

IN VIVO LACTATE KINETICS AT REST AND DURING RECOVERY FROM EXHAUSTIVE EXERCISE IN COHO SALMON (*ONCORHYNCHUS KISUTCH*) AND STARRY FLOUNDER (*PLATICHTHYS STELLATUS*)

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SUMMARY

A bolus injection of [¹⁴C]lactate was used to measure lactate turnover rates at rest and during recovery from exhaustive exercise in coho salmon (*Oncorhynchus kisutch*) and starry flounder (*Platichthys stellatus*). At rest, lactate turnover rate in salmon was almost double that in flounder (1.33 versus 0.76 $\mu\text{mol min}^{-1} \text{kg}^{-1}$), which reflected the higher blood lactate level in salmon (1.00 versus 0.12 mmol l^{-1}). From 2 to 4 h after exercise, when blood lactate levels were at their peak and constant, turnover rates were elevated in both species, though to a greater extent in salmon than in flounder (11.88 versus 2.27 $\mu\text{mol min}^{-1} \text{kg}^{-1}$). Lactate concentration and turnover rate were linearly correlated in both species. The higher turnover rate in salmon was solely a consequence of the higher blood lactate levels since, at similar blood lactate concentrations, turnover rates in flounder and salmon were the same. Therefore, the lower blood lactate levels in flounder after exercise were not a consequence of higher turnover. In neither species was the turnover rate adequate to account for the rate of lactate clearance from the muscle, suggesting a large portion was retained within the muscle and metabolized *in situ*. Furthermore, following injection of [¹⁴C]lactate, >80% of the total blood activity was recovered as lactate, indicating that little label was incorporated into other products (e.g. glucose). These data suggest that the Cori cycle plays a minimal role in the metabolism of lactate in salmon and flounder. Furthermore, at least in flounder, there was no correlation between the kinetics of lactate clearance and O₂ consumption, suggesting that the classical concept of 'O₂ debt' is not applicable in this species.

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INTRODUCTION

The accumulation of blood lactate in fish during recovery from exhaustive exercise is strikingly different from that generally observed in mammals. Following exhaustive exercise in man, blood [lactate] peaks at 15–20 mmol l⁻¹ within minutes after cessation of activity and returns to resting levels in less than 60 min (Bergstrom, Guarnieri & Hultman, 1971; Sahlin, Alvestrand, Brandt & Hultman, 1978). In fish, blood lactate concentration typically peaks 2–4 h after exercise and up to 12–24 h is required for complete recovery (see Wood & Perry, 1985, for a review). The peak blood lactate level attained in fish depends on the species and appears to be correlated with swimming performance. In active, pelagic fish, such as trout (*Salmo gairdneri*; Black, Chiu, Forbes & Hanslip, 1959; Turner, Wood & Clark, 1983a; Holeton, Neumann & Heisler, 1983; Milligan & Wood, 1986), tuna (*Katsuwonus pelamis*; Perry *et al.* 1985) and dogfish (*Scyliorhinus stellaris*; Piiper, Meyer & Drees, 1972; Holeton & Heisler, 1983), peak blood lactate levels are similar to those observed in man, 15–20 mmol l⁻¹. However, in benthic species, such as plaice (*Pleuronectes platessa*; Wardle, 1978), starry flounder (*Platichthys stellatus*; Wood, McMahon & McDonald, 1977; Milligan & Wood, 1987), flathead sole (*Hippoglossoides elassodon*; Turner, Wood & Høbe, 1983b), sea raven (*Hemitripterus americanus*; Milligan & Farrell, 1986) and the big skate (*Raja ocellata*; C. M. Wood & M. S. Graham, unpublished results, cited in Wood & Perry, 1985), blood lactate rarely attains levels greater than 2 mmol l⁻¹, despite markedly elevated muscle levels (Turner *et al.* 1983b; Milligan & Wood, 1987).

