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THE ROLE OF BLOOD GLUCOSE IN THE RESTORATION OF MUSCLE GLYCOGEN DURING RECOVERY FROM EXHAUSTIVE EXERCISE IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AND WINTER FLOUNDER (*PSEUDOPLEURONECTES AMERICANUS*)

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Summary

The role of blood-borne glucose in the restoration of white muscle glycogen following exhaustive exercise in the active, pelagic rainbow trout (*Oncorhynchus mykiss*) and the more sluggish, benthic winter flounder (*Pseudopleuronectes americanus*) were examined. During recovery from exhaustive exercise, the animals were injected with a bolus of universally labelled [^{14}C]glucose *via* dorsal aortic (trout) or caudal artery (flounder) catheters. The bulk of the injected label (50–70 %) remained as glucose in the extracellular fluid in both species. The major metabolic fates of the injected glucose were oxidation to CO_2 (6–8 %) and production of lactate (6–8 %), the latter indicative of continued anaerobic metabolism post-exercise. Oxidation of labelled glucose could account for up to 40 % and 15 % of the post-exercise $\dot{M}_{O_2}$ in trout and flounder, respectively.

Exhaustive exercise resulted in a reduction of muscle glycogen stores and accumulation of muscle lactate. Glycogen restoration in trout began 2–4 h after exercise, whereas in flounder, glycogen restoration began within 2 h. Despite a significant labelling of the intramuscular glucose pool, less than 1 % of the infused labelled glucose was incorporated into muscle glycogen. This suggests that blood-borne glucose does not contribute significantly to the restoration of muscle glycogen following exhaustive exercise in either trout or flounder and provides further evidence against a prominent role for the Cori cycle in these species.

Introduction

During burst-type exercise in fish the working muscle derives energy almost

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exclusively from the anaerobic metabolism of glycogen. Consequently, there is a near depletion of the muscle glycogen store and an accumulation of lactate (Milligan and Wood, 1986). The pathway for the resynthesis of muscle glycogen in fish is not clear. Classically, muscle glycogen restoration has been described by the Cori cycle: lactate produced in the muscle is transported to the liver, *via* the blood, where it is converted into glucose which, in turn, is released to the blood and utilized by the muscle to support glycogen resynthesis (Newsholme and Leech, 1983). However, in recent years a growing body of evidence has accumulated suggesting that Cori cycle activity is quite low in fish. In plaice (*Pleuronectes platessa*; Wardle, 1978), rainbow trout (*Oncorhynchus mykiss=Salmo gairdneri*; Turner *et al.* 1983*a*; Milligan and Wood, 1986), flathead sole (*Hippoglossoides elassodon*; Turner *et al.* 1983*b*) and starry flounder (*Platichthys stellatus*; Milligan and Wood, 1987) a large portion (>80 %) of the lactate generated by the exercising muscle is retained within the working muscle mass. Furthermore, *in vivo* lactate turnover rates in the American eel (*Anguilla rostrata*; Cornish and Moon, 1985), skipjack tuna (*Katsumonus pelamis*; Weber *et al.* 1986), coho salmon (*Oncorhynchus kisutch*) and starry flounder (Milligan and McDonald, 1988) are not adequate to account for the observed rates of lactate clearance. More recently, Walsh (1989) estimated that in the gulf toadfish (*Opsanus beta*), hepatic gluconeogenesis accounts for, at most, only 2 % of the lactate clearance following exercise.

In the present study, we expand upon this body of evidence by investigating the role of blood-borne glucose in the metabolic recovery in rainbow trout and winter flounder (*Pseudopleuronectes americanus*) following exhaustive exercise. In particular, we are interested in the contribution of blood glucose to muscle glycogen resynthesis, and to aerobic and anaerobic metabolism. Several previous studies (see Wood and Perry, 1985, for a review) have demonstrated profound differences in the lactate and glycogen dynamics between sluggish, benthic species, such as flounder, and more active species, such as trout. These different post-exercise blood lactate dynamics may affect Cori cycle activity and, therefore, the importance of blood-borne glucose in the metabolic recovery of these two species.

Materials and methods

Experimental animals

Rainbow trout

Rainbow trout [*Oncorhynchus mykiss* (Walbaum), 100–250 g] of both sexes were obtained from Rainbow Springs Trout Hatchery, Thamesford, Ontario, at various times of the year. Animals were held in a large circular tank (400 l) and supplied with a continuous flow of aerated dechlorinated London tap water at seasonal temperatures (6–20 °C). Experiments were performed between November and February when the water temperature ranged from 6 to 10 °C, except for the oxygen consumption experiments, which were performed in June and July. In the summer months, fish were acclimated to 8±1 °C for at least 2 weeks prior to experiments. During holding, fish were fed twice weekly with

commercial trout pellets. Food was withheld for 5 days prior to experimentation in order to minimize any possible dietary influence on exercise and recovery (Walton and Cowey, 1982).

Animals were anesthetized in a 1:10 000 solution of MS-222 (tricaine methane sulfonate; Sigma) and fitted with dorsal aortic cannulae according to the method of Soivio *et al.* (1972). The catheters (PE 50 polyethylene tubing) were filled with heparinized (50 i.u. ml^{-1} sodium heparin; Fisher Scientific Co.) Cortland saline (Wolf, 1963). Following surgery, fish were placed in darkened acrylic boxes continually supplied with well-aerated dechlorinated water at the experimental temperature. Fish were allowed to recover for at least 24 h prior to experimentation.

Winter flounder

Winter flounder [*Pseudopleuronectes americanus* (Walbaum), 150–500 g] of both sexes were collected by otter trawl from Passamoquoddy Bay, New Brunswick, in August. Fish were held outdoors in a large (10 000 l) fiberglass tank continuously supplied with sea water (29 ‰) at ambient temperature (12–14 °C). Fish were held for at least 7 days prior to experiments, during which time they were not fed.

Caudal artery catheters were surgically implanted as described by Watters and Smith (1973) while the fish were anesthetized 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 ambient temperature and allowed to recover for at least 48 h before experiments.

Experimental protocol

Trout were exercised by chasing them around a large circular tank (300 l) for 5 min, while flounder were chased for 10 min in a shallow rectangular tank (80 l). Previous studies have shown that this form of exercise leads to exhaustion and a significant reduction in muscle glycogen (Milligan and Wood, 1986, 1987). At the end of the exercise period, fish were immediately returned to the fish boxes and allowed to recover.

In these experiments, individual fish were terminally sampled at rest, immediately after (time 0) exercise, as well as at 2, 4, 6 and 8 h post-exercise. Trout were also sampled at 12 h post-exercise. Two hours prior to sampling, fish were given a bolus injection of 5 μCi 100 g^{-1} of universally labelled [^{14}C]glucose (ICN Radiochemicals; specific activity 7 mCi $mmol^{-1}$) *via* the dorsal aortic or caudal artery catheter. The bolus was washed in with a double volume of 0.9 % NaCl. Blood glucose and blood lactate mix within the same pool and since it has been shown that 2 h is adequate for labelled lactate to mix completely with the blood lactate pool (Milligan and McDonald, 1988), glucose injections were given 2 h prior to

sampling. At the appointed times post-exercise, a blood sample (trout 200 μl; flounder 1000 μl) was drawn from the catheter and the volume replaced with saline. Fish were then grasped firmly and quickly killed by a cephalic blow. The liver and samples of white muscle from the dorsal epaxial muscle mass (1–2 g) were removed and immediately frozen in liquid nitrogen. The time between tissue sampling and freezing was 30 s for muscle and 60–90 s for liver. The tissue samples were stored either at −80 °C in a freezer (trout) or in liquid nitrogen (flounder) and analyzed within 1 week. Blood samples were deproteinized in either 800 μl (trout) or 1000 μl (flounder) of ice-cold 6 % perchloric acid (PCA), centrifuged and refrigerated until analyzed. The deproteinized extract was analyzed for lactate and glucose concentrations and ^{14}C radioactivity. Muscle and liver samples were analyzed for glycogen, lactate, glucose and ^{14}C radioactivity.

