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RESEARCH ARTICLE

Lean, mean, lipolytic machines: lipid mobilization in rainbow trout during graded swimming

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ABSTRACT

The mobilization of mammalian lipid reserves is strongly stimulated during exercise to reach a maximum at moderate intensities, but the effects of swimming speed on fish lipolysis have never been quantified. Continuous infusion of 2-[^{3}H]glycerol was used to measure the rate of appearance of glycerol or lipolytic rate (R_a glycerol) in rainbow trout kept at rest, or during graded exercise in a swim tunnel up to critical swimming speed (U_{crit}). Results show that R_a glycerol is 1.67±0.18 µmol kg^{-1} min^{-1} in control animals, and remains at a steady level of 1.24±0.10 µmol kg^{-1} min^{-1} in exercising fish at all swimming intensities. Baseline lipolytic rate provides more than enough fatty acids from lipid reserves to accommodate all the oxidative fuel requirements for swimming at up to 2 body lengths per second (BL s^{-1}), and more than 50% of the energy needed at U_{crit} (3.4±0.1 BL s^{-1}). Such 'excess lipolysis' also means that trout sustain high rates of fatty acid reesterification. Maintaining steady lipolysis at rest and throughout graded swimming is strikingly different from mammals that stimulate R_a glycerol by twofold to fivefold to support exercise. Instead, trout act like 'lipolytic machines' that do not modulate R_a glycerol even when their metabolic rate triples – a strategy that eliminates the need to increase lipolytic rate during exercise. This study also supports the notion that maintaining a high rate of reesterification (or triacylglycerol/fatty acid cycling) may be a mechanism widely used by ectotherms to achieve rapid membrane remodelling in variable environments.

KEY WORDS: Fish lipolysis, Exercise, Fuel metabolism, *In vivo* metabolite fluxes

INTRODUCTION

Fish store more than 85% of their energy reserves as triacylglycerol (TAG), mainly because lipids can yield more ATP per gram than any other fuel (Weber, 2011). They make predominant use of these large reserves to support swimming (Magnoni et al., 2006; Richards et al., 2002), particularly when it is sustained during long migrations (Magnoni et al., 2006; Mommsen et al., 1980). Stored TAG are made available to energy metabolism through lipolysis, an essential process regulating the strategic release of the constituent fatty acids that fuel prolonged work (Frayn, 2010; Tocher, 2003). *In vivo* lipolysis is measured by continuous tracer infusion as the rate of appearance of glycerol in the circulation (R_a glycerol; Wolfe and Chinkes, 2005). This method is routinely used in human medicine (Kim et al., 2015) and it has been validated for fish (Bernard et al., 1999; Haman and Weber, 1996).

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Only two studies investigating *in vivo* lipolysis in rainbow trout are presently available (Bernard et al., 1999; Magnoni et al., 2008b). They reveal that circulating noradrenaline inhibits the lipolytic rate in trout instead of activating it as in mammals, whereas adrenaline has a stimulating effect in both groups of animals. Fish may therefore rely on the counterplay between the two catecholamines to modulate lipolysis (Magnoni et al., 2008b; Montpetit and Perry, 1998). Unfortunately, lipid mobilization of exercising trout has only been measured during sustained, low-intensity swimming. Steady exercise at ∼50% of critical swimming speed (U_{crit}) has no stimulating effect on baseline R_a glycerol, even when it is sustained for 4 days (Bernard et al., 1999). This observation is very surprising because mammals performing equivalent exercise show a threefold to fivefold increase in lipolysis (Issekutz et al., 1967; Klein et al., 1996; Wolfe et al., 1990). They are also known to reach a maximal lipolytic rate at intermediate work intensities, before showing a marked reduction when exercise becomes more strenuous (Romijn et al., 1993). This lipolytic peak occurs when the rates of mammalian fatty acid oxidation are the highest (Brooks, 1998). For fish, no information is available on the relationship between exercise intensity and the rates of lipolysis or lipid oxidation. It is unknown whether intense exercise causes the inhibition of R_a glycerol in fish as it classically occurs in mammals. Therefore, the goals of this study were to quantify the lipolytic rate of rainbow trout during graded swimming from rest to U_{crit}, and to determine whether fish and mammals modulate lipolysis similarly to support exercise of different intensities. We hypothesized that rainbow trout would stimulate lipolysis to a peak with increasing work intensity, but subsequently lower its rate when reaching U_{crit}, as ATP production from carbohydrates becomes dominant (Richards et al., 2002).

