The Journal of Experimental Biology 216, 4549-4556

doi:10.1242/jeb.091058

RESEARCH ARTICLE

Lactate kinetics of rainbow trout during graded exercise: do catheters affect the cost of transport?

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SUMMARY

Changes in lactate kinetics as a function of exercise intensity have never been measured in an ectotherm. Continuous infusion of a tracer is necessary to quantify rates of lactate appearance (R_a) and disposal (R_d), but it requires double catheterization, which could interfere with swimming. Using rainbow trout, our goals were to: (1) determine the potential effects of catheters and blood sampling on metabolic rate ($\dot{M}_{O_2}$), total cost of transport (TCOT), net cost of transport (NCOT) and critical swimming speed (U_{crit}), and (2) monitor changes in lactate fluxes during prolonged, steady-state swimming or graded swimming from rest to U_{crit}. This athletic species maintains high baseline lactate fluxes of 24 μmol kg^{-1} min^{-1} that are only increased at intensities >2.4 body lengths (BL) s^{-1} or 85% U_{crit}. As the fish reaches U_{crit}, R_a is more strongly stimulated (+67% to 40.4 μmol kg^{-1} min^{-1}) than R_d (+41% to 34.7 μmol kg^{-1} min^{-1}), causing a fourfold increase in blood lactate concentration. Without this stimulation of R_d during intense swimming, lactate accumulation would double. By contrast, steady-state exercise at 1.7 BL s^{-1} increases lactate fluxes to ~30 μmol kg^{-1} min^{-1}, with a trivial mismatch between R_a and R_d that only affects blood concentration minimally. Results also show that the catheterizations and blood sampling needed to measure metabolite kinetics in exercising fish have no significant impact on $\dot{M}_{O_2}$ or TCOT. However, these experimental procedures affect locomotion energetics by increasing NCOT at high speeds and by decreasing U_{crit}.

Key words: lactate turnover, carbohydrate metabolism, locomotion energetics, fish exercise, critical swimming speed, U_{crit}, continuous tracer infusion, respirometry, *Oncorhynchus mykiss*.

Received 13 May 2013; Accepted 29 August 2013

INTRODUCTION

As a glycolytic end-product, oxidative fuel and gluconeogenic substrate, lactate is one of the most dynamic intermediates of cell metabolism (Brooks, 1991; Gladden, 2004; Philp et al., 2005). Animals process lactate at high rates even under resting, normoxic conditions, and in mammals, many studies have shown that inherently high baseline lactate fluxes are strongly stimulated during exercise (Bergman et al., 1999; Donovan and Brooks, 1983; Issekutz et al., 1976; Stanley et al., 1985; Van Hall et al., 2003; Weber et al., 1987). Little is known about lactate fluxes in fish because adequate methods to quantify these fluxes accurately under controlled exercise conditions have only recently become available. Previous studies of fish lactate kinetics report turnover rates that were estimated by bolus injection (Cameron and Cech, 1990; Milligan and McDonald, 1988; Weber, 1991; Weber et al., 1986), an obsolete method with significant limitations (Omlin and Weber, 2010; Wolfe, 1992). They show that the lactate turnover rate of rainbow trout doubles during prolonged, low-intensity swimming (Weber, 1991). Although no measurements have been made at higher swimming speeds, a threefold to 10-fold increase over baseline has been observed during recovery from exhausting exercise for channel catfish (*Ictalurus punctatus*), coho salmon (*Oncorhynchus kisutch*) and starry flounder (*Platichthys stellatus*) (Cameron and Cech, 1990; Milligan and McDonald, 1988). Continuous tracer infusion techniques have been adapted and validated for fish (Haman et al., 1997; Haman and Weber, 1996). They have been used to quantify the rates of metabolite appearance (R_a) and disposal (R_d) accurately under non-steady state conditions. In particular, continuous infusion has been used to characterize the effects of swimming on glucose and lipid kinetics (Bernard et al., 1999; Magnoni et al., 2008; Shanghavi and Weber, 1999). Unfortunately, these fish measurements only deal with prolonged, low-intensity swimming. Therefore, nothing is known about the differential effects of higher intensity exercise on the rates of lactate production and disposal when significant changes in blood lactate concentration are observed.

Measuring metabolite kinetics by continuous infusion during swimming may be problematic because two catheters exiting from the snout of the fish are necessary (Haman and Weber, 1996). The hydrodynamic drag associated with these catheters may interfere with locomotion, and blood sampling may decrease capacity for oxygen transport. Therefore, cannulated fish may have a different metabolic rate ($\dot{M}_{O_2}$), a higher cost of transport [total (TCOT) or net (NCOT)] (Schmidt-Nielsen, 1972) and a lower critical swimming speed (U_{crit}) (Farrell, 2008) than non-catheterized animals. The cost of transport is the amount of energy (or oxygen) used to move one unit body mass by one unit distance. TCOT is the total amount of energy needed to power movement, including the cost of sustaining life in resting tissues. By contrast, NCOT only accounts for the cost of locomotion, but it also excludes all maintenance costs incurred at rest. To allow meaningful comparisons between fish studies, exercise intensity

Table 1. Morphological characteristics and metabolic rate (M_{O_2}) of rainbow trout in the different treatment groups

	Graded swimming			
	Control	Sham	Lactate kinetics	Steady-state swimming
N	6		8	6
Body mass (g)	338.8±14.7		369.0±10.1	487.6±11.4
Body length (cm)	32.4±0.7		33.6±0.4	35.4±0.4
Resting $\dot{M}_{O_2}$ (μmol kg^{-1} min^{-1})	64.7±5.8	61.4±4.6	57.4±2.5	–
Highest $\dot{M}_{O_2}$ (μmol kg^{-1} min^{-1})	247.7±1.2 (2)	224.0±38.7 (4)	201.7±9.5 (5)	–