In experiments with flounder, oxygen consumption ($\dot{M}_{O_2}$), carbon dioxide excretion ($\dot{M}_{CO_2}$) and $^{14}CO_2$ excretion to the water were monitored during the 2 h equilibration period. The water to the box was shut off, the volume adjusted to 5 l and the box sealed. Water was recirculated in the box by means of a Masterflex peristaltic pump (Cole-Palmer) at a rate of 1.0 l min^{-1}. At the end of 1 h, the box was flushed for 10 min and then closed again for 50 min. Water samples (10 ml) were taken at the beginning and end of each period and P_{O_2}, total CO_2 and $^{14}CO_2$ radioactivity were measured. During the experiment, water temperature was maintained by bathing the boxes in flowing sea water. At the end of the 2 h equilibration period, blood and tissue samples were taken as previously described.

In trout held as described above, $\dot{M}_{O_2}$, $\dot{M}_{CO_2}$ and $^{14}CO_2$ excretion to the water were measured in a separate series of experiments in June and July. One hour before exercise, the inflow to the box was closed and the water recirculated within the box by means of a Masterflex pump. Water samples (5 ml) were taken at the beginning and end of the 1 h control period and P_{O_2} and $[CO_2]$ were measured to determine resting $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$. A blood sample (100 μl) was also taken at the end of the 1 h period to determine resting glucose and lactate levels. The fish were then transferred to the circular swimming tank and vigorously exercised for 5 min. Final blood samples (200 μl) were taken 2, 4 or 6 h post-exercise. Two hours prior to taking the blood sample the fish were given a bolus injection of 5 μCi 100 g^{-1} of [^{14}C]glucose *via* the dorsal aortic catheter. During this 2 h interval, the flow to the boxes was closed for 1 h followed by 10 min of flushing with fresh water and then closed again for 50 min. Water samples (10 ml) were taken at the beginning and the end of each period and P_{O_2}, $[CO_2]$ and $^{14}CO_2$ radioactivity were measured. Blood was analyzed for [lactate], [glucose] and ^{14}C radioactivity.

Analytical techniques and calculations

Whole-blood lactate was determined enzymatically on 100 μl of deproteinized extract using Sigma lactate assay reagents. For analysis of whole-blood glucose, the extract was neutralized with 3.0 mol l^{-1} K_2CO_3 (10 μl per 100 μl) and the $KClO_4$ salt precipitated. Glucose was measured in the supernatant as described by Bergmeyer (1965).

To determine tissue lactate levels, muscle and liver samples (180–250 mg) were homogenized in 1 ml of 6 % ice-cold perchloric acid using a Polytron homogenizer (Brinkman instruments) at setting no. 6 (23 500 revs min^{-1}) for 30 s. The homogenates were then transferred to 1.5 ml microcentrifuge tubes and centrifuged for 10 min at 10 000 g. Following neutralization of the supernatant with 3.0 mol l^{-1} K_2CO_3 and precipitation of the $KClO_4$ salt, lactate and free glucose levels were measured enzymatically as described above for blood.

Muscle and liver glycogen were isolated as described by Hassid and Abraham (1957) and analyzed for glucose after digestion with amyloglucosidase.

[^{14}C]glucose, [^{14}C]lactate and [^{14}C]glycogen in the various tissues were also determined. Tissue [^{14}C]glycogen was determined by adding a 500 μl sample of the resuspended glycogen to 5 ml of Beckman Ready Safe scintillation cocktail. Samples were stored in the dark overnight to reduce chemiluminescence and counted the following day in either a Beckman (LS 3801) or LKB Rack-Beta liquid scintillation counter.

Blood and tissue [^{14}C]glucose and [^{14}C]lactate were separated using ion exchange chromatography. The neutralized perchloric acid tissue extracts (500 μl) were gently layered onto a 10 cm×0.5 cm plastic column (Biorad) containing 1 g of resin (Dowex-1, Cl^- form; Sigma) which had been washed with 5 ml of distilled H_2O (dH_2O), 6 ml of 1.0 mol l^{-1} sodium acetate and 4 ml of dH_2O, in series. The sample was washed onto the column with 5.0 ml of dH_2O. Two 2.5-ml fractions were collected and contained [^{14}C]glucose. [^{14}C]lactate was eluted with 2×5.0 ml of 0.5 mol l^{-1} formic acid. Each fraction (2.5 ml) was collected separately. To determine the ^{14}C radioactivity, 5 ml of scintillation cocktail (Ready Safe, Beckman) was added to the 2.5 ml samples containing either [^{14}C]glucose or [^{14}C]lactate previously separated. Samples were kept in the dark overnight and counted the next day. Preliminary addition/recovery experiments showed that approximately 95 % of the [^{14}C]glucose and 96 % of the [^{14}C]lactate added onto the column was recovered.

[^{14}C]glucose incorporation into glycogen (GLY) and lactate (LA) was calculated using blood specific activities and ^{14}C radioactivity (cts min^{-1} g^{-1} tissue) in glycogen or lactate according to the following equations:

$$\frac{\mu\text{mol glucose incorporated into GLY or LA}}{\text{gram tissue}} = \frac{\text{cts min}^{-1}\text{ in GLY or LA per gram tissue}}{\text{blood specific activity}}, \quad (1)$$

where blood glucose specific activity = cts min^{-1} μmol^{-1}. (2)

White muscle and liver values were corrected for trapped extracellular fluid (ECF) using ECFV given for trout and starry flounder (Milligan and Wood, 1986, 1987).

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 PHM73 acid–base analyzer. Total [CO_2] in water (in mmol l^{-1}) was measured by

gas chromatography (Shimadzu 8A or Varian 3300). Water (1 ml) was added to a 5 ml gas-tight Hamilton syringe containing 0.5 ml of 0.1 mol l^{-1} HCl equilibrated with ultra-pure helium. The syringe was then filled to the 5 ml mark with helium, placed on an orbital shaker (130 revs min^{-1}) at room temperature and allowed to equilibrate for at least 30 min prior to analysis. A 2 mmol l^{-1} $NaHCO_3$ standard, treated the same way as the water samples, was run at frequent intervals throughout the day. All samples and standards were run in duplicate.

For analysis of total $^{14}CO_2$, water samples (1 ml) were acidified with 500 μl of 1.0 mol l^{-1} HCl and the liberated $^{14}CO_2$ was collected using CO_2 gas traps. CO_2 traps consisted of a glass scintillation vial (20 ml) sealed with a rubber septum fitted with a small plastic well containing glass fiber filter paper wetted with 200 μl of 1.0 mol l^{-1} methylbenzonium hydroxide (Sigma). The vials were vigorously shaken (250 revs min^{-1}) for 1 h, and the filter paper was removed and added to 5 ml of scintillation cocktail and counted. Duplicate samples were analyzed. Preliminary experiments indicated that the efficiency of these traps was approximately 95 %.

