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LACTATE METABOLISM IN RAINBOW TROUT

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Summary

We have investigated the metabolic fate of blood lactate in resting rainbow trout and in fish recovering from a bout of exhaustive exercise. At rest and during recovery from exercise, the majority of blood lactate was oxidized, the proportion increasing with increasing oxygen consumption. It is estimated that, during recovery from exhaustive exercise, lactate released from the muscle has the potential to fuel a significant portion of oxidative metabolism. The bulk of the remaining blood lactate reappeared in the muscle lactate pool, probably *via* direct uptake by the muscle. There was a significant incorporation of blood lactate into the muscle glycogen pool, providing strong evidence for *in situ* glycogenesis as the mode for muscle glycogen replenishment. To investigate the role of the liver in blood lactate clearance, trout were functionally hepatectomized by ligation of the hepatic portal circulation. The exercise performance of hepatectomized fish was equal to that of sham-operated fish and controls, indicating that muscle relies primarily on endogenous fuel stores. Furthermore, blood lactate levels immediately after exercise were greater and muscle metabolic recovery was faster in hepatectomized fish than in sham-operated fish and controls. These observations suggest that glycogen resynthesis in trout muscle may be retarded because of a non-recoverable loss of substrate (i.e. lactate) from the muscle, because the lactate released is utilized by the liver. These results are discussed in view of what is known about these processes in other ectothermic vertebrates.

Introduction

Exhaustive, burst-type exercise in vertebrates is fuelled by anaerobic metabolism, which results in a depletion of glycogen and accumulation of lactate in the white or fast-twitch muscle. Over the past few years, it has become apparent that the fate of this lactate varies among the vertebrates: in mammals, an oxidative fate predominates (Brooks and Gaesser, 1980), whereas in reptiles, amphibians and fish, glycogenesis dominates (Withers *et al.* 1988; Gleeson and Dalessio, 1990; Pagnotta and Milligan, 1991; Fournier and Guderley, 1992; Girard and Milligan, 1992). In the reptiles, although the major fate of lactate is glycogenesis, lactate ‘shuttles’ from the white to red muscle fibres, where it is incorporated into the red muscle glycogen pool (Gleeson, 1991). In amphibians, glycogenic removal of lactate dominates over oxidative metabolism and involves primarily the skeletal muscle (Withers *et al.* 1988; Fournier and Guderley, 1992).

Key words: lactate, glycogenesis, metabolism, exercise, rainbow trout, *Oncorhynchus mykiss.*

Recent studies with a pleuronectid fish, winter flounder, suggest that *in situ* glycogenic removal of lactate dominates in fish (Pagnotta and Milligan, 1991; Girard and Milligan, 1992). Pleuronectids are slow and rather sluggish benthic fish and several previous studies have demonstrated clear differences between these fish and more active species, such as salmonids, in lactate dynamics after exercise. For example, after exhaustive exercise in flatfish, blood [lactate] rarely exceeds 1–2mmoll^{-1}, despite a 10- to 15-fold greater concentration of lactate in the muscle. In contrast, in salmonids after exercise, blood lactate levels of 15–20mmoll^{-1} are not uncommon and are typically half that in the muscle. Thus, the amount of lactate appearing in the blood space of pleuronectids represents less than 1% of the total produced, whereas in salmonids, that in the blood space amounts to 10–20% of the total. For these reasons, pleuronectid species have been termed 'lactate non-releasers' and salmonids have been termed 'lactate releasers' (see Wood and Perry, 1985, for a review). Because of these differences, we carried out this study to examine the metabolic fate of this 'released' lactate in rainbow trout, a representative salmonid. Specifically, we examined the contribution of blood lactate to oxidative metabolism and its role in muscle glycogen resynthesis. The latter was of particular interest as Turner and Wood (1983) have suggested that the lactate released by the muscle is actively taken back up and used as a substrate for glycogen resynthesis. Our final objective was to clarify what role, if any, the liver plays in blood lactate disposal.

