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GAS EXCHANGE, METABOLITE STATUS AND EXCESS POST-EXERCISE OXYGEN CONSUMPTION AFTER REPETITIVE BOUTS OF EXHAUSTIVE EXERCISE IN JUVENILE RAINBOW TROUT

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Summary

Juvenile rainbow trout (approximately 6 g) were exercised to exhaustion in two 5 min bouts given 6 h apart. Resting levels of whole-body lactate and glycogen were restored prior to the second bout. The rate of O_2 consumption increased about threefold 5 min after each bout of exercise, while recovery time decreased from 4 h after the first bout to 2–3 h after the second. The excess post-exercise oxygen consumption, i.e. 'oxygen debt', was significantly reduced by 40 % after the second exercise bout, despite almost identical rates of lactate clearance and glycogen resynthesis. The rates of CO_2 and ammonia excretion increased sixfold and threefold, and recovery times decreased from 4–6 h to 3 h and from 3 h to 1.5 h, respectively. After the first bout, whole-body lactate levels peaked at 5 min post-exercise at about 8.5 times pre-exercise levels. After the second bout, lactate levels peaked at 0 min post-exercise and fell more rapidly during recovery. Whole-body glycogen levels decreased by 70 % and 80 % and ATP levels decreased by 75 % and 65 % after the first and second bouts, respectively, while glucose levels increased about 1.5-fold immediately after both bouts. Creatine phosphate levels decreased by 70 % and 80 % after the first and second bouts, respectively. After 6 h of recovery, creatine phosphate levels were higher after the second bout than after the first. These findings suggest that exhaustive exercise may cause a 'non-specific' increase in metabolic rate not directly related to the processing of metabolites, which is reduced upon a subsequent exercise bout. This is in contrast with the classical 'oxygen debt hypothesis', which states that the oxygen debt and lactate clearance are linked. Furthermore, it appears that two sequential exercise bouts are sufficient to induce a 'training effect', i.e. improved rates of metabolic recovery.

Introduction

After exhaustive sprint exercise, the whole-body O_2 consumption ($\dot{M}_{O_2}$) of

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salmonids is elevated above resting levels for a number of hours (Hochachka, 1961; Brett, 1964; Wieser *et al.* 1985; Milligan and McDonald, 1988). Recently, we have shown in juvenile rainbow trout that the fast 'alactic' component of this excess post-exercise O_2 consumption (EPOC) can be satisfactorily accounted for by measured changes in whole-body ATP and creatine phosphate levels and reasonable estimates of the costs of increased cardiac work, ventilatory work and the refilling of body O_2 stores (Scarabello *et al.* 1991*a*). However, the much larger slow component (80%) of EPOC is far greater than can be explained by hypotheses based on either oxidation or glycogen resynthesis as the principal fate(s) of lactate. The cost of post-exercise recovery is clearly larger and more complex than suggested by the classical 'oxygen debt hypothesis' (Hill and Lupton, 1923; Margaria *et al.* 1933). As in mammals (Gaesser and Brooks, 1984; Bangsbo *et al.* 1990), other explanations for EPOC must now be sought.

One possible explanation is that a bout of chasing to exhaustion might induce a non-specific increase in $\dot{M}_{O_2}$ during the recovery period, which is not directly associated with the processing of metabolites, but due to the psychological aspects of exercise stress. If this were the case, then this non-specific effect might be reduced after a second bout given shortly after recovery from the first because of the animal's previous experience with the disturbance. An interval of 6 h was chosen as offering sufficient time for clearance of lactate, return of $\dot{M}_{O_2}$ close to resting levels (Scarabello *et al.* 1991*a*,*b*) and restoration of control plasma catecholamine levels (Milligan and Wood, 1987).

Pearson *et al.* (1990) have recently demonstrated that rainbow trout subjected to 33 sprint training sessions over 9 weeks exhibited less depletion of endogenous fuels and an enhanced rate of metabolic recovery after exhaustive exercise at the end of the training protocol. We wondered if any of these improvements might be seen after a single 'training' session (i.e. the first exercise bout). We hypothesized that recovery following a second bout of exhaustive exercise could be enhanced compared to that following the first, perhaps because of an attenuation of non-specific costs. If this explanation were valid, a lower EPOC per unit lactate clearance or glycogen resynthesis would occur after the second bout. Measurements of CO_2 and ammonia excretion were also made after the two exercise bouts to see whether these other two respiratory gases, about which much less is known, exhibited the same trends as O_2. An additional goal of the present study was to compare in detail the degree of ATP, creatine phosphate, glycogen and glucose depletion, lactate accumulation, and their rates of restoration, after the first and second exercise bouts. These results have implications to field situations where repeated exercise bouts may occur during fish stocking procedures, 'catch and release' angling and predator–prey interactions.

Materials and methods

Animals

Juvenile rainbow trout [*Oncorhynchus mykiss* (Walbaum); N=200] were

obtained from Aqua Farms Ltd (Feversham, Ontario) and were immediately divided randomly into two groups. One group was held in a large 400 l circular tank with fresh dechlorinated Hamilton tap water (hard water) for the metabolite study and the other group was held in a similar tank with synthetic soft water (see below) for the gas exchange study. In both cases the water was well aerated and maintained at 15±1°C for 3–4 weeks of acclimation. Fish were fed daily with 1.5 Gr. (granule size) trout pellets (Martin Feed Mills, Don Mills, Ontario).

