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EFFECTS OF SUSTAINED SWIMMING ON HEPATIC GLUCOSE PRODUCTION OF RAINBOW TROUT

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Summary

The rate of hepatic glucose production ($R_a$glucose) was measured by continuous infusions of 6-[^{3}H]glucose in live rainbow trout (*Oncorhynchus mykiss*) before, during and after swimming for 3 h at 1.5 body lengths s^{-1} in a swim tunnel. Contrary to expectation, we found that sustained swimming causes a 33 % decline in the $R_{a,glucose}$ of trout (from 7.6±2.1 to 5.1±1.3 μmol kg^{-1} min^{-1}, means ± S.E.M., N=7), even though exercise of the same intensity elicits a two- to fourfold increase in all the mammalian species investigated to date. Measurements of catecholamine levels show that circulating [epinephrine] decreases by 30 % during exercise (from 4.7±0.3 to 3.3±0.4 nmol l^{-1}, N=8), suggesting that this hormone is partly responsible for controlling the decline in $R_a$glucose. The inhibiting effect of swimming on hepatic glucose production persists for at least 1 h after the cessation of exercise. In addition, rainbow trout can maintain a steady blood glucose concentration throughout sustained exercise by closely matching hepatic glucose production with peripheral glucose utilization, even though this species is generally considered to be a poor glucoregulator. This study provides the first continuous measurements of glucose kinetics during the transition from rest to work in an ectotherm and it suggests that circulating glucose is not an important fuel for aerobic locomotion in trout.

Key words: glucose kinetics, hepatic glucose release, liver glycogen, gluconeogenesis, glucose, *in vivo* substrate flux, aerobic exercise, rainbow trout, *Oncorhynchus mykiss*.

Introduction

Mammals tightly control circulating glucose levels by continuously matching rates of hepatic glucose production with peripheral utilization (Pagliassotti et al., 1994). Such a regulatory process plays a fundamental role because this metabolic fuel represents an essential source of energy for the nervous system as well as for other tissues. During submaximal exercise, the rate of hepatic glucose production ($R_a$glucose) is usually strongly stimulated to replace the additional glucose consumed by locomotory muscles. The nature of this response has been well characterized in mammals (Wasserman and Cherrington, 1991), but little information is available for teleosts, a group of vertebrates known to tolerate wide fluctuations in glycaemia (Driedzic and Hochachka, 1978; Palmer and Ryman, 1972). Baseline glucose kinetics have been quantified in a variety of fish species, including the American eel, sea bass and rainbow trout (for a review, see Weber and Zwingelstein, 1995), and the effects of rapid changes in environmental conditions on $R_a$glucose of trout have also been investigated recently. Acute hypoxia causes a brief stimulation of hepatic glucose production leading to hyperglycaemia, whereas a rapid decrease in water temperature triggers a strong decline in $R_a$glucose, but without affecting glucose concentration (Haman et al., 1997b). To date, all the measurements of $R_a$glucose in fish have been carried out in the resting state, and the effects of swimming on glucose fluxes are unknown. Maximal exercise and anaerobic metabolism have been the focus of most studies of carbohydrate utilization in teleosts (Moyes and West, 1995) even though wild salmonids spend most of their time swimming at submaximal velocities (Beamish, 1978; Quinn, 1988; Weihs, 1973). The limited data presently available on aerobic swimming suggest that lipids are oxidized preferentially, whereas the contribution of carbohydrates remains small (Driedzic and Hochachka, 1978; Moyes and West, 1995; Walton and Cowey, 1982). Recent results from Kieffer et al. (1998) show that strenuous swimming causes a significant increase in the total carbohydrate use of juvenile trout (from 15 to 25 % of $\dot{M}_{O_2}$ in animals acclimated to 15 °C), but the respective contributions of muscle glycogen and circulating glucose could not be separated using their experimental approach. In contrast, the effects of sustained exercise on the $R_a$glucose of mammals have been thoroughly investigated. Rats (Brooks and Donovan, 1983), dogs (Weber et al., 1996) and humans (Weber et al., 1990) increase glucose production by two- to fourfold during exercise to help meet the higher energy demands of working muscles. By analogy, we have hypothesized that the hepatic

glucose release of fish would be strongly increased during sustained swimming. Therefore, our goal was to quantify $R_a$glucose by continuous tracer infusion before, during and after submaximal exercise in rainbow trout. Circulating catecholamine levels were also measured to determine whether they could play a role in the regulation of hepatic glucose release *in vivo*. Finally, the effects of exercise on glycogen reserves were evaluated in an attempt to partition the relative contributions of glycogenolysis and gluconeogenesis to total glucose production.