The same individuals were measured twice in random order for the control and sham-catheterized conditions. Sample size (*N*) is indicated on the first line, except for the highest $\dot{M}_{O_2}$ measured, where *N* is in parentheses (because not all individuals within each group were able to reach the highest speed).
Values are means ± s.e.m.

is traditionally standardized as $\%U_{crit}$ or as swimming speed in body lengths (BL) per second. However, it is unclear whether non-instrumented and catheterized animals swimming at the same $\%U_{crit}$ or at the same speed have the same $\dot{M}_{O_2}$. Therefore, the goals of this study were to: (1) determine whether double catheterization and blood sampling have an effect on $\dot{M}_{O_2}$, TCOT, NCOT or U_{crit} in rainbow trout, (2) apply continuous tracer infusion methods to measure the effects of steady swimming on lactate fluxes and (3) determine the relationship between exercise intensity and the rates of lactate production and disposal, using a graded swimming protocol.

MATERIALS AND METHODS

Animals

Male and female rainbow trout [*Oncorhynchus mykiss* (Walbaum)] were purchased from Linwood Acres Trout Farm (Campbellcroft, ON, Canada) (see Table 1). They were held in a 1300 liter flow-through tank containing dechlorinated, well-oxygenated water at 13°C for at least 2 weeks before experiments. Fish were kept under a 12 h:12 h light:dark photoperiod and fed commercial floating pellets (Martin Mills, Elmira, ON, Canada) three times a week until satiation. The effects of exercise were measured either during prolonged, steady-state swimming at 1.7 BL s^{-1} or during graded swimming (U_{crit} protocol). Fish used for graded exercise were randomly divided into two sub-groups: control/sham-catheterization (to measure swimming energetics only) and actual catheterization (to measure swimming energetics and lactate kinetics). To avoid training or fatigue effects in the first sub-group, locomotion energetics were measured in random order for the control (no catheters) and sham-catheterized conditions in the same individuals. The steady-state swimming group was catheterized to measure lactate kinetics only. All procedures were approved by the Animal Care Committee of the University of Ottawa and adhered to the guidelines established by the Canadian Council on Animal Care for the use of animals in research.

Catheterizations

Fish were fasted for at least 24 h before surgery. They were anesthetized with ethyl-*N*-aminobenzoate sulfonic acid (MS-222; 60 mg l^{-1}) in well-oxygenated water. The animals used to measure lactate kinetics were doubly cannulated in the dorsal aorta using PE-50 catheters (Intramedic, Clay-Adams, Sparks, MD, USA), as detailed elsewhere (Haman and Weber, 1996). The catheters were kept patent by flushing with Cortland saline (Wolf, 1963) containing 50 U ml^{-1} heparin (Sigma-Aldrich, St Louis, MO, USA). Only animals with a hematocrit >20% after recovery from surgery were used in tracer experiments. For the sham-catheterized group, the two catheters were sutured to the palate, but they were not inserted in the dorsal aorta. The aim of sham-catheterization was to produce the same hydrodynamic drag experienced during actual tracer experiments, but without affecting the vasculature or drawing blood samples.

Swim tunnel respirometry

All experiments were carried out at 13°C in a 90 liter swim tunnel respirometer (Loligo Systems, Tjele, Denmark) filled with the same quality water as the holding tank. A 'honeycomb' grid was placed before the swimming chamber to promote laminar flow. The fish always swam in the anterior part of the chamber (kept dark) to avoid the posterior part (brightly lit). The swim tunnel was calibrated with a flow probe (Global Water Geotech, Denver, CO, USA) to establish the linear relationship between water velocity (cm s^{-1}) and motor speed (rpm). Swimming speeds were corrected for solid blocking as in Claireaux et al. (Claireaux et al., 2006). $\dot{M}_{O_2}$ was measured by intermittent flow respirometry using galvanic oxygen probes connected to a DAQ-PAC-G1 instrument controlled with AutoResp software (version 2; Loligo Systems). The oxygen probes were calibrated before measurements using N_2-saturated water (0% O_2) and air-saturated water (20.9% O_2). Before experiments, each fish was placed in the swim tunnel overnight for acclimation to the experimental setup. During this period, water velocity was kept at 0.5 BL s^{-1}, a low speed requiring no swimming but enabling the fish to rest at the bottom of the respirometer. U_{crit} and the effects of graded exercise on $\dot{M}_{O_2}$, cost of transport and lactate kinetics were quantified using a stepwise U_{crit} protocol (Jain et al., 1997) with velocity increments of 0.2 BL s^{-1} every 20 min. Graded swimming experiments were terminated at exhaustion, when the fish was unable to remove itself from the rear grid.