Whole blood was also analyzed for total CO_2 using either gas chromatography (100 μl of blood) or a Corning model 965A total CO_2 analyzer (50 μl of blood). $^{14}CO_2$ was collected from 100 μl of blood using the CO_2 traps described.

O_2 consumption and CO_2 excretion were calculated according to the equations described by Milligan and McDonald (1988).

The contributions of muscle and liver to total body mass were determined for trout. Fish (N=3) were killed, wiped dry, weighed and the entire white muscle mass and liver were carefully dissected out and weighed individually.

Statistical analysis

Values are presented as means±S.E.M. Statistical differences (P<0.05) were assessed using Student's two-tailed t-test, unpaired design.

Results

Metabolic disturbances

Exhaustive exercise resulted in a significant depletion of white muscle glycogen in both trout and flounder (Fig. 1A). Immediately after exercise, white muscle glycogen levels in the trout were reduced by approximately 90 % and showed no signs of recovery until 2–4 h post-exercise. Glycogen stores were replaced during the next 2 h, so that, by 6 h after exercise, resting levels were obtained. In the flounder, glycogen stores were reduced by approximately 66 % and showed immediate signs of recovery, with resting levels obtained by 4 h after exercise. White muscle glycogen levels in the flounder continued to increase, so that, by 8 h, they were significantly greater (P<0.05) than resting levels.

Associated with the reduction of muscle glycogen stores was a significant accumulation of lactate (Fig. 2A). In both species, muscle lactate significantly (P<0.05) increased after exercise, but the pattern of lactate accumulation was

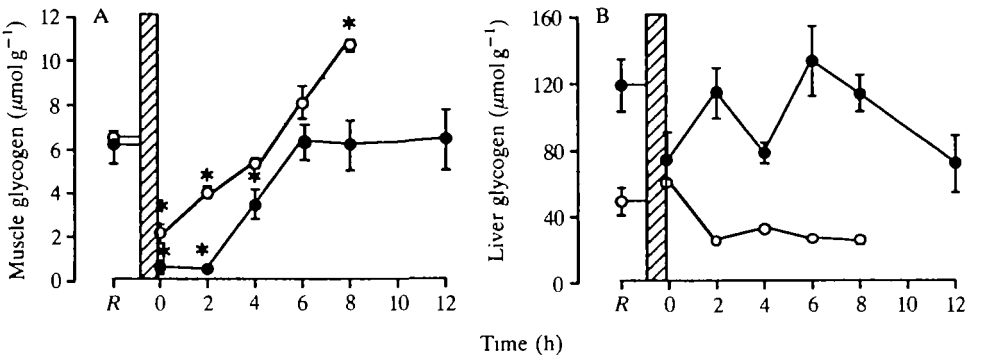

Fig. 1. Changes in glycogen levels in white muscle (A) and liver (B) prior to and during recovery from exhaustive exercise in rainbow trout (●) and winter flounder (○). Means±1 S.E.M. Hatched bars indicates period of exercise, *R* indicates rest, 0 immediately after exercise. $N=12$ at rest, 6 at 0 h and 2 h, 4 at 4 h, 5 at 6 h, 8 at 8 h and 3 at 12 h for trout. $N=5$ at all times except $N=4$ at 0 h in flounder. * Indicates a significant ($P<0.05$) difference from the corresponding rest value.

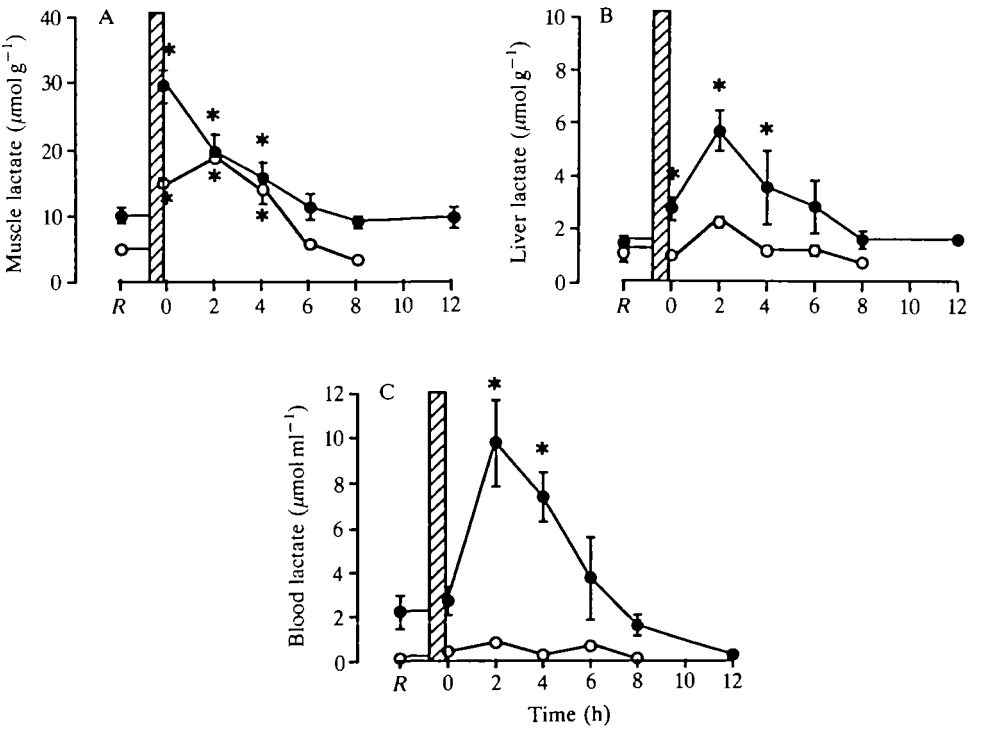

Fig. 2. Muscle (A), liver (B) and blood (C) lactate levels prior to and following exhaustive exercise. Other details as in the legend of Fig. 1 except $N=6$ at rest, 5 at 0 h and 2 h, 4 at 6 h and 7 at 8 h in trout (●) and $N=3$ at 0 h in flounder (○).

different in the two species. In the trout, lactate peaked immediately after exercise then declined until resting levels were obtained by 6 h after exercise. However, in flounder, muscle lactate levels did not peak until 2 h after exercise and, as in the trout, reached resting levels by 6 h after exercise.

Liver glycogen content was quite variable in trout (Fig. 1B) and, therefore, no significant effect of exercise was observed. The effect of exercise on liver lactate levels (Fig. 2B) was very different in the two species. In flounder, liver lactate levels were virtually unaffected by exercise. However, in trout, liver lactate significantly increased ($P<0.05$) after exercise, peaked at 2 h post-exercise and returned to resting levels by 6 h after exercise, paralleling the changes in blood lactate.

The most striking difference between the two species is the pattern of lactate accumulation in the blood (Fig. 2C) after exercise. Blood lactate levels 2 h after exercise were $9.71\pm1.91\,\mu mol\,ml^{-1}$ ($N=5$) in trout and $0.80\pm0.11\,\mu mol\,ml^{-1}$ ($N=5$) in flounder. Despite the transfer of some lactate from the white muscle to the blood, the level of lactate in the muscle remained much higher than that in blood throughout the recovery process in both species (Fig. 2A *vs* 2C).