MATERIALS AND METHODS

Animals

Adult rainbow trout [*Oncorhynchus mykiss* (Walbaum 1792)] of both sexes with a Fulton's condition factor K of 1.14±0.02 (N=16) [K=(10^5×M_b)/L^3), where M_b is body mass in g and L is total body length in mm] were purchased from Linwood Acres Trout Farm (Campbellcroft, ON, Canada) (see Table 1 for physical characteristics). The fish were held in a 1200 litre flow-through tank in dechlorinated Ottawa tap water maintained at 13°C, on a 12 h:12 h light:dark photoperiod, and were fed 5 days a week with Profishent floating fish pellets (Martin Mills, Elmira, ON, Canada). They were acclimated to these conditions for a minimum of 2 weeks before experiments. The animals were randomly divided into a control group kept at rest and an exercise group that performed graded swimming. All procedures were approved by the Animal Care Committee of the University of Ottawa in accordance with the guidelines established by the Canadian Council on Animal Care.

Catheterization

Fish were anaesthetized with ethyl 3-aminobenzoate methanesulfonate (MS-222; 60 mg l^{-1} buffered with sodium bicarbonate, 0.2 g l^{-1}) and

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Table 1. Mean physical characteristics and haematocrit of adult rainbow trout

	Control group	Exercise group	All fish
Sample size (*N*)	8	8	16
Body mass (g)	343±32	391±22	367±20
Length (cm)	31.3±1.0	32.0±0.5	31.7±0.5
Haematocrit (%)	16.1±2.0	18.1±1.5	17.1±1.2

Values are means±s.e.m.

doubly cannulated in the dorsal aorta with BTPE-50 catheters (Instech Laboratories, Plymouth Meeting, PA, USA) as previously described (Haman and Weber, 1996). During surgery, the catheters were kept patent by flushing with Cortland saline containing 50 U ml^{-1} heparin (Sigma-Aldrich, St Louis, MO, USA). Lower heparin levels of 25 U ml^{-1} were used during metabolic measurements to avoid stimulating lipolysis (Olivecrona and Bengston-Olivecrona, 1999). Fish were left to recover overnight in the swim-tunnel respirometer chamber at a water velocity of 0.5 body lengths per second (BL s^{-1}), a weak current that reduces stress and enhances the flow of water over the gills, but does not require swimming to maintain body position while resting at the bottom of the chamber (Choi and Weber, 2015).

Respirometry

All the experiments (resting controls and exercise) were performed in a 90 litre swim-tunnel respirometer (Loligo Systems, Tjele, Denmark) supplied with the same quality water as the holding tank and kept at 13°C. Metabolic rate ($\dot{M}_{O_2}$) 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 probes were calibrated before each experiment using air-saturated water (20.9% O_2).

Continuous tracer infusion

The catheters were made accessible above the swim-tunnel lid by channeling them through a water-tight port. The rate of appearance of glycerol (R_a glycerol) was quantified by continuous infusion of 2-[^{3}H] glycerol [American Radiolabelled Chemicals, St Louis, MO, USA; 925 GBq $mmol^{-1}$ (see Wolfe and Chinkes, 2005)], using procedures validated for measuring the lipolytic rate of rainbow trout *in vivo* (Bernard et al., 1999; Magnoni et al., 2008b). 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. Glycerol kinetics were measured by administering the tracer through the infusion catheter using a calibrated syringe pump (Harvard Apparatus, South Natick, MA, USA) (Haman and Weber, 1996). Exact infusion rate (~1 ml h^{-1}) was determined individually for each fish to adjust for differences in body mass. Infusion rates averaged 10,707± 485 Bq kg^{-1} min^{-1} (*N*=16) and were selected to reach isotopic steady state in less than 45 min (Magnoni et al., 2008b). These trace amounts only accounted for <0.001% of endogenous glycerol production. Graded swimming experiments consisted of step-wise U_{crit} tests as detailed previously (Teulier et al., 2013). Fish from the exercise group were kept at a resting water velocity (0.5 BL s^{-1}) during the first hour of tracer infusion before starting to swim. Exercise was initiated at 0.8 BL s^{-1} and intensified by 0.2 BL s^{-1} every 20 min until exhaustion. The point of exhaustion was determined when the fish could no longer sustain swimming, and was unable to remove itself from the rear grate of the swim tunnel. Metabolic rate and glycerol kinetics of the control group were monitored at rest for the same total duration as for the exercise group.