Materials and methods

Experimental animals

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] (150–350g) of both sexes were purchased from Rainbow Springs Trout Hatchery, Thamesford, Ontario, at various times of the year. Fish were held indoors in a large (400l) circular plastic tank continuously supplied with aerated dechlorinated London city water maintained at 14±1°C. During holding, fish were fed commercial trout pellets every other day. Food was withheld for 5 days prior to experimentation in order to minimize any dietary influence on metabolism (Walton and Cowey, 1982).

To facilitate blood sampling and injection of radiolabel, a catheter (PE 50 tubing) was surgically implanted into the dorsal aorta of anaesthetized (1:10000, MS 222) trout as described by Soivio *et al.* (1972). Trout were then placed in 6l darkened acrylic boxes and allowed to recover for at least 48h prior to experimentation.

To assess the role of the liver in post-exercise recovery, a second group of trout was functionally hepatectomized by ligation of the blood supply to the liver. After placement of the dorsal aorta catheter and while the fish was still anaesthetized, the liver was exposed by making a 2cm longitudinal incision that extended posteriorly from a point midway between the left pectoral fin and a midventral line. A 15cm length of 000 surgical silk was passed around the hepatic portal vein and the vessel was ligated. Prior to closure with silk sutures, the wound was dusted with the antibiotic oxytretracycline hydrochloride. In sham-operated fish, the hepatic portal vein was exposed, but not ligated. Control fish had only dorsal aorta catheters implanted. Fish were allowed to recover for 48h prior to experimentation.

Experimental protocol

Trout were exercised by chasing them around a large (250l) circular tank for 5min. Previous studies have shown that this form of exercise leads to exhaustion and a significant reduction in muscle glycogen and accumulation of lactate (Milligan and Wood, 1986*b*). At the end of exercise, fish were returned to the acrylic boxes and allowed to recover.

Metabolic fate of lactate

In these experiments, fish were terminally sampled at rest (i.e. fish were not exercised), immediately after exercise (time 0) and at 2, 4, 6 and 8h post-exercise. Two hours prior to sampling, fish were injected with $5\,\mu Ci 100\,g^{-1}$ of universally labelled L-[^{14}C]lactate (sodium salt, specific activity 90 $mCi mmol^{-1}$; ICN Radiochemicals, Montreal) *via* the dorsal aorta catheter. The injection was washed in with a double volume of 0.9% NaCl. The injections were given 2h prior to exercise since this has been shown to be adequate time for labelled lactate to equilibrate within the blood lactate pool (Milligan and McDonald, 1988). Trout sampled at time 0 were not injected with the label.

In order to measure $^{14}CO_2$ excreted, during the 2h period between label injection and sampling, the fish box volume was set to 6l, water flow was shut off, the box was sealed and water in the box was recirculated with a Masterflex pump (Cole-Palmer Instruments, Chicago, IL) at a rate of $1.0\,l\,min^{-1}$. At the end of 1h, the box was flushed for 10min and then closed again for 50min. Water samples (10ml) were taken at the beginning and end of each period for analysis of $^{14}CO_2$ and total CO_2 excretion. Experimental temperature (14±1°C) was maintained by bathing the boxes in flowing water.

At the end of the 2h period, blood (1.0ml) was withdrawn from the catheter with 500 μl gas-tight Hamilton syringes and the volume was replaced with saline. The fish were then grasped firmly and quickly killed with a cephalic blow. The liver, the heart and a sample of epaxial white muscle were removed and freeze-clamped in liquid nitrogen. The elapsed time between killing the fish and sampling the tissues was 30s for muscle and 45–60s for liver and heart. The samples were frozen at −80°C in a freezer and analyzed within 1 week. Tissue samples were analyzed for total ^{14}C radioactivity and radiolabel incorporation into lactate, glycogen (except blood), glucose, protein and lipid (except heart) pools.

