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Endurance swimming activates trout lipoprotein lipase: plasma lipids as a fuel for muscle

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Summary

Fish endurance swimming is primarily powered by lipids supplied to red muscle by the circulation, but the mechanism of delivery remains unknown. By analogy to mammals, previous studies have focused on non-esterified fatty acids (NEFA bound to albumin), but lipoproteins have not been considered as an energy shuttle to working muscles. The effects of exercise on fish lipoprotein lipase (LPL) have never been investigated. We hypothesized that LPL and circulating lipoproteins would be modified by prolonged swimming. Because LPL is naturally bound to the endothelium, we have used heparin to release the enzyme in the circulation and to characterize reserve capacity for lipoprotein catabolism. The effects of exercise (4 days at 1.5 body lengths s^{-1} in a swim tunnel) were measured for red muscle LPL, post-heparin plasma LPL, and lipoprotein concentration/composition. Red muscle LPL activity increased from 18±5 (rest) to 49± 9 nmol fatty acids min^{-1} g^{-1} (swimming). In resting fish, heparin administration caused a 27-fold increase in plasma LPL activity that reached a maximum of 1.32± 0.67 μmol fatty acids min^{-1} ml^{-1} plasma. This heparin-induced response of plasma LPL was not different between resting controls and exercised fish. Heparin or prolonged swimming had no effect on the concentration/composition of lipoproteins that contain 92% of the energy in total plasma lipids. We conclude that (1) red muscle LPL is strongly activated by endurance swimming, (2) rainbow trout have a high reserve capacity for hydrolyzing lipoproteins, and (3) future studies should aim to measure lipoprotein flux because their concentration does not reflect changes in flux. These novel characteristics of fish LPL imply that lipoproteins are used as a metabolic shuttle between fat reserves and working muscles, a strategy exploiting an abundant source of energy in rainbow trout.

Key words: sustained swimming, aerobic exercise, fish metabolism, lipoproteins, lipolysis, red muscle, heparin, rainbow trout, *Oncorhynchus mykiss*.

Introduction

In fish, endurance swimming is primarily supported by lipids supplied to red muscle through the circulation, but the actual mechanism of delivery remains unknown (Lauff and Wood, 1996; Moyes and West, 1995; Richards et al., 2002; Rome, 1998). By analogy with mammals, most fish studies have investigated the metabolic role of non-esterified fatty acids (NEFA), assuming that these lipids are responsible for transporting energy from adipose stores to locomotory muscles (Ballantyne et al., 1996; Bernard et al., 1999; Booth et al., 1999; Weber et al., 2002). This assumption is probably not justified, however, because (1) NEFA only account for a small percentage of the energy in plasma lipids (Babin and Vernier, 1989; Plisetskaya, 1980), and (2) endurance swimming has no effect on the turnover rate or concentration of plasma NEFA (Bernard et al., 1999). Although lipoproteins are used for transport to the gonads during egg production (Babin and Vernier, 1989), they have not been considered as a possible energy shuttle to working muscles. This is surprising because they carry most of the energy in the circulation (Moyes and West, 1995) and could theoretically play an important role in powering muscles during swimming. Lipoprotein lipase (LPL; EC 3.1.1.34) is the enzyme controlling the mobilization of lipoproteins and it has been well characterized in fish tissues (Lindberg and Olivecrona, 2002). Its potential role in orchestrating the supply of lipid fuel to working muscles has never been investigated. LPL is naturally bound to proteoglycans in the endothelium, but it can be released in the circulation by injecting heparin that has a higher affinity for the enzyme (Cryer, 1981). In this study, we have used heparin as a tool to characterize reserve capacity for lipoprotein catabolism, and to help us determine how exercise might modify the activity of this key enzyme. We could only find two studies where heparin had been used to investigate LPL *in vitro* (Lindberg and Olivecrona, 1995) or *in vivo*, but in a single fish in the resting state (Skinner and Youssef, 1982).

In wild sockeye salmon, we have previously proposed that lipoproteins are used to support swimming because their concentration changes dramatically during migration (Magnoni et al., 2006). However, it is unclear whether this response to migration is only caused by exercise or by a combination of stresses including swimming, fasting, reproduction and osmoregulation. To exclude confounding factors, this study

investigates the effects of endurance swimming on lipoprotein metabolism of rainbow trout under controlled laboratory conditions. Previous work had shown that the catabolism of very low density lipoproteins (VLDL) yields smaller particles such as VLDL-remnants or LDL (Havel, 1987; Zechner, 1997), and that monounsaturated fatty acids are a preferred substrate for oxidation (Henderson and Sargent, 1985; Kiessling and Kiessling, 1993; Sidell et al., 1995; Weber et al., 2002). We hypothesize that both LPL (enzyme) and plasma lipoproteins (substrate) are modified by prolonged exercise. It is predicted that endurance swimming will (1) activate LPL in red muscle, and (2) alter circulating lipoprotein classes (high-, low- and very low density lipoproteins: HDL, LDL and VLDL, respectively), components (triacylglycerol, phospholipids and NEFA), and composition (individual fatty acids). Our goals were to measure the effects of endurance exercise on LPL activity and on key characteristics of circulating lipoproteins. We have examined whether red muscle LPL and post-heparin plasma LPL are modified by several days of continuous swimming.