Materials and methods

Animals

Rainbow trout *Oncorhynchus mykiss* (Walbaum) of both sexes (709±54 g; 29–43 cm) were purchased from Linwood Acres Trout Farm (Campbellcroft, Ontario, Canada) and held at 13 °C in a 1300 l flow-through tank. They were kept in dechloraminated, well-oxygenated water under a 12 h:12 h L:D photoperiod. The animals were fed Purina trout chow (40 % protein, 20 % carbohydrate and 10 % lipid) three times a week until satiation and were acclimated to these conditions for a minimum of 6 weeks before experiments.

Catheterizations

A double cannulation of the dorsal aorta was performed under anaesthesia (0.1 g l^{-1} ethyl-*N*-aminobenzoate sulphonic acid, MS-222, buffered with 0.2 g l^{-1} sodium bicarbonate) (for details, see Haman and Weber, 1996). Catheterized animals were allowed to recover from surgery for 36–48 h in a swim tunnel with a low water current (11 cm s^{-1}), just sufficient to maintain oxygen saturation. At this flow rate, the animals were not swimming, but rested on the floor of the swim tunnel.

Swim tunnel

Measurements were carried out in a modified Blazka-type swim tunnel (Beamish, 1978). It consisted of a polyvinylchloride tube (1.5 m long and 0.2 m in diameter) immersed in a 550-l flow-through tank. Two 'honeycomb' grids were placed inside the tube to delimit a 28 l swimming compartment (0.9 m long) and to ensure laminar flow. The upper part of this compartment was closed by a transparent lid with a longitudinal slit allowing access to the catheters. Water flow was powered by an electric trolling motor (Mini Kota), and exact velocity was calibrated as described previously (Bernard et al., 1999). To avoid bright light, the experimental fish typically maintained a swimming position in the upstream region of the chamber that was kept under low light conditions.

Tracer infusion experiments

The rate of appearance of glucose ($R_a$glucose) was measured by continuous infusion of 6-[^{3}H]glucose (for details, see Haman and Weber, 1996; Haman et al., 1997a). The infusate was prepared daily by drying the isotope under N_2 and resuspending it in Cortland saline. While the fish was resting quietly on the floor of the swim tunnel, a priming dose equivalent to 90 min of infusion was injected, and the tracer infusion was started at 1 ml h^{-1} using a calibrated syringe pump (Harvard Apparatus, South Natick, MA, USA). Infusion rates averaged 74 286±8800 disints min^{-1} kg^{-1} min^{-1} (mean ± S.E.M., N=7). The rate of appearance of glucose was quantified before, during and after 3 h of swimming at 1.5 body lengths s^{-1} (BL s^{-1}). Isotope infusion was started 1 h before the onset of exercise and was continued for 1 h post-exercise to monitor recovery. Nineteen blood samples (0.15 ml each) were withdrawn during every infusion.

Blood sample analysis in tracer experiments

Immediately after sampling, haematocrit was measured and the blood was centrifuged. Plasma was stored at −20 °C for a maximum of 2 days before lactate and glucose concentrations were measured using standard spectrophotometric methods (Beckman DU 640 spectrophotometer) and glucose activity was counted (Packard Tri-Carb 2500 scintillation counter). Glucose activity was quantified by drying 30 µl of plasma under N_2, resuspending it in 1 ml of water, and counting in ACS II scintillant (Amersham, Oakville, Ontario, Canada). Rates of appearance (R_a) and disappearance (R_d) of glucose were first calculated separately using the non-steady-state equations of Steele (1959). Because mean R_a and R_d values were not significantly different from each other at any time during the experiments (P>0.05), only mean R_a values obtained using the steady-state equation are reported in the present paper (Steele, 1959).