Muscle glucose levels in trout (Fig. 3A) increased after exercise and remained elevated during the next 8 h before returning to resting levels. Like liver glycogen, liver glucose levels in trout (Fig. 3B) were quite variable, but tended to increase

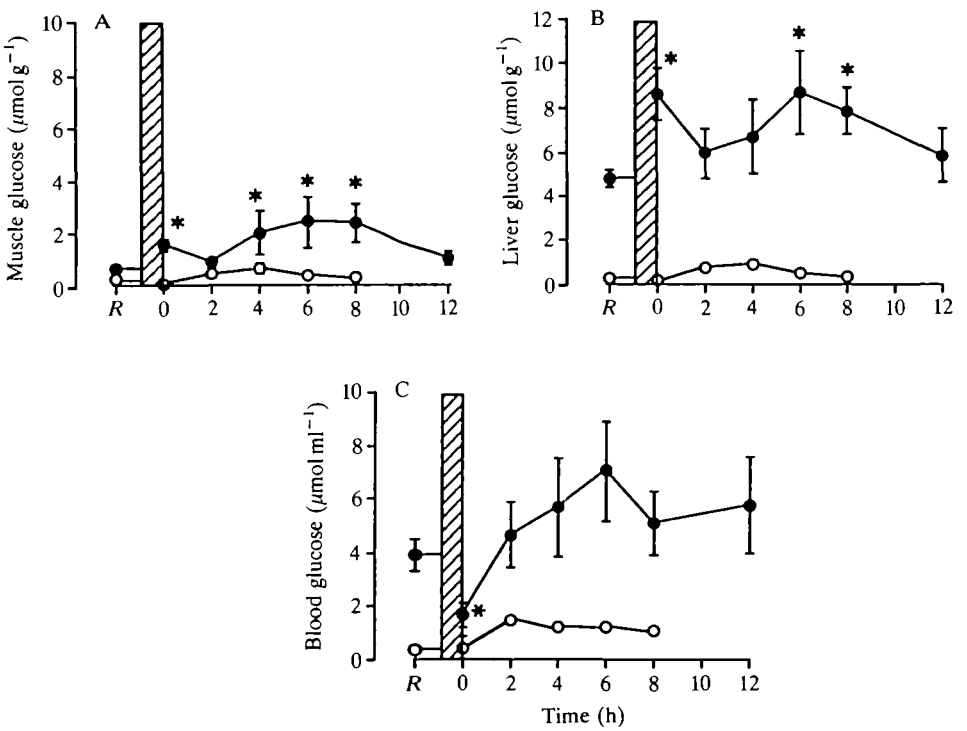

Fig. 3. Muscle (A), liver (B) and blood (C) glucose levels prior to and after exhaustive exercise. Other details as in the legend of Fig. 2. Trout (●), flounder (○).

after exercise. Blood glucose in trout (Fig. 3C) decreased significantly ($P<0.05$) immediately after exercise but quickly returned to resting levels.

For technical reasons, PCA extracts of samples taken at rest and immediately after exercise in flounder were refrigerated up to 1 month before glucose analysis. Addition/recovery experiments have shown that glucose degrades significantly in PCA extracts refrigerated for 1 month (S. Girard, personal communication); therefore, the rest and time 0 values for muscle, liver and blood glucose (Fig. 3A, 3B, 3C, respectively) are underestimates of the true values. For this reason, glucose values at rest and immediately after exercise cannot be compared to values at other times during recovery. However, during the recovery period, tissue glucose levels in flounder remained fairly constant.

Fig. 4A,B show oxygen consumption ($\dot{M}_{O_2}$) and carbon dioxide excretion ($\dot{M}_{CO_2}$) during recovery from exercise in trout and flounder, respectively. At all times during recovery, $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ values were much higher in trout than in flounder. In trout, $\dot{M}_{O_2}$ increased twofold and $\dot{M}_{CO_2}$ increased threefold after

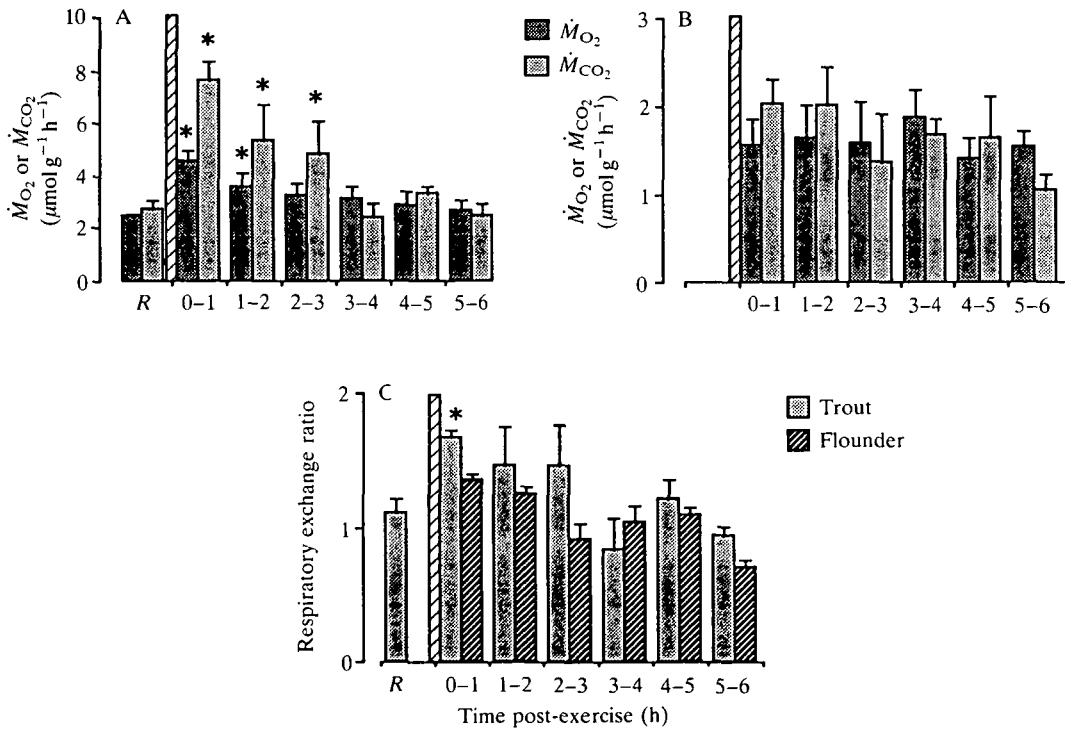

Fig. 4. Oxygen consumption ($\dot{M}_{O_2}$) (dark bars) and carbon dioxide excretion ($\dot{M}_{CO_2}$) (light bars) at rest and following exercise in trout (A) and flounder (B). (C) Trout (stippled bars) and flounder (striped bars) respiratory exchange ratios. Means ±1 S.E.M. Hatched bar indicates period of exercise. *R* indicates rest, 0 indicates immediately after exercise. $N=12$ at 1 h prior to exercise, 4 h at 0–1, 1–2, 4–5 and 5–6 h after exercise and 3 at 2–3 and 3–4 h after exercise in trout. $N=5$ for flounder. * Indicates a significant ($P<0.05$) difference from the corresponding pre-exercise value.

exercise, which resulted in a significant increase ($P<0.05$) in the repiratory exchange ratio (RER) (Fig. 4C). Oxygen consumption and carbon dioxide excretion returned to resting levels after 2 and 3 h, respectively (Fig. 4A).