Materials and methods

Animals

Female rainbow trout *Oncorhynchus mykiss* Walbaum [body mass 388±85 g, total body length (*BL*) 31±3 cm, *N*=54] were obtained from Linwood Acres Trout Farm (Campbellcroft, Ontario, Canada). They were kept in a 1300 l flow-through holding tank in dechloraminated, well-oxygenated water at 13°C under 12 h:12 h L:D photoperiod. The same water quality and photoperiod were used during all the measurements, and the animals were acclimated to these conditions for at least 1 month before experiments. In the holding tank, routine swimming speed was <0.3 *BL* s^{-1}). Trout were fed floating fish pellets (Martins Mills, Elmira, ON, Canada) five times a week until satiation [see Weber et al. (Weber et al., 2002) for FA composition]. The animals were fasted for 48 h before starting experiments to eliminate circulating chylomycrons (Wallaert and Babin, 1994).

Exercise experiments

Fish were randomly divided into two groups: control and exercise. Pairs of animals were always measured simultaneously to be able to correct for potential effects of fasting. Exercising fish were placed in a modified Blazka-type swim tunnel (see Bernard et al., 1999). Resting fish were measured in a tube with similar dimensions to the exercise chamber of the swim tunnel. For acclimation to the experimental setup, the animals were kept at rest in a weak water current (11 cm s^{-1}) for 24 h. For the following 4 days, the control fish was kept at rest, but the exercising fish had to swim at 1.5 *BL* s^{-1} (46 cm s^{-1}). At the end of the experiment, both fish were rapidly removed from water and killed by a sharp blow to the head. Blood (5 ml) was sampled from the caudal artery within 1 min after death using EDTA as anticoagulant (1 mg ml^{-1}). Plasma was separated by centrifugation (5000 ***g*** for 10 min at 13°C) and used for the analysis of circulating lipoproteins. Red muscle (~2 g) from the caudal region of the lateral line was dissected in <2 min and stored at –80°C for LPL analysis.

Heparin experiments

A single catheter was surgically implanted in the dorsal aorta using buffered ethyl-*N*-aminobenzoate sulphonic acid as anaesthetic (MS-222, Sigma, St Louis, MO, USA) and sodium citrate as anticoagulant (13 μmol ml^{-1}). The animals were allowed to recover for 24 h in opaque Plexiglas™ chambers (see Haman and Weber, 1996). Resting fish were injected with heparin (200, 600 or 1000 U kg^{-1} body mass; Hepalean, Organon, Toronto, ON, Canada) and blood (0.5 ml) was collected before and after heparin injection. Plasma was immediately separated and stored at –80°C for subsequent analyses of LPL activity, as well as circulating triacylglycerol (TAG) and glycerol concentrations (Sigma kits, St Louis, MO, USA).

Exercise + heparin experiments

Animals implanted with a dorsal aorta catheter were randomly divided into two groups: resting controls and swimming. Surgical procedures and swimming conditions were the same as above. After 4 days of rest or exercise, blood samples (0.5 ml) were obtained through the catheter before and 1 h after the administration of 600 U heparin kg^{-1} body mass to measure plasma LPL activity.