Synthetic soft water was used to facilitate measurements of CO_2 excretion into the medium. These measurements are normally quite difficult to make in Hamilton hard water because of high background levels of total CO_2 (mainly bicarbonate ion). Hard water conditions were (approximately) Ca^{2+}, 2 mequiv l^{-1}; Na^+, 0.6 mequiv l^{-1}; Cl^-, 0.8 mequiv l^{-1}; titratable alkalinity to pH 4.0, 1.8 mequiv l^{-1}; pH 8.0. Soft water conditions were (approximately) Ca^{2+}, 47 μequiv l^{-1}; Na^+, 68 μequiv l^{-1}; Cl^-, 95 μequiv l^{-1}; titratable alkalinity, 130 μequiv l^{-1}. Soft water pH levels were reduced with 0.1 mol l^{-1} HCl to an average of 6.2–6.3 to maintain lower background levels of total CO_2 (10–30 μmol l^{-1}).

Exercise protocol

Fish were fasted for only 1 day prior to experimentation so as not to compromise their ability to restore glycogen levels after exercise (Scarabello *et al.* 1991*b*). They were removed from the holding tank, weighed (average approximately 6 g) and acclimated overnight in small flow-through respirometers. These were blackened 20 ml syringe barrels fitted with three-way stopcocks at the inflow and outflow (for P_{O_2} sampling) and were perfused with the appropriate water at a flow of approximately 0.75 l h^{-1} by a Gilson Minipuls peristaltic pump. The water temperature was 15±1°C.

The following morning, each fish was individually exercised in a 10 l bucket by vigorously chasing it by hand for 3 min, followed by further stimulation for 2 min by touching a 9 V battery to the animal's tail. At the end of the 5 min, fish no longer responded when handled and were returned to the respirometers for recovery. After 6 h of recovery, a second identical exercise bout was given and recovery monitored over the next 18 h. Control fish (non-exercised) were sampled over the full 24 h period.

Gas exchange study

Rates of O_2 consumption ($\dot{M}_{O_2}$), CO_2 excretion ($\dot{M}_{CO_2}$) and ammonia excretion ($\dot{M}_{amm}$) were measured in the same fish both pre- and post-exercise through the entire 24 h period. Synthetic soft water was used. Water samples for P_{O_2} measurement were taken in 1 ml glass syringes and measured immediately with a Radiometer E5046 P_{O_2} electrode connected to a Cameron Instruments OM-200 oxygen meter. P_{O_2} measurements were converted to total O_2 content using solubility tables provided by Boutilier *et al.* (1984). Total CO_2 content was determined by gas chromatography (Shimadzu GC-8A gas chromatograph with

Shimadzu C-R3A integrator) using methods described by Playle *et al.* (1990). Total ammonia content was determined within a few days on a stored sample (frozen after sampling at −30°C) using a modification of the salicylate–hypochlorite method of Verdouw *et al.* (1978). Gas exchange rates were calculated as the product of flow rate through the respirometer and the difference in gas content between inflow and outflow water samples and were expressed on a wet body mass basis.

The mean of three samples per fish was obtained prior to exercise. Tests demonstrated that the minimum mixing time to yield a representative P_{O_2} measurement was 5 min; thus, the first post-exercise determination was made 5 min (0.08 h) after the end of exercise (when the fish was returned to the chamber). Thereafter, samples were taken at 0.5, 1, 1.5, 2, 3, 4, 6, 6.08, 7, 7.5, 8, 9, 10, 12, 14, 16 and 24 h. The areas under the curves for all three rates of gas exchange ($\dot{M}_{O_2}$, $\dot{M}_{CO_2}$, $\dot{M}_{amm}$) after both exercise bouts, relative to pre-exercise levels, were measured for each individual using a digitizer pad (GTCO Digi-pad) connected to a Zenith Data Systems microcomputer.

Metabolite study

Fish held in hard water were treated in the same manner as those acclimated to soft water and exercised as described. However, at a particular time in the recovery (0.08, 1, 3, 6, 6.08, 7, 9 or 12 h), $\dot{M}_{O_2}$ only was measured. Immediately thereafter, fish were freeze-clamped with pre-cooled (−196°C) aluminium tongs and immersed in liquid nitrogen for simultaneous whole-body metabolite measurements. Two control samples (*C*1 and *C*2) were also taken at times corresponding to 'pre-exercise' and '12 h of recovery'. These control fish were not exercised but were simply left in the respirometer for the appropriate time. A group of fish was also sampled (freeze-clamped) immediately post-exercise (0 h). A corresponding $\dot{M}_{O_2}$ measurement could not be made for those fish. Fish that struggled excessively (more than three tailflaps) were discarded. The area under the mean $\dot{M}_{O_2}$ recovery curve was measured as described above for the gas exchange study. It should be noted that, as a result of an accident, the second control sample (*C*2) had to be repeated. The measurements reported here were taken about 2 weeks after all the other measurements.

Each freeze-clamped whole fish was subsequently powdered in a mortar and pestle under liquid nitrogen, freeze-dried at −60°C under vacuum for 48 h and stored in an evacuated dessicator at −80°C. The freeze-dried tissue was then either extracted with 6% perchloric acid and neutralized with 2.5 mol l^{-1} K_2CO_3 (pH>7) for the determination of lactate, ATP and creatine phosphate, or extracted with 0.2 mol l^{-1} acetate buffer (pH 4.8) and neutralized with Trizma base (0.3 mol l^{-1}; Sigma) for the determination of glycogen and glucose. All metabolites were assayed fluorometrically (Fluoro-micro-photometer, American Instruments Co., Maryland) using the enzymatic methods of Bergmeyer (1965).

All values in both studies are reported as means±1 S.E.M. Sample size is at least

8 unless otherwise stated. For the gas exchange study, significance was tested using a two-tailed Student's *t*-test at $P<0.05$, paired design. A Bonferroni procedure for multiple comparisons was also employed. For the metabolite study, significance was tested using a two-tailed Student's *t*-test at $P<0.05$, unpaired design.