Blood sampling and analyses

Blood samples (0.1 ml each) were drawn 50, 55 and 60 min after starting the tracer infusion and every 20 min thereafter until the end of each experiment. The amount of blood sampled from individual fish accounted for <10% of total blood volume. Each blood sample was immediately deproteinized in 0.2 ml perchloric acid (6% wt/wt) and centrifuged (5 min; 13,000 ***g***). Supernatants were then kept at −20°C until analyses. Each sample was divided into three aliquots to measure lactate concentration (in µmol ml^{-1}), glycerol concentration (in µmol ml^{-1}) and radioactivity in plasma glycerol (in Bq ml^{-1}). Glycerol and lactate concentrations were measured by spectrophotometry as previously described (Teulier et al., 2013; Weber et al., 1993). Glycerol radioactivity was quantified by scintillation counting (Perkin-Elmer TriCarb 2910TR, Perkin-Elmer, Inc., Waltham, MA, USA) in Bio-Safe II™ scintillation cocktail (Research Products International, Mt Prospect, IL, USA). Previous work had determined that tritium from infused 2-[^{3}H]-glycerol partitions into circulating H_2O, glucose and glycerol (Bernard et al., 1999). To quantify the activity present only in glycerol, glucose activity was eliminated by incubating the samples with ATP and hexokinase to phosphorylate free glucose that was trapped by ion-exchange column chromatography [DOWEX-1 (formate form) and DOWEX-50 (HCl form)] (see Weber et al., 1993). The samples were then dried under N_2 to eliminate tritiated H_2O and resuspended in dH_2O, leaving [2-^{3}H]glycerol as the only labelled compound. Preliminary measurements with known amounts of labelled glycerol showed that 10.6±0.9% (measured in duplicate) was lost during the ion-exchange separation by column chromatography and all glycerol activity values were corrected accordingly.

Calculations and statistics

Glycerol specific activity (in Bq $µmol^{-1}$; see Table 2) was calculated as glycerol radioactivity (in Bq ml^{-1}) divided by glycerol concentration (in $µmol^{-1}$ ml^{-1}). The steady-state equation of Steele (1959) was used to calculate lipolytic rate (R_a glycerol) because specific activity varied little over time and the high turnover rate relative to pool size allows for rapid equilibration of the tracer within the glycerol pool. Under these conditions, the steady-state equation provides the most accurate estimates of glycerol flux (Beylot et al., 1987; Magnoni et al., 2008b; Wolfe et al., 1990). Two-tailed *t*-tests were used to compare mean values for $\dot{M}_{O_2}$ and the parameters of glycerol metabolism between the control and exercise groups (Table 2). The effects of time (resting group) or swimming speed (exercise group) on $\dot{M}_{O_2}$, cost of transport (COT), metabolite concentrations and R_a glycerol were assessed by one-way ANOVA with repeated measures (RM-ANOVA) followed by the Holm–Sidak *post hoc* test to determine which means were significantly different from baseline. When the assumptions of normality or equality of variances were not met, the

Table 2. Resting metabolic rate ($\dot{M}_{O_2}$) and parameters of glycerol metabolism in the control and exercise groups

	Control group	Exercise group
Resting $\dot{M}_{O_2}$ (µmol O_2 kg^{-1} min^{-1})	49.4±0.4	57.5±2.4*
Resting [glycerol] (µmol ml^{-1})	0.18±0.01	0.13±0.01
Resting specific activity in glycerol (Bq $µmol^{-1}$)	11,304±1758	11,006±2011
Resting R_a glycerol (µmol kg^{-1} min^{-1})	1.67±0.18	1.49±0.37

R_a glycerol, rate of appearance of glycerol or lipolytic rate. Values are means± s.e.m. (*N*=8). *Significant difference from control group (*P*<0.05).

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data were normalized by transformation ($\log_{10}$, square or square root) before parametric analysis. If transformation was unsuccessful, Friedman's nonparametric RM-ANOVA on ranks was performed. In all the analyses, values for individual fish that were different from the mean by more than 2 standard deviations were excluded as outliers. All values presented are means±s.e.m., and a level of significance of $P<0.05$ was used in all tests.