Wardle (1978) has suggested that these low blood lactate levels are due to a lactate non-release phenomenon at the level of the muscle cell. An alternative explanation for these low blood lactate levels is that lactate is turned over much more quickly in the benthic fish than in the active fish. Studies of lactate turnover in mammals have repeatedly shown that measurement of blood lactate concentration alone does not accurately reflect changes in lactate kinetics (Eldridge, 1975; Donovan & Brooks, 1983; Brooks, 1985; Stanley *et al.* 1985; Mazzeo, Brooks, Schoeller & Budinger, 1986). For a given blood lactate concentration, turnover can be 2–4 times greater during exercise than during rest in man (Mazzeo *et al.* 1986).

Thus, the purpose of the present study was to measure lactate turnover rates in an active species (coho salmon, *Oncorhynchus kisutch*) and a benthic species (starry flounder, *Platichthys stellatus*) to test the hypothesis that increased turnover rates are responsible for the lower blood lactate levels observed in benthic fish after exhaustive exercise. Turnover rates were measured using [¹⁴C]lactate by the bolus injection method (Hetenyi, Perez & Vranic, 1983). To satisfy the criteria of dynamic steady state required by this method, lactate turnover after exercise was measured from 2 to 4 h into recovery, when lactate levels had peaked and were constant (see Milligan & Wood, 1986, 1987). Also, measurements of oxygen consumption and carbon dioxide excretion were made both prior to and following exercise as a means of comparing metabolic performance of the two species.

MATERIALS AND METHODS

*Experimental animals**Coho salmon*

Adult coho salmon (*Oncorhynchus kisutch*) (150–400 g) were obtained from Swecker Salmon Farm, Inc., Rochester, WA in September and November 1986. Fish were transported in fresh water to Friday Harbor Laboratories where, over a 1-week period, they were acclimated to 29‰ sea water at 9–11°C. Following acclimation, fish were held either indoors in a large circular tank where they had been seawater-acclimated or outdoors in large aquaria continually supplied with aerated ($P_{O_2} = 145$ mmHg; 1 mmHg = 133.32 Pa) fresh running sea water at 9–11°C. Fish were offered commercial fish pellets weekly, but they did not feed.

For lactate turnover studies, salmon were anaesthetized in a 1:10 000 solution of MS 222 (Sigma) and the dorsal aorta was chronically cannulated using the method of Soivio, Westman & Nyholm (1972). The catheters were filled with heparinized (50 i.u. ml⁻¹) Cortland saline (Wolf, 1963) adjusted to 160 mmol l⁻¹ NaCl. Fish were allowed to recover for at least 48 h before experiments in 5-l darkened Lucite fish boxes continually supplied with well-aerated sea water at 9–11°C.

Starry flounder

Adult starry flounder (*Platichthys stellatus*) (170–500 g) of both sexes were collected by otter trawl from Bellingham Bay, WA in September 1986. Fish were held either indoors in rectangular Plexiglas boxes or outdoors in large aquaria with sand-covered bottoms supplied with aerated ($P_{O_2} = 145$ mmHg) fresh running sea water at 9–11°C. During holding, fish fed on other small fish and invertebrates present in the tanks.

Caudal artery catheters were surgically implanted using the method described by Watters & Smith (1973) while fish were anaesthetized in a 1:10 000 solution of MS 222. To prevent infection, the wound was dusted with the antibiotic oxytetracycline hydrochloride (Syndel Labs, Vancouver) prior to closure with silk sutures. Catheters were filled with heparinized Cortland saline adjusted to 160 mmol l⁻¹ NaCl. Fish were then placed in 8-l darkened plastic tubs supplied with fresh flowing sea water at 9–11°C and allowed to recover for at least 48 h before experiments.

*Experimental protocol**Lactate turnover studies*

For each species, two experimental groups were employed; one was exercised (salmon, $N = 5$; flounder, $N = 6$) and the other left at rest (salmon, $N = 6$; flounder, $N = 6$).

Salmon were exercised by vigorously chasing them around a large circular tank (250 l) for 5 min, while flounder were chased for 10 min in a shallow rectangular tank (180 l). Previous studies have shown that this form of exercise leads to exhaustion and elevates blood lactate levels to 15–20 and 1–2 mmol l⁻¹ in trout and flounder,

respectively (Milligan & Wood, 1986, 1987). At the end of exercise, fish were returned to their boxes.