Blood glucose levels during recovery in trout from the $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ experiments were slightly higher [2 h: 6.88±0.93 (4); 4 h: 7.18±0.67 (3); 6 h: 6.61±1.04 (4) μmol ml^{-1}] and blood lactate levels were significantly lower (2 h: 4.2±0.84 (4); 4 h: 4.29±0.44 (3); 6 h: 3.95±0.39 (4) μmol ml^{-1}] than levels measured from the tissue sampling series (Figs 3C and 2C, respectively).

Distribution of [^{14}C]glucose

Metabolite specific activities in both species injected with [^{14}C]glucose at various times after exercise are shown in Table 1. In both flounder and trout, at any time after exercise, the majority of [^{14}C]glucose injected remained in the extracellular space (Fig. 5A,E). Even after 8 h of recovery in flounder and 12 h in trout, about 60 % of the injected label remained within the extracellular glucose pool. [^{14}C]glucose was also found in muscle and liver (Fig. 5A,E), with muscle showing the greater accumulation. In both species, there was significant incorporation of glucose carbon into the extracellular lactate pool, which decreased from approximately 7 % of the total injected label after 2 h of recovery to 4 % during the latter part of recovery (Fig. 5B,F). There was also incorporation of ^{14}C into lactate in muscle and liver, though it represented, at most, only 1 % of the total injected label.

Incorporation of ^{14}C into muscle glycogen was higher than the incorporation into liver glycogen in both flounder (Fig. 5C) and trout (Fig. 5G). However, at no time during recovery was the incoporation of the label into glycogen in muscle or liver in excess of 1 % of the total ^{14}C injected. $^{14}CO_2$ excreted into the water accounted for, at most, 8 % in flounder (Fig. 5D) and 6 % in trout (Fig. 5H) of the injected label at any time during recovery. Blood $^{14}CO_2$ accounted for less than 1 % of the total ^{14}C label injected.

In all tissues and at all times after exercise, trout incorporated more glucose into lactate than did flounder (Fig. 6). Despite the rapid accumulation of lactate in white muscle of both species following exercise (Fig. 2A), there was a continual production of lactate, as indicated by the incorporation data (Fig. 6A). Although in trout there was a net decline in liver lactate from 2 h post-exercise onwards (Fig. 2B), lactate was still being synthesized, as indicated by the increase in the label appearing as lactate (Fig. 6B). In contrast, glucose incorporation into liver lactate in flounder (Fig. 6B) remained fairly constant during recovery. Incorporation of [^{14}C]glucose into the extracellular lactate pool (Fig. 6C) decreased with time after exercise in both species, although more so in flounder than trout.

In trout, the incorporation of [^{14}C]glucose into muscle glycogen increased slowly up to 8 h after exercise (Fig. 7A). Even though no net glycogen synthesis was observed 2 h post-exercise in trout (Fig. 1A), a small amount of [^{14}C]glucose was incorporated into glycogen during the first 2 h after exercise. Even after muscle glycogen stores had been replenished (i.e. 6 h after exercise), the amount of label

Table 1. *Specific activities used to calculate glucose incorporation into tissue glycogen and lactate*

	Trout						Flounder					
	Glucose ($nCi\,\mu mol^{-1}$)	Glycogen ($nCi\,g^{-1}$)		Lactate ($nCi\,g^{-1}$)			Glucose ($nCi\,\mu mol^{-1}$)	Glycogen ($nCi\,g^{-1}$)		Lactate ($nCi\,g^{-1}$)		
Time post-exercise	Blood	Muscle	Liver	Blood	Muscle	Liver	Blood	Muscle	Liver	Blood	Muscle	Liver
0–2 h	32.5±	0.19±	9.12±	11.0±	1.12±	9.01±	31.13±	0.16±	0.77±	7.70±	0.27±	6.31±
	1.77	0.06	0.71	2.32	0.14	0.47	1.95	0.07	0.15	2.95	0.14	1.59
	(4)	(4)	(4)	(4)	(4)	(4)	(5)	(5)	(5)	(5)	(5)	(5)
2–4 h	51.81±	0.71±	21.5±	14.6±	1.13±	6.66±	36.53±	0.31±	5.91±	2.21±	0.81±	2.86±
	20.5	0.13	21.2	4.76	0.72	5.89	4.48	0.13	3.84	0.74	0.72	1.59
	(3)	(3)	(3)	(3)	(3)	(3)	(5)	(5)	(5)	(5)	(5)	(5)
4–6 h	19.72±	0.05±	2.20±	3.12±	0.29±	2.23±	45.5±	0.24±	3.32±	5.01±	0.15±	9.14±
	4.32	0.02	1.59	0.27	0.008	0.85	8.46	0.04	0.82	2.05	0.14	2.77
	(4)	(4)	(4)	(4)	(4)	(4)	(5)	(5)	(5)	(5)	(5)	(5)
6–8 h	50±	0.21±	3.24±	5.91±	0.78±	9.86±	43.33±	0.24±	2.56±	2.23±	0.65±	10.49±
	15.5	0.19	2.12	2.95	0.46	2.99	5.67	0.05	0.95	0.53	0.52	3.75
	(7)	(7)	(7)	(7)	(7)	(7)	(5)	(5)	(5)	(5)	(5)	(5)
10–12 h	31.62±	0.40±	5.84±	6.9±	1.31±	1.99±						
	0.82	0.09	2.61	0.62	0.84	0.73						
	(4)	(4)	(4)	(4)	(4)	(4)						

Means±1 S.E.M. (*N*).