Lactate kinetics

The catheters were made accessible through the swim tunnel lid by channeling them through a water-tight port. The rates of lactate appearance (R_a) and lactate disposal (R_d) were measured by continuous infusion of [U-^{14}C] lactate (New England Nuclear, Boston, MA, USA; 4.84 GBq mmol^{-1}). Infusates were freshly prepared immediately before each experiment by drying an aliquot of the solution obtained from the supplier under N_2 and resuspending in Cortland saline. Labeled lactate was infused for 1 h in resting fish to quantify baseline lactate kinetics. It was administered at a rate of 2029±227 Bq kg^{-1} min^{-1} (*N*=15) using a calibrated syringe pump (Harvard Apparatus, South Natick, MA, USA) at 1 ml h^{-1}. Under these conditions, isotopic steady-state is reached in <45 min (Omlin and Weber, 2010). Lactate (labeled + unlabeled) was infused at rates accounting for <0.002% of the endogenous R_a measured in resting fish. Tracer infusion was continued either for 4–5 h to complete a U_{crit} protocol (graded exercise experiments) or for 2 h at 1.7 BL s^{-1} (steady-state exercise experiments). The water was kept normoxic throughout the measurements

(10.64±0.07 mg O_2 l^{-1}). Blood samples (100 µl each) were drawn at the end of the initial resting period and at regular intervals during swimming (5 min before each stepwise velocity increment for graded exercise and every 10 min for steady-state exercise). The total amount of blood sampled from each fish accounted for <10% of blood volume. Samples were immediately deproteinized in 200 µl perchloric acid (6% w/w) and centrifuged for 5 min at 16,000 ***g*** (Eppendorf 5415C, Brinkmann, Rexdale, ON, Canada). Supernatants were kept frozen at −20°C until analyses.

Sample analyses

Blood lactate concentration was measured spectrophotometrically (Bergmeyer, 1985) using a SpectraMax Plus384 Absorbance Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). To measure activity, lactate was separated using ion exchange columns as decribed in Omlin and Weber (Omlin and Weber, 2010). Before passing through the columns, each deproteinized blood sample was neutralized with 1 mol l^{-1} potassium bicarbonate and diluted with 5 ml deionized H_2O. Preliminary experiments with known amounts of labeled lactate showed that 70% of total activity was recovered, and measured lactate activities were corrected accordingly. Radioactivity was measured by scintillation counting (Beckman Coulter LS 6500, Fullerton, CA, USA) in Bio-Safe II scintillation fluid (RPI Corp., Mount Prospect, IL, USA).

Calculations and statistics

U_{crit} (BL s^{-1}) was calculated according to (Brett, 1964):

$$U_{crit} = V_f + [(t_f / t_i) \times V_i] , \quad (1)$$

where V_f is the highest speed at which a full time interval was completed (BL s^{-1}), V_i is the speed increment between intervals (0.2 BL s^{-1}), t_f is the time spent swimming during the last interval causing exhaustion (min) and t_i is the full interval (20 min). The amount of energy needed to transport one unit body mass by one unit distance, or TCOT (Schmidt-Nielsen, 1972) was calculated from total $\dot{M}_{O_2}$ as follows:

$$\text{TCOT} = \text{total } \dot{M}_{O_2} / U , \quad (2)$$

where TCOT is in µmol O_2 kg^{-1} m^{-1}, $\dot{M}_{O_2}$ is in µmol O_2 kg^{-1} min^{-1} and U is the swimming speed in m min^{-1}. NCOT was calculated similarly, but from net $\dot{M}_{O_2}$ defined as swimming $\dot{M}_{O_2}$ minus resting $\dot{M}_{O_2}$. Resting $\dot{M}_{O_2}$ was obtained by averaging the 10 lowest values recorded during the night preceding the exercise measurements. R_a and R_d were calculated using the non-steady-state equations of Steele (Steele, 1959). Statistical comparisons were performed using one- or two-way repeated-measures ANOVA (RM ANOVA) with Dunnett's *post hoc* test to determine which means were different from baseline, or the Holm–Sidak test to compare treatments (SigmaPlot v.12, Systat Software, San Jose, CA, USA). When the assumptions of normality (Shapiro–Wilk test) or homoscedasticity (Levene's test) were not met, Friedman's RM ANOVA on ranks was used or the data were normalized by $\log_{10}$ transformation before parametric analysis. All values presented are means ± s.e.m. and P<0.05 was used as the level of significance in all tests.

RESULTS

Graded swimming

Metabolic rate

Resting rates of oxygen consumption were not different between treatments (P>0.05; Table 1). Metabolic rate increased progressively with swimming speed and was higher than resting $\dot{M}_{O_2}$ at all speeds (P<0.001; Fig. 1A). The exercise-induced increase in $\dot{M}_{O_2}$ was not significantly different between groups (P>0.05). The highest $\dot{M}_{O_2}$ was reached at the end of the graded exercise protocol (Table 1). Two individuals of the control group were able to reach the highest swimming speed of 3.6 BL s^{-1} (Fig. 1A). Fig. 1B shows changes in $\dot{M}_{O_2}$ as a function of exercise intensity expressed as $\%U_{crit}$. The three treatments were not different from each other (P>0.05) and the pooled data were fitted with a second-order polynomial regression (r^2=0.72, P<0.001,): $\dot{M}_{O_2}$=109.136–1.669($\%U_{crit}$)+0.0285($\%U_{crit}$)^2.