Lipoprotein lipase

LPL activity was measured in frozen samples within 4 weeks of sampling because preliminary experiments showed that it was not affected by freezing. For red muscle, 0.5 g tissue was homogenized in 9 vol. buffer (10 mmol l^{-1} Hepes, 1 mmol l^{-1} EDTA, 1 mmol l^{-1} dithiothreitol and 5 U heparin ml^{-1} at pH 7.4) using a ground glass homogenizer on ice. Homogenates were centrifuged (20 000 ***g***, 20 min at 4°C) and the clear phase between the top layer and the pellet was used for LPL analysis. The characteristics of the substrate used to measure LPL activity have been described in detail elsewhere (Bengtsson-Olivecrona and Olivecrona, 1991). A 20% lipid solution (Intralipid, Sigma, St Louis, MO, USA) was emulsified with tri[9,10(n)-^{3}H]oleate (Amersham, Buckinghamshire, UK). This type of emulsion has been commonly used to measure LPL in a variety of vertebrate tissues (Karpe et al., 1998; Lindberg and Olivecrona, 1995), and its suitability as an artificial substrate has been specifically demonstrated for rainbow trout plasma and tissues (Albalat et al., 2005; Albalat et al., 2006; Lindberg and Olivecrona, 2002). Briefly, radiolabeled trioleate (~1.7 MBq) was dried under N_2 and resuspended in 2 ml Intralipid solution and 8 ml Cortland saline. This mixture was sonicated for 5 min at 70% pulse mode and low setting (Branson Sonifier 450; Danbury, CT, USA). Each assay was carried out using a 50 μl aliquot of the emulsion as substrate, mixed with 50 μl preheated rat serum, 250 μl assay medium, and 100 μl plasma or red muscle homogenate. The reaction was stopped after 1 h incubation at 20°C by adding 3 ml methanol:chloroform:heptane (1.41:1.25:1 v/v/v) and 100 μl 0.1 mol l^{-1} NaOH. After centrifugation (1200 ***g***, 20 min at 20°C), 1 ml of the upper phase was counted in 10 ml Safety Solve cocktail (Research Products, Mount Prospect, IL, USA) using a liquid scintillation counter (Beckman Coulter CS6500, Palo Alto, CA, USA). All LPL determinations were performed in triplicate.

Lipoprotein analysis

Lipoprotein classes were separated by ultracentrifugation (Beckman TL Optima; Palo Alto, CA, USA) using a self-generated gradient (Optiprep, Axis-Shield, Oslo, Norway) (see Graham et al., 1996). Fresh plasma (3.2 ml), Optiprep gradient (0.8 ml) and buffered saline (0.7 ml) were layered in Optiseal ultracentrifuge tubes (Beckman Coulter, Palo Alto, CA, USA) before centrifugation (350 000 ***g***, 3 h at 13°C). The different lipoprotein fractions were collected: high-density lipoproteins (1.6 ml), LDL (1.6 ml) and VLDL (1.5 ml). The exact nature of the three lipoprotein fractions was confirmed with agarose gels (Paragon electrophoresis system, Beckman Coulter, Fullerton, CA, USA). Fractions were stored at –80°C for subsequent analysis. Protein content was measured using the Bradford reagent (Sigma). Three lipid classes [NEFAs, TAG and phospholipids (PLs)] were separated in each lipoprotein fraction following published methods (Magnoni et al., 2006), and their FA concentration and composition were measured by gas chromatography after methylation (NEFAs) or acid transesterification (TAG and PLs) (Abdul-Malak et al., 1989; Chapelle and Zwingelstein, 1984). Heptadecanoic acid (17:0) was used as an internal standard because preliminary experiments showed that this acid is absent from NEFA, TAG and PL of trout plasma. The fatty acid methyl esters obtained were analyzed on an Agilent Technologies 6890N gas chromatograph equipped with a fused silica capillary column (Supelco DB-23, 60 m×0.25 mm i.d., 0.25 μm film thickness), using hydrogen as carrier gas at constant pressure and linear flow of 43 cm s^{-1}. The system was equipped with an automatic injection system (Agilent Technologies 7683B Series). The following conditions were used during analysis: (a) oven temperature was programmed for 1 min at 130°C, up to 170°C at 6.5°C min^{-1}, up to 215°C at 2.75°C min^{-1}, held at 215°C for 12 min, up to 230°C at 40°C min^{-1}, and held at 230°C for 3 min, (b) injector temperature was 270°C using a 50:1 split ratio, and (c) detector temperature was 280°C. Each methyl ester was identified specifically by determining its exact retention time with an authentic standard (Supelco, Bellefonte, PA, USA). Only the fatty acids representing more than 1% of total fatty acids in each lipid fraction are included in calculations.

Statistical analyses

A Student's? *t*-test was used to evaluate the effects of swimming on LPL activity in red muscle. In all other cases, statistical differences were assessed using analysis of variance (ANOVA), or Kruskal–Wallis ANOVA on ranks when the assumption of normality or homoscedasticity was not met. When significant changes were detected by ANOVA, the Holm–Sidak method was used for pairwise comparisons. Percentages were transformed to the arcsine of their square root before statistical analysis and all values given are means ± standard error of the mean (s.e.m.).