RESULTS

Metabolic rate, critical swimming speed and cost of transport

$\dot{M}_{O_2}$ and blood lactate concentration of rainbow trout during graded swimming and during control, resting experiments are presented in Fig. 1. The first $\dot{M}_{O_2}$ value indicated for the exercise treatment (at a 'non-swimming' water velocity of 0.5 BL s^{-1}) is the resting metabolic rate for this group of fish. Graded exercise caused a large increase in $\dot{M}_{O_2}$ from a baseline value of 57.5±2.4 µmol O_2 kg^{-1} min^{-1} to a maximum of 153.5±16.5 µmol O_2 kg^{-1} min^{-1} at 3.0 BL s^{-1} ($P<0.05$; Fig. 1A). The $\dot{M}_{O_2}$ of resting controls remained stable at 49.4±0.4 µmol O_2 kg^{-1} min^{-1} throughout the experiment ($P>0.05$; Fig. 1B). Blood lactate concentration remained unchanged for both groups ($P>0.05$; Fig. 1) and averaged 1.8±0.2 µmol ml^{-1} in the control fish and 1.7±0.1 µmol ml^{-1} in the exercising fish. U_{crit} averaged 3.4±0.1 BL s^{-1}. Total oxygen COT was calculated from $\dot{M}_{O_2}$ as a function of swimming speed from 0.8 to 3.2 BL s^{-1} (Fig. 2). A maximum COT of 5.9±0.4 µmol O_2 kg^{-1} m^{-1} was observed at the lowest speed. Increasing exercise intensity then caused a progressive decrease in COT to reach a minimal value of 2.3±0.2 µmol O_2 kg^{-1} m^{-1} at 2.2 BL s^{-1} ($P<0.001$). No further decline in COT was elicited as swimming speed was increased from 2.2 to 3.2 BL s^{-1}.

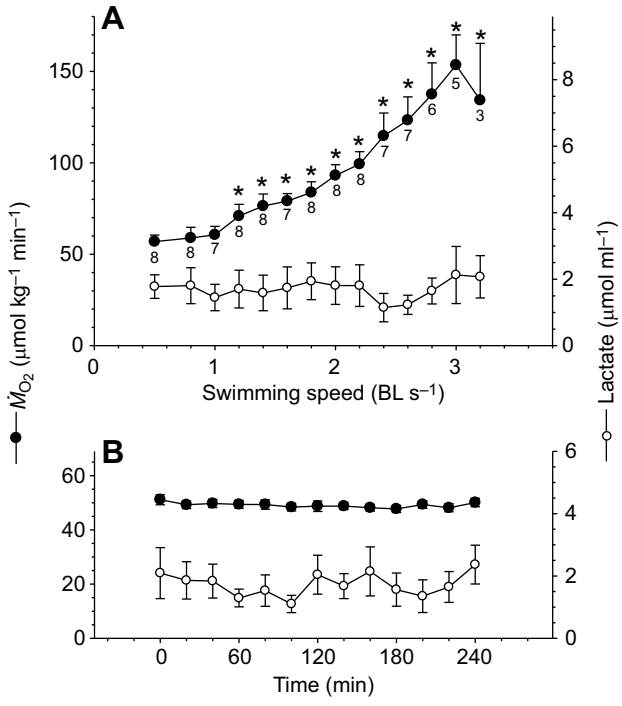

Fig. 1. Metabolic rate and blood lactate concentration of swimming trout and resting controls. (A) Graded swimming; (B) resting controls. Values are means±s.e.m. (*N* for individual means of swimming fish are given on the graph; *N*=8 for controls). The time scale of resting controls (B) also applies to swimming fish (A). *Significant differences from baseline values (measured at 0.5 BL s^{-1} or time=0; $P<0.05$).

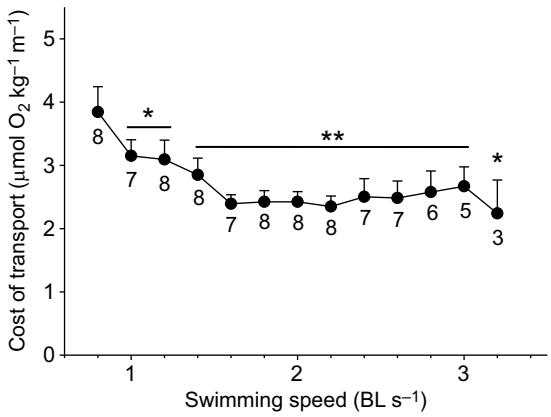

Fig. 2. Total cost of transport during graded exercise from the lowest swimming speed to critical swimming speed (U_{crit}). Values are means±s.e.m. with sample size *N*. Significant differences from cost of transport at the lowest speed of 0.8 BL s^{-1} are indicated with asterisks (*$P<0.05$; **$P<0.001$).

Glycerol metabolism

The rate of appearance of glycerol in the circulation (R_a glycerol) and plasma glycerol concentration were quantified in controls (Fig. 3) and swimming fish (Fig. 4). In control animals, R_a glycerol averaged 1.67±0.18 µmol kg^{-1} min^{-1} and glycerol concentration 0.18±0.01 µmol ml^{-1} throughout the 4 h of resting measurements. Only minor differences from the initial values of 2.41±0.42 µmol kg^{-1} min^{-1} (R_a glycerol) and 0.17±0.02 µmol ml^{-1} (glycerol concentration) were observed over time (Fig. 3). The R_a glycerol of controls was lower at 160 and 220 min ($P<0.05$), but not different from initial fluxes at all other times ($P>0.05$). Glycerol concentration remained steady at all times ($P>0.05$), except at