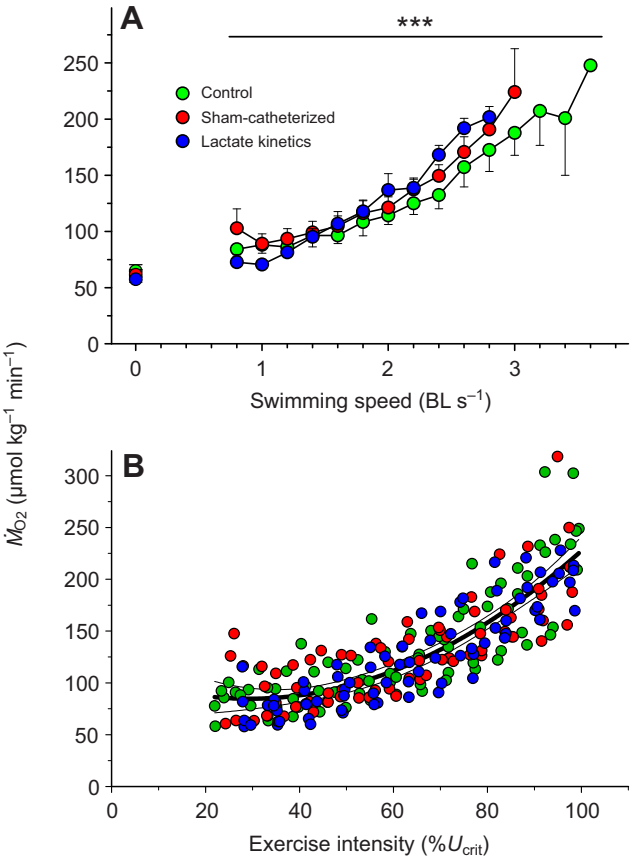

Fig. 1. Effects of graded swimming on the metabolic rate ($\dot{M}_{O_2}$) of rainbow trout. Three groups of fish were measured: non-catheterized controls, sham catheterized animals and catheterized animals during the measurement of lactate kinetics. (A) $\dot{M}_{O_2}$ as a function of swimming speed (in BL s^{-1}). Values are means ± s.e.m. For speeds <3 BL s^{-1}, sample sizes were N=6 for the control and sham-catheterized groups, and N=8 for the lactate kinetics group. Not all fish were able to swim at the higher speeds (where N<6). Asterisks indicate significant differences from resting values (***P<0.001). (B) $\dot{M}_{O_2}$ as a function of exercise intensity (expressed as $\%U_{crit}$). The three treatments were not significantly different from each other (P>0.05). The thick line was fitted by a second-order polynomial regression (r^2=0.72, P<0.001): $\dot{M}_{O_2}$=109.136–1.669($\%U_{crit}$)+0.0285($\%U_{crit}$)^2. Thin lines indicate confidence intervals (±99%).

Cost of transport and U_{crit}

Across speeds, treatment groups had the same TCOT (P=0.28; Fig. 2A), but a different NCOT (P<0.01; Fig. 2B). Maximum TCOT was measured at the lowest swimming speed of 0.8 BL s^{-1} for all groups (Fig. 2A). As exercise intensity was increased, TCOT became lower than maximal values for all swimming speeds above 1 BL s^{-1} (P<0.001). Averaged among groups, TCOT decreased from a maximum of 5.4 µmol O_2 kg^{-1} m^{-1} at 0.8 BL s^{-1} to a minimum of 3.1 µmol O_2 kg^{-1} m^{-1} at 2.2 BL s^{-1}. NCOT was also affected by exercise intensity (Fig. 2B) and was higher between 2.4 and 3.2 BL s^{-1}

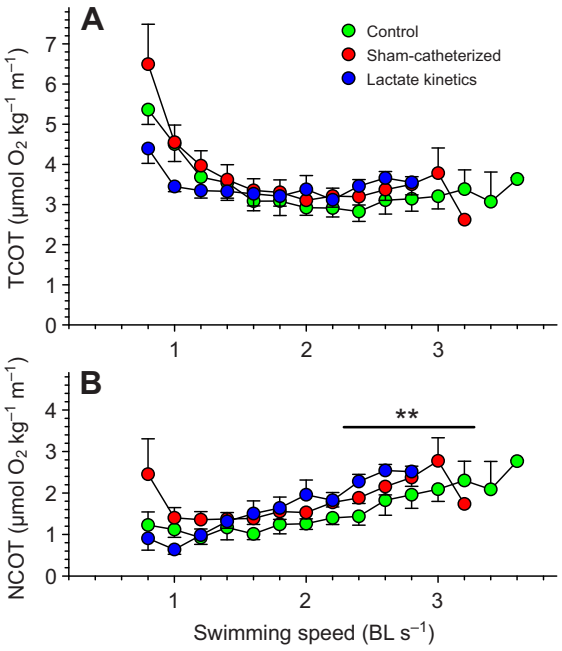

Fig. 2. Effects of graded swimming on the total and net cost of transport (TCOT and NCOT, respectively) in three groups of rainbow trout (non-catheterized controls, sham catheterized animals and catheterized animals during the measurement of lactate kinetics). Only the statistical differences between treatment groups are indicated directly on the graph (the effects of swimming speed are not). (A) TCOT was not different between groups, but it was lower at all speeds above 1 BL s^{-1} than at the lowest speed (0.8 BL s^{-1}) for all groups (P<0.001). (B) Between 2.4 and 3.2 BL s^{-1}, NCOT was lower in controls than in the other groups (**P<0.01) and it was higher than at 0.8 BL s^{-1} across groups (P<0.01).

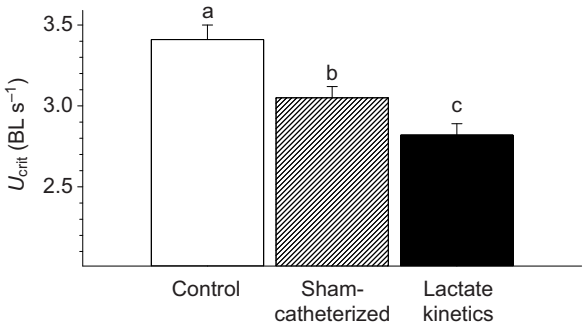

Fig. 3. Critical swimming speed (U_{crit}) in three groups of exercising rainbow trout (non-catheterized controls, sham catheterized animals and catheterized animals during the measurement of lactate kinetics). Values are means ± s.e.m. (N=6 for controls and sham-catheterized, N=7 for lactate kinetics). Different letters indicate significant differences between treatments (P<0.05).

than for the lowest swimming speed (P<0.01). Treatments had a significant effect on U_{crit} (P<0.05; Fig. 3), which was highest in controls (3.4 BL s^{-1}), intermediate in sham-catheterized animals (3.1 BL s^{-1}) and lowest for catheterized animals during the measurement of lactate kinetics (2.8 BL s^{-1}). Minimum TCOT was the same for all treatments (P>0.05), but occurred at different swimming speeds. Minimal NCOT was not different between controls and sham-catheterized animals (P>0.05), but it was higher for sham-catheterized animals than for lactate kinetics (P<0.05). Minimal TCOT was measured at higher swimming speeds (2.0–2.4 BL s^{-1}) than minimal NCOT (1.0–1.2 BL s^{-1}).

