EFFECT OF SPRINT TRAINING ON SWIM PERFORMANCE AND WHITE MUSCLE METABOLISM DURING EXERCISE AND RECOVERY IN RAINBOW TROUT (*SALMO GAIRDNERI*)

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Summary

Experimental fish were sprint trained by individual chasing for 30 s on alternate days for 9 weeks. Ten trained and 10 untrained animals were rapidly freeze-clamped at rest and 0, 1, 3 and 6 h after a 5-min chase. Swimming speed of 10 fish in each group was measured in a 2-min chase. Phosphocreatine (PCr), creatine, adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), glycogen, glucose-1-phosphate (G-1-P), glucose-6-phosphate (G-6-P), fructose-6-phosphate (Fru-6-P), glucose, glycerol-3-phosphate (Glyc-3-P), pyruvate and lactate were measured on extracts from freeze-dried white muscle. Trained fish swam 14 % further in 2 min, with 90 % of this difference occurring between 20 and 50 s. Trained fish accumulated 32 % more lactate, but showed no differences in glycogen or PCr depletion, and 22 % less ATP depletion, suggesting increased use of exogenous glucose. Glycogen repletion in early recovery, and lactate clearance between 1 and 3 h after exercise, were also enhanced in trained fish. Energy stores (ATP equivalents) were higher after exercise and especially during recovery in trained fish. Overall, sprint training minimizes endogenous fuel depletion during exhaustive swimming, even though swim speed (and distance) increases, and enhances the rate of metabolic recovery following the swim.

Introduction

Ninety percent of rainbow trout body musculature is white muscle (Johnston, 1981). This tissue is characterized by poor vascularization (Daxboeck, 1981), low mitochondrial density (Johnston, 1981) and low myoglobin content (see Johnston, 1980). It is fuelled, primarily, by anaerobic glycolysis. The high proportion of white fast twitch fibres in the muscle (compared with mammalian muscle) makes it a good model for biochemical studies (Dobson *et al.* 1987).

Exercise training protocols may be categorized as endurance (aerobic) or sprint

(anaerobic). Endurance-trained fish show elevated critical swim speeds (Farlinger and Beamish, 1978), increased fatigue resistance (Brett *et al.* 1958; Hammond and Hickman, 1966), skeletal muscle hypertrophy (Davison and Goldspink, 1977; Greer-Walker and Emerson, 1978) and enhanced anaerobic recovery (Lackner *et al.* 1988). They are also able to tolerate much higher oxygen debts (Hochachka, 1961) and larger muscle lactate loads (Hammond and Hickman, 1966). Despite the high proportion of white muscle in fish, anaerobic training has remained completely uninvestigated until very recently. Gamperl *et al.* (1988) have shown that it decreases growth and food consumption without altering body composition. Decreased solubility (more crosslinking) of white muscle collagen (A.K. Gamperl and H. Bruce, unpublished data) and elevated activities of the glycolytic enzymes phosphofructokinase (PFK), pyruvate kinase and glycogen phosphorylase (J. Bryant and E.D. Stevens, unpublished data) have also been demonstrated in sprint-trained trout.

In the present study, fish were sprint trained over a 9-week period, then terminally sampled at rest or during recovery from 5 min of intense exercise. Metabolite contents of the white muscle were measured to identify any possible training-induced metabolic advantage in the swim or recovery. We show that sprint training enhances burst swimming stamina, and provide evidence that training increases white muscle uptake of exogenous glucose in post-exercise glyconeogenesis. Higher energy stores are maintained in muscle of trained fish throughout recovery. The paper also contains the first measurements of several glycolytic intermediates in fish white muscle during recovery from exercise.

Materials and methods

Experimental animals

Rainbow trout (*Salmo gairdneri* Richardson) (9.97 ± 1.11 cm) were purchased from Humber Springs Hatchery, Orangeville, Ontario, and held in 70-l tanks supplied with continuously flowing (2.5 ± 0.9 l min^{-1}) well water (10.1 ± 0.8°C) under a 12 h:12 h photoperiod. Fish were fed pelleted food (Martin Feed Mills, Elmira, Ontario) twice daily to satiety.

Five days after introduction to the tanks, fish were randomly divided into experimental and control groups (60 fish each). Three days later training began.

Training

Experimental fish were chased, individually, at their maximum speed, for 30 s on alternate days over a 9-week period, for a total of 33 sessions. The protocol was that of Gamperl *et al.* (1988) modified by the use of an electric prod (13 V). Use of a swim tunnel was impossible as these fish will not swim at speeds of 7–8 lengths s^{-1}; they simply allow themselves to be swept into the rear barrier of the tunnel's swim compartment.

Tissue sampling

Ten control and 10 trained fish were terminally sampled at rest and at each of 0, 1, 3 and 6 h following an exhaustive 5-min chase. During recovery, fish were placed in darkened, 1.5-l flow-through tanks until sampled.

Resting levels of several metabolites, particularly phosphocreatine (PCr), adenosine triphosphate (ATP) and lactate are very difficult to measure accurately because of the effects of struggling and stress before and during the sampling procedure. Therefore, fish to be sampled at rest were transferred to a remote, isolated outdoor tank (100 l, 10 l of which was replaced every second day) at least 5 days prior to sampling. During this presampling period, the fish were given food containing diazepam (10 mg kg^{-1} body mass). Diazepam is a central nervous system depressant which acts on the reticular system (Baldessarini, 1985). Therefore, it should reduce the response to disturbance during sampling and avoid the hypoxia that is associated with anaesthetics (Houston *et al.* 1971) and may induce metabolite changes. At least 20 h prior to sampling, fish were transferred to a darkened 1-l tank supplied with water recirculated from the main tank. Fish were sampled after sunset.