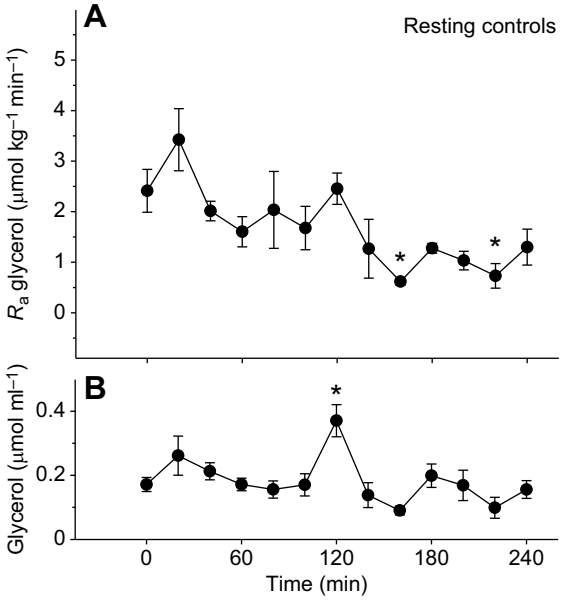

Fig. 3. Glycerol metabolism of resting (control) trout. (A) Rate of appearance of glycerol or lipolytic rate (R_a glycerol) and (B) blood glycerol concentration. R_a glycerol was measured by continuous infusion of 2-[^{3}H] glycerol. Values are means±s.e.m. (*N*=8). *Significant differences from baseline (time=0; $P<0.05$).

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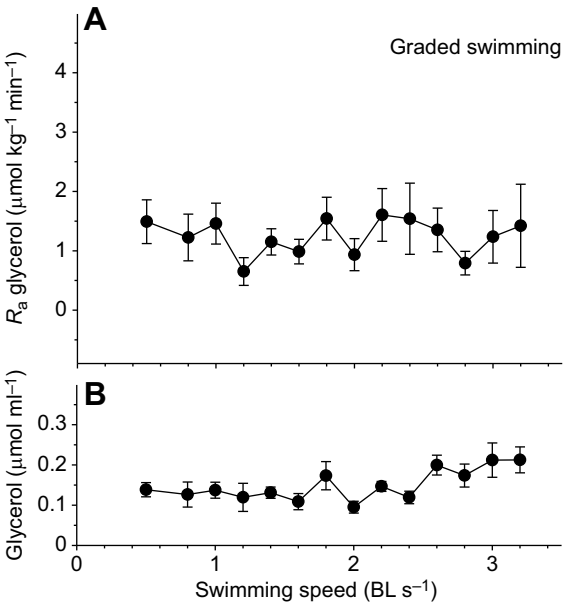

Fig. 4. Glycerol metabolism of trout during graded exercise up to critical swimming speed (U_{crit}). (A) Rate of appearance of glycerol or lipolytic rate (R_a glycerol) and (B) blood glycerol concentration. R_a glycerol was measured by continuous infusion of 2-[^{3}H] glycerol. Values are means±s.e.m. (individual sample sizes *N* are the same as in Fig. 1A). No significant effect of exercise was detected (P>0.05).