Hepatectomy and recovery from exercise

In this series of experiments, fish (control, sham-operated and hepatectomized) were terminally sampled at rest, immediately after exercise (time 0) or 6h into recovery. At sampling, 800 μl of blood was withdrawn from the catheter and the volume was replaced with saline; tissue (white muscle and liver) samples were obtained as described. Tissues were analyzed for glycogen (except blood), lactate and glucose.

To assess the degree to which blood flow to the liver was compromised by this hepatectomy procedure, a separate group of sham-operated and hepatectomized fish was injected with 2ml of saline coloured with blue food colouring *via* the dorsal aorta catheter. The dye was allowed to circulate for 20min, at which point the fish exhibited a

blue superficial colouring. The fish were then killed, and the liver was removed and weighed. Those portions of the liver that were visibly coloured blue were dissected and weighed. The percentage of total liver mass coloured in shams (N=4) and hepatectomized (N=3) fish was 100% and 34±14%, respectively.

Analytical techniques and calculations

A sample of whole blood (300 μl) was deproteinized in 1200 μl of 6% perchloric acid, set on ice and then centrifuged for 5min at 10000 ***g***. The supernatant was withdrawn and stored at 4˚C for up to 1 week prior to analysis of lactate and glucose. The pellet was stored separately and analyzed for total protein. The remaining blood was centrifuged for 5 min at 10000 ***g*** and the plasma was withdrawn and frozen at −80˚C for up to 1 week prior to analysis for ^{14}C radioactivity and total lipid content.

Frozen muscle, liver and heart were individually ground to a fine powder in liquid nitrogen using an insulated mortar and pestle. White muscle (100–200mg), liver (100–200mg) and heart (50mg) samples were homogenized on ice at 2500revsmin^{-1} for 3×10s using a Tissue Tearor (Biospec Products, Bartlesville, OK) in 1.0ml of 6 % perchloric acid. The homogenate was centrifuged for 5min at 10000 ***g*** and the supernatant analyzed for lactate and glucose. The pellet was analyzed for total protein.

Tissue lactate was determined enzymatically on 100 μl of deproteinized extract using Sigma lactate assay reagents. For analysis of tissue glucose, the perchloric acid extract was neutralized with 3mol l^{-1} K_2CO_3 (10 μl per 100 μl) and the $KClO_4$ salt was precipitated by centrifugation. Free glucose was measured in the supernatant as described by Bergmeyer (1965).

Tissue glycogen was isolated as described by Hassid and Abraham (1957) and analyzed for glucose after digestion with amyloglucosidase. Preliminary addition/recovery trials showed that 92±5% of the glycogen was recovered with this procedure.

To determine total protein, the perchloric acid pellets were washed three times with 900 μl of 6% perchloric acid and centrifuged for 5min at 10000 ***g***. Washed pellets were lyophilized using a Freeze-dryer 8 (Labconco, Kansas City, MO). The lyophilized pellets were digested in 3ml (muscle and liver), 1ml (heart) or 6ml (blood) of 2.5% KOH overnight at room temperature and mixed periodically until completely digested. Total protein content was measured by the Biuret method on 400 μl of the pellet digest mixed with 2.5ml of Sigma total protein reagent and incubated for 20min at 20˚C. Absorbance was measured spectrophotometrically at 540nm against bovine serum albumin (BSA) standards. A preliminary test yielded a recovery of 88±6% (N=4) of BSA washed and isolated by the procedure described.

To measure total lipid content, 100 μl plasma and 100mg of frozen liver and white muscle were homogenized in 3ml of ice-cold chloroform:methanol (2:1 v/v) for 3min. Lipids were extracted from the homogenates using the modified Folch method as described by Christie (1982). After centrifugation at 1500 ***g*** for 10min, a 150 μl sample of the lower chloroform layer was evaporated under a stream of dry nitrogen. To the dry lipid, 50 μl of 100% ethanol was added and the lipid concentration was determined by the sulphophosphovanillin reaction using Boehringer Mannheim reagents. Addition/

recovery trials showed a 92±8% (N=5) recovery of total lipid using this extraction procedure.