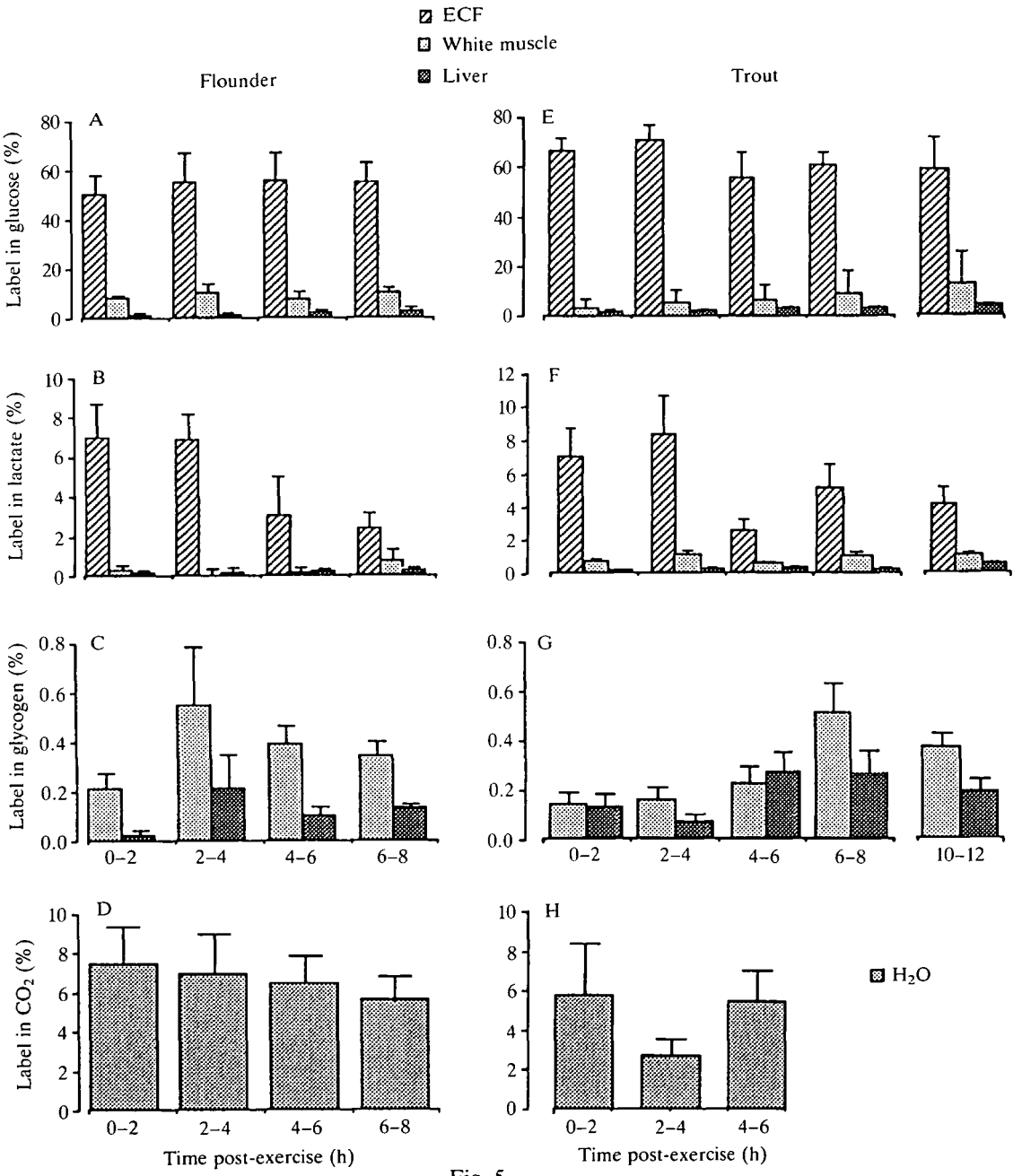

Fig. 5

incorporated into glycogen continued to increase in trout. Incorporation of [^{14}C]glucose into muscle and liver glycogen in the flounder (Fig. 7A and 7B, respectively) was lower than that in trout and remained fairly constant during the 8 h recovery period.

Fig. 5. Distribution of ^{14}C label in glucose (A,E), lactate (B,F), glycogen (C,G) and CO_2 and H_2O (D,H) during recovery from exhaustive exercise in flounder and trout, respectively, following the injection of [^{14}C]glucose. Means±1 S.E.M. Metabolites were measured in extracellular fluid (ECF) (except glycogen), muscle and liver. *N* as in Fig. 1 except for trout $N=4$ at 0–2 and 4–6 h, 3 at 2–4 h, 7 at 6–8 h and 2 at 8–12 h for blood glucose and $N=8$ for $^{14}CO_2$ measurements in H_2O except $N=6$ at 4 h after exercise. No measurments were taken for $^{14}CO_2$ in H_2O after 6 h post-exercise for trout. Distribution of label was estimated for each fish assuming (1) that extracellular fluid volume is 27 % in trout and 25 % in flounder (Milligan and Wood, 1986, 1987); (2) that white muscle constitutes 50 % of the body mass in trout and 42 % in flounder (S. Girard and C. L. Milligan, unpublished data); and (3) that liver is 1.4 % of the body mass in trout and 0.73 % in flounder (S. Girard and C. L. Milligan, unpublished data).

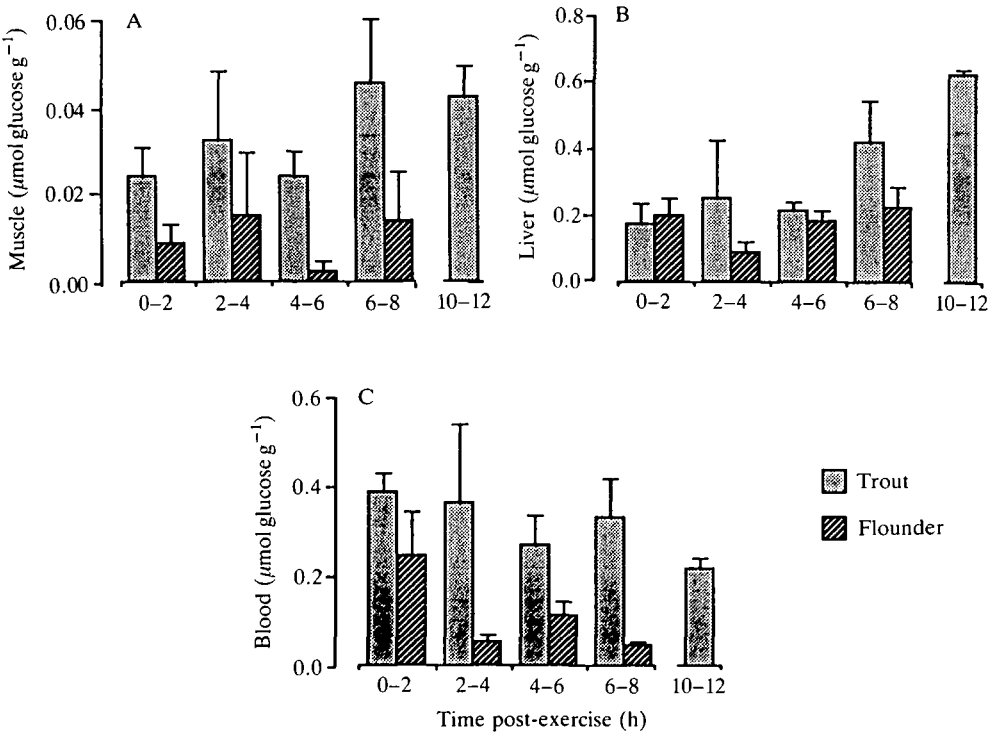

Fig. 6. Incorporation of [^{14}C]glucose into lactate in muscle (A), liver (B) and blood (C) during recovery from exercise in trout (stippled bars) and flounder (striped bars). Means±1 S.E.M. $N=4$ at 2 h, 3 at 4 h, 4 at 6 h, 7 at 8 h and 2 at 12 h for trout and $N=5$ for flounder. No measurements were taken after 8 h post-exercise in flounder.

Discussion

Metabolic response to exercise

The metabolic disturbances associated with exhaustive exercise in trout and flounder are typical of those reported previously for trout and related flatfish

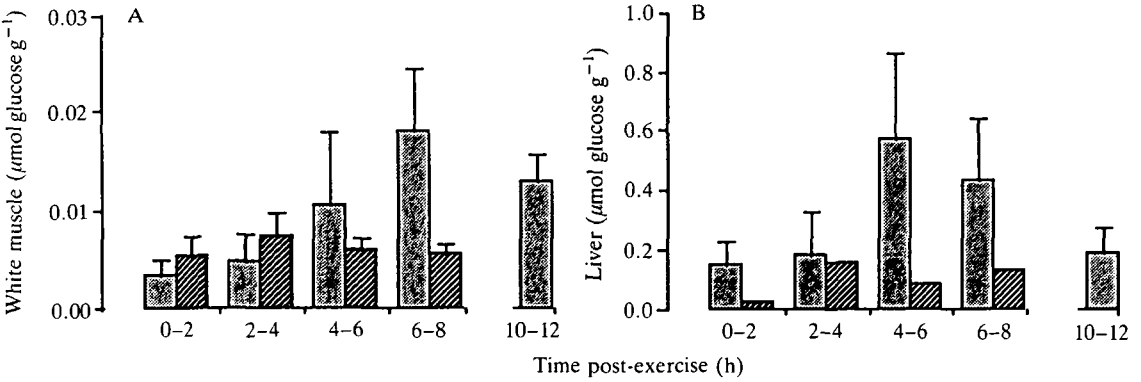

Fig. 7. Incorporation of [^{14}C]glucose into glycogen in muscle (A) and liver (B) during recovery from exercise in trout (stippled bars) and flounder (striped bars). Other details as in legend of Fig. 6.

species (see Wood and Perry, 1985, for a review). Exercise to exhaustion resulted in a reduction in muscle glycogen stores and an accumulation of lactate in muscle and blood. Blood lactate levels in the $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ experiments in trout were lower than levels measured in the tissue sampling series. Perhaps this variation is because the $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ experiments were performed in summer while the others were performed in winter, though we know of no study that indicates a seasonal influence on exercise performance, glycogen depletion or lactate accumulation.