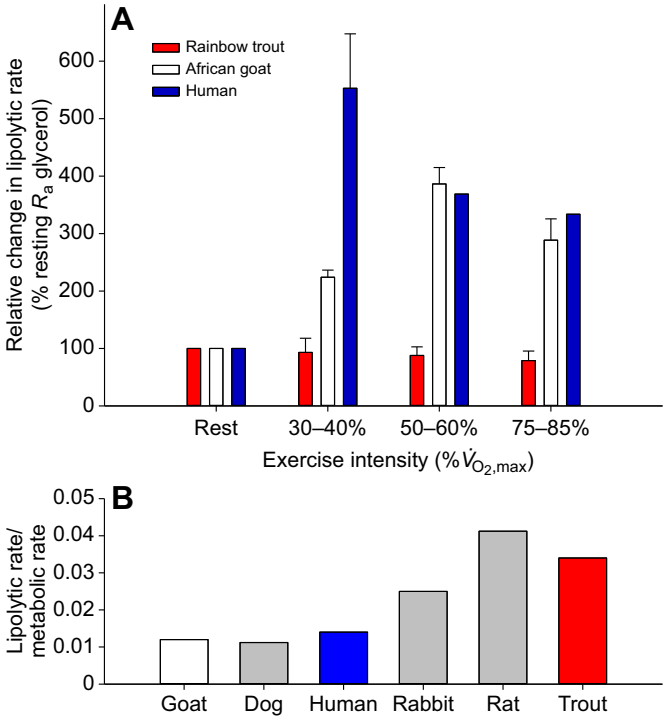

Fig. 5. Comparison of lipolytic capacities in trout and mammals. (A) Relative changes in lipolytic rate as a function of exercise intensity expressed as a percentage of resting R_a glycerol. Values are presented for rainbow trout (this study), African goat (calculated from Weber et al., 1993) and humans (calculated from Coggan et al., 2000; Klein et al., 1996; Wolfe et al., 1990). Mammals show a threefold to fivefold increase in lipolytic rate during exercise, whereas trout maintain a steady, baseline rate at all exercise intensities. Values are means (±s.e.m. when they could be obtained from the source papers). (B) Standardized baseline lipolytic rate (calculated as resting R_a glycerol/resting metabolic rate). Values were calculated for goat (Weber et al., 1993), dog (resting metabolic rate: Issekutz et al., 1967; R_a glycerol: Issekutz et al., 1975), human (Wolfe et al., 1990), rabbit (Weber and Reidy, 2012), rat (McClelland et al., 2001) and trout (present study). Trout maintain a higher baseline lipolytic rate relative to their metabolic rate than most mammals.

120 min, when it increased from baseline to 0.37±0.05 μmol ml^{-1} (P<0.05). In exercising fish (Fig. 4), R_a glycerol averaged 1.24±0.10 μmol kg^{-1} min^{-1} and glycerol concentration 0.13±0.01 μmol ml^{-1} throughout the experiments. Graded exercise had no effect on R_a glycerol at any swimming speed up to U_{crit} (P>0.05). Glycerol concentration was slightly increased with exercise intensity (P<0.05 for effect of speed in overall ANOVA), but the *post hoc* test was unable to identify which mean was significantly different from the resting value. Mean R_a glycerol and mean plasma glycerol concentration throughout the experiments were not different between the controls and the exercise group (P>0.05; Table 2).

Comparison of lipolytic responses in trout and mammals

Fundamental differences in the regulation of R_a glycerol between trout and mammals are illustrated in Fig. 5. Graded exercise causes a threefold to fivefold increase in the lipolytic rate of goats and humans, but has no stimulating effect in rainbow trout (Fig. 5A). In mammals, maximal rates of lipolysis are reached at intermediate exercise intensities of 30–60% $\dot{M}_{O_2,max}$. Because the metabolic fuel demands of fish and mammals greatly differ (ectotherm versus endotherm, and effects of body mass), baseline lipolytic rates were standardized as resting R_a glycerol/resting $\dot{M}_{O_2}$ to provide an energetically fair comparison (Fig. 5B). The standardized baseline lipolytic rate of rainbow trout is higher than for mammals measured to date except for rats.

Contribution of trout lipolysis to total oxidative fuel needs during exercise

In Fig. 6, the contribution of fatty acids to energy metabolism was calculated as a function of exercise intensity by assuming that all the fatty acids released by lipolysis were oxidized (=3 times mean R_a glycerol during exercise=3.72 μmol fatty acid kg^{-1} min^{-1}). The percentage of $\dot{M}_{O_2}$ accounted for by fatty acid oxidation was then computed for the two most abundant fatty acids available in trout plasma: palmitate (16:0) and oleate (18:1) (Bernard et al., 1999). These calculations show that lipolytic rate supplies more than enough fatty acids to support total metabolic rate up to 2 BL s^{-1} (values >100% $\dot{M}_{O_2}$), and 50% $\dot{M}_{O_2}$ at U_{crit} (Fig. 6).

DISCUSSION

This study shows that rainbow trout do not modulate *in vivo* lipolysis to cope with exercise of any intensity. Baseline lipolytic rate is steadily maintained throughout graded swimming from rest to U_{crit}. When standardized to metabolic rate, the resting lipolytic rate of trout is high compared with most mammals for which data currently exist, but it is neither stimulated by submaximal exercise nor inhibited by intense work. Baseline trout lipolysis releases enough fatty acids from lipid stores to power swimming up to 2 BL s^{-1}, and it can provide over 50% of the oxidative fuel required at U_{crit}. Therefore, these fish show a completely different strategy for lipid mobilization from mammals that boost lipolytic rate several fold above baseline at intermediate exercise intensities.