Fish were given a bolus injection of $1 \mu\text{Ci } 100 \text{ g}^{-1}$ of [^{14}C]lactate (New England Nuclear; specific activity = $178.8 \text{ mCi mmol}^{-1}$; universally labelled) *via* the dorsal aorta or caudal artery catheter at time 0. The bolus was washed in with an equal volume of heparinized saline. In the exercised group, the bolus injection was given 2 h after exercise and turnover followed until 4 h. Previous studies have shown that during this period blood lactate levels are at their peak and relatively constant (Milligan & Wood, 1986, 1987). Blood samples ($300 \mu\text{l}$) were taken prior to infusion, at 2 min after infusion, then at 5, 8, 10, 15, 30, 60, 90 and 120 min. In preliminary experiments, blood was sampled at 2-min intervals over the first 30 min post-infusion, and it was found that the above sampling protocol was adequate to define the early and rapidly declining portion of the lactate specific activity curve. The volume of blood sampled was replaced with saline. Samples were analysed for blood [lactate], lactate specific activity and plasma ^{14}C radioactivity.

O₂ consumption, CO₂ excretion studies

This series of experiments used non-cannulated fish. At least 24 h before experiments, fish were placed in either 5-l (salmon) or 8-l (flounder) air-tight boxes supplied with fresh flowing sea water ($9\text{--}11^\circ\text{C}$). One hour before exercise, the inflow to the box was closed, the volume set to 5 l for salmon and 8 l for flounder, and the water recirculated within the box by means of a Masterflex pump (Cole-Palmer) at a rate of 1.01 min^{-1} . At the end of the 1 h period, the box was flushed with fresh sea water. For 2 h following exercise, the flow was closed for 30 min intervals, interspaced by 10 min of flushing with fresh sea water. At the end of 2 h, the flow was restored, and then closed again for 30 min at 4 h after exercise. Water samples (5 ml) were taken at the beginning and end of each period and P_{O_2} and $[\text{CO}_2]$ were measured. Thus, O_2 consumption (\dot{M}_{O_2}) and CO_2 excretion (\dot{M}_{CO_2}) were measured for four 30-min periods 2 h after exercise, then again for one 30-min period 4 h after exercise. During the experiment, temperature was maintained by bathing the boxes in flowing sea water.

Analytical techniques and calculations

Whole blood lactate was measured in $200 \mu\text{l}$ of blood deproteinized in $400 \mu\text{l}$ of ice-cold 8% perchloric acid. Samples were mixed and allowed to sit on ice for 5 min, then centrifuged at 9000 g for 3 min. The supernatant ($100 \mu\text{l}$) was analysed for lactate enzymatically (L-lactate dehydrogenase/NADH) using Sigma reagents and the remainder ($50 \mu\text{l}$) was neutralized with 1.5 mol l^{-1} Trizma base (Sigma) and used to determine lactate specific activity. Lactate was separated by ion exchange chromatography. The neutralized extract was passed, in series, through two small columns (i.d. = 5 mm) containing ion exchange resins. The top column contained 0.5 g of Dowex 50 (H^+ form; 200–400 mesh, Sigma) and the lower 0.5 g of Dowex 1 (Cl^- form; 100–200 mesh, Sigma). The top column removed amino acids and the bottom column removed lactate (Katz, Okajima, Chenoweth & Dunn, 1981). The

columns were separated and lactate was eluted from the bottom column with 5 ml of 2 mol l^{-1} acetic acid. This method was found to give better than 95 % recovery of labelled lactate. The eluate containing lactate (5 ml) was mixed with scintillation fluor (10 ml; ACS, Amersham) and counted on a Beckman LS9000 liquid scintillation counter. ^{14}C radioactivity in plasma was measured on $50 \mu\text{l}$ samples mixed with 5 ml of water and 10 ml of ACS fluor and counted as described.