Results

Effects of sustained swimming

The effect of endurance swimming on the LPL activity of rainbow trout red muscle is presented in Fig. 1. Four days of continuous swimming at 1.5 *BL* s^{-1} caused a 2.7-fold increase in LPL activity (P=0.009). The capacity of this enzyme to hydrolyze fatty acids from TAG increased from 18±5 to 49±9 nmol FA min^{-1} g^{-1} wet tissue. The effects of prolonged swimming on circulating lipoproteins were examined in detail and this analysis is summarized in Figs 2 and 3. Fatty acid content, protein content and the fatty acid/protein ratio of the three lipoprotein classes (HDL, LDL or VLDL) are shown in Fig. 2. Endurance swimming had no measurable effect on these parameters (P>0.05). The relative fatty acid content of the different lipoprotein classes was the same (P>0.05), but their protein content (P=0.002) and their fatty acid/protein ratio (P<0.01) varied drastically. Protein content decreased in the following order: HDL>LDL>VLDL (Fig. 2B), whereas the opposite was true for the fatty acid to protein ratio (Fig. 2C).

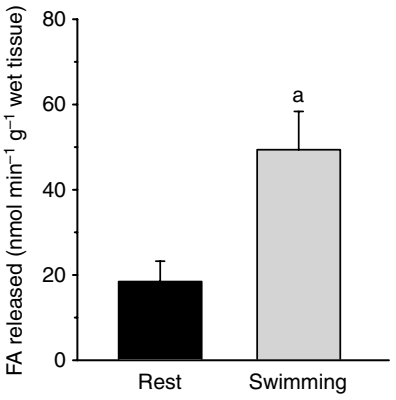

Fig. 1. Effect of endurance swimming (4 days at 1.5 *BL* s^{-1}) on lipoprotein lipase activity (LPL) in red muscle of rainbow trout. Values are mean ± s.e.m. (N=8). Letter a denotes a significant difference from the resting control (P<0.01).

Lipoproteins were also analyzed by separating their various components. The concentrations of PL, TAG and NEFA in the three classes of lipoproteins are shown in Fig. 3. Endurance swimming had no measurable effect on these parameters (P>0.05). Phospholipid was the main lipid component of HDL (63% of total fatty acids in HDL) and LDL (59% of total fatty acids in LDL), whereas TAG was the main lipid component in VLDL (46% of total fatty acids in VLDL). NEFAs only represented 6–11% of total FA in the various lipoprotein fractions. The detailed fatty acid composition of PLs, TAG and NEFAs in the three lipoprotein classes was also analyzed. Because no measurable effect of endurance swimming was detected on fatty acid composition (P>0.05), only pooled data for control and exercised fish are presented in Table 1, which groups individual fatty acids into the major classes: saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated (PUFAs). In all lipoproteins, PUFAs are the main fatty acids in PLs (63–71%) and TAG (47–51 %), whereas SFAs are dominant in NEFAs (64–80%). MUFAs are thought to provide the best fuel for oxidation (Sidell et al., 1995) and they account for an important fraction of TAG fatty acids (34–40%).

Effects of heparin

The time course of changes in plasma LPL activity after injection of heparin is presented in Fig. 4. Baseline LPL activity was 0.04±0.01 μmol FA released min^{-1} ml^{-1} plasma and it was

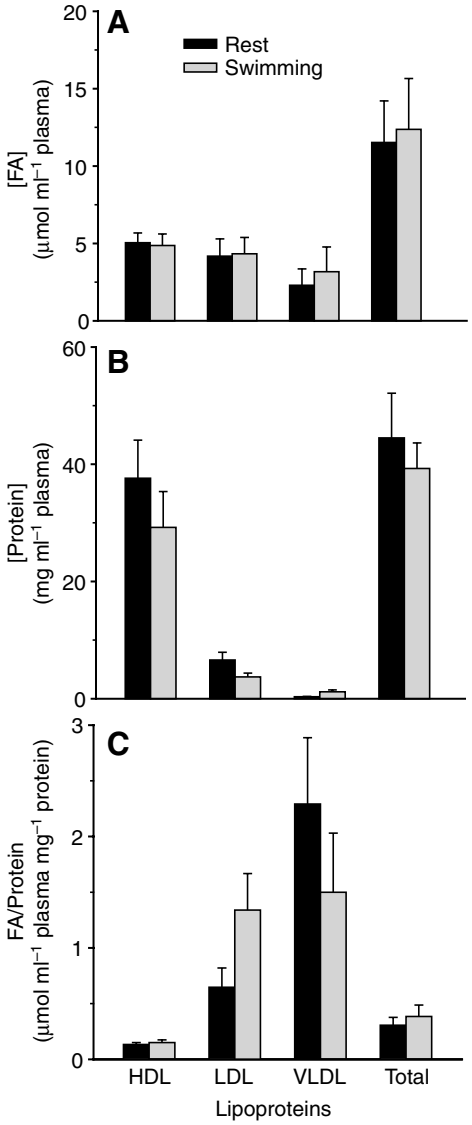

Fig. 2. Concentrations of fatty acids (FA) (A), proteins (B) and FA to protein ratio (C) in the plasma lipoproteins of rainbow trout after 4 days of resting or 4 days of sustained swimming at 1.5 *BL* s^{-1}. Values are mean ± s.e.m. (*N*=7). HDL, high density, LDL, low density and VLDL, very low density lipoproteins.