Tissue glycogen experiments

The effect of exercise on tissue glycogen stores was assessed on 16 fish from the same batch as the animals used for the measurement of $R_a$glucose. These individuals were also doubly cannulated and allowed to recover in the swim tunnel as described above, but no infusion was performed. They were randomly divided between a control and an exercise group, and were killed before or after 3 h of swimming at 1.5 BL s^{-1} by injecting an overdose of sodium pentobarbital (MTC Pharmaceuticals, Cambridge, Ontario, Canada) through one of the catheters. Liver, kidney, white muscle and red muscle samples were rapidly excised and freeze-clamped with aluminium tongs precooled in liquid nitrogen. All tissue samples were frozen within 4 min of death. They were stored at −80 °C until subsamples of 0.5–1.5 g were homogenized and glycogen content was determined using the amyloglucosidase hydrolysis method (Fournier and Weber, 1994). In the exercise group, four blood samples (0.2 ml each) were taken from each animal at 0 (rest), 15, 90 and 180 min of exercise to monitor plasma catecholamine levels during swimming. Immediately after sampling, the blood samples were centrifuged, and the plasma was frozen in liquid N_2 and stored at −80 °C until analysis. Catecholamine levels were determined on alumina-extracted plasma using high-pressure liquid chromatography (HPLC) with electrochemical detection (Woodward, 1982) and 3,4-dihydroxybensalamine hydrobromide as internal standard.

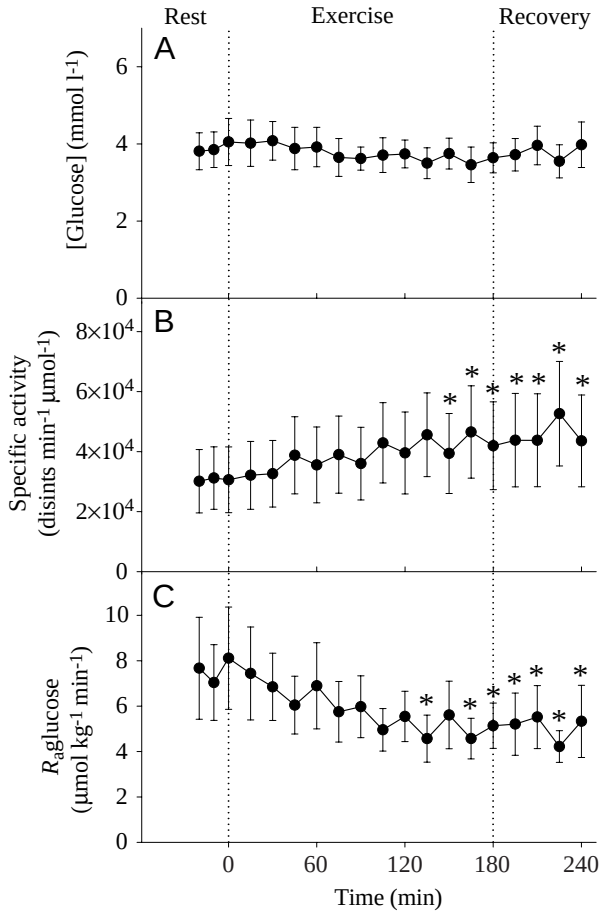

Fig. 1. Plasma glucose concentration (A), glucose specific activity (B) and the rate of hepatic glucose production ($R_a$glucose) (C) in rainbow trout at rest, during exercise at 1.5 BL s^{-1} and during recovery. Values are means ± S.E.M. (N=7). An asterisk denotes a significant difference from the mean resting value (P<0.05). Vertical dotted lines mark transitions between resting and swimming. BL, body length.

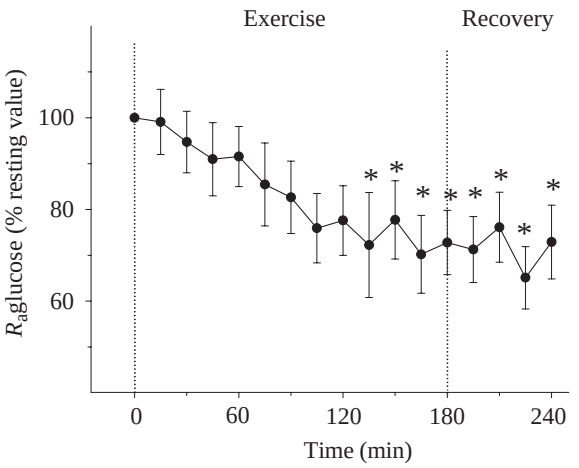

Fig. 2. Effect of 3 h of swimming at 1.5 BL s^{-1} on the rate of hepatic glucose production ($R_a$glucose) of rainbow trout. Values are presented as a percentage of resting rate and are means ± S.E.M. (N=7). An asterisk denotes a significant difference from the mean resting value (P<0.05). Vertical dotted lines mark transitions between resting and swimming. BL, body length.