Under the conditions of dynamic steady state, the rate of lactate appearance equals the rate of disappearance and is called turnover rate (Rt). Measurement of the turnover rate by bolus injection of labelled tracer requires that the metabolite be in dynamic steady state. Under these conditions, the turnover rate ($\mu\text{mol min}^{-1}$) can be estimated by dividing the injected dose (in counts min^{-1}) by the area under the specific activity decay curve [in $\text{min} (\text{counts min}^{-1}) \mu\text{mol}^{-1}$] (Katz *et al.* 1981). To calculate this area, the decay curve was first fitted with a multi-exponential function by use of the non-linear regression program, NONLIN (Metzler, Elfung & McEwan, 1974). The fitted curve was then integrated between time 0 and the time when the specific activity was at 5 % of the time 0 value; the latter estimated by back-extrapolating the curve to time 0. Metabolic clearance rate (MCR; ml min^{-1}) was calculated as the turnover rate divided by the steady-state lactate concentration. Both MCR and Rt are expressed in terms of kilograms body mass.

Water P_{O_2} was measured with a Radiometer P_{O_2} electrode (type E5036) maintained at the experimental temperature and connected to a Radiometer PHM 72 acid-base analyser. Total $[\text{CO}_2]$ in water (in mmol l^{-1}) was measured by gas chromatography (Shimadzu Gas Chromatograph, model 8A) using a Porapak Q column to separate CO_2 . 1 ml of water was added to a 5-ml gas-tight Hamilton syringe containing 0.5 ml of 0.1 mol l^{-1} HCl. The syringe was then filled to the 5 ml mark with CO_2 -free helium, placed on a shaker at room temperature and allowed to equilibrate for at least 30 min prior to analysis. Peak height was taken as a measure of total $[\text{CO}_2]$. Standards (2 and 4 mmol l^{-1} NaHCO_3 , treated the same as the seawater samples) were run at frequent intervals throughout the day. All samples and standards were run in duplicate.

Oxygen consumption (\dot{M}_{O_2}) was calculated according to the equation:

$$\dot{M}_{\text{O}_2} = \frac{(\text{P}_{\text{iO}_2} - \text{P}_{\text{fO}_2}) \times \alpha_{\text{O}_2} \times \text{box volume}}{\text{mass} \times \text{time}},$$

where i and f refer to initial and final measurements, respectively, P_{O_2} is in mmHg, box volume in l, mass in kg, time in h, and α_{O_2} (in $\text{mmol l}^{-1} \text{ mmHg}^{-1}$) is the solubility constant for oxygen in sea water at 9–11°C (Dejours, 1981). Similarly, CO_2 excretion (\dot{M}_{CO_2}) was calculated as:

$$\dot{M}_{\text{CO}_2} = \frac{([\text{CO}_2]_{\text{f}} - [\text{CO}_2]_{\text{i}}) \times \text{box volume}}{\text{mass} \times \text{time}},$$

where $[\text{CO}_2]$ is in mmol l^{-1} .

Significant differences between groups were assessed with Student's *t*-test, unpaired design, and to test differences within groups the paired design was used. All

differences, unless noted otherwise, are at the 5% level of significance. Lines were fitted using the least-squares method of linear regression and the significance of the correlation coefficient was assessed by regression analysis.

RESULTS

Blood lactate levels were constant in both flounder and salmon at rest and between 2 and 4 h after exercise (Fig. 1B,D,F,H), justifying the use of a dynamic steady-state model to estimate lactate turnover rates (Katz *et al.* 1981).

A three-compartment model was found to give best fits to the lactate specific activity decay curves. The decay curves of lactate specific activity were similar in both salmon and flounder at rest and at 2–4 h after exercise (Fig. 1A,C,E,G), with specific activity declining rapidly over the first 15 min after injection, and then more gradually.

Between 2 and 4 h after exercise, when blood lactate levels had peaked, lactate turnover rates in both salmon and flounder had increased significantly relative to resting values (Table 1). There was a significant positive correlation between blood