Results

Gas exchange study

The repetitive exercise bouts had similar effects on the rates of exchange of all three respiratory gases measured. $\dot{M}_{O_2}$ increased about threefold (6.3 to 20.1 μmol h^{-1} g^{-1} wet mass) after the first exercise bout and returned to pre-exercise levels in about 4 h (Fig. 1A). The second exercise bout resulted in a similar elevation of $\dot{M}_{O_2}$; however, only 2–3 h was required for recovery. $\dot{M}_{CO_2}$ increased almost sixfold (4.1 to 23.6 μmol h^{-1} g^{-1} wet mass) and required 4–6 h to return to pre-exercise levels (Fig. 1B). After the second bout, $\dot{M}_{CO_2}$ increased to the same degree but required only 3 h for recovery. In control fish, the overall instantaneous respiratory gas exchange ratio (RE) averaged 0.71. RE increased to 1.17 immediately after the first exercise bout and returned to control levels by 4–6 h. A similar increase in RE (1.20) was seen after the second exercise bout but there was a faster recovery (2–3 h). $\dot{M}_{amm}$ increased threefold after both exercise bouts (Fig. 1C; 0.6 to 1.8 and 0.7 to 2.0 μmol h^{-1} g^{-1} wet mass, after the first and second bouts, respectively). Recovery, however, was again faster after the second bout (1.5 h) than after the first (3 h).

The experimental design of the gas exchange study allowed us to use the data obtained from each fish to define its pre-exercise reference point for the calculation of how much more gas was exchanged (consumed or excreted) after each bout of exercise. This was measured as the area under the curve after both exercise bouts for each individual fish and each respiratory gas (Table 1). Note that had we used the appropriate mean values from the time-paired non-exercised fish as the reference points, the conclusions would have been the same. The EPOC was significantly reduced by 40 % after the second bout. Although the excess quantity of CO_2 excreted also decreased by 46 % after the second bout, this

Table 1. *The mean excess post-exercise O_2 consumption, CO_2 production and ammonia production (μmol g^{-1} wet mass) after two bouts of exhaustive exercise separated by 6 h*

	Bout 1	Bout 2	*N*
$\dot{M}_{O_2}$	14.5±1.9	8.8±0.9*	16
$\dot{M}_{CO_2}$	22.5±4.9	12.0±1.5	6
$\dot{M}_{amm}$	2.3±0.3	2.3±0.6	8

Values are means±S.E.M.

* Significantly different from corresponding value in bout 1; Student's *t*-test ($P<0.05$).

difference was not significant because of greater variability in the CO_2 measurements and the small number of fish tested. There was no significant difference in excess ammonia excretion after the two exercise bouts.

Metabolite study

Both resting ($8.7\,\mu mol\,h^{-1}\,g^{-1}$ wet mass) and post-exercise $\dot{M}_{O_2}$ levels ($18.1\,\mu mol\,h^{-1}\,g^{-1}$ wet mass) were similar to, but statistically different from, those recorded in the gas exchange study. Again, $\dot{M}_{O_2}$ declined more rapidly after the second bout. The total EPOCs based on the mean $\dot{M}_{O_2}$ recovery curves were 10.8 and $7.5\,\mu mol\,g^{-1}$ wet mass after the first and second exercise bouts, respectively. The significance of this difference cannot be assessed statistically because EPOC was not measured on an individual fish basis in the metabolite study. Nevertheless, it followed the same trend (difference 31%) as in the gas exchange study (difference 41%), where the phenomenon of lower EPOC after the second bout was statistically significant.

Whole-body lactate levels (Fig. 2A) increased from 1.2 to $9.0\,\mu mol\,g^{-1}$ wet mass immediately after the first exercise bout and reached $10.3\,\mu mol\,g^{-1}$ wet mass at 5 min post-exercise. These two values were not significantly different from one another. Thereafter, levels began to drop and by 6 h they were no longer significantly different from control. The net lactate clearance during this period was $8.1\,\mu mol\,g^{-1}$ wet mass. Immediately after the second bout, lactate levels increased to $9.1\,\mu mol\,g^{-1}$ wet mass but they began to decline immediately. Lactate recovery was faster after the second bout. By 5 min, levels had dropped by $1.2\,\mu mol\,g^{-1}$ wet mass, significantly below the corresponding 5 min sample after the first bout. In addition, the 9 and 12 h values were significantly lower than the corresponding 3 and 6 h values. Overall, the amount of lactate cleared during recovery from the second bout ($8.0\,\mu mol\,g^{-1}$ wet mass) was the same as after the first, but it was cleared more quickly after the second bout.

Whole-body glycogen levels (Fig. 2B) dropped to 30% of resting levels after the first exercise bout (5.7 to $1.7\,\mu mol\,g^{-1}$ wet mass), remained constant for about 1 h and then started to increase. By 3 h, glycogen had returned to pre-exercise levels. Total glycogen repletion was $4.3\,\mu mol\,g^{-1}$ wet mass. After the second exercise bout, the extent of glycogen depletion was similar to that of the first bout (5.9 to $1.3\,\mu mol\,g^{-1}$ wet mass) and control levels were reached by 12 h. Glycogen repletion ($5.0\,\mu mol\,g^{-1}$ wet mass) was also similar to that after the first bout. Although the second control sample ($C2$) was significantly higher than $C1$ (Fig. 2B), we believe that this point is not a true representation of glycogen levels at the end of

Fig. 1. Changes in the rates of (A) O_2 consumption ($\dot{M}_{O_2}$; $N=16$) (B) CO_2 excretion ($\dot{M}_{CO_2}$; $N=6$) and (C) ammonia excretion ($\dot{M}_{amm}$; $N=8$) following two sequential 5 min bouts of exhaustive exercise (EX1, EX2; arrows) in juvenile rainbow trout. ▲ indicates non-exercised controls, ● indicates exercised samples. Means±1 S.E.M. Resting values immediately prior to exercise were the mean of three samples per fish. The first sample after exercise was made at 5 min. * indicates a value significantly different from the respective pre-exercise level for each bout (Student's t-test $P<0.05$).