Statistical analyses

Changes in glucose and lactate concentrations, glucose specific activity, $R_a$glucose, haematocrit and catecholamine levels were assessed by two-way analysis of variance (ANOVA) and Dunnett's multiple-comparison test after $\log_{10}$-transformation when the normality test failed. In Fig. 2, the $R_a$glucose data given as a percentage of resting values were transformed to the arcsine of their square root before analysis using the Kruskal–Wallis analysis of variance on ranks. Tissue glycogen concentrations and total glycogen reserves were assessed using unpaired t-tests. All values presented are means ± S.E.M.

Results

Plasma lactate concentration and haematocrit remained constant throughout the experiments (P>0.1). Lactate concentration averaged 1.11±0.03 mmol l^{-1} (N=19 sampling times) and haematocrit averaged 26.1±0.3 % (N=19). Each of the 19 values was the mean for seven animals.

Effects of exercise on glucose kinetics

Glucose concentration, specific activity and rate of appearance throughout the experiments are plotted in Fig. 1. Plasma glucose concentration averaged 3.78±0.47 mmol l^{-1} (N=19) over time and did not change during or after exercise (P=0.84). Glucose specific activity increased above resting levels at the end of the exercise period and during recovery (P<0.002). The rate of appearance of glucose averaged 7.6±2.1 µmol kg^{-1} min^{-1} (N=7) in the resting state and decreased during the experiments (P<0.05). Exercise caused a progressive decline in $R_a$glucose, which became significantly lower than resting values during the last 30 min of swimming. $R_a$glucose remained below resting levels throughout recovery, when it averaged 5.1±1.3 µmol kg^{-1} min^{-1}. Fig. 2 shows the effects of exercise on hepatic glucose production plotted as a percentage of resting $R_a$glucose. All the mean values after 120 min of swimming were significantly lower than resting rates (P<0.05).

Tissue glycogen reserves

Glycogen concentrations in the liver, kidney, white muscle and red muscle of non-exercised and exercised fish are presented in Table 1. The exercise protocol had no effect on the glycogen concentration of the kidney (P=0.45) and white muscle (P=0.16), but caused a significant decrease in the liver (P<0.05) and in red muscle (P<0.01). Mass-specific glycogen reserves of all measured tissues were calculated by multiplying concentration by tissue mass and dividing by total body mass (Table 1). Exercise caused significant glycogen depletion only in the red muscle (P<0.01).

Table 1. *Mean mass, glycogen concentration and glycogen reserves of the whole fish and its tissues in the non-exercised and the exercised groups*

	Mass (g)	
	Non-exercised	Exercised
Whole body	936±66	890±66
Liver	11.5±1.0	10.7±1.6
Kidney	5.6±0.4	5.2±0.5

	Glycogen concentration (μmol glucosyl units g^{-1} wet mass tissue)		Glycogen reserves (μmol glucosyl units g^{-1} fish)	
	Non-exercised	Exercised	Non-exercised	Exercised
Liver	118.33±4.58	107.41±1.84*	1.49±0.18	1.27±0.12
Kidney	40.45±1.68	38.22±1.29	0.25±0.03	0.22±0.02
White muscle	59.36±2.01	54.11±3.08	29.67±0.99	27.03±1.53
Red muscle	137.29±9.38	58.73±2.43*	6.86±0.47	2.93±0.12*

Red muscle and white muscle mass were assumed to be 5 % and 60 % of body mass, respectively (Moyes and West, 1995).
The sample size for each tissue is N=8 except for red muscle pre-exercise (N=4) and the exercised group (N=6).
* denotes a statistically significant difference between exercised and non-exercised groups (P<0.05).

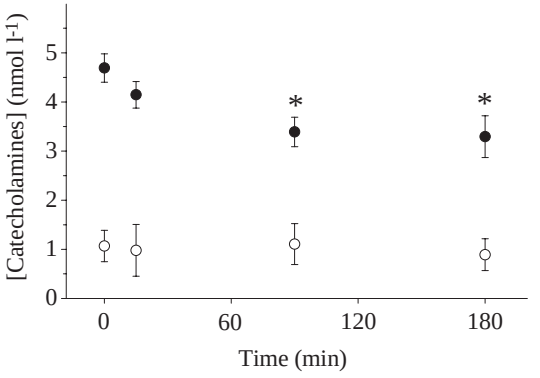

Fig. 3. Plasma concentrations of epinephrine (filled circles) and norepinephrine (open circles) in rainbow trout before (time 0) and during swimming at 1.5 BL s^{-1}. Values are means ± S.E.M. (N=8). An asterisk denotes a value significantly different from the resting value (P<0.05). BL, body length.