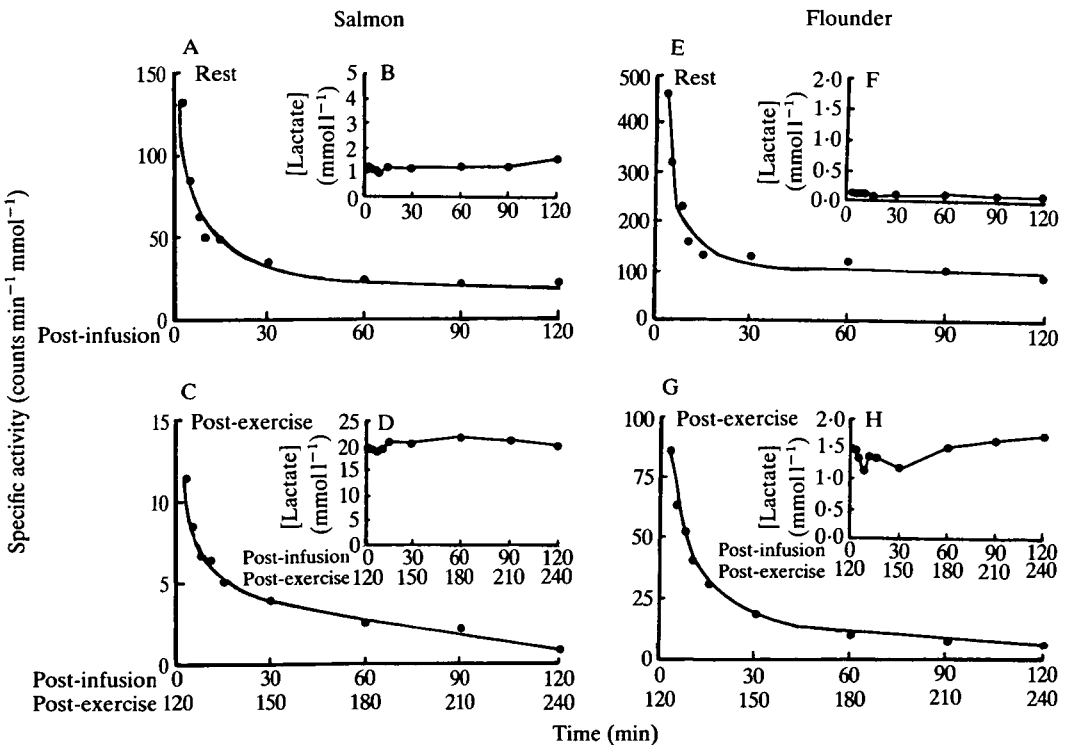


Fig. 1. Typical decay curves of lactate specific activity for coho salmon (A,C) and starry flounder (E,G) at rest and between 2 and 4 h after exercise. Curves were fitted with the sum of three exponential functions. Insets (B,D,F,H) show blood lactate concentration during the sampling period. Data are from fish representative of each group.

Table 1. Lactate turnover and metabolic clearance rates in coho salmon and starry flounder at rest and during recovery from exhaustive exercise

	[Lactate] (mmol l ⁻¹)	Turnover rate ($\mu\text{mol min}^{-1} \text{kg}^{-1}$)	Metabolic clearance rate (ml min ⁻¹ kg ⁻¹)
Rest			
Salmon (<i>N</i> = 6)	1.00 \pm 0.11	1.33 \pm 0.14	1.39 \pm 0.15
Flounder (<i>N</i> = 6)	0.12 \pm 0.02	0.76 \pm 0.14	7.48 \pm 1.30
After exercise			
Salmon (<i>N</i> = 5)	16.77 \pm 2.17	11.88 \pm 0.97	0.80 \pm 0.40
Flounder (<i>N</i> = 6)	1.33 \pm 0.10	2.27 \pm 0.83	2.14 \pm 0.64

[lactate] and turnover rates in both salmon ($R_t = 1.55 + 0.56[\text{lactate}]$, $r = 0.87$, $N = 11$; $P < 0.005$) and flounder ($R_t = 0.66 + 1.19[\text{lactate}]$, $r = 0.69$, $N = 12$; $P < 0.025$).

During the 2 h after infusion of label, in both salmon and flounder, 80–90% of the ¹⁴C counts in the plasma were recovered as [¹⁴C]lactate, indicating that there was little interconversion of labelled lactate into other blood-borne products (e.g. glucose, alanine). This was observed in fish at rest as well as at 2–4 h after exercise. The metabolic clearance rate (MCR) of lactate, the amount of blood cleared of lactate per minute, decreased significantly in both salmon and flounder after exercise, though to a greater extent in the latter (Table 1).