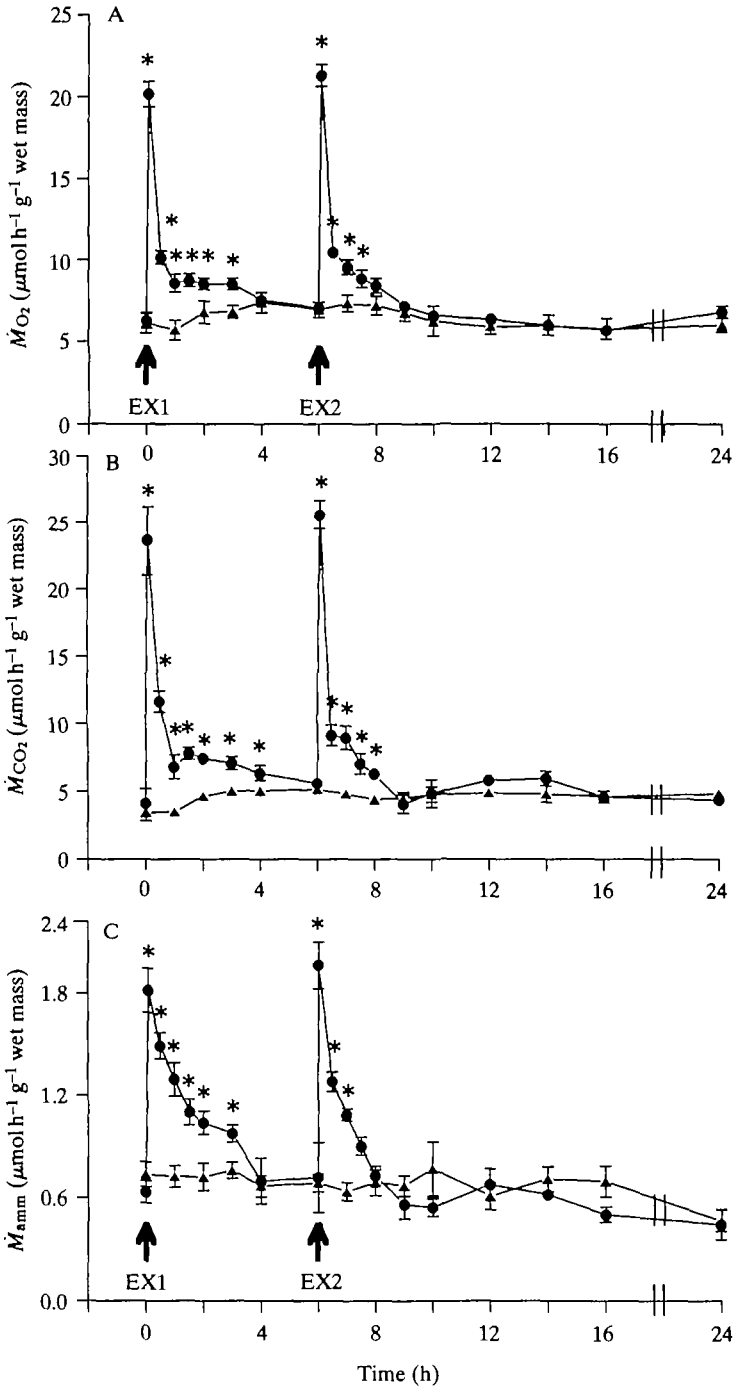

Fig. 1

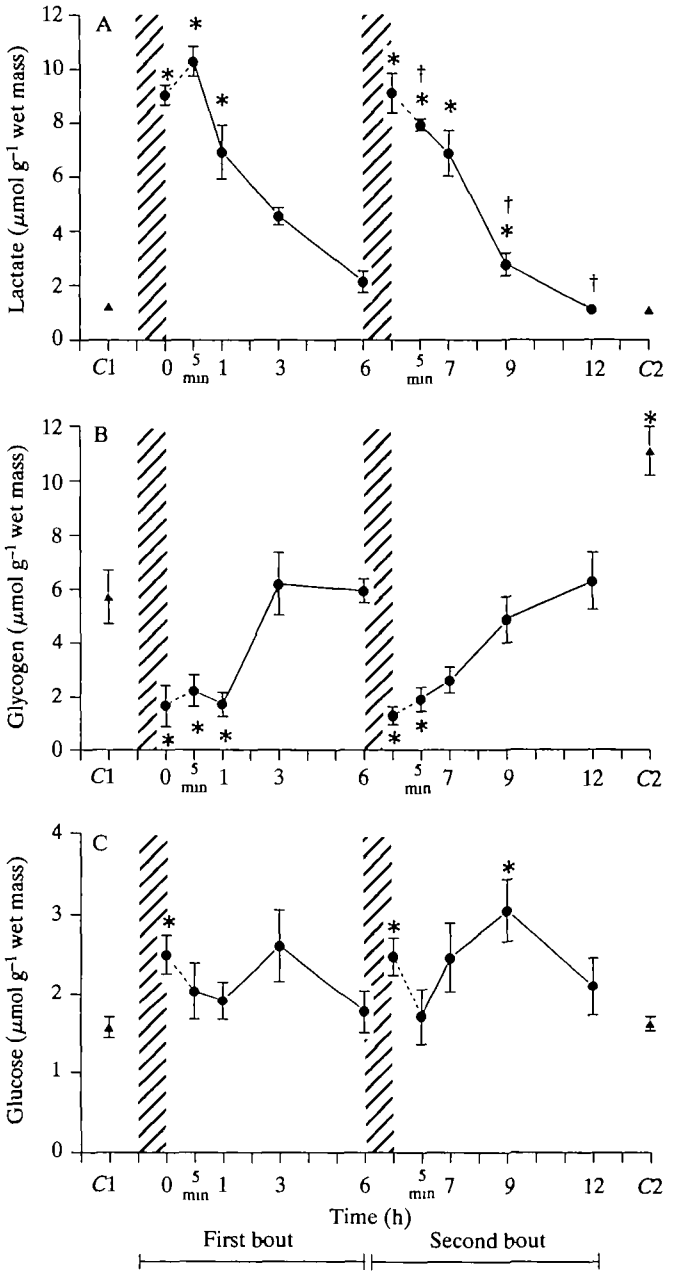

Fig. 2

the experiment because this group was run about 2 weeks after the other groups. It is likely that the mass-specific glycogen reserves of the fish had changed by that time. However, all other variables measured showed no differences between *C*1 and *C*2.

Fig. 2. Changes in whole-body lactate levels (A), glycogen levels (B) and glucose levels (C) following two sequential bouts of exhaustive exercise in juvenile rainbow trout. ▲, labelled *C*1 and *C*2, are non-exercised controls. ● indicates exercised samples. Means (N=8)±1 S.E.M. Hatched bars indicate the two 5 min exercise bouts. Note that, for clarity, an expanded time scale has been used between the zero and 5 min sample points. The time scale is linear only between 5 min and 6 h, and between the second 5 min and 12 h. * indicates values significantly different from *C*1. † indicates values significantly different from corresponding sample time after the first exercise bout (Student's t-test $P<0.05$). Note that, in B, *C*2 was significantly different from *C*1. See text for details.

Changes in whole-body glucose levels were similar after both exercise bouts (Fig. 2C). Immediately after exercise, glucose level was significantly elevated but by 5 min it had returned to pre-exercise levels. There was a tendency for a secondary rise at 3 h post-exercise, but this was only significant after the second bout. Whole-body creatine phosphate (Fig. 3A) decreased to 30 % of resting levels after the first bout (6.1 to 1.8 μmol g^{-1} wet mass) but had almost recovered by 5 min. Levels continued to rise, overshot resting levels and peaked at 1 h (at 8.8 μmol g^{-1} wet mass). By 6 h, creatine