Total CO_2 in water was measured on a 1ml water sample by gas chromatography as described by Milligan and McDonald (1988) using a Varian 3300 gas chromatograph.

To assess ^{14}C incorporation into various metabolite pools, samples of tissues and water were counted to determine total tissue activity. Samples of frozen, powdered muscle (50–100mg), liver (50–100mg), heart (20–40mg) or plasma (50 μl) were digested in 1.0ml of Soluene-350 (Canberra-Packard, Downer's Grove, IL) in sealed glass scintillation vials. Digestion was accelerated by heating the vials to 54°C for 6h. Hionic fluor (10ml; Canberra-Packard) was added to the cooled vials and the vials were stored in the dark overnight to reduce chemiluminescence.

Tissue [^{14}C]lactate and [^{14}C]glucose were separated by HPLC using ion exclusion. The HPLC system consisted of a Beckman System Gold programmable multi-solvent pump and absorbance detector set to 208nm, connected to a Varian 4270 integrator and controlled by a NEC PC-8300 module. The samples were eluted with 8mmol l^{-1} H_2SO_4 (HPLC grade) delivered at a rate of 0.5ml min^{-1} through a Bio-Rad cation–H^+ precolumn and a Bio-Rad Aminex HPX-87H ion-exclusion column (300mm×7.8mm) maintained at 54°C. Adipic acid was added to 600 μl of perchloric acid extract to yield a final concentration of 20mmol l^{-1}, the samples were filtered through a 0.2 μm nylon filter and 20 μl was injected onto the column. Lactate and glucose peaks were identified by the injection of known standards. Eluent fractions of 0.5ml were collected with a Gilson 203 fraction collector, added to 5ml of Ready-Safe fluor (Beckman) and stored overnight in the dark prior to counting. Addition/recovery trials showed that 97±1% (N=5) of labelled glucose and lactate were recovered and that the glucose and lactate peaks neither overlapped nor co-eluted with other labelled metabolites.

To determine label incorporation into tissue protein, glycogen and lipid pools, samples of the protein (200 μl), glycogen (500 μl) and lipid (100 μl of the chloroform layer) digests were added to 5ml of Ready Safe fluor and stored in the dark overnight prior to counting.

Label incorporation into excreted CO_2 was measured in duplicate samples of 1.0ml of water injected into a CO_2 trap. The trap consisted of a 20ml glass scintillation vial sealed with a rubber septum and containing 1ml of 0.1mol l^{-1} HCl. A plastic well, containing filter paper soaked with 1mol l^{-1} methylbenzonium hydroxide (Sigma), was suspended from the septum. The traps were shaken on an orbital shaker at 100revs min^{-1} for at least 1 h, and the filter paper was removed and added to 5ml of Ready Safe fluor in a scintillation vial. Preliminary experiments using [^{14}C]$NaHCO_3$ indicated that the $^{14}CO_2$ trapping efficiency of this system was 95±5% (N=6).

All samples were counted on a Canberra Packard 1900 TR or Beckman LS 3801 liquid scintillation counter using automatic quench correction. All radioactivity data are expressed as disints min^{-1}.

All tissue metabolite and ^{14}C radioactivity data were corrected for trapped extracellular fluid using tissue extracellular fluid volume (ECFV) estimates for rainbow trout determined by Milligan and Wood (1986*a*) and Munger *et al.* (1991).

Blood [^{14}C]lactate incorporation into tissue and glycogen pools was corrected for

carbon recycling using [^{14}C]glucose incorporation data from the comparable study of Pagnotta and Milligan (1991), as described by Gleeson and Dalessio (1990) and Girard and Milligan (1992) according to the following equation:

$$\mu\text{mol } ^{14}\text{C[lactate] into glycogeng}^{-1} = \frac{(\text{disintsmin}^{-1} \text{ in glycogeng}^{-1})_{LA} - [(\mu\text{mol } ^{14}\text{C[glucose] in glycogeng}^{-1})_{GLU} \times (\text{blood glucose SA})_{LA}]}{(\text{blood lactate SA})_{LA}}, \quad (1)$$

where the subscripts LA and GLU indicate that the data came from the present study or from that of Pagnotta and Milligan (1991), respectively, and SA refers to specific activity, in $\text{disintsmin}^{-1}\,\mu\text{mol}^{-1}$.