All fish were freeze-clamped around the entire body at the dorsal fin with tongs pre-cooled in liquid nitrogen and were immediately immersed in nitrogen. Following removal from the liquid nitrogen, the clamped portion of the body was excised, wrapped in aluminium foil and stored at $-80\,°C$. In all cases the time required to clamp was less than 5 s, and was usually less than 3 s. The tongs have brass jaws weighing 115 g each (surface area 6.2 cm^2). Muscle freezing rate was measured using a thermocouple placed beside the spine of an intact, 47 g, dead fish. Core temperature dropped from $18\,°C$ to $-5\,°C$ within 10 s after clamping. Thermocouple position was verified by dissection after thawing.

Swimming distance

Differences in the amount of work done (swim distance) by trained and untrained fish during the swim bout could alter muscle metabolite content after exercise. We therefore measured the distance covered by 10 fish of each group during a 2-min chase. The animal was forced to swim unidirectionally in the training track. The track was long (4.27 m) and narrow (10 cm). The path of each fish was mapped by hand on scale diagrams of the exercise arena, over consecutive 10-s intervals. The narrowness of the track prevented meandering and ensured accuracy of the distance estimates. Distances were calculated using a digitizer and converted to body lengths.

Metabolite extraction and assays

White muscle was separated from the samples, under nitrogen, broken into small pieces (0.5 g), freeze-dried (16 h) and stored desiccated at $-80\,°C$. Freeze-dried muscle was powdered and dissected free of connective tissue and other non-muscular elements. Samples of prepared muscle were extracted with 0.5 mol l^{-1}

$HClO_4$ (1.0 mmol l^{-1} ethylene diamine tetraacetic acid), and neutralized (2.2 mol l^{-1} $KHCO_3$). Extracts were analyzed enzymatically (Bergmeyer, 1965) for PCr, creatine, ATP, adenosine diphosphate (ADP), adenosine monophosphate (AMP), and the glycolytic intermediates glucose, glucose-1-phosphate (G-1-P), glucose-6-phosphate (G-6-P), fructose-6-phosphate (Fru-6-P), glycerol-3-phosphate (Glyc-3-P), pyruvate and lactate, as described by Harris *et al.* (1974). Two additional samples of prepared muscle were used for duplicate determination of glycogen content. Muscle glucose and hexose monophosphates were destroyed by incubating in 0.1 mol l^{-1} NaOH at 80°C for 10 min. The extract was neutralized and glycogen was degraded to glucose with amyloglucosidase in a citrate buffer. Glucose was analyzed enzymatically as described by Bergmeyer (1965). Our results are reported as μmol g^{-1} dry muscle and literature values were converted to these units for comparison. Muscle water content was 76 % (range 74–79 %); measured as differences between wet and dry masses of muscle from three untrained fish. Training does not affect whole-body water content (Gamperl *et al.* 1988).

Calculations

Anaerobic ATP turnover rate was calculated as described by Spriet *et al.* (1987):

$$ATP \text{ turnover} = 1.5\Delta[\text{lactate}] + \Delta[\text{PCr}] + 2\Delta[\text{ATP}] - \Delta[\text{ADP}].$$

Energy stores (ATP equivalents) were calculated as:

$$[\text{ATP}] + [\text{PCr}] + 2[\text{glucose}] + 3([\text{glycogen}] + [\text{G-6-P}] + [\text{Fru-6-P}]).$$

Data analysis

Analysis of variance (ANOVA) of metabolite data was performed using a two-factor design (training and recovery time). Significant time effects were identified using Duncan's multiple-range test. When interactive effects or a significant noninteractive training effect was indicated by the ANOVA, training effects were tested for by using a simple effects test (Steel and Torrie, 1980).

Differences in swimming distance and changes of metabolite content between trained and untrained fish were examined using Student's *t*-tests (two-tailed). The 0.05 level of significance was used throughout, and all data are presented as a mean±S.E.M.

Results

Swimming distance

Trained fish swam 14 % further during the first 2 min of exercise (Fig. 1). Ninety percent of this difference was due to the maintenance of higher speeds by trained fish between 20 and 50 s. Both groups maintained similar low speeds thereafter.
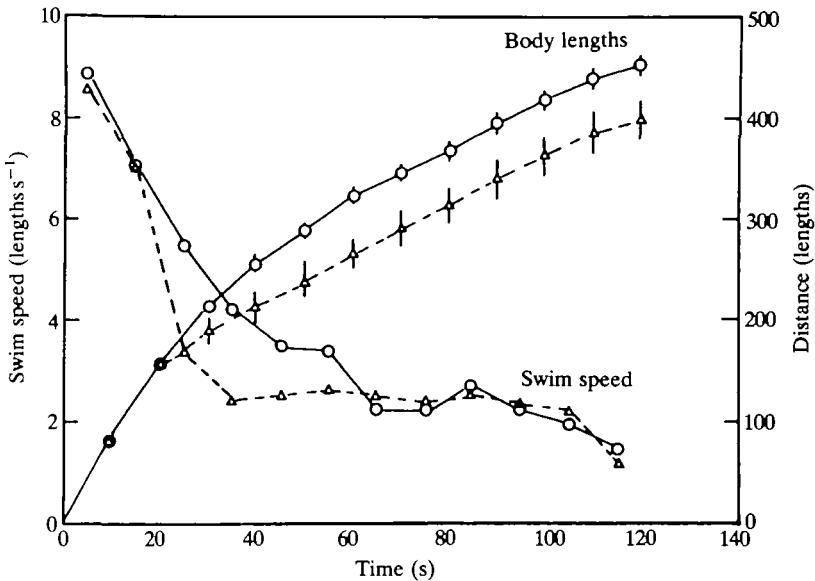
Fig. 1. The decrease in swimming speed (body lengths s^{-1}) and increase in cumulative distance (body lengths) during 2-min of intense exercise in trained (○) and untrained (△) rainbow trout, *Salmo gairdneri*. Vertical bars denote standard errors of means (N=10).