Trout lipolysis is not affected by exercise

Submaximal and intense exercise have no impact on the intrinsically high lipolytic rate of rainbow trout. Throughout graded swimming, the R_a glycerol of exercising fish averages 1.24 μmol kg^{-1} min^{-1}

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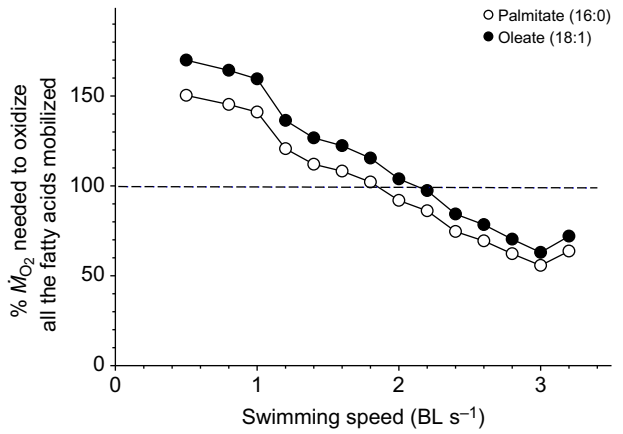

Fig. 6. Capacity of trout lipolysis to fuel oxidative metabolism with fatty acids as a function of exercise intensity. Values are expressed as percentage of the fish metabolic rate accounted for by the oxidation of all the fatty acids made available by lipolysis (=3 times mean R_a glycerol of swimming fish or 3.72 µmol fatty acids kg^{-1} min^{-1}). These values were calculated as if all the fatty acids oxidized were either palmitate (16:0) or oleate (18:1) because these two acids are the most abundant in trout lipid reserves. Lipolysis can provide all the energy necessary to support total metabolic rate up to 2 BL s^{-1}, and more than 50% of the energy needed at critical swimming speed (U_{crit}).

and remains unchanged from baseline values of the same individuals (Fig. 4) or from separate control fish kept in the resting state for the same duration (Fig. 3, Table 2). These results highlight an essential metabolic difference in the way fish and mammals regulate fuel selection. During the transition from rest to moderate exercise, dogs (Issekutz et al., 1975), goats (Weber et al., 1993) and humans (Klein et al., 1996; Wolfe et al., 1990) stimulate lipolytic rate by threefold to fivefold to provide more fatty acids to working muscles as their principal oxidative fuel (Fig. 5A). When exercise becomes more strenuous, ATP production starts relying more on carbohydrates (Brooks, 1998; Roberts et al., 1996) and lipolysis becomes inhibited (Romijn et al., 1993). This pattern is not observed in fish because they keep high and constant lipolytic rates at all times (Figs 4 and 5).

High sustained lipolytic rate in trout

The ratio of R_a glycerol to $\dot{M}_{O_2}$ calculated for resting conditions is higher in trout than in mammals measured to date, except for the rat (Fig. 5B). Such proportionately high rates of resting lipolysis are sufficient to cover the energy needs of trout exercise up to 2 BL s^{-1}, thereby meeting all oxidative fuel requirements for routine swimming (Fig. 6). Baseline trout lipolysis can even provide over 50% of the energy required at U_{crit}, suggesting that no stimulation of R_a glycerol is ever necessary in exercising salmonids. By contrast, mammals may be forced to stimulate R_a glycerol by several fold to support exercise because of their comparatively low resting lipolytic rate (standardized to resting $\dot{M}_{O_2}$; see Fig. 5B). Multiple papers now show that rainbow trout sustain much higher fluxes of glycerol (present study; Bernard et al., 1999; Magnoni et al., 2008b), fatty acids (Bernard et al., 1999; Weber et al., 2003) and TAG (Magnoni et al., 2008a) than strictly necessary to fuel energy metabolism (Weber et al., 2016). Together, these results demonstrate that a large fraction of the released fatty acids are therefore reesterified to prevent exceeding the solubilizing capacity of albumin-like proteins. This is particularly true at rest, but also while swimming up to 2 BL s^{-1} (top half of Fig. 6) because oxidizing 100% of the fatty acids made available by lipolysis would require much higher metabolic rates than actually measured here *in vivo* (Fig. 1, Table 2).

Potential role of sustained reesterification

Simultaneous lipolysis and reesterification form the TAG/fatty acid cycle, a substrate cycle that consumes ATP (Reidy and Weber, 2002). It is unclear why resting fish would need to keep this cycle active, but they are unlikely to expend energy on such a process if it has no physiological purpose. One intriguing hypothesis for the 'excess lipolysis' observed in fish is that it continually supplies fatty acids of various chain lengths and levels of unsaturation that could be conveniently used to remodel membrane phospholipids. Under changing thermal and osmotic conditions, the ability to stabilize membrane fluidity and the activity of membrane-bound proteins is crucial for survival (Gonzalez et al., 2013; Guderley et al., 1997; Williams and Hazel, 1995), particularly in the highly variable environments that some anadromous salmonids routinely encounter (Anttila et al., 2015; Hulbert and Else, 2000; Spares et al., 2015). Rainbow trout may therefore maintain high rates of lipolysis to ensure rapid and continuous restructuring of membrane phospholipids (Bernard et al., 1999; Magnoni et al., 2008a). Such a strategy has the convenient advantage that no upregulation of lipolysis is necessary to support swimming.

