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THE IMPACT OF ENDURANCE TRAINING ON ARTERIAL PLASMA K^+ LEVELS AND SWIMMING PERFORMANCE OF RAINBOW TROUT

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Summary

Arterial plasma K^+ and lactate concentrations ($[K^+]_a$ and $[lactate]_a$), as well as blood oxygenation status, were measured in relation to increasing swimming speeds in rainbow trout *Oncorhynchus mykiss*. Neither $[K^+]_a$ nor $[lactate]_a$ changed at swimming speeds below 1.5 BL s^{-1}, where BL is total body length. Between 1.5 and 2.0 BL s^{-1}, $[K^+]_a$ started to increase, and above 2.0 BL s^{-1} both $[K^+]_a$ and $[lactate]_a$ increased with swimming speed. Training shifted the onset of these increases to higher swimming speeds and increased the critical swimming speed (U_{crit}) from 2.4 to 3.0 BL s^{-1}. Blood oxygen content showed no changes in control fish, whereas in trained fish it increased by 22 % at the final swimming speed. From the $[K^+]_a$ data, we suggest that no loss of K^+ occurred from the working muscle at low swimming speeds, allowing an unlimited endurance, whereas moderate and higher speeds were probably associated with a loss of K^+ from the working muscles, indicating a limited endurance.

Key words: potassium, lactate, rainbow trout, *Oncorhynchus mykiss*, swimming, endurance, muscle fatigue, Pa_{O_2}, haematocrit.

Introduction

Migratory fish species such as trout can swim for months without rest (e.g. Davie *et al.* 1986), and other species such as tuna are actually dependent on continuous swimming for respiration and buoyancy. The life style of many fish thus differs from that of mammals, which divide their life into active and resting periods.

Continued swimming in fish relies almost exclusively on the red muscle, which in trout constitutes less than 5 % of the trunk muscle mass (Davie *et al.* 1986). Thus, a small fraction of the skeletal muscle mass is repeatedly exposed to propagating action potentials and a concomitant Na^+ influx and K^+ efflux. To sustain such muscle activity, the regulation of levels of these ions must suffice to maintain their electrochemical potentials. In mammals, the onset of exercise is always reported to be associated with increases in arterial and venous plasma K^+ concentrations ($[K^+]_a$ and $[K^+]_v$) and a negative arterio-venous difference in plasma K^+ concentration ($[K^+]_{a-v}$) across the working muscle, implying a loss of K^+ (e.g. Hallén, 1996; Lindinger, 1995; Sjøgaard *et al.* 1985; Vøllestad *et al.* 1994). These changes are quantitatively correlated to the intensity of the exercise (Hallén, 1996; Lindinger, 1995; Lindinger and Sjøgaard, 1991; Rolett *et al.* 1990; Sjøgaard *et al.* 1985; Sjøgaard, 1990; Vøllestad *et al.* 1994). Both an increase in $[K^+]_a$ and a loss of K^+ from the muscle lead to a decrease in the membrane potential of the muscle cell, and it is suggested that this may contribute to the development of muscle fatigue (Bangsbo *et al.* 1996; Sjøgaard *et al.* 1985; Sjøgaard, 1990, 1996). At a specific work intensity, the changes in both $[K^+]_a$ and $[K^+]_v$ are decreased following training, and both endurance and the maximal rate of oxygen uptake ($\dot{V}_{O_2max}$) are improved in mammals (Green *et al.* 1993; Madsen *et al.* 1994; McKenna *et al.* 1993; McKenna, 1995; Tibes *et al.* 1976; Kjeldsen *et al.* 1990*a*). Studies on swimming trout reported increases in $[K^+]_a$ at swimming velocities of 1–1.5 BL s^{-1} (Nielsen *et al.* 1994; Nielsen and Lykkeboe, 1992; Thomas *et al.* 1987), i.e. at swimming speeds that the fish is supposed to be capable of sustaining. However, these studies only dealt with a limited range of swimming velocities.

The aim of the present study was to examine $[K^+]_a$ and $[lactate]_a$ during sustained swimming in untrained and trained trout. This was achieved at a range of speeds from a low level of exercise to fatiguing exercise. In addition, arterial blood oxygen tension (Pa_{O_2}), tetramer haemoglobin concentration ($[Hb_4]_a$), oxygen concentration (Ca_{O_2}), haematocrit and pH (pHa) and the plasma concentration of cortisol were measured to evaluate the effects of swimming speed on these parameters.