Metabolites

Metabolite contents (μmol g^{-1} dry muscle) of trained and untrained fish at each sampling time are presented in Tables 1 and 2.

Rest

Muscle lactate levels were very low in resting untrained (6.4±1.0 μmol g^{-1}) and trained (6.2±1.1 μmol g^{-1}) fish (Table 2). Resting levels of adenine nucleotides and glycolytic intermediates were in the normal ranges for fish white muscle, but PCr content was low (41.5±4.8 μmol g^{-1} in untrained and 44.9±3.5 μmol g^{-1} in trained fish) relative to values in some recent reports.

Creatine content was significantly lower in trained (113±6.4 μmol g^{-1}) than in untrained (131.6±5.8 μmol g^{-1}) fish at rest, although PCr content was not altered. Muscle of trained fish also contained more glycogen (111.4±13.0 μmol g^{-1}) than that of untrained (96.1±8.7 μmol g^{-1}) animals. This difference was not statistically significant because of high variability.

Exercise

Glycogen, ATP and PCr were greatly depleted by exercise in both groups, but trained fish had more glycogen and ATP following the swim (Tables 1 and 2). Lactate accumulated to high levels during exercise in all fish, but was significantly higher in trained (110.8±5.8 μmol g^{-1}) than in untrained (85.0±5.7 μmol g^{-1})

Table 1. *High-energy phosphates in trained and untrained rainbow trout white muscle at rest and at 0, 1, 3 and 6 h after 5 min of strenuous exercise*

| | Untrained fish Time after exercise (h) | | | | | Trained fish Time after exercise (h) | | | | |
	Rest	0	1	3	6	Rest	0	1	3	6
Phosphocreatine	41.5 (4.8)	7.0* (1.2)	54.1 (2.8)	42.1 (7.6)	42.7 (6.3)	44.9 (3.5)	2.3* (0.3)	64.6* (1.6)	68.1*·† (6.2)	57.6*·† (7.0)
Creatine	131.6 (5.8)	144.9 (8.6)	92.3* (5.9)	115.2 (6.4)	110.9* (7.2)	113.0† (6.4)	148.5* (5.4)	106.6 (3.8)	92.5*·† (5.4)	110.8 (7.1)
ATP	27.25 (1.15)	4.09* (0.26)	11.46* (0.85)	18.07* (1.51)	22.13 (1.41)	28.07 (1.36)	9.16*·† (1.43)	16.89*·† (1.05)	33.16*·† (4.43)	25.57 (1.03)
ADP	3.28 (0.12)	2.17* (0.12)	1.86* (0.09)	2.11* (0.19)	3.00 (0.17)	2.93 (0.21)	2.96† (0.19)	2.04* (0.15)	2.28* (0.19)	2.72 (0.17)
AMP	0.25 (0.05)	0.60* (0.05)	0.23 (0.03)	0.38 (0.08)	0.27 (0.02)	0.24 (0.02)	0.52* (0.08)	0.22 (0.03)	0.25 (0.03)	0.28 (0.07)

All units are μmol g^{-1} dry muscle.

Bracketed values are standard errors ($N=10$).

* Significantly different ($P<0.05$) from corresponding rest value.

† Significant difference ($P<0.05$) between trained and untrained fish.

Table 2. *Metabolites in trained and untrained trout white muscle at rest, and at 0, 1, 3 and 6 h after 5 min of strenuous exercise*

	Untrained fish Time after exercise (h)					Trained fish Time after exercise (h)				
	Rest	0	1	3	6	Rest	0	1	3	6
Glycogen	96.1 (8.7)	15.8* (2.5)	17.6* (4.4)	28.4* (2.3)	50.5* (4.6)	111.4 (13.0)	39.4*† (6.5)	50.3*† (5.2)	67.0*† (5.7)	85.1*† (7.2)
Glucose-1-phosphate	0.65 (0.08)	1.06 (0.20)	0.36 (0.10)	0.89 (0.24)	0.95 (0.08)	0.54 (0.08)	1.68* (0.31)	0.73 (0.09)	0.84 (0.14)	0.97 (0.13)
Glucose	1.28 (0.18)	4.89* (0.56)	5.75* (0.40)	8.17* (0.32)	9.97* (0.62)	1.42 (0.15)	5.69* (0.54)	7.73*† (0.60)	8.73* (0.55)	11.25* (0.64)
Glucose-6-phosphate	1.77 (0.22)	14.79* (1.76)	8.66* (1.14)	7.08* (0.67)	4.66 (0.42)	1.27 (0.19)	14.84* (1.82)	15.51*† (1.37)	6.52* (0.72)	3.93 (1.18)
Fructose-6-phosphate	0.24 (0.03)	2.04* (0.28)	1.11* (0.15)	0.90* (0.10)	0.66 (0.09)	0.21 (0.03)	1.89* (0.24)	2.16*† (0.23)	0.89* (0.10)	0.70* (0.18)
Glycerol-3-phosphate	1.00 (0.18)	3.96* (0.19)	3.72* (0.62)	3.08* (0.43)	1.45 (0.24)	0.89 (0.14)	3.35* (0.36)	3.38* (0.24)	2.91* (0.27)	1.08 (0.13)
Pyruvate	0.20 (0.04)	1.59* (0.19)	1.22* (0.11)	0.39 (0.08)	0.27 (0.04)	0.26 (0.11)	1.79* (0.20)	1.39* (0.17)	0.39 (0.08)	0.32 (0.04)
Lactate	6.4 (1.0)	85.0* (5.7)	73.8* (4.4)	41.1* (5.3)	6.4 (1.4)	6.2 (1.1)	110.8*† (5.8)	103.3*† (7.9)	30.9* (3.8)	6.7 (0.8)