phosphate had dropped back to pre-exercise levels. After the second bout, a similar pattern was observed; creatine phosphate was depleted to 1.2 μmol g^{-1} wet mass at the end of exercise, overshot resting levels to 10.5 μmol g^{-1} wet mass during subsequent recovery and, at 12 h, was still at a level significantly above that of the 6 h sample (8.4 *versus* 5.7 μmol g^{-1} wet mass).

ATP changes were similar but not identical after both exercise bouts (Fig. 3B). ATP dropped to about 25 % of resting levels immediately after the first exercise bout (from 1.6 to 0.4 μmol g^{-1} wet mass) and to about 35 % of resting levels (from 1.7 to 0.6 μmol g^{-1} wet mass) after the second bout. ATP level started to recover immediately, requiring about 1 h to return to pre-exercise levels in each case.

Discussion

Effects on gas exchange

Resting and post-exercise $\dot{M}_{O_2}$ measurements in the present study (Fig. 1A) agree well with the findings of Wieser *et al.* (1985) in similar-sized rainbow trout, although their exercise protocol was different (electrical stimulation for 60 s). The greater relative increase in $\dot{M}_{CO_2}$ than in $\dot{M}_{O_2}$, and therefore in RE, after exhaustive exercise, is in accord with the findings of Steffensen *et al.* (1987) and Milligan and McDonald (1988) on adult trout and salmon, respectively. It is likely to result from a shift to increased carbohydrate metabolism and the titration of blood and tissue bicarbonate reserves by metabolic acid production.

After the second exercise bout, $\dot{M}_{O_2}$ increased to the same degree as after the first bout; however, recovery time was considerably faster (Fig. 1A) and EPOC was significantly reduced by 40 % (Table 1). Woodward and Smith (1985) reported