strongly stimulated 30–60 min after heparin administration, when maximal values were recorded (1.07±0.20 and 1.32±0.67 μmol FA released min^{-1} ml^{-1} plasma for heparin doses of 200 and 600 U kg^{-1}, respectively) (P<0.001). Activity stayed elevated above baseline for 2 h after heparin injection, but values measured from 4 to 48 h after injection were not different from baseline (P>0.05). Maximal stimulation of plasma LPL was already obtained at 200 U heparin kg^{-1} because no significant difference between the two doses were detected (P>0.05). This was confirmed by administration of 1000 U kg^{-1} in four additional fish (data not shown). Fig. 5 shows the effects of heparin administration on the concentrations of plasma TAG and plasma glycerol. Injecting 200 or 600 U heparin kg^{-1} had no detectable effect on the concentrations of TAG or glycerol (Fig. 5) (P>0.05).

Combined effects of swimming and heparin on plasma LPL

The effects of prolonged swimming and heparin on plasma LPL activity are presented in Fig. 6. Heparin had a major stimulating effect on plasma LPL activity (P<0.001), but this strong response was not significantly different between resting fish and those that had been swimming for 4 days at 1.5 *BL* s^{-1} (P>0.05).

Discussion

This study shows that lipoprotein lipase of fish red muscle is strongly activated during prolonged exercise, implying that lipoproteins are used as an energy shuttle to working muscles (Fig. 1). In rainbow trout, the existence of this fuel supply mechanism is further supported by the high reserve capacity for lipoprotein hydrolysis demonstrated here with heparin-induced stimulation of plasma LPL (Fig. 2). Contrary to expectation, the large increases in LPL activity elicited by endurance exercise (threefold) and heparin (27-fold) are not accompanied by changes in plasma lipoprotein concentration or composition (Figs 3 and 5, Table 1).

Effects of swimming on red muscle LPL

Fish LPL has been characterized in most tissues (red and white muscle, mesenteric fat, gonads and liver) where it is strongly modulated by seasonal cycles associated with fasting and reproduction (Black and Skinner, 1986; Black and Skinner, 1987; Fremont et al., 1987; Ibáñez et al., 2003; Lindberg and Olivecrona, 1995; Saera-Vila et al., 2005). Our study is the first

Table 1. *Percentages of total fatty acids per lipid class transported in high density, low density and very low density lipoproteins in rainbow trout plasma*

	HDL			LDL			VLDL		
Fatty acids	PL	TAG	NEFA	PL	TAG	NEFA	PL	TAG	NEFA
SFA	19.2 (2.1)	15.0 (2.3)	63.6 (9.4)	18.8 (2.4)	17.4 (4.3)	69.7 (6.6)	16.2 (3.5)	11.3 (4.3)	80.4 (6.9)
MUFA	17.5 (0.9)	34.4 (2.1)	22.4 (6.6)	17.4 (0.7)	35.2 (2.7)	16.5 (3.6)	12.9 (2.1)	39.8 (4.3)	16.8 (7.0)
PUFA	63.3 (2.6)	50.6 (3.1)	14.0 (2.9)	63.7 (2.9)	47.4 (4.6)	13.7 (4.0)	70.9 (5.1)	48.9 (5.8)	2.8 (1.1)

HDL, high density, LDL, low density and VLDL, very low density lipoproteins; PL, phospholipid; TAG, triacylglycerol; NEFA, non-esterified fatty acid; SFA, saturated, MUFA, monounsaturated and PUFA, polyunsaturated fatty acids.

Measurements were made on two groups of 7 fish after 4 days of resting or swimming. Because exercise had no significant effect, pooled values are reported as means (s.e.m.) (*N*=14).