Materials and methods

Experimental animals

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] all from the same stock were obtained from a commercial farmer and acclimated to 15 °C and a 12 h:12 h light:dark photoperiod. The fish were randomly divided into two groups. One group served

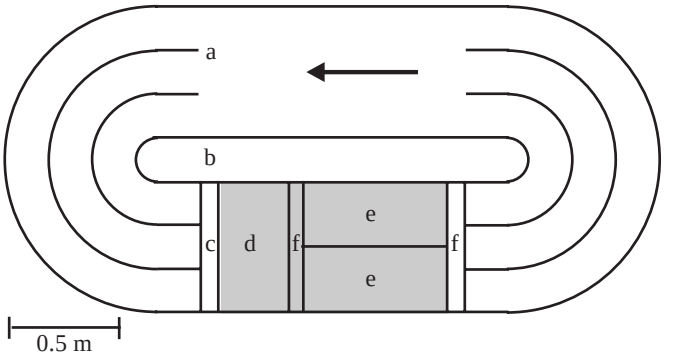

Fig. 1. An outline representing both the training and the test raceways viewed from above. In the first raceway (used for training), a constant water flow was created by a 6 kW centrifugal pump recirculating the water through a multibarrel injector. The water flow of the second raceway (used for the swim test) was created by a 5 kW four-blade propeller controlled by a frequency regulator. The water velocity was measured with a flow probe (Höntzsch μP-ASDI). (a) Turning vanes; (b) cooling section; (c) honeycomb layer for linearization of the flow; (d) swim section for training (grey-shaded area); length, 1.2 m; width, 0.6 m; depth, 0.5 m; (e) swim test sections; length, 0.7 m; width, 0.27 m; depth, 0.2 m; (f) fish retaining grids.

as controls and was maintained in aerated water, and the other group was placed in a training raceway (see Fig. 1 for a description). All fish were fed commercial trout pellets once daily. The fish in the training raceway were trained to swim for 23 h day^{-1} at 1.2–1.5 BL s^{-1}, where BL is total body length, over a 10 day period with regular increments in daily swimming time from 4 h day^{-1} initially to 23 h day^{-1} finally, which was then maintained for a minimum of 2 months. Fish were acclimated to these conditions on two occasions: October 1995 (approx. 300 g fish) and April 1996 (approx. 200 g fish). These fish are referred to as W95 and S96 respectively.

Swim test

The swimming capacity of the fish was measured in the test raceway (see Fig. 1 for a description). In this raceway, the water velocity was adjustable from 0 to approximately 100 cm s^{-1}. To allow sampling of arterial blood, the fish were anaesthetized (benzocaine, 0.1 g l^{-1}) and fitted with a dorsal aortic cannula (Soivio *et al.* 1975). The fish were then placed in the swim chamber of the test raceway and allowed to recover until the next day, at a water velocity of 10 cm s^{-1} (routine swimming).

The swim test *per se* consisted of hourly step increases in water speed corresponding to 0.5 BL s^{-1} until the fish fatigued (defined as the inability of the fish to stay free of the back grid of the swim chamber).

A blood sample was withdrawn into a syringe without anticoagulant and analysed for a number of parameters (see below) 45–50 min after each step increase in water speed or immediately when fatigue occurred. The blood volume was replaced with heparinized physiological saline (100 i.u. ml^{-1}). Finally, the fish was killed by a sharp blow to the head, and the total length (cm) and mass (g) were measured.

Analytical procedures

For the W95 fish, approximately 0.6 ml of blood was withdrawn at all swimming speeds, and 150 μl was immediately centrifuged at 10^4 revs min^{-1} for 2 min in an Eppendorf tube to separate the plasma. Plasma $[K^+]$ was measured in duplicate (2×20 μl) in a Radiometer FLM3 flame photometer, and 10 μl was used for determination of plasma lactate levels (Sigma 735-10). The remaining plasma was frozen in heparin-coated Eppendorf tubes for determination of cortisol levels (Diagnostic Product Corporation, Coat-a-Count). Blood haematocrit was measured in heparinized microhaematocrit tubes (centrifuged at 10^4 revs min^{-1} for 10 min), $[Hb_4]_a$ was measured using the cyanmethaemoglobin method (Zijlstra *et al.* 1983), pHa was measured with a Radiometer BMS2 Mk2 system connected to a PHM73 unit, P_{aO_2} was measured with a Radiometer 5046 thermostatically controlled oxygen electrode, and C_{aO_2} was measured by the Tucker (1967) method. In the smaller S96 fish, P_{aO_2}, C_{aO_2} and pHa were only measured at the routine and at the final swimming speed, reducing the blood sample at intermediate swimming speeds to 0.3 ml.