All units are μmol g^{-1} dry muscle.

Bracketed values are standard errors ($N=10$).

* Significantly different ($P<0.05$) from corresponding rest value.

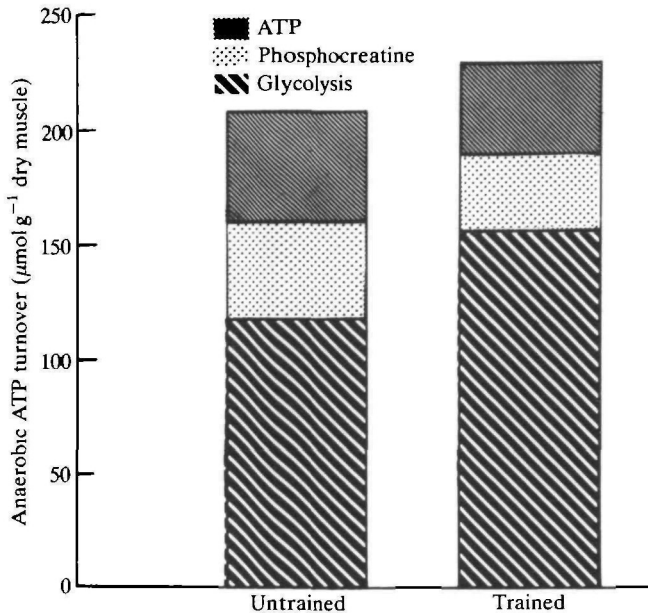† Significant difference ($P<0.05$) between trained and untrained fish.

Fig. 2. Calculated anaerobic ATP turnover in untrained and trained rainbow trout, *Salmo gairdneri*, during 5 min of intense exercise. Calculations were made assuming 1.5 mol of ATP are produced per mol of lactate produced, 1 mol of ATP is produced per mol of phosphocreatine hydrolysed, and $2(\Delta[ATP]) - \Delta[ADP]$ mol of ATP are produced in adenylate pool depletion.

animals. Contents of glucose and all measured glycolytic intermediates also increased significantly following exercise, but no training effects were observed. Accumulations of lactate and glycolytic intermediates accounted for all (104 %) of the measured decrease in glycogen in trained fish, but only 75 % in untrained fish.

ADP content decreased significantly in muscle of untrained fish, but was maintained at the resting level in trained fish immediately after exercise. AMP content increased significantly during exercise in both groups.

Calculated anaerobic ATP turnover was not significantly affected by training, but the relative contributions of its components were. Trained fish used more ATP from glycolysis and less from ATP depletion than did untrained fish (Fig. 2). PCr depletion did not significantly differ between groups.

Recovery

Phosphocreatine recovered to levels in excess of rest (130 % in untrained, 144 % in trained fish) in less than 1 h. In trained fish, this excess was maintained for at least 6 h, while it was transient in untrained fish, returning to rest levels by 3 h after exercise. ATP recovered significantly in both groups during the first hour. In trained fish, ATP content overshot the resting level by 3 h after exercise, but returned to it by 6 h. In contrast, ATP in untrained fish rose continuously, only reaching resting levels by 6 h. The fast recovery of trained fish seems to result from

a smaller amount of depletion during exercise, as training did not significantly affect the rate of repletion. In both groups, ADP content fell for the first hour after exercise, but recovered to resting values by 6 h. AMP recovery was complete in less than 1 h.

Glycogen repletion was significant but incomplete after 6 h, reaching 52 % and 76 % of rest levels in untrained and trained fish, respectively (Table 2). Glycogen content increased more during the first hour of recovery in trained than in untrained fish, although the content after 1 h was not significantly different from the post-exercise value in either case. Lactate content remained at the post-exercise level for the first hour in both groups (Table 2). It then quickly dropped in trained fish, reaching untrained levels by 3 h. Recovery of both groups was complete in 6 h. Muscle lactate disappearance during the first hour of recovery could account for only 35 % of the glycogen repletion in trained fish. In contrast, the glycogen repletion was more than accounted for (311 %) by lactate disappearance in untrained fish. Between 1 and 3 h after exercise, all glycogen repletion could be accounted for by lactate disappearance (217 % in trained, 154 % in untrained fish), but between 3 and 6 h only 79 % of the repletion in untrained and 67 % of the repletion in trained fish could be lactate-derived.

Muscle glucose rose continuously during recovery, reaching levels double those immediately after exercise and almost 10 times above resting levels by 6 h in both groups. Trained fish had significantly more muscle glucose at 1 h after exercise (Table 2).