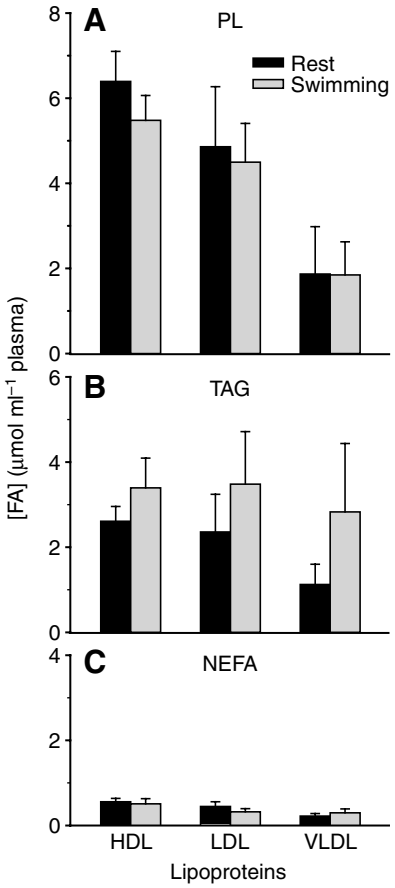

Fig. 3. Concentrations of phospholipids (PL) (A), triacylglycerol (TAG) (B), and non-esterified fatty acids (NEFA) (C) in the plasma lipoproteins of rainbow trout after 4 days of rest or swimming at 1.5 *BL* s^{-1}. To allow comparisons between lipid classes, all concentrations are given in μmol fatty acids ml^{-1} plasma. Absolute PL and TAG concentrations can be obtained by dividing the values presented in A by 2 (2 fatty acids per PL) and in B by 3 (3 fatty acids per TAG). Values are mean ± s.e.m. (*N*=7). HDL, high density, LDL, low density and VLDL, very low density lipoproteins.

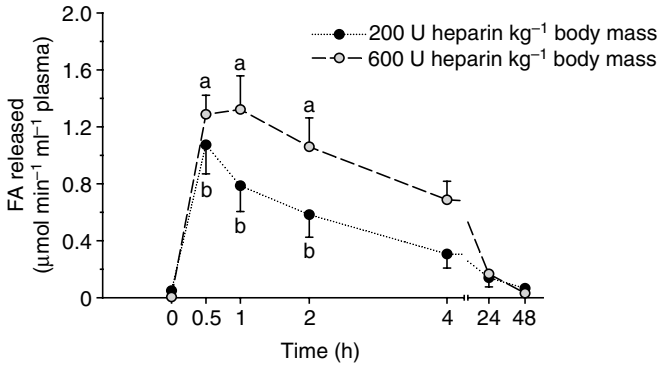

Fig. 4. Time course of changes in lipoprotein lipase activity of rainbow trout plasma after injection of 200 or 600 U heparin kg^{-1} body mass. Values are mean ± s.e.m. (*N*=8). Letters show significant differences from control values at time 0 (before heparin administration).

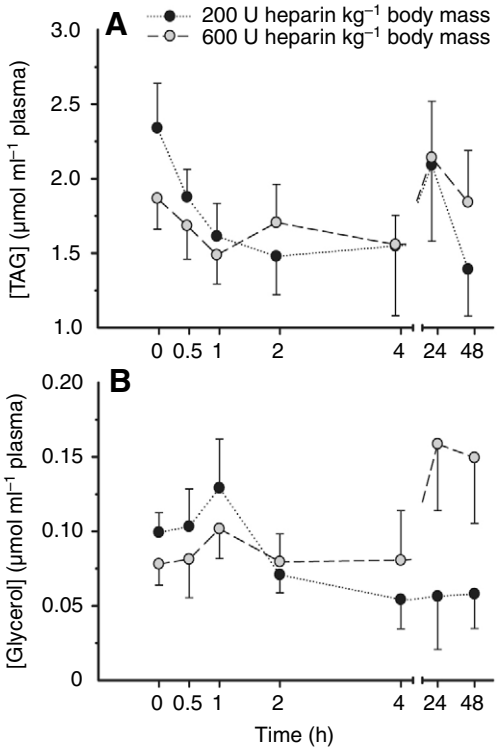

Fig. 5. Concentrations of triacylglycerol (TAG) (A) and glycerol (B) in rainbow trout plasma after injection of 200 or 600 U heparin kg^{-1} body mass. Values are means ± s.e.m. (*N*=8).

to examine the effects of sustained swimming on this enzyme, and it shows that LPL is stimulated by exercise in lateral red muscle, which is the engine for endurance swimming. The threefold increase in LPL activity observed in trout muscle after prolonged exercise is consistent with the response reported for several mammals, including rats [two- to threefold (Bagby et al., 1986; Ladu et al., 1991a; Oscai et al., 1982)], dogs [twofold (Budohoski, 1985)] and humans [threefold (Lithell et al., 1984)]. *In vitro* experiments show that the addition of LPL to co-culture systems containing lipoprotein-secreting hepatocytes and muscle cells of fish increases muscle FA uptake by 60% (Alam et al., 2004).