Calculations and data presentation

The swimming speed was calculated as water velocity divided by total fish length. The critical swimming speed (U_{crit}) was calculated as described by Brett (1964). Erythrocyte tetramer haemoglobin concentration ($[Hb_4]_{aE}$) was calculated from $[Hb_4]_a$ and the haematocrit, ignoring trapped plasma. The condition factor of the fish was calculated as 100×body mass/(total length)^3. Values are given as means ± S.D. unless stated otherwise. Student's unpaired or paired *t*-tests were used where appropriate, and the significance level was taken as 5 %.

Results

Fish condition

The fish increased in both length and mass during the acclimation period; the mean final mass and length and the condition factor are given in Table 1. Mass and length were significantly larger in the W95 groups than in the S96 groups, and the condition factor tended to be larger in the trained groups. All fish appeared healthy.

Swimming performance

The critical swimming speeds of the S96 fish are shown in Fig. 2. All of the control and five of the eight trained fish fatigued during the swim test. The mean U_{crit} was 2.4±0.6 BL s^{-1} (N=8) for control fish, and this was lower (P<0.02) than the underestimate of 3.0±0.3 BL s^{-1} (N=8) for trained fish (estimated from U_{crit} of fatigued fish and the highest attained swimming speed of the remaining fish). The highest water velocity of the test raceway was too low to exhaust the larger W95 fish consistently, rendering calculation of U_{crit} for these fish impossible. However, four out of six control fish and two out of seven trained fish fatigued, and the remaining fish, two control and five trained, were still

Table 1. *Mass, length and condition factor for trained and control fish of the W95 and S96 series*

	Total body length, BL (cm)	Body mass, M_b (g)	Condition factor, $100\times M_b/BL$
Trained, W95 (N=7)	34.6±2.8	576±114	1.40±0.19
Control, W95 (N=6)	34.8±3.0	536±150	1.24±0.10
Trained, S96 (N=8)	29.8±1.0*	398±46*	1.50±0.14
Control, S96 (N=8)	30.3±1.7*	391±67*	1.40±0.10

No differences were found within the two series between trained and control fish.

Differences between trained and control fish across the two series are marked with an asterisk (P<0.02).

Values are means ± S.D.

swimming at a mean velocity of 2.7 BL s^{-1}. Thus, it appeared that the trained W95 fish also performed better and that the U_{crit} values were probably close to the values measured for the S96 control and trained fish.

Arterial plasma [K⁺] and [lactate]

Before the onset of the swim test, $[K^+]_a$ was approximately 2.2 mmol l^{-1}, and it did not change at swimming speeds below 1.5 BL s^{-1} (Fig. 3). At swimming speeds above 1.5 BL s^{-1}, $[K^+]_a$ started to increase, and training tended to shift this increase to higher swimming speeds (Fig. 3). Training thus tended to widen the speed range over which baseline $[K^+]_a$ was maintained. $[K^+]_a$ at fatigue did not differ between control and trained fish or between W95 and S96 fish. The combined mean $[K^+]_a$ at fatigue of 3.37±0.47 mmol l^{-1} (N=19) was significantly higher than the value of 2.88±0.52 mmol l^{-1} at the preceding swimming speed (P<0.01, Student's unpaired t-test). Neither trained nor control fish showed any change in $[lactate]_a$ at swimming speeds below 2.0 BL s^{-1} (Fig. 4). (The aberrant value obtained for a single control fish was probably due to contamination of the test tube). At higher swimming speeds, $[lactate]_a$ increased and, like $[K^+]_a$, the increase occurred at lower swimming speeds for the control than for the trained group. At fatigue, however, there was no difference in $[lactate]_a$ between trained and control fish, nor between W95 and S96 fish. The combined mean value of 4.07±2.45 mmol l^{-1} (N=19) at fatigue was, however, significantly higher than the mean of 1.74±1.68 mmol l^{-1} at the preceding swimming speed (P<0.01, Student's unpaired t-test).