Relative to flounder, glycogen restoration in trout was delayed; there were no signs of recovery until 2–4 h after exercise, whereas in flounder, glycogen resynthesis began within 2 h. Previous studies have shown that, at least in trout, muscle glycogen synthesis is delayed until muscle pHi is restored to a level compatible with glyconeogenesis (Milligan and Wood, 1986). During the period of net glycogen synthesis in trout (2–6 h) and flounder (0–4 h) the relationships between glycogen repletion and lactate depletion were 1.94:1 and 2.32:1, respectively, which is close to the 2:1 expected if lactate were converted to glycogen.

Muscle glycogen synthesis was previously reported to be a slow process in fish; Black *et al.* (1962) and Milligan and Wood (1986) reported that, in trout, glycogen restoration required 12–24 h. Similarly, Wardle (1978) found that 50–80 % of the muscle glycogen stores in plaice were replenished within the first 8 h but complete restoration required up to 24 h. However, results from the present study indicate that glycogen resynthesis may be a faster process as muscle glycogen levels were restored within 6 h in trout and 4 h in flounder. Recently, Pearson *et al.* (1990) and Scarabello *et al.* (1991) have reported similar recovery times for muscle glycogen in juvenile trout. There is no clear explanation for the differences in the time required for glycogen resynthesis in the various studies, but differences in water temperature, feeding history, size and age of fish may be contributing factors.

A rather curious observtion is the continued increase in muscle glycogen levels in flounder during recovery such that, by 8 h, glycogen levels were significantly higher than those in resting fish. One possible explanation for this apparent overshoot in muscle glycogen in flounder is that the resting levels may be underestimates of true resting glycogen levels. While there was no apparent struggling (i.e. no tail flaps) while sampling resting fish, the muscles may have contracted isometrically, leading to some glycogenolysis.

The greater accumulation of lactate in trout blood relative to flounder, reflects, in part, the greater production in the muscle. However, this is not the only explanation, as muscle lactate in trout was only double that in flounder, whereas blood lactate levels were 10 times greater, suggesting a greater release of lactate from the muscle. This quantitative difference in blood lactate accumulation is now considered typical of these types of fish; trout, an active pelagic fish has been termed a 'lactate releaser', whereas flounder, a benthic, sluggish fish, has been termed a 'lactate non-releaser'. However, in both species, muscle lactate was higher than blood lactate at all times after exercise, indicating that there is a significant retention of lactate in the muscle. It is estimated that, in trout, only 10 % and, in flounder, less than 3 % of the lactate produced in the muscle is released to the blood (Milligan and Wood, 1986, 1987). Although the underlying mechanism(s) for this lactate retention by the muscle is not clear, it is not due to a tissue perfusion limitation but rather to some membrane-related phenomenon (Wardle, 1978; Turner *et al.* 1983*a*,*b*).

Estimates of resting $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ are typical of those reported previously for salmonids (Brett, 1972; Milligan and McDonald, 1988). In trout, $\dot{M}_{O_2}$ had returned to rest levels within 2 h after exercise, despite the fact that tissue lactate levels were still elevated and glycogen repletion had not yet begun. Similar observations were made for salmon and flounder (Milligan and McDonald, 1988) and juvenile trout (Scarabello *et al.* 1991), suggesting that elevated post-exercise oxygen consumption (EPOC) in fish is not correlated to lactate clearance. In fact, neither Milligan and McDonald (1988) nor Scarabello *et al.* (1991) were able to show any correlation between either lactate turnover or lactate accumulation and EPOC.

Respiratory exchange ratios (RER) greater than 1 are typically observed after exhaustive exercise in fish (Fig. 4C). In addition to aerobically produced CO_2, metabolic acid, an end product of anaerobic metabolism, titrates tissue bicarbonate stores, leading to the release of CO_2 (Burggren and Cameron, 1980; Milligan and Wood, 1986) and consequently CO_2 production is in excess of O_2 consumption.

Metabolic fate of blood glucose during recovery from exercise

The metabolic fate of the injected [^{14}C]glucose was qualitatively identical in both trout and flounder, with quantitative differences probably reflecting the greater metabolic rate in trout compared to flounder (e.g. Fig. 4). Because of exchange reactions between labelled and unlabelled glucose carbons (recycling *via* the liver; Katz, 1979), the estimates of label incorporation into various end

products (lactate, glycogen) reported in the present study tend to underestimate the true incorporation by an unknown amount. Katz *et al.* (1974) and Katz (1979) have suggested methods for correcting data for carbon recycling. However, given the low glucose turnover rates in fish (Cornish and Moon, 1985), the error in our estimates is probably small and we have chosen to leave our data uncorrected. Thus, our estimates of label incorporated err on the conservative side.

At any time during recovery from exhaustive exercise, 70–80 % of the injected label was recovered in both trout and flounder; the remaining 20–30 % of the label probably resides in tissues (e.g. heart, kidney, red muscle) and metabolites (e.g. protein, tricarboxylic acid cycle intermediates; e.g. Brooks and Gaesser, 1980) not measured in this study. The bulk of the injected labelled glucose (50–70 %) remained as glucose in the extracellular space in both species. The major metabolic fates of the injected glucose were oxidation to CO_2 (6–8 %) and production of lactate (6–8 %). This is in marked contrast to the situation in the rat, where, following exhaustive exercise, only 1–2.5 % of the injected [^{14}C]glucose is recovered as lactate and 25–30 % is incorporated into CO_2 (Brooks and Gaesser, 1980). The greater incorporation of label into CO_2 in rats relative to trout and flounder is probably due to the fact that fish derive more metabolic energy from protein than from carbohydrate (Walton and Cowey, 1982; Moon, 1988).

The percentage of labelled glucose appearing as CO_2 is underestimated because some of the $^{14}CO_2$ produced no doubt equilibrated with the tissue total CO_2 pools (Brooks and Gaesser, 1980), which were not measured in the present study. However, the label in blood total CO_2 was less than 1 % of that excreted to the water and, since tissue total CO_2 is generally much lower than that of blood (Milligan and Wood, 1986), the error is probably not very great. In fact, in the lizard *Dipsosaurus dorsalis*, only 2 % of the label was recovered in tissue bicarbonate pools, compared to 14 % in expired CO_2 (Gleeson and Dalessio, 1989). The low incorporation of labelled glucose into CO_2 observed for both trout and flounder is consistent with the general view that fish oxidize glucose slowly (Walton and Cowey, 1982; Cornish and Moon, 1985). In fact, in the American eel it was estimated that if all the oxygen consumed were solely for the oxidation of glucose, only 8 % of the glucose turned over would undergo complete oxidation (Cornish and Moon, 1985).