Tissue uptake of fatty acids from circulating lipoproteins is thought to be limited by the rate of TAG hydrolysis and, therefore, it is mainly regulated by LPL activity (Nilsson-Ehle, 1980). Numerous hormones, including insulin, catecholamines, glucocorticoids and thyroxine, are well-known modulators of LPL. The relative distribution of their various receptors is responsible for tissue-specific responses (Mead et al., 2002). The activation of mammalian LPL by exercise is linked to increased mRNA levels in skeletal muscle (Kiens et al., 2004), with catecholamines and insulin acting as the most probable hormonal modulators (Chernick et al., 1986; Enerback and Gimble, 1993; Ladu et al., 1991b; Lithell et al., 1981; Seip et al., 1997). In addition to systemic regulation by hormones, local signals associated with contractile activity have been implicated in LPL modulation. In rats, a significant role for contractile activity is supported by experiments in which electrical stimulation was performed unilaterally. Stimulated muscles

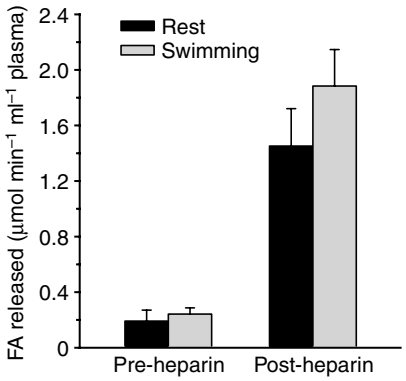

Fig. 6. Plasma lipoprotein lipase activity before and 1 h after administration of 600 U heparin kg^{-1} body mass in resting (control) and swimming rainbow trout (4 days at 1.5 *BL* s^{-1}). Heparin increased LPL activity in both groups ($P<0.001$), but this effect was not different between resting and swimming fish ($P>0.05$). Values are means ± s.e.m. ($N=9$).

showed a threefold increase in LPL activity, whereas contralateral (rested) muscles did not respond (Hamilton et al., 1998). Taken together, current data on the exercise-induced up-regulation of mammalian LPL do not allow assessment of the relative importance of these various mechanisms. For fish, even less information is available, but a significant role for insulin modulation of muscle LPL appears doubtful. Recent experiments show that *in vivo* administration of insulin causes the activation of adipose tissue LPL in rainbow trout, but has no measurable effect on red muscle (Albalat et al., 2006). The exercise-induced activation of LPL observed in our study is therefore probably associated with changes in circulating catecholamines or contractile activity. For example, prolonged exercise causes a decrease in circulating epinephrine levels (Shanghavi and Weber, 1999) that may be involved in LPL activation. Even though further studies are needed to characterize exact regulation mechanisms in fish, results clearly show that red muscle LPL is recruited during prolonged swimming, making lipoproteins available as a fuel for locomotion.

Effects of heparin on plasma LPL

This study characterizes the activation of plasma LPL by heparin in intact fish. It provides a time course of changes in circulating LPL activity for different doses (200–1000 U heparin kg^{-1}) and shows that a maximal response is reached ~1 h after injecting 600 U kg^{-1}. We are aware of only one other study investigating this issue; it reports the partial response of an individual fish after injection of 100 U kg^{-1} (Skinner and Youssef, 1982). The maximal LPL activity measured here was much higher than in this previous study and it occurred later (60 *vs* 35 min after injection). Such discrepancy is not simply due to the higher doses used here, but mainly to the actual substrate for the LPL assay. Intralipid-based emulsions (this study) are considered a better imitation of real lipoproteins than those made with gum arabic (as in Skinner and Youssef, 1982) that yield sub-optimal hydrolysis of radiolabeled trioleate (Bengtsson-Olivecrona and Olivecrona, 1991). This view is further supported by *in vitro* measurements of plasma LPL on Intralipid emulsions that give values of up to 0.8 μmol FA min^{-1} ml^{-1} (Lindberg and Olivecrona, 2002), approaching maximal activities reported here (1.32 μmol FA min^{-1} ml^{-1}; Fig. 4). Under baseline conditions, LPL is mostly bound to the endothelium, and plasma LPL activity is therefore very low (here, only 3% of peak, post-heparin values; see Fig. 4). The injection of heparin causes a drastic increase in activity by (1) releasing the enzyme in plasma, and (2) inhibiting its normal uptake by the liver for degradation (Chajek-Shaul et al., 1988). The impressive 27-fold increase in activity observed here in plasma after release of the enzyme by heparin demonstrates that rainbow trout tissues have a remarkable reserve capacity for lipoprotein hydrolysis.

Combined effects of swimming and heparin on plasma LPL

One of the goals of this study was to determine whether the changes in tissue LPL caused by exercise would be measurable in post-heparin plasma. Contrary to expectation, the observed increase in red muscle LPL (Fig. 1) was not mirrored by post-heparin plasma LPL (Fig. 6). However, close examination of the data reveals a non-significant trend towards an increase in the exercise group that would be consistent with our red muscle results. It is also conceivable that the activation of red muscle LPL is accompanied by inhibition of the enzyme in other tissues, yielding no overall change in post-heparin plasma LPL. Alternatively, the effect of exercise on red muscle LPL may not be large enough to influence LPL activity in post-heparin plasma because trout red muscle only represents 7% of total body mass. For example, rats using a larger muscle mass than the trout of our experiments show a significant increase in post-heparin plasma LPL after exercise (Hamilton et al., 1998).