The incorporation of blood lactate into tissue metabolite pools was calculated from tissue specific activities according to the equation:

$$\mu\text{mol blood } ^{14}\text{C[lactate] into metabolite tissue pool g}^{-1} = \frac{\text{metabolite disintsmin}^{-1}\,\text{g}^{-1}}{\text{blood metabolite SA}}. \quad (2)$$

The distribution of ^{14}C isotope in white muscle, liver, heart and extracellular fluid was calculated as a percentage of total isotope injected, assuming white muscle, heart, liver and extracellular fluid constitute 50%, 0.08%, 1.4% (Pagnotta and Milligan, 1991) and 27% (Milligan and Wood, 1986*a*) of body mass, respectively.

Significant differences (P<0.05) between groups were assessed with analysis of variance coupled with Dunnett's multiple-comparisons test (Zar, 1974).

Results

The metabolic changes associated with exhaustive exercise are typical for this species and are shown in Table 1. There was a significant depletion of muscle glycogen and accumulation of lactate in the muscle, blood, liver and heart after exercise (Table 1). Although the changes were not significant, because of the large variability, total lipid levels in muscle and plasma tended to decline immediately after exercise (Table 1). By 8 h into recovery, most metabolites measured in most tissues examined had returned to resting levels, indicating the fish had recovered from exercise.

Metabolite specific activities are shown in Table 2. At rest and during recovery from exercise both the blood lactate and glucose pools were in equilibrium with the heart and liver pools, since the specific activities of the metabolites were not different. However, at all times the specific activities of muscle lactate and glucose were significantly lower than that in blood, indicating a disequilibrium between the muscle and blood pools (Table 2). This disequilibrium extended to the muscle lipid pool, but not to the liver lipid pool. Despite the large increase in the muscle lactate pool after exercise, there was no reduction in muscle lactate specific activity compared to the resting state.

At rest and during recovery from exercise, 60–70% of the injected label was recovered in the water, muscle, extracellular fluid, liver and heart (Fig. 1). At rest, the bulk of the