Effects of exercise intensity on lactate metabolism

Swimming speed was progressively increased over 4 h, following a classic stepwise U_{crit} protocol (Fig. 4A). Both R_a (P<0.001) and R_d (P<0.01) were strongly stimulated over time as exercise intensity increased (Fig. 4B). Mean R_a values above 2.4 BL s^{-1} (or above 85% U_{crit}) were higher than baseline (P<0.05). R_a increased from a baseline level of 24.2 μmol kg^{-1} min^{-1} to a maximum of 40.4 μmol kg^{-1} min^{-1}. R_d increased from 24.6 μmol kg^{-1} min^{-1} to a maximum of 34.7 μmol kg^{-1} min^{-1}. Lactate concentration increased from a baseline value of 1.3 mmol l^{-1} to a maximum of 5.1 mmol l^{-1} with exercise intensity (P<0.001; Fig. 4C). Mean blood lactate concentrations for speeds above 2.0 BL s^{-1} were higher than baseline (P<0.05).

Steady-state swimming

Metabolic rate

The first 60 min were monitored at rest to quantify baseline lactate kinetics. The transition from rest to steady-state swimming was made progressively over 30 min before maintaining a constant speed of 1.7 BL s^{-1} for 90 min (Fig. 5A). Metabolic rate increased from resting levels of ~80 μmol O_2 kg^{-1} min^{-1} to a maximum of 126.8 μmol O_2 kg^{-1} min^{-1} after 40 min of exercise (Fig. 5B). $\dot{M}_{O_2}$ was maintained above resting values between 40 and 80 min (P<0.05) before declining to 99.9 μmol O_2 kg^{-1} min^{-1} over the last 30 min.

Steady exercise and lactate metabolism

Blood lactate concentration increased from a resting value of 0.7 to ~1.4 mmol l^{-1} during the first 30 min of steady swimming at 1.7 BL s^{-1}, and stayed at that level until the end of the experiment (P<0.05; Fig. 6A). Both R_a and R_d increased over time (P<0.001; Fig. 6B) from baseline values of 22.4 (R_a) and 23.7 μmol kg^{-1} min^{-1} (R_d) to maximal levels of 30.9 (R_a) and 29.8 μmol kg^{-1} min^{-1} (R_d). Mean R_a and R_d were higher than baseline between 30 and 50 min of steady-state swimming (P<0.05), but returned to resting values for the last 60 min of exercise (P>0.05).

DISCUSSION

This study is the first to characterize the relationship between exercise intensity and lactate kinetics in an ectotherm. It shows that the lactate fluxes of rainbow trout are stimulated at speeds greater than 2.4 BL s^{-1} (or ~85% U_{crit}), when lactate production starts diverging from lactate disposal. At these high exercise intensities, the change in R_a stops being matched by the increase in R_d, leading to a significant accumulation of glycolytic end-product in the circulation. By contrast, steady-state submaximal exercise causes R_a and R_d to increase similarly from ~20 to ~30 μmol kg^{-1} min^{-1}, with a trivial mismatch between production and disposal that affects blood concentration only minimally (from 0.7 to 1.4 mmol l^{-1}). Earlier measurements by bolus injection had underestimated true lactate fluxes, but the same relative effect of steady, low-intensity swimming was observed (Weber, 1991). The present results show that catheterization has no impact on metabolic rate and cost of transport below 85% U_{crit}. At these submaximal speeds, swimming energetics are not affected by the catheters or by sampling blood, and, therefore, all the parameters of metabolite kinetics measured by continuous tracer infusion also apply to non-instrumented fish. Above 85% U_{crit}, catheterization increases NCOT, and direct comparisons between intact and instrumented animals swimming at the same speed should be made with caution.