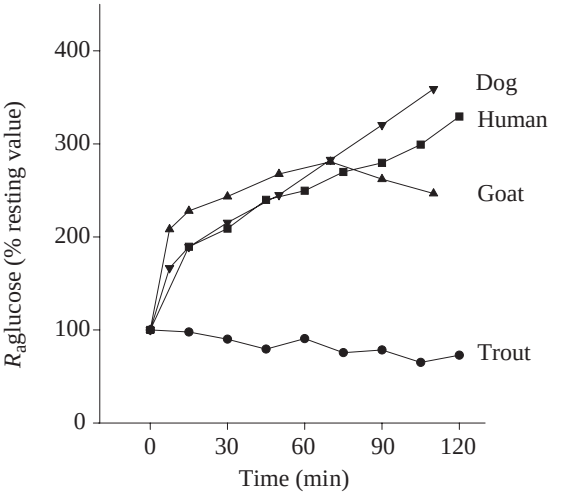

Fig. 4. Relative effects of sustained exercise (40–50 % $\dot{M}_{O_2max}$) on the rate of hepatic glucose production ($R_a$glucose) in rainbow trout (this study) and in mammals calculated from published values for dogs (Weber et al., 1996), goats (Weber et al., 1996) and humans (Coggan et al., 1995).

Plasma catecholamine levels

Plasma norepinephrine and epinephrine concentrations before and during exercise are plotted in Fig. 3. Swimming caused no change in norepinephrine levels (P=0.92), which averaged 1.0±0.4 nmol l^{-1} over time. However, there was a significant decline in epinephrine concentration at 90 and 180 min of exercise (P<0.001). Epinephrine concentration was 4.7±0.3 nmol l^{-1} at rest and decreased to 3.3±0.4 nmol l^{-1} after 3 h of exercise.

Discussion

This study is the first to provide continuous *in vivo* measurements of glucose kinetics during the transition from rest to exercise in an ectotherm. Contrary to expectation, we found that sustained swimming does not stimulate hepatic glucose release in rainbow trout, but that it causes a significant decrease. Fig. 4 summarizes the effects of submaximal exercise on the $R_a$glucose of several vertebrate species and shows that the responses of mammals and rainbow trout to the same work intensity are in opposite directions. From previous investigations of exercising salmonids of identical size, we can estimate that 1.5 BL s^{-1} corresponds to 50 % $\dot{M}_{O_2max}$ or 70 % of critical swimming speed (U_{crit}) (Beamish, 1978; Kiceniuk and Jones, 1977; Webb, 1971). Submaximal exercise causes a major increase in the $R_a$glucose of dogs (Weber et al., 1996), goats (Weber et al., 1996) and humans (Coggan et al., 1995), whereas a 33 % decrease was observed in the present study

after 3 h of swimming (Figs 1, 2). Furthermore, the effects of exercise were longer-lasting in rainbow trout than previously observed in mammals. The low $R_a$glucose recorded at the end of swimming persisted throughout the 60 min of recovery (Fig. 1C), whereas a 30 min post-exercise resting period is sufficient for $R_a$glucose to return to basal levels in mammals (Weber et al., 1990, 1996).

Possible reasons for the decrease in R_a*glucose*

It could be argued that glucose production was stimulated at the beginning of our experiments because the animals were stressed and not really at rest before swimming. However, this scenario is unrealistic because the mean $R_a$glucose measured in the swim tunnel before exercise ($7.6\pm2.1\ \mu mol\ kg^{-1}\ min^{-1}$) was similar to resting values from previous experiments in which trout were kept quietly in opaque acrylic boxes for more than 36 h ($9.0\pm0.7\ \mu mol\ kg^{-1}\ min^{-1}$, Haman and Weber, 1996; $8.6\pm1.3\ \mu mol\ kg^{-1}\ min^{-1}$, Haman et al., 1997b) and in which they had true resting metabolic rates ($41\ \mu mol\ O_2\ kg^{-1}\ min^{-1}$, Haman et al., 1997b). Instead, changes in liver perfusion or in the concentration of circulating hormones may be responsible for the observed decrease in $R_a$glucose. Sustained swimming is known to cause a decline in liver blood flow by constricting the coeliacomesenteric artery (Randall and Daxboeck, 1982), and this cardiovascular response could lead to a reduction in hepatic glucose release into the circulation. A decrease in circulating catecholamine levels could also contribute to a reduction in $R_a$glucose during exercise. Swimming had no effect on plasma norepinephrine levels, but epinephrine levels declined by 30 % (Fig. 3). These findings are compatible with results from another study in which the plasma catecholamine levels of catheterized rainbow trout also decreased during prolonged exercise (Butler et al., 1986). Such hormonal changes are known to reduce rates of glycogenolysis and gluconeogenesis in isolated hepatocytes (Wright et al., 1989), and they may have the same effects *in vivo*.