Table 1. *Tissue metabolites prior to and following exercise in rainbow trout*

	Time (h)					
	Rest (*N*=5)	0 (*N*=6)	2 (*N*=5)	4 (*N*=4)	6 (*N*=5)	8 (*N*=5)
White muscle						
Glycogen[a]	9.96±1.58	2.62±1.18*	4.85±1.61*	5.36±1.09*	6.98±1.47	5.86±1.49
Lactate[a]	5.07±1.02	27.02±1.94*	19.81±4.62*	10.42±0.81	4.57±1.39	1.53±0.23
Glucose[a]	0.68±0.16	2.06±0.31*	2.27±0.74*	2.10±0.23*	1.75±0.19	1.10±0.46
Pyruvate[a]	0.19±0.06	0.41±0.11	0.30±0.12	0.18±0.01	0.11±0.07	0.08±0.03
Lipid[b]	299.15±58.18	111.28±41.00	106.91±24.19	145.39±31.47	103.46±63.52	200.6±55.65
Protein[b]	376.06±67.6	393.26±51.27	326.31±42.26	462.03±58.44	379.33±53.69	381.73±35.32
Blood						
Lactate[a]	0.83±0.21	5.78±0.61*	16.52±2.74*	13.66±3.13*	11.75±3.05*	5.30±2.25
Glucose[a]	2.70±0.15	5.91±1.31*	7.01±1.06*	4.36±1.08	5.75±0.77*	5.20±0.55
Lipid[b,c]	34.11±10.86	10.15±5.82	26.91±9.75	30.80±12.26	38.53±11.75	9.39±5.63
Protein[b]	225.02±15.07	208.91±38.66	328.10±83.27	299.46±6.40	228.77±45.34	201.47±70.39
Liver						
Glycogen[a]	107.88±45.82	139.41±27.06	54.09±14.85	59.47±8.95	64.32±45.85	36.53±11.25
Lactate[a]	1.32±0.25	7.49±2.79*	6.92±2.17	3.65±0.89	3.83±0.90	1.53±0.23
Glucose[a]	2.20±0.53	7.18±1.21*	5.45±0.81*	5.25±1.29	3.74±0.58	3.33±0.24
Pyruvate[a]	0.10±0.03	0.20±0.07	0.13±0.03	0.14±0.03	0.08±0.01	0.10±0.01
Lipid[b]	55.86±8.95	55.66±8.74	70.14±6.68	84.25±13.29	64.25±13.45	84.15±4.36*
Protein[b]	167.43±39.07	159.03±24.64	141.49±28.4	156.78±26.04	117.4±20.01	166.69±27.69
Heart						
Glycogen[a]	33.43±7.30	42.87±7.58	45.21±5.97	37.86±2.62	40.61±5.31	33.97±2.81
Lactate[a]	2.33±1.71	11.88±8.61*	8.91±1.84*	3.61±0.89	4.06±0.74	2.54±1.05
Glucose[a]	2.55±1.10	6.47±2.29	6.28±1.93	4.70±0.16	3.06±0.43	2.06±0.60
Protein[b]	213.48±38.96	178.81±39.92	152.07±34.97	224.15±72.41	142.05±36.61	182.29±67.18

[a]Expressed as μmol g^{-1} tissue; [b]expressed as mg g^{-1} tissue; [c]measured in plasma.
* indicates a significant (P<0.05) difference from corresponding value in resting fish.

Table 2. *Metabolite specific activities in various tissues prior to and following exercise in rainbow trout*

	Time (h)				
	Rest (*N*=5)	2 (*N*=5)	4 (*N*=4)	6 (*N*=5)	8 (*N*=5)
Blood					
Lactate	57.85±37.47	4.33±0.60	5.78±0.93	8.26±2.58	15.57±4.82
Glucose	4.20±0.97	1.45±0.52	3.47±1.35	2.47±1.04	4.14±0.74
Lipid[a]	0.45±0.34	0.18±0.12	0.11±0.06	0.04±0.01	0.87±0.78
White muscle					
Glycogen	0.06±0.02	0.39±0.13	0.78±0.31	0.18±0.07	0.29±0.18
Lactate	1.81±0.31†	1.31±0.26†	1.57±0.40†	3.24±0.70†	2.99±0.84†
Glucose	0.18±0.11†	0.64±0.44†	0.43±0.31†	0.54±0.09†	1.07±1.03†
Lipid	0.01±0.01†	0.01±0.01†	0.01±0.01†	0.01±0.01†	0.01±0.01†
Liver					
Glycogen	0.02±0.01	0.01±0.003	0.05±0.04	0.01±0.005	0.17±0.06
Lactate	10.16±3.34	3.77±1.60	8.40±5.08	6.18±1.03	7.63±2.72
Glucose	7.48±4.23	2.36±1.08	3.19±0.80	4.96±2.04	4.85±0.98
Lipid	0.45±0.06	0.18±0.07	0.26±0.08	0.56±0.27	0.39±0.06
Heart					
Glycogen	0.02±0.001	0.02±0.001	0.02±0.002	0.01±0.004	0.17±0.06
Lactate	23.12±8.62	5.83±0.44	19.72±10.08	7.71±1.64	3.77±1.29
Glucose	8.67±2.72	2.60±0.73	6.14±0.99	5.18±0.66	7.67±2.09