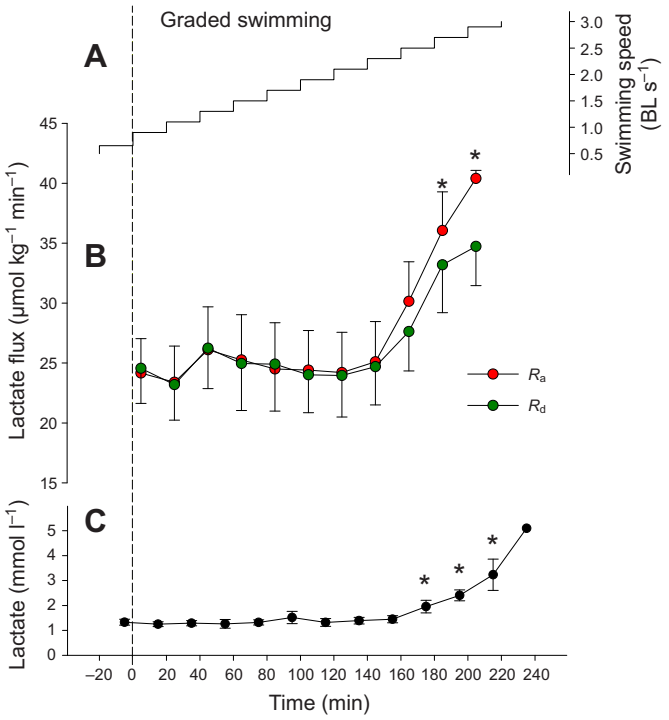

Fig. 4. Effects of graded exercise on lactate metabolism in rainbow trout: (A) changes in swimming speed, (B) lactate fluxes and (C) blood lactate concentration. B shows the rates of lactate appearance (R_a, in red) and disposal (R_d, in green). Exercise was started at time 0 (dashed line) and tracer infusion at −60 min. Values are means ± s.e.m. (for lactate fluxes, N=7 before 180 min, and N=4 and 2 for the last two points). Asterisks indicate significant differences from baseline (P<0.05).

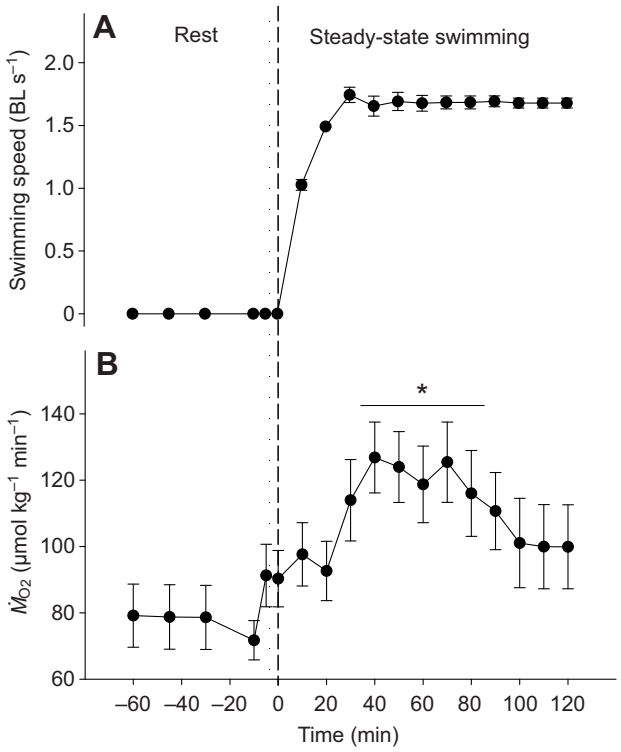

Fig. 5. Changes in (A) swimming speed and (B) $\dot{M}_{O_2}$ over time for rainbow trout during steady-state exercise experiments. Values are means ± s.e.m. (N=7). Asterisk indicates a significant difference from resting $\dot{M}_{O_2}$ (*P<0.05). Dashed line indicates the time at which exercise was started.

Lactate production during swimming

Below 85% U_{crit}, swimming has no effect on the R_a and R_d of rainbow trout (Fig. 4). At higher speeds, glycolysis is sharply stimulated, causing an increase in lactate production from 24 to 40 μmol kg^{-1} min^{-1} (Fig. 4B). This 67% rise in R_a was measured at the highest speed allowing metabolite flux measurements in a swimming fish. Trout may be able to upregulate R_a more strongly than reported here, as previous studies have suggested several-fold changes for flounder, salmon and catfish between rest and recovery from exhausting exercise (Cameron and Cech, 1990; Milligan and McDonald, 1988). However, these fluxes measured post-exercise were estimated by bolus injection and may need to be confirmed with more reliable methods.

The stimulation of lactate flux is stronger in mammals than in trout: submaximal exercise induces a sixfold increase in dogs (Issekutz et al., 1976), thoroughbred horses (Weber et al., 1987) and humans (Bergman et al., 1999). Moreover, humans can increase lactate production by 22-fold over resting values during a graded exercise protocol similar to what was used here for fish (Stanley et al., 1985). Trout may only be able to show a modest relative increase in flux because their metabolic scope is much smaller than that of mammals (Brett, 1972). Also, greater stimulation of lactate fluxes may not be possible for trout because their baseline levels could already be quite high. This notion is supported by the fact that the $R_a/\dot{M}_{O_2}$ ratios of trout and humans are similar during intense exercise (8.9 for trout *versus* 6.4 for humans), but much higher in resting trout (19.5) than in resting humans (only 2.9) (Stanley et al., 1985; present study).

Intense exercise stimulates lactate disposal

Above 85% U_{crit}, R_d increases by 41% (Fig. 4B). Without this response, circulating lactate would reach twice the concentration actually observed at the end of exercise (Fig. 4C). Therefore, increasing the rate of lactate disposal during intense swimming plays an important role in reducing the lactate load on the circulation, a metabolic strategy previously noticed during exposure to hypoxia [fig. 5 in Omlin and Weber (Omlin and Weber, 2010)]. Such a response is rather surprising at a time when anaerobic glycolysis is stimulated. As the only two pathways available for lactate clearance, how could gluconeogenesis and/or oxidation contribute to the increase in R_d? The effects of swimming on gluconeogenesis have never been measured directly in fish, but several tracer studies suggest that this pathway is not stimulated by exercise (reviewed in Moyes and West, 1995). Hepatic glucose production actually decreases during submaximal swimming, but it is unclear whether gluconeogenesis or glycogenolysis is responsible for this decline (Shanghavi and Weber, 1999). Intuitively, stimulating gluconeogenesis during swimming would seem undesirable because glucose synthesis is energetically costly [6 ATP per glucose (Clark et al., 1974)]. Overall, current information suggests that the stimulation of lactate disposal reported here is not accounted for by gluconeogenesis, but by an increase in lactate oxidation. Highly aerobic tissues such as heart, red muscle, kidney and brain can readily use lactate as an oxidative fuel (Bilinski and Jonas, 1972; Soengas and Aldegunde, 2002), and they are probably responsible for increasing lactate clearance during exercise. For example, perfused trout heart experiments show that lactate oxidation is stimulated when cardiac work or lactate availability becomes elevated (Lanctin et al., 1980; Milligan and Farrell, 1991). In addition, important physiological roles for various lactate shuttles