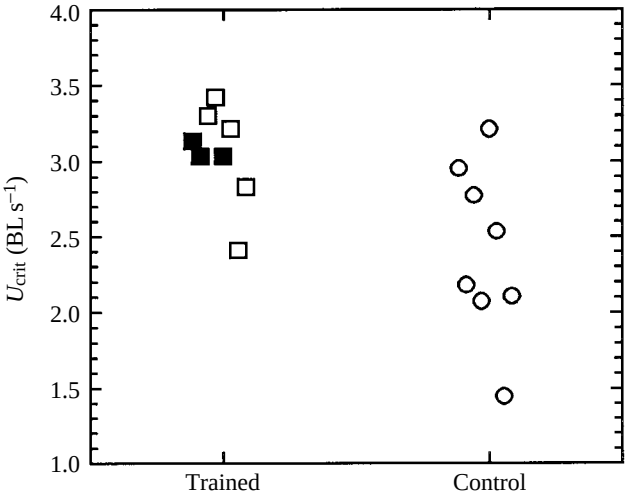

Fig. 2. The critical swimming speed (U_{crit}) of trained (squares) and control (circles) trout in group S96. In the trained group, three of the fish were not fatigued (filled symbols) at the highest water velocity. BL, total body length.

Respiratory parameters

Initial and final blood respiratory parameters are given in Table 2. There were no differences between the W95 and S96 groups in either the initial or final values of any of the variables measured, and data for the W95 and S96 groups were therefore

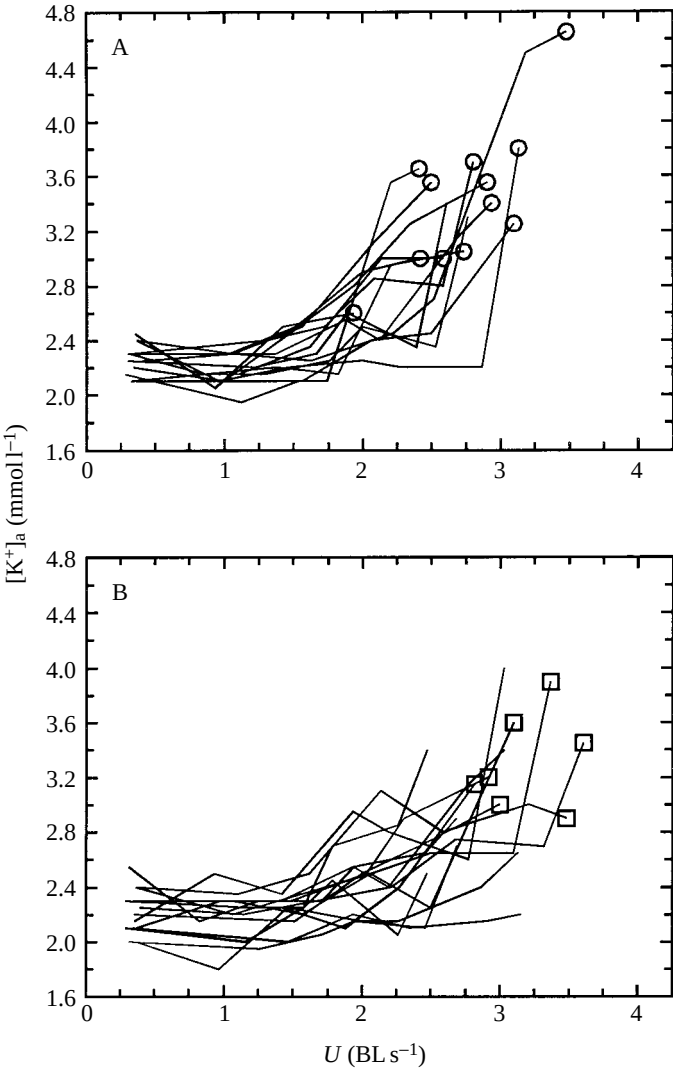

Fig. 3. Values of $[K^+]_a$ at increasing swimming speeds (U) for control (A) and trained (B) trout. Each curve represents the values for an individual fish from groups W95 and S96. A symbol at the highest speed indicates that the fish fatigued at this speed. BL, total body length.