G-1-P recovered completely in 1 h in both groups. G-6-P and Fru-6-P recovered in 6 h, but the pattern was influenced by training. In trained fish, post-exercise muscle contents were maintained for at least 1 h, while in untrained fish, they dropped immediately after exercise (Table 2). Levels at 3 and 6 h were similar in both groups. Post-exercise levels of Glyc-3-P, in both groups, were maintained for the first 3 h after exercise, but recovery was complete in 6 h. Pyruvate levels recovered completely in 3 h in both groups.

Energy stores were substantially higher in trained than in untrained fish throughout recovery (Fig. 3). Immediately after exercise, energy stores in trained fish were 60 % higher. By 1 h after exercise, the stores were 89 % higher because of an increase in glycogen recovery and the maintenance of post-exercise G-6-P levels.

Discussion

The resting state

The difficulty in obtaining accurate resting values for several metabolites in fish is reflected in the wide range of reported values. For example, reports of resting white muscle lactate levels in rainbow trout range from 6.4 ± 1.0 (present study) to $61.1\pm23.0\ \mu\text{mol g}^{-1}$ dry muscle (Stevens and Black, 1966).

In recent years, improved sampling techniques, such as rapid freeze-clamping in liquid nitrogen, have led to reports of lower lactate values and higher glycogen and
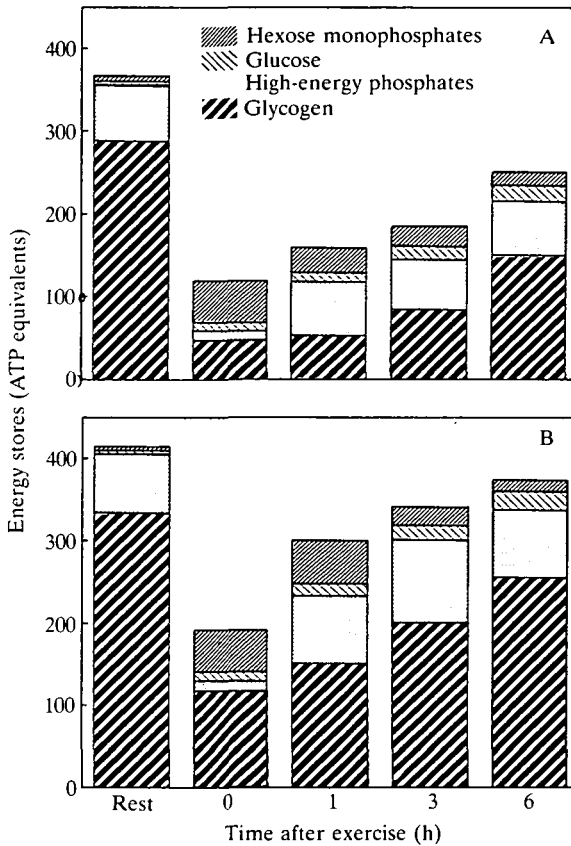
Fig. 3. Energy stores, as ATP equivalents, in untrained (A) and trained (B) rainbow trout white muscle, at rest and during recovery from 5 min of intense exercise. Calculations were made assuming 1 mol of ATP is produced per mol of phospho-creatine, 3 mol of ATP are produced per mol of glycogen, glucose-6-phosphate or fructose-6-phosphate, and 2 mol of ATP are produced per mol of glucose. High-energy phosphates denote sums of ATP and phosphocreatine, and hexose monophosphates indicate sums of glucose-6-phosphate and fructose-6-phosphate.

PCr values at rest (e.g. Dobson *et al.* 1987; Parkhouse *et al.* 1988*a*,*b*; present study).

In the present study, the use of orally administered diazepam and a rapid freeze-clamping technique resulted in the lowest resting lactate values reported to date for rainbow trout. Glycogen contents were equivalent to the highest reported values (93.4±24.0 μmol g^{-1} in Dobson *et al.* 1987; 97.2±4.2 μmol g^{-1} in Parkhouse *et al.* 1988*a*). However, PCr content was about half that reported in most other studies (Driedzic *et al.* 1981; Milligan and Wood, 1986; Dobson *et al.* 1987; Parkhouse *et al.* 1988*b*). Because of the high affinity of creatine kinase for ADP, and its high activity, PCr is the first fuel to be depleted (Driedzic and Hochachka, 1978). It appears that, despite the diazepam, significant PCr hydrolysis occurred

during sampling. A single tail-flip is sufficient to decrease the PCr content of trout white muscle by 50 % (Dobson and Hochachka, 1987) but is unlikely to elevate lactate levels significantly. Owing to the speed of PCr recovery, and the total lack of struggling by fish sampled 1 h after exercise, PCr measurements from these animals (54.1 ± 2.8 in untrained, $64.6\pm1.6\,\mu$mol g^{-1} in trained) are probably more representative of true resting levels.

Resting ATP content in this study is close to the $31.27\pm0.46\,\mu$mol g^{-1} dry muscle reported by Parkhouse *et al.* (1988*b*), but almost double the $15.8\pm1.5\,\mu$mol g^{-1} of Milligan and Wood (1986). This difference is difficult to explain, as stress-related hydrolysis of ATP in the latter case is unlikely, given their high PCr values ($105\pm9\,\mu$mol g^{-1}). Our AMP and ADP values (Table 1) agree well with previous studies (Dobson *et al.* 1987; Parkhouse *et al.* 1988*a*), as do the levels of resting glycolytic intermediates (Dobson *et al.* 1987; Parkhouse *et al.* 1988*a*).

