose and lactate to support their energy demands, particularly during recovery from exercise. Assuming lactate is the major carbon source for glycogen synthesis, this lesser oxidation of lactate and of glucose derived from lactate might allow for an increased proportion of lactate converted into muscle glycogen after the second and third bouts of exercise. This might also explain the higher proportion of glycogen replenished during recovery from these exercise bouts. Irrespective of whether this explanation holds, our findings clearly suggest that several aspects of fuel metabolism differ between the first and subsequent bouts of exercise and that this must be taken into consideration when attempting to explain the increased sparing of muscle glycogen in response to several bouts of exercise. It is not clear, however, the extent to which these differences might partly result from possible differences in work output between the first and subsequent exercise bouts. Moreover, different hormonal responses might also exist between the first and subsequent exercise bouts and explain also, at least in part, the differences in the pattern of glycogen metabolism between consecutive sprints.

In conclusion, our results corroborate earlier findings that glycogen repletion is only partial in fasted rats recovering from a single bout of high-intensity exercise. However, this study indicates that mechanisms exist to ensure that muscles from fasted rats can replenish completely their stores of glycogen if subjected to more than one bout of high-intensity exercise. Rats, therefore, resemble many other vertebrate species in that without food intake they can also protect their muscle glycogen against sustained depletion by replenishing completely their glycogen stores post-exercise to support the energy demands associated with fight-or-flight behaviour. Since, following high-intensity exercise, muscle glycogen repletion from endogenous carbon sources is only partial in other mammal species, such as humans, it remains to be established whether our findings in the rat are typical of mammals in general.

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