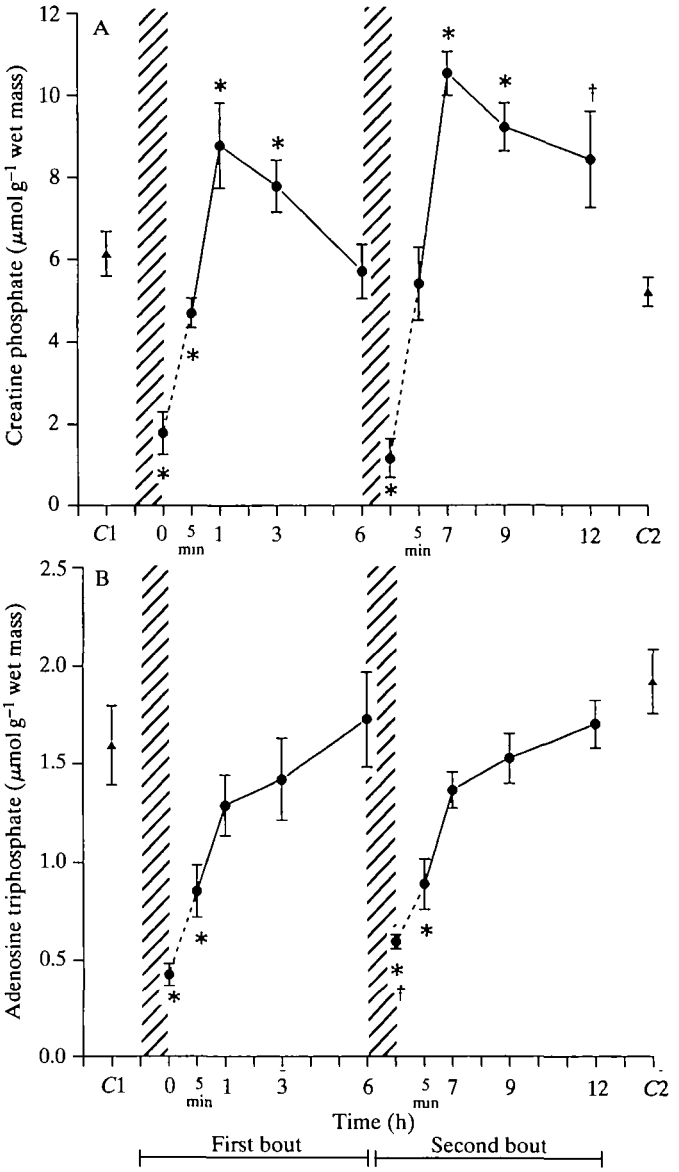

Fig. 3. Changes in whole-body creatine phosphate levels (A) and adenosine triphosphate levels (B) following two sequential bouts of exhaustive exercise in juvenile rainbow trout. ▲, labelled *C*1 and *C*2, are non-exercised controls. ● indicates exercised samples. Means (N=8)±1 S.E.M. * indicates values significantly different from *C*1. † indicates values significantly different from the corresponding sample time after the first exercise bout (P<0.05). Other details as in Fig. 2.

that rainbow trout (trained for 6 weeks to swim at 1.5 body lengths per second) had smaller increases of $\dot{M}_{O_2}$ with exercise, possibly as a result of familiarity with the training routine, which may make the exercise less stressful. In contrast,

Hochachka (1961) reported that trained trout consumed more O_2 after exhaustive exercise associated with the greater glycogen utilization and lactate accumulation. In both cases, the training regimes were essentially aerobic. In the present study, glycogen utilization remained the same after the second bout, which may explain the similar elevations in $\dot{M}_{O_2}$, but the faster recovery times may be due to familiarity with the exercise protocol.

$\dot{M}_{CO_2}$ also increased to the same degree after both exercise bouts (Fig. 1B); however, recovery was considerably faster after the second bout. Although the difference was not statistically significant, the mean excess post-exercise CO_2 excretion was 46 % lower after the second bout (Table 1). This is likely to have resulted from a combination of decreased $\dot{M}_{O_2}$ (i.e. decreased aerobic CO_2 production) and slightly lower net lactate production (Fig. 2A) and net ATP depletion (Fig. 3B) (i.e. less metabolic acid production and therefore less titration of bicarbonate reserves) after the second bout. Differences in catecholamine dynamics could also have contributed to this effect. Plasma catecholamines enhance oxygen delivery to the tissues (Milligan and Wood, 1987) and regulate bicarbonate entry into fish red blood cells (the rate-limiting step in CO_2 excretion; Wood, 1991).

The threefold increase in $\dot{M}_{amm}$ after both exercise bouts (Fig. 1C) was approximately proportional to the increase in $\dot{M}_{O_2}$ (Fig. 1A) rather than to the increase in $\dot{M}_{CO_2}$ (Fig. 1B). Blood and white muscle total ammonia levels have been shown to increase significantly after exercise both in rainbow trout weighing 30–55 g (Mommsen and Hochachka, 1988) and in adult trout (Wright and Wood, 1988). An increase in ammonia in the muscle after exercise is thought to occur as a result of adenylate pool depletion and/or increased protein catabolism. That the excess ammonia excretion (2.3 μmol g^{-1} wet mass; Table 1) was greater than the observed ATP depletion (approximately 1 μmol g^{-1} wet mass; Fig. 3B) points to the importance of increased protein catabolism after exercise. The majority is retained in the muscle, where it is utilized to convert IMP back into AMP in the purine nucleotide cycle (Mommsen and Hochachka, 1988). A small amount, however, is released into the blood and excreted at the gills (Fig. 1C; see also Milligan and Wood, 1986*a*) or metabolized by the liver (Mommsen and Hochachka, 1988). It is not clear how ammonia production may be altered by the repetitive exercise bouts. Although the mean $\dot{M}_{amm}$ recovery rate appeared to be faster after the second bout (Fig. 1C), the areas under the curve exhibited no difference when assessed on an individual fish basis (Table 1).

Metabolite study

Resting lactate and ATP levels in the present study are comparable with the findings of Wieser *et al.* (1985) as well as with our own recent studies (Scarabello *et al.* 1991*a*,*b*) in rainbow trout fry of similar size. Similar resting glycogen and creatine phosphate levels were reported by Dobson and Hochachka (1987) in white muscle of 50 g rainbow trout, but their resting lactate levels were about

threefold higher. Pearson *et al.* (1990) reported 50 % lower lactate levels and 100 % higher ATP levels than in the present study in resting rainbow trout fed with diazepam to reduce sampling disturbance. Interestingly, their creatine phosphate levels were about only 75–80 % of those in the present study (assuming white muscle constitutes 55 % of whole-body mass). Differences in total creatine levels, which were not measured in the present study, could be a factor here. Changes in metabolite levels caused by exhaustive exercise in the present investigation were similar to those in our own recent studies on comparably sized rainbow trout fry (Scarabello *et al.* 1991*a*,*b*). Scarabello *et al.* (1991*a*) provide a detailed comparison with values in the literature. We conclude that resting and immediately post-exercise measurements are in general agreement with previously reported literature values, despite differences in fish size, seasonal variation and method of sampling. However, a notable difference from some studies occurred in the rate of glycogen repletion (Fig. 2B). The rapid rate of glycogen repletion observed after both exercise bouts contrasts with many previous studies on white muscle in adult rainbow trout (e.g. Black *et al.* 1962; Milligan and Wood, 1986*b*; Dobson and Hochachka, 1987; Pearson *et al.* 1990). It also contrasts with the slow and only partial repletion seen in trout fry starved for several days prior to testing (Scarabello *et al.* 1991*a*). However, we have shown a comparable rate of repletion in fry starved for only 1 day (Scarabello *et al.* 1991*b*) as they were in the present experiment. These studies show that, at least in fry, the initial glycogen reserves may be important in determining the rate and extent of glycogen repletion after exercise.