Lipoprotein concentration and composition

We were unable to demonstrate significant effects of endurance exercise on the concentration or the composition of circulating lipoproteins. It might be argued that the non-significant trends towards a decrease in the FA/protein ratio and increase in TAG concentration observed for VLDL (Fig. 2C and Fig. 3B) may have become significant with a larger sample size than used here. However, demonstrating such effects of swimming does not appear practical or necessary for two reasons: (1) sensitivity analysis reveals that sample sizes of more than 40 would be needed to reach significance with a minimal statistical power of 0.8 (SigmaStat 3.1, Systat Software Inc. 2004), and (2) by itself, a change in concentration would not be sufficient to prove a role for lipoproteins as a muscle fuel because mismatches between changes in flux and concentration are common occurrences in animals, including rainbow trout (see Haman et al., 1997). The large increase in muscle LPL activity caused by swimming (Fig. 1) implies that the rate of lipoprotein turnover is stimulated and, therefore, that lipoprotein concentration is a poor indicator of lipoprotein flux. This observation reinforces the necessity for developing more sensitive methods to analyze the effects of endurance swimming on lipoprotein metabolism, and, in particular, to quantify lipoprotein flux. The exercise-induced activation of LPL is not accompanied by changes in NEFA concentration and, therefore, locomotory muscles must be able to take up NEFA rapidly after

lipoprotein hydrolysis as previously suggested (Cryer, 1981; Merkel et al., 2002). Because prolonged swimming had no measurable effect on the fatty acid composition of circulating lipoproteins, this study does not support the hypothesis that any specific fatty acid is preferentially used by locomotory muscles (selectivity).

The 27-fold increase in plasma LPL activity caused by heparin (Fig. 4) does not have any measurable effect on plasma TAG concentration (Fig. 5), the main substrate for the enzyme. In contrast, plasma TAG concentration of mammals is decreased by endurance exercise (Ensign et al., 2002; Hardman, 1998), and *in vivo* heparin administration has a marked lipolytic effect on circulating TAG (Skoglund-Andersson et al., 2003). The high lipoprotein concentrations of fish compared to mammals (Babin and Vernier, 1989) may be responsible for the concentration inertia observed here in trout. Because concentration stays constant, tissue uptake of lipoprotein-derived fatty acids must be regulated by changes in LPL activity rather than by a mass action effect of its substrate (Nilsson-Ehle, 1980).

Most lipoprotein studies report concentrations in mass % (mg/100 ml). However, this unit fails to reveal quantitative differences in energy content because lipoprotein classes contain different amounts of protein, as well as cholesterol. To avoid this problem, Fig. 3 expresses concentrations of the different lipid components in μmol FA ml^{-1} plasma (because fatty acids contain most of the energy in lipoproteins). This figure shows that the great majority of the energy circulating in the plasma lipids of rainbow trout resides in PLs (55%) and TAG (37%), whereas NEFAs only make a minor contribution (8% of the energy). Within each lipoprotein class, fatty acid content is similar, but protein content is highly variable (HDL>LDL>VLDL). Therefore, the FA/protein ratio ranges between ~0.2 for HDL and ~2.0 for VLDL (Fig. 2). Using protein content from Fig. 2B (in mg protein ml^{-1} plasma: HDL=33, LDL=5 and VLDL=0.7) and published values for percentage protein in each lipoprotein class by mass [% protein: HDL=45, LDL=30 and VLDL=13 (Babin and Vernier, 1989)], we can estimate that 70% of all trout lipoproteins are HDL, 21% are LDL and the remaining 9% are VLDL.

Conclusion

Red muscle LPL is activated by endurance swimming and rainbow trout show a very high reserve capacity for the hydrolysis of circulating lipoproteins. These novel characteristics of trout LPL and the fact that lipoproteins contain 92% of the energy in plasma lipids imply that lipoproteins are used as an energy shuttle between fat reserves and working muscles. Such a mechanism contrasts with the classic mammalian strategy where lipid fuel is supplied by NEFA–albumin complexes. Because lipoprotein concentration does not reflect changes in flux, direct measurements of lipoprotein kinetics will be needed as soon as adequate methods are developed.

List of abbreviations

HDL	high-density lipoprotein
LDL	low-density lipoprotein
LPL	lipoprotein lipase
MUFA	monounsaturated fatty acid
NEFA	non-esterified fatty acid
PUFA	polyunsaturated fatty acid
SFA	saturated fatty acid
TAG	triacylglycerol
VLDL	very low density lipoprotein

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