Exercise

The post-exercise lactate content of untrained fish (Table 2) was lower than in studies using similar exercise protocols ($115\pm16.7\,\mu$mol g^{-1} in Black *et al.* 1962; $216.7\pm23.5\,\mu$mol g^{-1} in Stevens and Black, 1966; $153\pm15.9\,\mu$mol g^{-1} in Milligan and Wood, 1986; $149.6\pm2.0\,\mu$mol g^{-1} in Dobson *et al.* 1987). The reason is unclear, but may relate to differences in exercise protocols.

The increases in levels of glycolytic intermediates (Table 2) and AMP, and the decreases in ATP (Table 1), agree well with existing reports (Dobson *et al.* 1987; Parkhouse *et al.* 1988*a*). ADP content decreased with exercise in untrained fish but did not change in trained fish until the recovery phase. ADP is an intermediate in the pathway of adenylate depletion (ATP to IMP). Its level depends on the relative rates of several reactions (Driedzic and Hochachka, 1978), making it difficult to identify why training induces differences.

Recovery

Glycogen recovery after exercise is slower in fish than in mammals. Black *et al.* (1960, 1962) reported insignificant recovery in the 6 h following a 15-min chase, and less than half complete recovery in 24 h. Milligan and Wood (1986), however, demonstrated a substantial repletion in 8 h, after chasing trout for 6 min, and we found significant glycogen recovery in untrained fish in 6 h. Our fish were exercised for only 5 min, while Black *et al.* (1962) chased theirs for 15 min. Milligan and Wood (1986) argued that repletion is delayed until intracellular pH has recovered to a critical level. The 10 min difference in exercise duration between the present study and that of Black *et al.* (1960, 1962), however, would have a minimal effect on white muscle pH, as the tissue is largely depleted of fuel in less than 5 min. Red muscle, fuelled by aerobic metabolism, probably provides the vast majority of subsequent propulsion. We cannot explain why glycogen recovery was so protracted in the studies of Black *et al.* (1960, 1962), but our data support the view that glycogen repletion in trout white muscle is a faster process than their work indicates.

Lactate recovery time was within the usual range, being complete in less than 6 h. Black *et al.* (1962) reported almost complete recovery in 6 h, and Milligan and Wood (1986) show that it requires between 4 and 8 h.

Dobson and Hochachka (1987) reported a large post-exercise lactate accumulation in white muscle and concluded that this indicated continued glycolytic activity. They argued that the derived energy was used to replete ATP and PCr stores. Our results do not support their contention (Table 2), nor do several other studies (Black *et al.* 1962; Turner *et al.* 1983; Milligan and Wood, 1986). As lactate oxidation yields 18 mol of ATP per mol of substrate, an undetectable amount of it could account for the repletion.

Training

Swimming performance

Sprint training improves endurance at burst speeds (Fig. 1) and trained fish achieve higher accelerations in the first 50 ms of a fast start (A. K. Gamperl, D. Schnurr and E. D. Stevens, unpublished data). As fish rely on sprint swimming in nature (e.g. in predator–prey interactions), these differences may represent an adaptive advantage.

Metabolites at rest

Boobis *et al.* (1983) demonstrated that sprint training increases glycogen stores in man. Our results (Table 2) suggest that this may occur in fish, but high variability precluded statistical significance. Trained fish did not use more glycogen, but preserved higher post-exercise levels. Perhaps increased glycogen stores at rest, resulting in a higher post-exercise level, is an adaptation for conserving energy for a second exercise bout.

Metabolites during exercise

The high variability in the glycogen measurements may also be masking a training-induced elevation in glycogen depletion which would explain the higher post-exercise lactate levels in trained than in untrained fish (Table 2). Alternatively, the difference in lactate accumulation could be ascribed to increased use of exogenous glucose by trained fish or loss of some glycolytic carbon in the untrained fish.

Trained fish would need to sequester 13 μmol of glucose per g of dry white muscle from exogenous sources during the 5 min of exercise to account for the 30 % difference in lactate accumulation. Given a blood glucose concentration of 4 mmol l^{-1} (Milligan and Wood, 1986), and 102 ml kg^{-1} min^{-1} blood flow in exercised white muscle (Neuman *et al.* 1983), and assuming 100 % removal of glucose from the blood, only 2.0 μmol g^{-1} can be accounted for. These numbers are based on untrained fish. The actual uptake by trained fish may be higher, as capillary proliferation (which would allow more blood flow and, consequently, glucose delivery) is stimulated by local hypoxia (Hudlicka, 1985), which certainly would have occurred in our training regime. Increased hexokinase (HK) activity

would also result in greater glucose uptake. Sprint-trained rats show elevated maximal HK activity (Staudte *et al.* 1973), but sprint-trained rainbow trout do not (J. Bryant and E. D. Stevens, unpublished data). Although *in vitro* HK is inhibited by high G-6-P concentrations (Sols, 1981), and G-6-P levels are high in exercise and recovery, the effects *in vivo* and after sprint training are unknown.