Opposing effects of adrenaline and noradrenaline may explain steady lipolysis

In mammals, adrenaline and noradrenaline stimulate lipolysis (Romijn et al., 1993) and the circulating levels of both hormones increase during exercise (Pedersen and Hoffman-Goetz, 2000). Catecholamines were not measured here due to limitations in the total volume of blood that could be drawn. However, previous studies have characterized the effects of swimming on fish catecholamines. In trout, blood adrenaline and noradrenaline levels fall below resting values at low to moderate exercise intensities (Butler et al., 1986; Shanghavi and Weber, 1999), and both show a sharp increase during and after more strenuous swimming (Butler et al., 1986; Milligan, 1996). Because trout lipolysis is stimulated by adrenaline (Fabbri et al., 1998; Magnoni et al., 2008b; Shanghavi and Weber, 1999), but reduced by noradrenaline (Magnoni et al., 2008b) via inhibitory β-adrenoceptors in adipocytes (van den Thillart et al., 2002; Vianen et al., 2002), the coordinated changes in these hormone levels elicited by swimming may leave trout lipolytic rate independent of exercise at any intensity. Constant lipolysis may be maintained *in vivo* via differential activation of adrenoceptor subtypes (van den Thillart et al., 2002) and tissue-specific differences in receptor expression (Fabbri et al., 1998).

Critical swimming speed and cost of transport

Exercising rainbow trout reached a U_{crit} of 3.4±0.1 BL s^{-1} (N=8). It could be argued that their true U_{crit} may be higher because no significant increase in blood lactate concentration was observed at the highest swimming speeds (Fig. 1A). It is unlikely that U_{crit} was underestimated here, however, because lower U_{crit} were measured in previous studies on doubly cannulated trout of the same size, tested with the same swimming protocol, but accompanied by elevated blood lactate concentrations [U_{crit}=2.9±0.2 BL s^{-1} (N=7) (Omlin et al., 2014), 2.8±0.1 BL s^{-1} (N=7) (Teulier et al., 2013) and 2.3±0.1 BL s^{-1} (N=10) (Choi and Weber, 2016)]. The observed changes in COT with increasing speed (Fig. 2) are consistent with previous studies on doubly cannulated trout when metabolite kinetics were quantified (Choi and Weber, 2016; Teulier et al., 2013). In all cases, rainbow trout showed the highest COT at low swimming speeds before falling to minimal values above 1 BL s^{-1}. It is interesting to note that preferred swimming speeds reported for salmonids range between 1 and 2 BL s^{-1} (Brett, 1964; Tudorache et al., 2011; Webb,

1971a,b) and coincide with the minimal COT observed here (Fig. 2). Salmonids migrate at these intermediate speeds because exercising more slowly increases COT and migration time, whereas higher speeds would have to rely on very limited carbohydrates reserves (Weber, 2009). To ensure adequate fitness and performance, selecting an optimal speed that maximizes swimming efficiency without using carbohydrates is essential, particularly for species that stop feeding during migration (Kadri et al., 1995; McCormick et al., 1998; Quinn and Myers, 2004; Spares et al., 2015).

Conclusions

We show that rainbow trout mobilize their lipid reserves in a drastically different way from mammals. They maintain a high baseline lipolytic rate at all exercise intensities rather than modulating it to match instantaneous needs for oxidative fuel. These animals behave like 'lipolytic machines' that hydrolyze TAG reserves at a rate constantly exceeding the fatty acid requirements of energy metabolism. Such a strategy eliminates the need to stimulate lipolysis by several fold during exercise as classically observed in mammals. The counteracting effects of trout adrenaline and noradrenaline could play a role in maintaining a steady lipolytic rate, independent of fluctuations in metabolic rate. This study also supports the notion that maintaining a high rate of TAG/fatty acid cycling may be a mechanism widely used by ectotherms to achieve rapid membrane remodelling in variable environments.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: E.D.T., J.-M.W.; Methodology: E.D.T., J.-M.W.; Formal analysis: E.D.T., J.-M.W.; Investigation: E.D.T.; Resources: J.-M.W.; Writing - original draft: E.D.T.; Writing - review & editing: E.D.T., J.-M.W.; Supervision: J.-M.W.; Funding acquisition: J.-M.W.

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