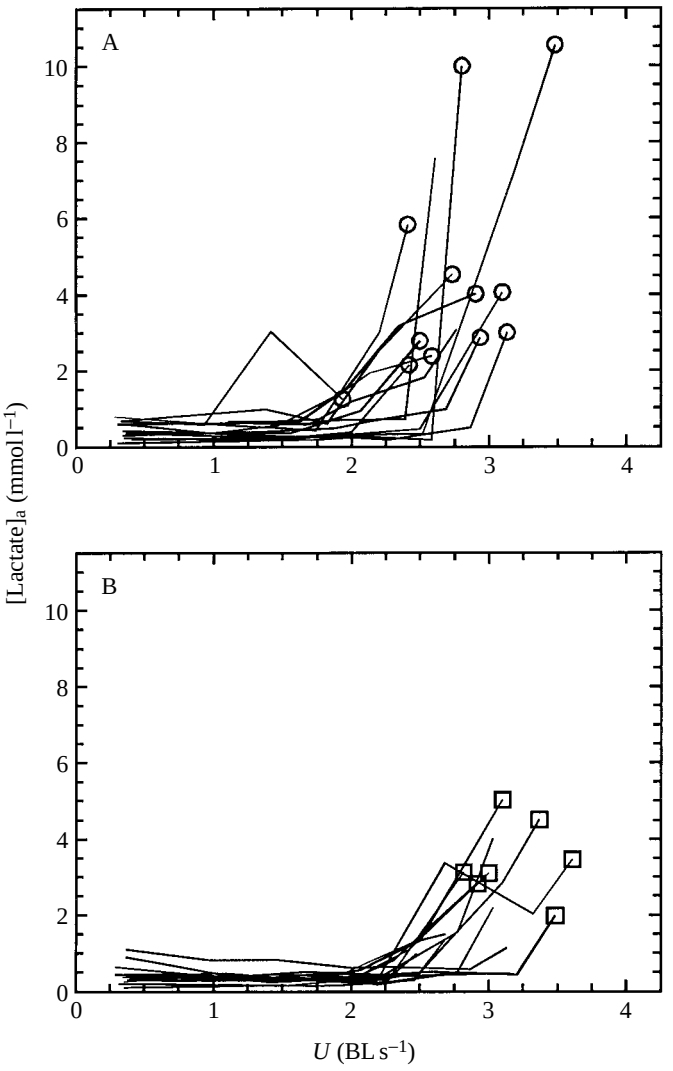

Fig. 4. Arterial plasma [lactate] at increasing swimming speeds (U) of control (A) and trained (B) trout. Each curve represents the values for an individual fish from groups W95 and S96. A symbol at the highest speed indicates that the fish fatigued at this speed. BL, total body length.

combined. In spite of the replacement of blood samples with saline, there was no decrease in either haematocrit or $[Hb_4]_a$. Pa_{O_2} tended to be slightly higher at the final swimming speed than at the start of the swim test, and for trained fish a significant increase in Ca_{O_2} occurred. There were no changes in $[Hb_4]_{aE}$, and the mean level of 4.5 mmol l^{-1} suggests the absence of any catecholamine stimulation of the erythrocytes (Holk and Lykkeboe, 1995). For trained fish, pHa did not differ between the initial and final sample. For control fish, however, the final pHa was slightly, but significantly, lower than the initial pHa (Table 2). Plasma cortisol concentrations did not differ between trained and control fish or between W95 and S96 groups, and combined means were therefore calculated for the routine and the highest swimming speeds. This revealed an increase in mean cortisol concentation from 131±136 nmol l^{-1} (N=22) at the routine swimming speed to 288±143 nmol l^{-1} (N=23) at the highest swimming speed (P<0.001). The value at routine swimming speed is within the range found for cannulated trout in other studies, and the level at the highest swimming speed is similar to the levels reported immediately after burst exercise (Milligan, 1996).

Discussion

Performance

Swimming at 1.2–1.5 BL s^{-1} for 23 h day^{-1} for 2 months induced an increase in the critical swimming speed of rainbow trout from a mean value of 2.4 BL s^{-1} to 3.0 BL s^{-1} (Fig. 2). In the context of blood O_2 transport, this is in keeping with the 22 % higher arterial blood oxygen content at the highest swimming speed of the trained trout (Table 2). Thus, the training intensity and duration sufficed to cause a marked improvement in swimming performance, and the U_{crit} values recorded for both control and trained fish are among the highest reported for surgically operated trout weighing 400–1000 g (Butler *et al.* 1992; Farrell *et al.* 1990, 1991; Gallaugher *et al.* 1995; Kiceniuk and Jones, 1977; Thomas *et al.* 1987; Thorarensen *et al.* 1993, 1996).