If lactate dehydrogenase (LDH) became saturated with pyruvate during exercise in untrained fish, the excess substrate could be catalyzed by glutamate–pyruvate transaminase (GPT) to form alanine, and leave the glycolytic pathway. Unlike the LDH reaction, alanine synthesis from pyruvate does not produce NAD to balance the cell's redox state. Consequently, it is an advantage to maximize lactate production from pyruvate. Perhaps the higher lactate yield in trained fish is due to a reduction in the amount of pyruvate shunted to alanine. Unfortunately, we have no data on the relative activities of LDH in trained and untrained fish or on alanine content of white muscle before and after exercise. The activities of LDH and GPT in rainbow trout white muscle, however, are 340 and $2 \text{i.u.} \text{g}^{-1}$, respectively (Gaudet *et al.* 1975). The disparity suggests that this pathway's flux is relatively small in trout. We believe that enhanced glucose uptake in trained fish may contribute to the disparity in glycogenolysis accounted for by lactate production between the treatments, especially in view of the evidence suggesting its use in recovery (see below).

Metabolites during recovery

Only 35 % of the $10.9 \mu\text{mol g}^{-1}$ glycogen repletion in trained fish during the first hour of recovery can be explained by lactate disappearance, while untrained fish showed no repletion during this time. Trained fish may provide the missing substrate through enhanced glucose uptake. The lack of decrease in G-6-P and Fru-6-P (which is in equilibrium with G-6-P) suggests anabolic activity leading from glucose to glycogen. The benefits of such a system are apparent. Glycogen synthesis from glucose requires an investment of only two ATP per glucosyl unit, while three are required when lactate is the substrate.

Glyconeogenesis from lactate is also delayed, in trout, until intracellular pH rises from its post-exercise level (Milligan and Wood, 1986). In the present study, glycogen levels in untrained fish did not recover at all in the first hour after exercise (Table 2). Sprint training may have increased muscle buffering capacity, an effect known to occur in humans (Parkhouse *et al.* 1985), and/or resulted in enhanced H^+ efflux from muscle. Increased tissue perfusion (see above) could account for the latter. Either scenario would result in increased intracellular pH and an earlier start of lactate-fuelled glyconeogenesis.

The maintenance of high levels of G-6-P and Fru-6-P for 1 h after exercise (Table 2) is also advantageous to trained fish. Glycolysis remains primed and glucosyl units are effectively stored in the pathway. Quantitatively, at 1 h after exercise, trained fish have $7.9 \mu\text{mol g}^{-1}$ glucosyl units more than untrained fish stored in these compounds, corresponding to $23.7 \mu\text{mol g}^{-1}$ ATP production. The 15.54 ± 1.37 glucosyl units stored as G-6-P and Fru-6-P amounts to 16 % of the total

stored energy (ATP equivalents) in trained fish at this early stage of recovery (Fig. 3).

The rapid decrease in muscle lactate content of trained *versus* untrained fish between 1 and 3 h (Table 2) is similar to that found by Lackner *et al.* (1988). They attributed the decrease to clearance, *via* the blood, for oxidation by other tissues. In actuality it could be explained by any combination of increases in clearance, oxidation and glyconeogenesis. Increased perfusion (see above) could explain higher efflux. The greater muscle-to-blood electrochemical gradient caused by the higher lactate load in trained fish would also contribute, assuming that efflux is not carrier-dependent. Hammond and Hickman (1966) showed that endurance-trained trout removed lactate *via* the blood more quickly than did untrained fish. Increased oxidation could result from higher mitochondrial densities in sprint-trained trout, a possibility now being explored. It is not possible to ascertain from our data whether trained fish were using more lactate in glyconeogenesis between 1 and 3 h after exercise.

The cumulative effect of the training adaptations is seen in the higher energy stores of trained fish after exercise and throughout recovery (Fig. 3). The trained fish are in much better condition to cope with a second exercise bout and, in fact, swim significantly farther (and faster) in a second 2-min chased swim 2 h after the initial one (E. Luiker and E. D. Stevens, unpublished data). Stevens and Black (1966) concluded that trout (untrained) were poorly adapted to intermittent exercise, even though this is probably the type most critical to survival (i.e. predator–prey interactions). Metabolically, wild fish are probably intermediate between the trained and untrained groups. Perhaps the poor survivorship (Miller, 1958) sometimes observed in hatchery, relative to wild, fish is in part due to metabolic differences.

In conclusion, sprint training increases endurance in trout at sprint swim speeds. Glycogen repletion may be enhanced both temporally and in efficiency by the use of exogenous glucose early in recovery. Maintenance of post-exercise G-6-P and Fru-6-P levels keeps glycolysis primed and stores glucosyl units in the glycolytic pathway. Training also enhances lactate clearance between 1 and 3 h after exercise, allowing recovery from the higher loads to be completed in the same time as in untrained fish. Overall, training minimizes endogenous fuel depletion and enhances the rate of recovery.

References

Baldessarini, R. J. (1985). Drugs and the treatment of psychotic disorders. In *The Pharmacological Basis of Therapeutics*, 7th edn, chapter 17 (ed. A. E. Goodman, L. S. Goodman, T. W. Rall and F. Murad). New York, Toronto, London: Macmillan.

BERGMEYER, H. U. (1965). *Methods of Enzymatic Analysis.* New York: Academic.

BLACK, E. C., CONNOR, A. R., LAM, K. AND CHIU, W. (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.

BLACK, E. C., ROBERTSON, A. C., HANSLIP, A. R. AND CHIU, W. (1960). Alterations in glycogen, glucose, and lactate in rainbow and kamloops trout, *Salmo gairdneri,* following muscular activity. *J. Fish. Res. Bd Can.* **17**, 487–500.