Resting \dot{M}_{O_2} and \dot{M}_{CO_2} values in salmon were about double those in flounder (Fig. 2A,C). In neither species was the mean respiratory exchange ratio, RER, significantly different from 1.0 prior to exercise (Fig. 2B,D). Exercise to exhaustion resulted in approximately two- and four-fold increases in \dot{M}_{O_2} in flounder and salmon, respectively. Similar increases in CO_2 excretion were seen in both species. Consequently, in neither species did RER change significantly after exercise, except for a slight increase observed 0–30 min post-exercise in flounder (Fig. 2D). \dot{M}_{O_2} remained elevated for 4 h after exercise in salmon, but had returned to rest levels by this time in flounder. In both species, \dot{M}_{CO_2} had decreased to near rest levels by 4 h into recovery, though in flounder \dot{M}_{CO_2} was lower than at rest, resulting in a reduction in RER (Fig. 2D).

DISCUSSION

Lactate turnover rates at rest reported here for salmon and flounder are similar to those reported for the American eel (*Anguilla rostrata*) at 12–15°C (0.30–1.39 $\mu\text{mol min}^{-1} \text{kg}^{-1}$; Cornish & Moon, 1985) and rainbow trout (*Salmo gairdneri*) at 9–10°C (2–3 $\mu\text{mol min}^{-1} \text{kg}^{-1}$; Dunn & Hochachka, 1987), but are two orders of magnitude lower than those reported for skipjack tuna (*Katsuwonus pelamis*) at 25°C (112–431 $\mu\text{mol min}^{-1} \text{kg}^{-1}$; Weber, Brill & Hochachka, 1986). It should be noted that resting blood lactate concentrations in the tuna study ranged from 10 to

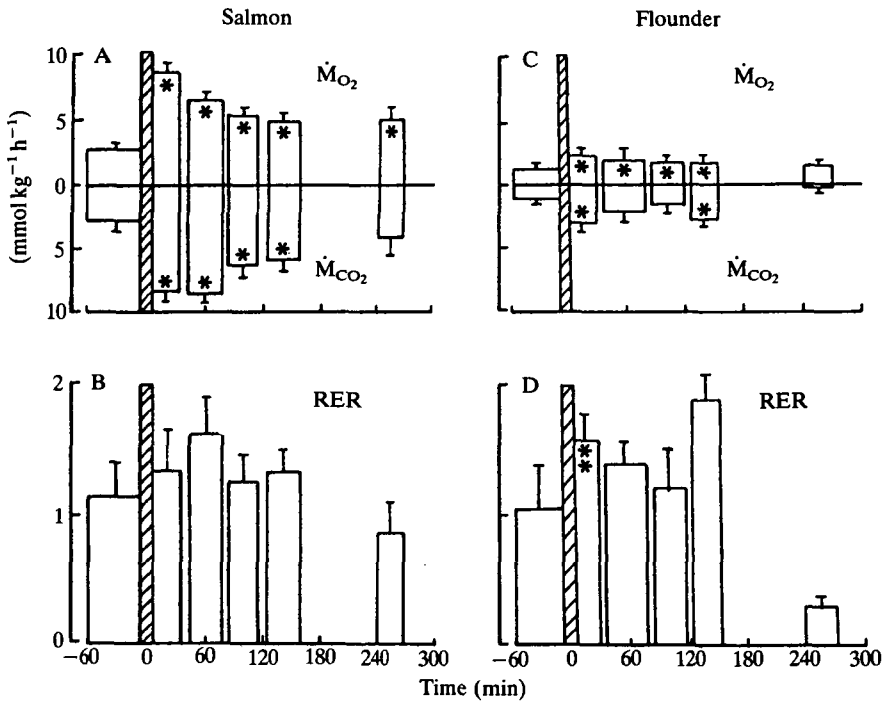


Fig. 2. Oxygen consumption (\dot{M}_{O_2}), CO₂ excretion (\dot{M}_{CO_2}) and respiratory exchange ratio (RER) at rest and following exhaustive exercise in coho salmon (A,B; $N = 8$) and starry flounder (C,D; $N = 7$). Means \pm 1 S.E.M. * indicates a significant difference from the corresponding pre-exercise value ($P < 0.05$); ** indicates a significant difference from 1.00 ($P < 0.05$); hatched bars indicate periods of exercise; time 0 indicates immediately after exercise; -60 indicates 60 min prior to exercise.