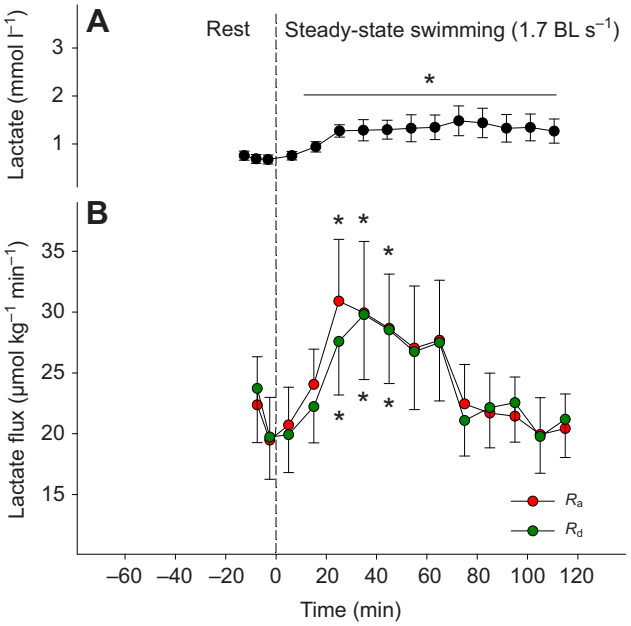

Fig. 6. Changes in (A) blood lactate concentration and (B) lactate fluxes in rainbow trout during steady-state swimming at 1.7 BL s^{-1}. Rates of lactate appearance (R_a) and disposal (R_d) are shown in red and green, respectively. Values are means ± s.e.m. (N=6). Asterisks indicate significant differences from baseline at time 0 (*P<0.05). Dashed line indicates the time at which exercise was started.

have been demonstrated in mammals (Brooks, 1998; Gladden, 2004). Exercising fish may also rely on lactate shuttles to transport the end-product from white muscle to aerobic tissues for oxidation. In trout, however, inter-tissue lactate shuttles may be constrained by white muscle lactate retention – a phenomenon that has intrigued fish biologists for decades (Turner and Wood, 1983; Wang et al., 1997). We have recently demonstrated that white muscle has a very limited capacity to export lactate because this tissue shows minimal expression of monocarboxylate transporters, even after exercise (Omlin and Weber, 2013). Presumably, R_a and R_d could be stimulated much more than observed here during exercise if white muscle expressed monocarboxylate transporters at the higher levels prevalent in mammalian glycolytic fibers. The spatial separation of fish white and red muscles also precludes the intramuscular lactate shuttle between adjacent glycolytic and oxidative fibers, which is well characterized within mixed mammalian muscles (Brooks, 1998; Van Hall, 2000).

Previous experiments by bolus injection underestimated lactate fluxes

Continuous tracer infusion is the preferred method to quantify *in vivo* metabolite fluxes accurately in humans and animals (Wolfe, 1992). Its application to fish (Omlin and Weber, 2010; present study) shows that the lactate fluxes of rainbow trout are approximately three times higher than previously estimated by bolus injection (Weber, 1991). This is because the bolus injection method relies on problematic estimates of surface areas to calculate flux (flux=dose injected/surface area under the specific activity decay curve). Overestimation of surface area under the decay curve can happen for a number of reasons: (1) curve fitting for early sampling points is extremely inaccurate because specific activity changes very rapidly just after the injection of the bolus; (2) ^{14}C recycling can artificially increase specific activities in the right-hand side of the decay curve (later sampling times); and (3) a single catheter is used for bolus injection of the tracer and subsequent blood sampling; therefore, residual bolus activity on the catheter walls can increase specific activity in sampled blood by contamination. Finally, the bolus injection technique assumes steady-state conditions, and each experiment only yields a single measurement of flux, two important restrictions that do not apply to continuous tracer infusion. For all of these reasons, bolus injection has been virtually abandoned as a practical tool to quantify metabolite kinetics.