Table 2. *Initial and final arterial blood parameters for trained and control fish in groups W95 and S96*

	Trained		Control	
	Initial	Final	Initial	Final
Pa_{O_2} (mmHg)	89±28	97±21	92±18	98±17
Haematocrit (%)	25.3±4.7	28.5±5.1	22.9±4.0	23.4±3.7†
$[Hb_4]_a$ (mmol l^{-1})	1.13±0.21	1.27±0.24	1.04±0.18	1.05±0.16†
$[Hb_4]_{aE}$ (mmol l^{-1})	4.47±0.14	4.49±0.17	4.54±0.20	4.48±0.17
Ca_{O_2} (mmol l^{-1})	4.21±0.99	5.21±1.08*	4.25±0.89	4.27±0.72†
pH_a	7.83±0.06	7.84±0.05	7.86±0.07	7.78±0.06*

Values are means ± S.D., N=19.

*Significant difference between initial and final value, P<0.02; †significant difference between initial or final values of control and trained trout, P<0.02.

$[Hb_4]_a$, arterial tetramer haemoglobin concentration; $[Hb_4]_{aE}$, erythrocyte tetramer haemoglobin concentration.

1 mmHg = 0.133 kPa.

$[K^+]_a$ and [lactate]$_a$

The $[K^+]_a$ homeostasis was improved after training. This was revealed by a shift of the increase in $[K^+]_a$ to higher swimming speeds for trained fish (Fig. 3), and is comparable to the finding of a blunted increase in $[K^+]_a$ at a fixed work intensity following training in humans (Green *et al.* 1993; Kjeldsen *et al.* 1990*a*; McKenna *et al.* 1993; Madsen *et al.* 1994; Tibes *et al.* 1976). In the studies of Green *et al.* (1993), McKenna *et al.* (1993) and Madsen *et al.* (1994), the blunted increase in $[K^+]_a$ in humans was associated with a 15 % increase in the concentration of Na^+/K^+-ATPase in the vastus lateralis, whereas Kjeldsen *et al.* (1990*a*,*b*) found an improved regulation of $[K^+]$ without a concomitant increase in the concentration of Na^+/K^+-ATPase in the muscle. The results of the present study are in accordance with the latter report, since training did not lead to an increased concentration of Na^+/K^+-ATPase (measured as [^{3}H]ouabain binding site concentration) in either red or white muscle in trout (K. Holk, O. B. Nielsen and T. Clausen, in preparation).

Low to moderate swimming speeds in trout are based on an almost exclusive use of red muscle (Bone *et al.* 1978; Davison and Goldspink, 1984; Johnston and Moon, 1980). Training at these speeds is reported to lead to an increase in red muscle mass due to an increase in both fibre number and fibre volume (Davie *et al.* 1986; Davison and Goldspink, 1977) and, predictably, this reduces the activity level of each red fibre at a given swimming speed. This, together with the results of the present study and the observation that concentrations of Na^+/K^+-ATPase in red and white muscle are maintained (K. Holk, O. B. Nielsen and T. Clausen, in preparation), explains the widened speed range for $[K^+]_a$ homeostasis in trained fish.

There was no increase in [lactate]$_a$ at swimming speeds below 2 BL s^{-1} (Fig. 4), and it might be argued that this could be due to the retention of lactate in the white muscles (Wang *et al.* 1997). However, in studies where trout were chased, the blood lactate concentration did increase from 0.5 to 5–10 mmol l^{-1} within 5 min (Pagnotta *et al.* 1994; Milligan, 1996). As the blood samples in the present study were withdrawn 45–50 min after the step increases in swimming speed, an increase in muscle lactate concentration would probably have been reflected in an increase in [lactate]$_a$. At swimming speeds above 2 BL s^{-1}, plasma lactate levels started to increase, and training shifted the onset of this increase to higher swimming speeds. Thus, the speed range over which the increased energy demand was covered aerobically and without a priming production of lactate was higher for the trained fish, and this further supports the assumption of an increase in red muscle mass.

Swimming-related increases in $[K^+]_a$ have been reported in a number of studies of trout (Thomas *et al.* 1987; Nielsen *et al.* 1994; Nielsen and Lykkeboe, 1992). Although the same trends were found throughout, these studies reported increases in $[K^+]_a$ at lower swimming speeds than was found in the present study. This was probably due to differences in experimental protocols, and the following discussion of $[K^+]$ and [lactate] changes considers only data obtained from the present study.