28 mmol l⁻¹ and this, in conjunction with the higher temperature, may be a contributing factor to the higher turnover rates observed in tuna. In comparison, mammalian lactate turnover rates at rest (15–200 $\mu\text{mol min}^{-1} \text{kg}^{-1}$; Donovan & Brooks, 1983; Stanley *et al.* 1985; Mazzeo *et al.* 1986) are 10–100 times greater than those observed in the present study.

In dynamic steady state, by definition, the rate of removal of lactate from the blood must be equal to the rate of lactate appearance. In the present study, this condition was met between 2 and 4 h after exercise when the coefficient of variation of blood lactate concentration was less than 10% for both species. During this period, lactate turnover rates increased about 10-fold in salmon and about 3.5-fold in flounder relative to resting values (Table 1). The higher lactate turnover rate in salmon was due almost solely to the higher blood lactate levels in these fish, for at the same blood lactate concentration (e.g. flounder after exercise *versus* salmon at rest), lactate turnover rates did not differ between the two species. Therefore, the lower blood lactate levels observed after exercise in flounder (and other benthic fish, see Wood & Perry, 1985) are not due to a higher turnover of lactate, but rather appear to be due to

Table 2. Predicted and observed times for lactate clearance following exhaustive exercise in coho salmon and starry flounder

	Turnover rate ($\mu\text{mol min}^{-1} \text{kg}^{-1}$)	[Lactate] (mmol kg^{-1})	Predicted lactate clearance time (h)	Observed lactate- clearance time (h)
Flounder	2.3	4.0*	28	12*
Salmon	12.0	15.5†	21	8–12†

* Milligan & Wood (1987).
† From rainbow trout, Milligan & Wood (1986).

a 'non-release' of lactate from the muscle mass (Wardle, 1978; Turner *et al.* 1983b; Milligan & Wood, 1987). The underlying mechanism is not known.

If the turnover rates from the present study are considered together with estimated lactate loads for exhaustive exercise for both species (from previous studies) then it is possible to predict the total time required for lactate removal and compare it with the actual clearance time (Table 2). For salmon we have assumed that the load and clearance time are similar to those of the rainbow trout studied previously (Milligan & Wood, 1986). The calculation predicts that at peak turnover rates salmon would require 21 h and flounder 28 h to clear the post-exercise lactate load. However, based on previous observations, lactate clearance is complete in about half the predicted time in flounder and one-third the predicted time in salmon (Table 2). These calculations indicate that a large portion of the lactate produced as the result of exhaustive exercise is not released from muscle to blood during recovery but rather is metabolized *in situ*. This, in conjunction with the observation that only 10–20 % of the ^{14}C label in lactate was incorporated into other blood-borne compounds, suggests that the Cori cycle (shuttle of lactate from the muscle to the liver *via* the blood, where it is converted to glucose and then transported back to the muscle) plays a minimal role in the disposal of lactate in salmon and flounder. Similarly, in tuna, only 5 % of the ^{14}C label infused as lactate appeared as [^{14}C]glucose (Weber *et al.* 1986); in eels, only 1 % of the label appeared as [^{14}C]glucose (Cornish & Moon, 1985).

In both salmon and flounder, there was a linear relationship between lactate turnover rate and mean blood lactate concentration. A similar relationship has been reported for skipjack tuna (Weber *et al.* 1986) and rainbow trout (Dunn & Hochachka, 1987). However, in mammals (dogs: Eldridge, T'So & Chang, 1974; rats: Donovan & Brooks, 1983; humans: Mazzeo *et al.* 1986) the relationship between lactate turnover and blood lactate concentration is not linear, but rather curvilinear in a manner suggesting that lactate removal is a saturable process (Mazzeo *et al.* 1986), presumably at the level of tissue uptake. It is not clear, however, if the absence of curvilinearity in both the present study and that of Weber *et al.* (1986) on tuna indicates that lactate removal from the blood of fish is a non-saturable process. The only data available on lactate uptake mechanisms by fish tissues is on toadfish (*Opsanus beta*) hepatocytes, in which lactate uptake is by passive diffusion, i.e. a

non-saturable process (Walsh, 1987). Whether the same is true in other fish tissues, or in hepatocytes from other species, has yet to be examined.