BOOBIS, L. H., WILLIAMS, C. AND WOOTON, S. A. (1983). Influence of sprint training on muscle metabolism during brief maximal exercise in man. *J. Physiol., Lond.* **342**, 36–37 (Abstract).

BRETT, J. R., HOLLANDS, M. AND ALDERDICE, D. F. (1958). The effect of temperature on the cruising speed of young sockeye and coho salmon. *J. Fish. Res. Bd Can.* **15**, 587–605.

DAVISON, W. AND GOLDSPINK, G. (1977). The effect of prolonged exercise on the lateral musculature of the brown trout (*Salmo trutta*). *J. exp. Biol.* **70**, 1–12.

DAXBOECK, C. (1981). A study of the cardiovascular system of the rainbow trout (*Salmo gairdneri*) at rest and during swimming exercise. PhD thesis, University of British Columbia, Vancouver.

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.

DOBSON, G. P., PARKHOUSE, W. S. AND HOCHACHKA, P. W. (1987). Regulation of anaerobic ATP generating pathways in trout fast-twitch skeletal muscle. *Am. J. Physiol.* **253**, R186–R194.

DRIEDZIC, W. R. AND HOCHACHKA, P. W. (1978). Metabolism in fish during exercise. In *Fish Physiology,* vol. VII (ed. W. S. Hoar and D. J. Randall), pp. 503–541. New York, London: Academic Press.

DRIEDZIC, W. R., McGUIRE, G. AND HATHEWAY, M. (1981). Metabolic alterations associated with increased energy demand in fish white muscle. *J. comp. Physiol.* **141**B, 425–432.

FARLINGER, S. AND BEAMISH, F. W. H. (1978). Changes in blood chemistry and critical swimming speed of largemouth bass, *Micropterus salmoides,* with physical conditioning. *Trans. Am. Fish. Soc.* **107**, 523–527.

GAMPERL, A. K., BRYANT, J. AND STEVENS, E. D. (1988). Effect of a sprint training protocol on growth rate, conversion efficiency, food consumption, and body composition of rainbow trout (*Salmo gairdneri* Richardson). *J. Fish Biol.* **33**, 861–870.

GAUDET, M., RACICOT, J. G. AND LERAY, C. (1975). Enzyme activities of plasma and selected tissues in rainbow trout *Salmo gairdneri* Richardson. *J. Fish Biol.* **7**, 505–512.

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. AND 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.

HARRIS, R. C., HULTMAN, E. AND NORDESJO, L.-O. (1974). Glycogen, glycolytic intermediates and high-energy phosphates determines in biopsy samples of musculus quadriceps femoris of man at rest. *Scand. J. clin. Lab. Invest.* **33**, 109–120.

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

HOUSTON, A. H., MADDEN, J. A., WOODS, R. J. AND MILES, H. M. (1971). Some physiological effects of handling and tricaine methane–sulfonate anesthetization upon the brook trout, *Salvelinus fontinalis. J. Fish. Res. Bd Can.* **28**, 625–633.

HUDLICKA, O. (1985). Development and adaptability of microvasculature in skeletal muscle. *J. exp. Biol.* **115**, 215–228.

JOHNSTON, I. A. (1980). Specialization of fish muscle. In *Development and Specializations of Muscle. Soc. exp. Biol. Seminar Series Symp.* **7** (ed. D. F. Goldspink), pp. 123–148. Cambridge: Cambridge University Press.

JOHNSTON, I. A. (1981). Structure and function of fish muscles. *Symp. zool. Soc. Lond.* **48**, 71–113.

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.

MILLER, R. B. (1958). The role of competition in the mortality of hatchery trout. *J. Fish. Res. Bd Can.* **15**, 27–45.

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

NEUMAN, P., HOLETON, G. F. AND HEISLER, N. (1983). Cardiac output and regional blood flow in gills and muscles after exhaustive exercise in rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **105**, 1–14.

PARKHOUSE, W. S., DOBSON, G. P. AND HOCHACHKA, P. W. (1988a). Control of glycogenolysis in rainbow trout muscle during exercise. *Can. J. Zool.* **66**, 345–351.

PARKHOUSE, W. S., DOBSON, G. P. AND HOCHACHKA, P. W. (1988b). Organization of energy provision in rainbow trout during exercise. *Am. J. Physiol.* **254**, R302–R309.

PARKHOUSE, W. S., McKENZIE, D. C., HOCHACHKA, P. W. AND OVALLE, W. K. (1985). Buffering capacity of deproteinized human *vastus lateralis* muscle. *J. appl. Physiol.* **58**, 14–17.

SOLS, A. (1981). Multimodulation of enzyme activity. *Curr. Topics Cell Regul.* **19**, 77–101.

SPRIET, L. L., SODERLUND, K., BERGSTROM, M. AND HULTMAN, E. (1987). Anaerobic energy release in skeletal muscle during electrical stimulation in men. *J. appl. Physiol.* **62**, 611–615.

STAUDTE, H. W., EXNER, G. U. AND PETTE, D. (1973). Effects of short-term high intensity (sprint) training on some contractile and metabolic characteristics of fast and slow muscle of the rat. *Pflügers Arch. ges. Physiol.* **344**, 159–168.

STEEL, R. G. D. AND TORRIE, J. H. (1980). *Principles and Procedures of Statistics: A Biometrical Approach.* 2nd edn. New York: McGraw-Hill.

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.

TURNER, J. D., WOOD, C. M. AND CLARK, D. (1983). Lactate and proton dynamics in the rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **104**, 247–268.