The swimming-speed-related steady-state values of $[K^+]_a$ and [lactate]$_a$ could be separated into three sequences. First, at low swimming speeds (up to 1.5 BL s^{-1}), both $[K^+]_a$ and [lactate]$_a$ were maintained. Thus, the rate of oxidative phosphorylation kept pace with the increased power output, which was probably derived solely from red muscle activation. There was either no loss of K^+ from the working muscle or an exact matching of Na^+/K^+-ATPase activity in other tissues to maintain arterial plasma K^+ homeostasis. Rainbow trout can, however, sustain swimming speeds below 1.5 BL s^{-1} for weeks, rendering a continuous loss of K^+ from the red muscle at these swimming speeds unlikely, since drainage of K^+ must sooner or later result in inexcitability of the muscle cells.

In humans, the onset of exercise is associated with an increase in $[K^+]_v$ and $[K^+]_a$ and a negative $[K^+]_{a-v}$, the latter partly or fully vanishing within 2–3 min (Hallén *et al.* 1994; Hallén, 1996; Lindinger, 1995; Lindinger and Sjøgaard, 1991; Sjøgaard, 1986, 1990; Tibes *et al.* 1976; Vøllestad *et al.* 1994). Dynamic work at intensities below 50 % of $\dot{V}_{O_2max}$ in humans can be maintained for several hours, but it may be associated with a continuous loss of K^+ from the working muscles (Hallén *et al.* 1994; Sjøgaard, 1986, 1990, 1996). $\dot{V}_{O_2}$ as a percentage of $\dot{V}_{O_2max}$ for trout can be estimated from the relationship between swimming speed and $\dot{V}_{O_2}$ given by Brett (1964) (setting $\dot{V}_{O_2max}=\dot{V}_{O_2}$ at U_{crit}). A swimming speed of 1.5 BL s^{-1} then corresponds to 39 % of $\dot{V}_{O_2max}$ in trained trout and to 57 % of $\dot{V}_{O_2max}$ in control trout. Thus, sustained swimming at 1.5 BL s^{-1} taxes the aerobic capacity by approximately 50 % in trout, but apparently does not lead to any loss of K^+ from the working muscle.

Second, at intermediate swimming speeds (1.5–2.0 BL s^{-1}), a small increase in $[K^+]_a$ occurred, whereas [lactate]$_a$ was maintained. Thus, the rate of oxidative phophorylation still sufficed to keep pace with the energy turnover. At these swimming speeds, red muscle is still the main source for thrust generation, but white muscle activity also contributes (Bone *et al.* 1978; Davison and Goldspink, 1984; Jayne and Lauder, 1994; Johnston and Moon, 1980). Owing to the experimental design, the present study gave no information concerning the time course of the changes in $[K^+]_a$ following a step increase in swimming speed. The increased $[K^+]_a$ may thus either represent a new steady-state value or $[K^+]_a$ may still have been increasing.

Exercise above 50 % of $\dot{V}_{O_2max}$ in humans is associated with increased $[K^+]_a$ and $[K^+]_v$, a loss of K^+ from the working muscle and accumulation of K^+ in inactive tissues (Hallén *et al.* 1994; Lindinger, 1995; Rolett *et al.* 1990; Sjøgaard *et al.* 1985; Sjøgaard, 1990, 1996; Vøllestad *et al.* 1994). Thus, active muscle is depleted of K^+. Using the same assumptions as above for trout, a swimming speed of 2.0 BL s^{-1} can be estimated to represent 54 % of $\dot{V}_{O_2max}$ for trained trout and 78 % of $\dot{V}_{O_2max}$ for control trout. Swimming was sustainable for more than 1 h at these work intensities, but $[K^+]_a$ increased and, since a continuous loss of K^+ from the working muscle could

not be dismissed, a finite endurance corresponding to findings in humans is predictable.