It is possible to calculate the oxygen required to restore glycogen and high-energy phosphates. The first bout required about 26 ATP to restore glycogen (i.e. 6.5 per glucosyl unit; McGilvery, 1983); about 4 ATP were required to restore ATP itself (resynthesis from IMP is assumed, i.e. 3~P/ATP; Scarabello *et al.* 1991*a*); and about 4 ATP equivalents were needed for the creatine phosphate pool, giving a total of 34 ATP or 5.7 μmol g^{-1} O_2 (i.e. P:O=3; Astrand and Rodahl, 1970). The measured EPOC was 14.5 μmol g^{-1} wet mass in the gas exchange study and 10.8 μmol g^{-1} wet mass in the metabolite study. The second bout had a similar metabolic requirement but the EPOC values were 8.8 and 7.5 μmol g^{-1} wet mass in the gas exchange and metabolite studies, respectively.

The differences between the calculated EPOC and the measured EPOC suggest a non-metabolic cost of recovery from exercise that is lower in the second bout. In accord with our original hypothesis, part of the EPOC appears to be 'non-specific', perhaps reflecting the psychological aspects of exercise stress ('panic') exerting an influence on respiratory gas exchange and tissue metabolism. This non-specific energy demand can apparently be reduced after only one 'training' exercise bout. A simple explanation of the phenomenon would be learning, i.e. that the fish is less psychologically disturbed by the second bout. However, this does not appear to be a complete explanation because, at the metabolite level, the pattern of recovery differed. The second exercise bout resulted in similar *absolute* changes (both respiratory and metabolic) compared to the first bout. However, the *rate of*

recovery from exercise was increased, in particular for lactate (Fig. 2A), creatine phosphate (Fig. 3A) and the respiratory gases (Fig. 1).

Improved recovery from exercise after only *one* 'sprint training' session has never been shown before. Stevens and Black (1966) examined the metabolic response of rainbow trout re-exercised at intervals up to 1 h, and found almost additive effects. They concluded that 'rainbow trout are not well adapted to tolerate frequent exercise of short duration'. However, in contrast to the present study, their protocol imposed the second exercise bout before any significant recovery from the first bout had occurred. The present results suggest that, as long as full metabolism correction from the first bout has occurred, a second bout of exercise is better tolerated. This finding may be significant in terms of fish handling practices in hatchery and stocking operations, in establishing 'catch and release' sport fishing regulations, and in understanding the ability of fish to survive repeated predator attacks in the wild.

We can only speculate about the mechanism(s) responsible for these improved changes. Improved ability to recover from exhaustive exercise has been shown in a number of long-term endurance-type training studies (Hochachka, 1961; Hammond and Hickman, 1966; Lackner *et al.* 1988), as well as after prolonged sprint training (Pearson *et al.* 1990). Because of the much faster time course involved in the present study (i.e. bouts given 6 h apart), it is not likely that the same mechanisms observed with long-term training are involved, for example structural changes such as hypertrophy of muscle (Greer Walker and Emerson, 1978). Furthermore, Lackner *et al.* (1988) and Pearson *et al.* (1990) reported that after long-term training glycolysis continued for a longer period after the termination of exercise; exactly the opposite of the present study (Fig. 2A).

Recently, Wood (1991) has suggested that the cost of correcting ion and fluid volume shifts after exhaustive exercise makes a significant contribution to EPOC in trout. It may be that this factor becomes less important after a second bout. The involvement of 'stress hormones' (catecholamine, cortisol) is another possibility. Although Wood (1991) concluded that adrenaline and noradrenaline mobilization was probably not an important direct factor in stimulating $\dot{M}_{O_2}$ in fish, there is no definitive evidence on this point. If the stress hormones are important, then either a lower mobilization after the second bout or a reduced tissue response to their mobilization could decrease the costs and increase the speed of recovery. In this regard, Woodward and Smith (1985) reported that for the last 3 weeks of a course of 6 weeks of forced swimming, trained trout had lower levels of adrenaline, noradrenaline and cortisol, coupled with 15–20% lower $\dot{M}_{O_2}$, compared to untrained trout subjected to the same 6 weeks of forced swimming. However, the involvement of catecholamines and cortisol in the responses to sprint training and repetitive bouts of exercise is not yet known and requires further study.

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References

ASTRAND, P. O. AND RODAHL, K. (1970). *Textbook of Work Physiology*. New York: McGraw-Hill Book Co., pp. 301–303.

BANGSBO, J., GOLLNICK, P. D., GRAHAM, T. E., JUEL, C., KIENS, B., MIZUNO, M. AND SALTIN, B. (1990). Anaerobic energy production and O_2 deficit–debt relationship during exhaustive exercise in humans. *J. Physiol., Lond.* **422**, 539–559.

BERGMEYER, H. V. (1965). *Methods In Enzymatic Analysis*. New York: Academic Press, Inc.

BLACK, E. C., CONNER, A. R., LANE, K. C. AND CHIU, W. G. (1962). Changes in glycogen, pyruvate, and lactate in rainbow trout (*Salmo gairdneri*) during and following muscular activity. *J. Fish Res. Bd Can.* **19**, 409–436.