Glucoregulation

Frequent blood sampling allowed circulating glucose concentration to be closely monitored. Rainbow trout were able to maintain a steady glucose concentration during and after sustained exercise (Fig. 1A) even though glucose fluxes decreased by more than 30 % (Fig. 1C). This homeostatic response was achieved because the progressive decrease in hepatic glucose production (R_a) was accompanied by a parallel and synchronized decline in glucose uptake by peripheral tissues (R_d). Such tight matching between the rates of appearance and disappearance of glucose is rather surprising in an animal that is often described as a poor glucoregulator (Driedzic and Hochachka, 1978; Van den Thillart, 1986). The effects of sustained swimming on the blood glucose levels of rainbow trout have only been measured by a few others (Nielsen et al., 1994; Zelnik and Goldspink, 1981). In these experiments, temporary hyperglycaemia was observed at the beginning of exercise before blood glucose levels returned to baseline values within a few minutes. The high plasma cortisol levels reported in these studies suggest that the transient increase in blood glucose concentration may have been caused by stress rather than exercise *per se* (Mazeaud and Mazeaud, 1981). In the present study, cortisol levels were not measured, but we show that rainbow trout have the capacity to control blood glucose concentration during submaximal exercise when $R_a$glucose changes significantly over time.

Circulating glucose as an oxidative fuel to support swimming

Because the rates of production (R_a) and disappearance (R_d) of glucose were never statistically different, the graphs showing the decline in R_a (Figs 1C, 2) also depict how R_d was affected by exercise. The observed decreases in R_a and R_d show that circulating glucose is probably not an important fuel for the locomotory muscles of rainbow trout during sustained exercise. Reducing the rates of hepatic glucose production and of glucose disappearance from the blood compartment would not make sense if the rate of glucose oxidation by contracting muscles were increased during swimming. Furthermore, no example in which the rates of disappearance and oxidation change in opposite directions are presently known, and R_d glucose can often be used as an approximation of oxidation rate during exercise (Coggan et al., 1990; Paul and Issekutz, 1967).

Sources of circulating glucose

All the glucose released into the circulation can be accounted for by two processes: gluconeogenesis and glycogenolysis. The relative contribution of gluconeogenesis can therefore be estimated by subtracting glycogenolysis from total glucose production ($R_a$glucose). In this context, we have tried to measure glycogenolysis by estimating glycogen depletion in the liver and kidney during exercise. Unfortunately, no significant change could be demonstrated because of the high variability among individuals (Table 1). Given the observed means and variances in pre-exercise glycogen reserves of liver and kidney, we can determine that the minimal detectable difference ($P<0.05$) in combined hepatic and renal glycogen reserves is $609\ \mu mol\ glucosyl\ units\ kg^{-1}$ fish (Zar, 1996). Any actual depletion below this value would have remained undetected in our experiments. Therefore, this amount of $609\ \mu mol\ glucosyl\ units\ kg^{-1}$ represents the maximal possible contribution of glycogenolysis to total glucose production during the 3 h of exercise. By measuring the surface area under the $R_a$glucose curve during exercise (Fig. 1C), we can determine that total glucose production for 3 h of swimming was $1062\ \mu mol\ glucosyl\ units\ kg^{-1}$ fish. Therefore, the maximum possible contribution of glycogenolysis to $R_a$glucose was 57 % (609/1062) and, by deduction, at least 43 % of $R_a$glucose was accounted for by gluconeogenesis.

In conclusion, this study shows that prolonged submaximal exercise causes a decline in the $R_a$glucose of rainbow trout of more than 30 % and that circulating epinephrine may be partly responsible for controlling this response. Furthermore, trout

are able to maintain a steady blood glucose concentration by matching rates of glucose production (R_a) and peripheral glucose utilization (R_d) throughout exercise. The observed reduction in the $R_a$glucose of swimming trout is strikingly different from the two- to fourfold increase that takes place in mammals exercising at the same intensity. These results show that circulating glucose is probably not an important oxidative fuel for sustained locomotion in fish.

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