The metabolic clearance rate (MCR) of lactate, the amount of blood cleared of lactate per minute, was lower after exercise than at rest in both salmon and flounder (Table 1). This decline in MCR indicates that blood flow is probably redistributed away from lactate-utilizing tissues (e.g. red muscle, liver, kidney) towards lactate-producing tissues (e.g. white muscle). Post-exercise blood flow data are not available for either flounder or salmon, although in rainbow trout blood flow to the white muscle mass was increased after exhaustive exercise (Neumann, Høleton & Heisler, 1983). During heavy exercise in humans (Mazzeo *et al.* 1986; Stanley *et al.* 1985) and rats (Donovan & Brooks, 1983), lactate MCR also declined, and this was correlated with a reduction in the proportion of cardiac output serving lactate-utilizing tissues (Mazzeo *et al.* 1986).

Estimates of resting O_2 consumption in the present study are typical of those previously reported for salmonids (Brett, 1972) and flatfish (Duthie, 1982). CO_2 excretion has not been measured previously in these species; nonetheless, the estimates are also probably reasonable since they were similar in magnitude to the oxygen consumption measurements. This being the case, the respiratory exchange ratio (RER) of 1 indicates that carbohydrates were the main metabolic fuel for both species both prior to and following exercise (Newsholme & Leech, 1983). In both species, \dot{M}_{O_2} and \dot{M}_{CO_2} were elevated after exercise, though more so in salmon than in flounder (Fig. 2). Classically, the persistent elevation of O_2 consumption after the cessation of exercise has been termed 'O₂ debt' and attributed to metabolism of lactate produced during exercise. In recent years, however, many data have accumulated from a wide range of species (several species of amphibians, reptiles and mammals; see Gaesser & Brooks, 1984, for a review) which indicate there is little correlation between the kinetics of lactate removal and elevated oxygen consumption. This suggests that the elevated post-exercise oxygen consumption (EPOC; Gaesser & Brooks, 1984) is due to factors other than lactate metabolism. The results of the present study, at least from flounder, are in agreement with these observations, since oxygen consumption had returned to resting rates while blood lactate levels and turnover rate were still elevated (Table 1; Fig. 2C). In salmon, the relationship is less clear cut, but \dot{M}_{O_2} declined despite almost constant blood lactate levels, suggesting a similar phenomenon.

In both salmon and flounder, the relative increases in \dot{M}_{O_2} and \dot{M}_{CO_2} were similar, resulting in a maintenance of RER at a value of 1. Only in flounder 0–30 min after exercise did RER significantly exceed 1 (Fig. 2D). In both flounder and salmon, \dot{M}_{CO_2} fell more than \dot{M}_{O_2} during the latter part of recovery (Fig. 2A,C), resulting in an RER value of less than 1. The release of metabolic acid into the blood during exercise acidifies blood bicarbonate, leading to a release of CO_2 (see Milligan & Wood, 1986). The excretion of this CO_2 could explain the elevation of RER in flounder. As a consequence, the animals acquire a 'CO₂-debt' (Burggren & Cameron, 1980) which, during replenishment, would lead to a reduction in CO_2 excretion and hence RER.

In conclusion, this study has shown that after exercise the low levels of blood lactate that are typical of benthic fish, such as flounder, are not a consequence of an increased turnover rate, but are more likely to be due to a non-release phenomenon at the level of the muscle. Furthermore, the elevated turnover rates after exercise in both salmon and flounder cannot account for the observed rates of lactate clearance during recovery, suggesting that a significant portion of the lactate produced in the muscle is metabolized *in situ* and that the role played by the Cori cycle in lactate metabolism is of minimal importance in both species.

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