BOUTILIER, R. G., HEMING, T. A. AND IWAMA, G. K. (1984). Appendix: Physicochemical parameters for use in fish respiratory physiology. In *Fish Physiology*, vol. 10A (ed. W. S. Hoar and D. J. Randall), pp. 403–430. New York: Academic Press.

BRETT, J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish Res. Bd Can.* **21**, 1183–1226.

DOBSON, G. P. AND HOCHACHKA, P. W. (1987). Role of glycolysis in adenylate depletion and repletion during work and recovery in teleost white muscle. *J. exp. Biol.* **129**, 125–140.

GAESSER, G. A. AND BROOKS, G. A. (1984). Metabolic basis of excess post-exercise oxygen consumption: a review. *Med. Sci. Sports Exerc.* **16**, 29–43.

GREER WALKER, M. AND EMERSON, L. (1978). Sustained swimming speeds and myotomal muscle function in the trout, *Salmo gairdneri*. *J. Fish Biol.* **13**, 475–481.

HAMMOND, B. R., HICKMAN, C. P., JR (1966). The effect of physical conditioning on the metabolism of lactate, phosphate and glucose in rainbow trout, *Salmo gairdneri*. *J. Fish Res. Bd Can.* **23**, 65–83.

HILL, A. V. AND LUPTON, H. (1923). Muscular exercise, lactic acid and the supply and the utilization of oxygen. *Q. Jl Med.* **16**, 135–171.

HOCHACHKA, P. W. (1961). The effect of physical training on oxygen debt and glycogen reserves in trout. *Can. J. Zool.* **39**, 767–776.

LACKNER, R., WIESER, W., HUBER, M. AND DALLA VIA, J. (1988). Responses of intermediary metabolism to acute handling stress and recovery in untrained and trained *Leuciscus cephalus* (Cyprinidae, teleostei). *J. exp. Biol.* **140**, 393–404.

MARGARIA, R., EDWARDS, H. T. AND DILL, D. B. (1933). The possible mechanisms of contracting and paying the oxygen debt and the role of lactic acid in muscular contraction. *Am. J. Physiol.* **106**, 689–715.

MCGILVERY, R. W. (1983). *Biochemistry. A Functional Approach*. Toronto: Saunders.

MILLIGAN, C. L. AND MCDONALD, D. G. (1988). *In vivo* lactate kinetics at rest and during recovery from exhaustive exercise in coho salmon (*Oncorhynchus kisutch*) and starry flounder (*Platichthys stellatus*). *J. exp. Biol.* **135**, 119–131.

MILLIGAN, C. L. AND WOOD, C. M. (1986*a*). Intracellular and extracellular acid–base status and H^+ exchange with the environment after exhaustive exercise in the rainbow trout. *J. exp. Biol.* **123**, 93–121.

MILLIGAN, C. L. AND WOOD, C. M. (1986*b*). Tissue intracellular acid–base status and the fate of lactate after exhaustive exercise in the rainbow trout. *J. exp. Biol.* **123**, 123–144.

MILLIGAN, C. L. AND WOOD, C. M. (1987). Regulation of blood oxygen transport and red cell pHi after exhaustive activity in rainbow trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). *J. exp. Biol.* **133**, 263–282.

MOMMSEN, T. P. AND HOCHACHKA, P. W. (1988). The purine nucleotide cycle as two temporally separated metabolic units: a study on trout muscle. *Metabolism* **37**, 552–556.

PEARSON, M. P., SPRIET, L. L. AND STEVENS, E. D. (1990). Effect of sprint training on swim performance and white muscle metabolism during exercise and recovery in rainbow trout (*Salmo gairdneri* Richardson). *J. exp. Biol.* **149**, 45–60.

PLAYLE, R. C., MUNGER, R. S. AND WOOD, C. M. (1990). Effects of catecholamines on gas exchange and ventilation in rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **152**, 353–367.

SCARABELLO, M., HEIGENHAUSER, G. J. F. AND WOOD, C. M. (1991*a*). The oxygen debt hypothesis in juvenile rainbow trout after exhaustive exercise. *Respir. Physiol.* **84**, 245–259.

SCARABELLO, M., WOOD, C. M. AND HEIGENHAUSER, G. J. F. (1991*b*). Glycogen depletion as an

experimental test of the oxygen debt hypothesis in juvenile rainbow trout. *Can. J. Zool.* **69**, 2562–2568.

STEFFENSEN, J. F., TUFTS, B. L. AND RANDALL, D. J. (1987). Effect of burst swimming and adrenaline infusion on O_2 consumption and CO_2 excretion in rainbow trout, *Salmo gairdneri*. *J. exp. Biol.* **131**, 427–434.

STEVENS, E. D. AND BLACK, E. C. (1966). The effect of intermittent exercise on carbohydrate metabolism in rainbow trout, *Salmo gairdneri*. *J. Fish Res. Bd Can.* **23**, 471–485.

VERDOUW, H., VAN ECHTELD, C. J. A. AND DEKKERS, E. M. J. (1978). Ammonia determination based on indophenol formation with sodium salicylate. *Water Res.* **12**, 399–402.

WIESER, W., PLATZER, U. AND HINTERLEITNER, S. (1985). Anaerobic and aerobic energy production of young rainbow trout (*Salmo gairdneri*) during and after bursts of activity. *J. comp. Physiol.* **155**B, 485–492.

WOOD, C. M. (1991). Acid–base and ion balance, metabolism, and their interactions after exhaustive exercise in fish. *J. exp. Biol.* **160**, 285–308.

WOODWARD, J. J. AND SMITH, L. S. (1985). Exercise training and stress response in rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol.* **26**, 435–447.

WRIGHT, P. A. AND WOOD, C. M. (1988). Muscle ammonia stores are not determined by pH gradients. *Fish Physiol. Biochem.* **5**, 159–162.