Effects of catheters and blood sampling on swimming performance

We have tested whether applying the continuous tracer infusion technique has an impact on key physiological indices of performance: $\dot{M}_{O_2}$, TCOT, NCOT and U_{crit}. TCOT and NCOT were quantified separately because they are both helpful, but in different contexts. For example, TCOT is useful to determine the cost of migration. By contrast, NCOT only takes into account the energy used to power movement and excludes maintenance costs incurred by all tissues including muscle. In biomechanics, NCOT is particularly useful to quantify the efficiency of muscle contraction. It has been shown that instrumenting aquatic animals with catheters, tracking systems or individual markers can affect locomotion energetics (Bannasch et al., 1994; Culik and Wilson, 1991; Gauthier-Clerc et al., 2004). However, these devices only impact locomotion at high speeds because hydrodynamic drag forces increase with the square of velocity (Biewener, 2003). In this study, we have quantified the cost of transport from measured rates of oxygen consumption, but have ignored the contribution of anaerobic metabolism. To estimate the potential error introduced by this approach, we have calculated the relative importance of anaerobic compared with aerobic metabolism at the highest swimming speed for which lactate kinetics could be measured. Assuming that carbohydrate was the only fuel consumed and that R_d was either completely oxidized or not oxidized at all, we have determined a range of potential errors. At 2.8 BL s^{-1}, metabolic rate was 202 μmol O_2 kg^{-1} min^{-1} (Fig. 1A), or 33.7 μmol glucose kg^{-1} min^{-1} or 1212 μmol ATP kg^{-1} min^{-1}. If 100% of R_d was oxidized, anaerobic metabolism would be 5.7 μmol lactate kg^{-1} min^{-1} (=net lactate production=R_a–R_d; Fig. 4B) or 11.4 μmol ATP kg^{-1} min^{-1}, and would only account for <1% of aerobic metabolism (=11.4/1212). If 0% of R_d were oxidized (a very unlikely scenario), anaerobic metabolism would be 40.4 μmol lactate kg^{-1} min^{-1} (=R_a) or 80.8 μmol ATP kg^{-1} min^{-1}, and would account for 6.7% of aerobic metabolism (=80.8/1212). Therefore, cost of transport could have been underestimated by 1 to 6.7% at the highest swimming speed. Results show that two catheters exiting from the snout of the fish do not significantly increase $\dot{M}_{O_2}$ or TCOT at any swimming speed tested in our study (Figs 1, 2). However, catheterized animals have a higher NCOT than non-instrumented controls (+21% for sham catheterized and +29% for lactate kinetics) when they travel faster than 2.4 BL s^{-1}, and their U_{crit} is reduced (Fig. 2B, Fig. 3A). The higher NCOT observed in instrumented animals is not due to differences in resting $\dot{M}_{O_2}$ because catheterization has no effect on this parameter (Table 1), indicating that the stress of surgery is very low. All metabolite fluxes previously measured by continuous tracer infusion in swimming fish were not influenced by double catheterization because the experiments were performed at low, sustainable speeds (<1.5 BL s^{-1}) and NCOT is only affected above 2.4 BL s^{-1} (Bernard et al., 1999; Magnoni et al., 2008; Shanghavi and Weber, 1999).

In rainbow trout, the relationship between cost of transport and swimming speed is U-shaped (Fig. 2A), as predicted by various

models derived exclusively from hydrodynamic theory (Pettersson and Hedenström, 2000; Wakeman and Wohlschlag, 1981). This finding is highly consistent with empirical observations made in other fish species including European sea bass (Claireaux et al., 2006), Atlantic cod (Syme et al., 2008), Pacific bonito (Sepulveda et al., 2003), several flatfish (Duthie, 1982) and zebrafish (Palstra et al., 2010). Here, optimal swimming speed (U_{opt}=speed with minimal cost of transport) was 2.1 BL s^{-1} for TCOT (Fig. 2A) and 1.1 BL s^{-1} for NCOT (Fig. 2B). This interesting difference has been commonly reported in the fish literature. It indicates that maximal aerobic efficiency (Webb, 1971) is achieved at ~70% U_{crit}, whereas maximal muscle efficiency occurs at ~30–40% U_{crit} (Luna-Acosta et al., 2011; Palstra et al., 2008; Palstra et al., 2010).

Critical swimming speed was significantly reduced by the experimental manipulations necessary to measure metabolite kinetics. On their own, the drag forces elicited by the two catheters decreased U_{crit} by 11%. When the stress of sampling blood was added to this mechanical interference, U_{crit} was further decreased by 6% (−17% compared with non-instrumented controls; Fig. 3A), possibly through a reduction in oxygen transport. Another study reported no effect of cannulation on U_{crit} (Butler et al., 1992), but the measurements were made on brown trout implanted with a single catheter that may have caused less drag than the two catheters of our experiments. It may also be easier to demonstrate significant effects of hydrodynamic drag in rainbow trout because they have a better swimming capacity (U_{crit} of 2.8–3.4 BL s^{-1}) than brown trout, which are less athletic (U_{crit} of ~2.2 BL s^{-1}) (Butler et al., 1992).

Conclusions

This study is the first to show how the lactate kinetics of an ectotherm change with exercise intensity, and quantifies the rates of lactate production and disposal in rainbow trout, from rest to U_{crit}. This aerobic species maintains high baseline lactate fluxes of 24 μmol kg^{-1} min^{-1} that are only increased at speeds greater than 2.4 BL s^{-1} or ~85% U_{crit}. When the fish accelerates to reach U_{crit}, R_a is more strongly stimulated than R_d (+67% *versus* +41%) and this mismatch causes a fourfold increase in blood lactate concentration. Without this stimulation of R_d, the accumulation of end-product would double and impose an extra load on the circulation. Increased lactate oxidation by aerobic tissues (red muscle, heart, kidney and brain) is probably responsible for the higher R_d observed during intense swimming. Results also show that the hydrodynamic drag from double catheterization and blood sampling needed to measure metabolite kinetics in swimming trout have no significant impact on $\dot{M}_{O_2}$ or TCOT. However, these experimental procedures affect locomotion energetics by increasing NCOT at speeds >2.4 BL s^{-1} and by decreasing U_{crit}.

ACKNOWLEDGEMENTS

We thank Bill Fletcher and Christopher Lavergne for taking care of the animals.

AUTHOR CONTRIBUTIONS

All authors conceived and designed the experiments. They were all involved in interpreting the results and writing the article. L.T. and T.O. carried out the measurements.

COMPETING INTERESTS

No competing interests declared.

FUNDING

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) to J.-M.W [Discovery grant 105639-2012 and Research Tools and Instruments grant 390071-2010]. L.T. was the recipient of an International Mobility Fellowship from Région Rhône-Alpes (France) and a Travelling Fellowship from The Company of Biologists Ltd (UK).

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