Third, at swimming speeds higher than 2.0 BL s^{-1}, a further increase in $[K^+]_a$ and an increase in $[lactate]_a$ occurred. This suggests a loss of K^+ from either one or both of the fibre types and a rate of oxidative phosphorylation insufficient to keep pace with the increased energy turnover. The S96 fish were unable to swim continuously for 1 h at speeds of approximately 3.2 BL s^{-1} for trained fish and 2.7 BL s^{-1} for control fish. We interpreted this inability to result from muscle fatigue rather than to a reluctance to swim. At this time, $[K^+]_a$ had increased to a mean value of 3.4 mmol l^{-1}, i.e. 150 % of the value found at routine swimming speed. For comparison, depending on the type and intensity of exercise, fatigue in humans is reported to be associated with an increase in $[K^+]_a$ from approximately 4 mmol l^{-1} at rest to between 5.3 and 8.1 mmol l^{-1} at fatigue, i.e. an increase of 130–200 %, and this increase is suggested to be a contributing factor in the development of muscle fatigue (Bangsbo *et al.* 1996; Clausen, 1996; Clausen and Nielsen, 1994; Madsen *et al.* 1994; McKenna *et al.* 1993; Medbø and Sejersted, 1990; Sjøgaard *et al.* 1985; Sjøgaard, 1990, 1996). At the time of fatigue, $[lactate]_a$ in trout had increased to 4 mmol l^{-1}. However, the increased $[lactate]_a$ was associated with only a small decrease in pHa at the highest swimming speed for the control fish, while pHa was unchanged in trained fish. The increase in $[lactate]_a$ was associated with either no decrease or only a modest decrease in pHa, indicating a complete (trained fish) or an almost complete (control fish) compensation of the lactacidosis at the time of fatigue.

In summary, a tight regulation of $[K^+]_a$ was apparent at low swimming speeds, where thrust was generated by red muscle only, without any increase in $[K^+]_a$. Moderate and high swimming speeds challenged the $[K^+]_a$ homeostasis and led to a proportional increase in $[K^+]_a$ and to fatigue. In these cases, thrust was generated by the red muscle, probably supplemented by some white muscle activity. The energy demand was covered aerobically at swimming speeds below 2 BL s^{-1}. At higher swimming speeds, $[lactate]_a$ started to increase. However, $[lactate]_a$ at fatigue was lower than the values seen after burst exercise and, together with the perfect (trained fish) or near-perfect (control fish) compensation of the ensuing acidosis, it seems unlikely that a metabolic acidosis contributed to the development of fatigue.

Respiratory status

Blood oxygen saturation was at no time threatened. $P_{a_{O_2}}$ was maintained or slightly increased at the fatiguing speed compared with the values at routine speed (Table 2), and this finding is comparable to the maintenance of $P_{a_{O_2}}$ above 120 mmHg (16 kPa) at rest and at all swimming speeds reported by Kiceniuk and Jones (1977). In a number of studies, however, a decrease in arterial $P_{a_{O_2}}$ and a corresponding decrease in oxygen saturation at swimming speeds exceeding 85 % of $\dot{V}_{O_2max}$ have been reported (e.g. Thomas *et al.* 1987; Thorarensen *et al.* 1993, 1996). In all studies, water P_{O_2} remained high at all water velocities, leaving the discrepancy in $P_{a_{O_2}}$ unexplained. Throughout the swim test, blood oxygen content was maintained (in control fish) or somewhat increased (in trained fish) (Table 2). A decrease in this variable was predictable due to the substitution of the blood samples with saline, but a minor increase in urine flow rate (Wood and Randall, 1983) and a release of red blood cells from the spleen (Wells and Weber, 1990) probably compensated for this, particularly in the trained fish. The splenic release was, however, smaller than in trout exposed to exhaustive burst exercise (e.g. Nielsen and Lykkeboe, 1992; Milligan and Wood, 1986). In addition, the probable absence of a catecholamine response in the blood (maintained erythrocyte $[Hb_4]$), at the same time as the increased cortisol concentration at the highest swimming speeds, indicated an enhanced adrenergic responsiveness of the erythrocytes (Perry *et al.* 1996), and suggested that the oxygen delivery system, even at fatigue, was not overly taxed.

In conclusion, swimming performance was improved and the onset of increases in $[K^+]_a$ and $[lactate]_a$ shifted to higher swimming speeds as a consequence of 2 months of swim training in rainbow trout. The energy demand was supplied strictly aerobically at low and moderate swimming speeds, but was supplemented anaerobically at higher swimming speeds. The oxygen content of arterial blood was not in jeopardy at any swimming speed in the present study. At low swimming speeds, there was no increase in $[K^+]_a$, and thus no apparent net loss of K^+ from the working muscle, which is consistent with the unlimited endurance at these swimming speeds. Moderate swimming speeds led to increases in $[K^+]_a$, and the possibility of a continuous loss of K^+ from the working muscle could not be dismissed. A finite endurance at these swimming speeds is therefore predictable.

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