Although a slow process, glucose oxidation could account for a considerable portion of the metabolic rate during recovery from exercise. Based upon the measured $^{14}CO_2$ excretion and blood glucose specific activities, the contribution of blood glucose to oxidative metabolism was estimated for both trout and flounder, assuming 6 mmol of oxygen consumed per mmol of glucose oxidized. These calculations, summarized in Table 2, indicate that, in trout, as much as 40 % of the post-exercise $\dot{M}_{O_2}$ could be attributed to labelled glucose oxidation while, in flounder, glucose oxidation could account for, at most, only 15 % of $\dot{M}_{O_2}$. A caveat to this interpretation is the assumption that all the labelled CO_2 results from complete glucose oxidation. Some $^{14}CO_2$ is liberated when pyruvate is oxidized to acetyl CoA, which can then enter numerous pathways (e.g. oxidation in the Krebs

Table 2. *Contribution of oxidation of labelled glucose to total $\dot{M}_{O_2}$ during recovery from exercise in winter flounder and rainbow trout*

Time post-exercise	$\dot{M}_{O_2}$ (μmol g^{-1})	μmol glucose oxidized	% $\dot{M}_{O_2}$
Flounder			
0–2 h	3.21±0.67 (5)	0.36±0.1 (5)	13.53±3.74 (5)
2–4 h	3.48±0.18 (5)	0.26±0.08 (5)	7.21±1.32 (5)
4–6 h	2.9±0.26 (5)	0.23±0.07 (5)	7.79±1.47 (5)
6–8 h	2.94±0.47 (5)	0.24±0.04 (5)	11.63±4.7 (5)
Trout			
0–2 h	8.49±0.49 (4)	2.42±0.67 (4)	30.09±13.19 (4)
2–4 h	6.4±0.38 (3)	1.15±0.24 (3)	18.49±7.84 (3)
4–6 h	5.58±0.85 (5)	2.28±0.57 (4)	35.56±11.71 (4)

For details, see text.
Means±1 S.E.M. (*N*).

cycle; synthesis of fatty acids or acetate) and, thus, may lead to overestimates of the contribution of glucose to oxidative metabolism. This overestimation is probably small, given that acetate production is generally quite low in fish and fatty acid synthesis would be minimal during recovery from exhaustive exercise (Walton and Cowey, 1982). Nonetheless, it is quite clear that $^{14}CO_2$ production from glucose is three times greater in trout than in flounder, which is consistent with the nearly threefold greater metabolic rate in trout than flounder (Fig. 4).

Despite the elevation in oxygen consumption, there was considerable anaerobic metabolism post-exercise, as indicated by the recovery of a substantial amount of the labelled glucose as lactate in the extracellular space. At the time of peak blood lactate levels, 2 h post-exercise, the labelled lactate represented only about 5 % of the total extracellular lactate in trout, but as much as 25 % of the total in flounder. Though substantial, the post-exercise lactate production is not adequate to explain the continued elevation of blood lactate observed during recovery; rather, the 'delayed-release' phenomenon described by Batty and Wardle (1979) is the more likely explanation of this slow rise. The site of this continued lactate production is not clear; labelled lactate was recovered in both the white muscle and liver. However, it is not known if this reflects *in situ* production or uptake from the blood. In liver, the latter is probably the case, since, at least in trout, the pattern of lactate accumulation in the liver parallels that of the blood and trout hepatocytes are capable of lactate oxidation (Walsh *et al.* 1988). The situation appears to be more complex is flounder as, at all times sampled, liver [lactate] is greater than

blood [lactate]. Whether this implies a fundamental difference in liver lactate metabolism between trout and flounder remains to be seen. In muscle, *in situ* production probably predominates since at all times the muscle [lactate] was greater than blood [lactate]. Furthermore, in flounder, *in vivo* experiments with [^{14}C]lactate tend to suggest that white muscle does not take up lactate (S. Girard and C. L. Milligan, unpublished observations). The continued post-exercise lactate production in the muscle, as shown by the isotopic data, despite a net reduction in muscle [lactate] (Fig. 2), indicates that the muscle lactate pool is not static, but is in a dynamic state. Our estimate of the amount of label incorporated into lactate tends to err on the conservative side, since any carbon recycling *via* the liver (e.g. lactate produced from labelled glucose elsewhere is taken up by the liver and reconverted to glucose) would tend to underestimate the true incorporation. However, hepatic enzyme activities and *in vitro* observations from isolated hepatocytes suggest that liver tends to oxidize lactate rather than utilize it as a gluconeogenic substrate (Johnston and Moon, 1979; Walsh *et al.* 1988; Walsh, 1989). Thus, the error introduced by carbon recycling is probably minimal.

In flounder, muscle glycogen resynthesis begins immediately after the cessation of exercise and continues through to 8 h, whereas, in trout, net glycogen resynthesis is delayed, occurring 2–6 h post-exercise (Fig. 1A). Even during periods when there was no net glycogen synthesis (e.g. 0–2; 6–8, 10–12 h post-exercise in trout; Fig. 1), there was still incorporation of the label into glycogen. This observation suggests that glycogen is not a static substrate pool that is drawn upon in time of need and replenished once that need has been fulfilled; rather, it is in a dynamic state of flux. The regulation of glycogen metabolism is obviously complex.

Although there was significant labelling of the intramuscular glucose pool (approximately 50 % of the free glucose was labelled) in both species, the labelled glucose accounted for only 0.2–0.6 % of the glycogen restored (Table 3). This

Table 3. *Contribution of blood glucose to muscle glycogen resynthesis in winter flounder and rainbow trout during recovery from exhaustive exercise*

Time post-exercise	ΔGlycogen (μmol g^{-1})	% Resynthesized from blood glucose
Flounder		
0–2 h	1.87	0.28
2–4 h	1.3	0.56
4–6 h	2.74	0.21
6–8 h	2.64	0.21
Trout		
2–4 h	2.96	0.16
4–6 h	2.86	0.36

ΔGlycogen and the percentage resynthesized from blood glucose were calculated from the mean values at each time interval.

indicates that blood-borne glucose is not the major substrate for the post-exercise restoration of muscle glycogen in either trout or flounder, and argues further against a prominent role for the Cori cycle in these fish. Studies by Gleeson and Dalessio (1989, 1990) also showed that the liver may not play a predominant role in supplying substrate for muscle glycogen resynthesis during recovery in the lizard. Rather, reptilian muscle replenishes glycogen by utilizing lactate directly as a gluconeogenic substrate (Gleeson and Dalessio, 1990). A similar scenario has been described for the American toad (*Bufo americanus*; Withers *et al.* 1988). This situation is quite different from that described for the rat (Brooks and Gaesser, 1980) where, following exhaustive exercise, approximately 24 % of the injected [^{14}C]glucose label was recovered in the muscle glycogen pool. The strategy of utilizing lactate for glycogen resynthesis seen in fish, toads (Withers *et al.* 1988) and lizards (Gleeson and Dalessio, 1989, 1990) may be due in part to the low metabolic rate of these animals and to their greater dependence on anaerobic metabolism for routine locomotion. Thus, it may be advantageous for them to retain lactate in the muscle for glyconeogenesis and support the process through oxidation of some of the lactate and non-carbohydrate substrates, rather than have locomotor capacity diminished because of low glycogen levels. The metabolic pathway utilized for muscle glycogen resynthesis from lactate *in situ* remains to be determined.

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