Means ± 1 S.E.M. (*N*=5).
Specific activities are expressed as disintsmin^{-1} nmol^{-1} or disintsmin^{-1} μg^{-1}.
[a]Measured in plasma.
† indicates a significant (P<0.05) difference from blood or plasma value at that time.

label (approximately 36%) was recovered in the water (as CO_2), with the muscle, liver and ECF accounting for 22%, 7% and 6% of the injected label, respectively. The total activity recovered in the heart accounted for no more than 0.25% of the total injected, either at rest or during recovery from exercise. After exercise, the tissue distribution of label changed, with more of the label being found in the muscle, particularly during the first 2h of recovery (30%). There was also a near doubling of the proportion of the label found in the ECF from 2h into recovery onwards.

When the label distribution by metabolite within a tissue is examined, some interesting trends emerge (Fig. 2). First, in all tissues examined, the proportion of total label accounted for increased during recovery from exercise. For example, in heart and plasma, at rest, only 50% of the total tissue activity was accounted for, but during recovery from exercise, the label accounted for increased to 90–100% (Fig. 2). Similar, though less dramatic, trends were observed in the white muscle and liver: at rest, only 30% of the total tissue activity was accounted for, but during recovery from exercise, we could account for 45–50% of tissue activity. Although label accountability remained high in muscle, liver and plasma throughout the recovery process, that in the

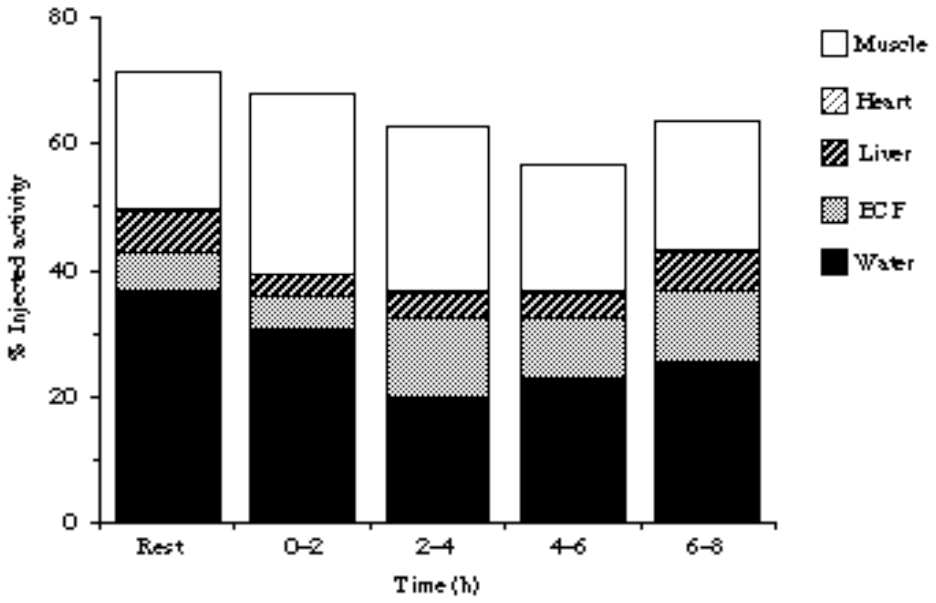

Fig. 1. Percentage of injected ^{14}C activity recovered in various tissues of rainbow trout prior to (rest) and following exhaustive exercise. For the sake of clarity, only means are shown. *N* as given in Table 1. ECF, extracellular fluid.

heart steadily declined with time, so that by 6–8h, only 20% of tissue activity was recovered.

The increase in tissue label accountability in muscle, plasma and heart is due almost entirely to the accumulation of labelled lactate. In muscle, the proportion of label as lactate increased from 24% at rest to 42% by 2h into recovery, despite the high endogenous lactate levels at this time (Table 1). Similarly in plasma and heart, the proportion of the label as lactate increased from 30% and 16% to 60–65% after exercise, respectively.

In liver, the story is somewhat different. At any given time, the majority of the label recovered was in the protein pool. After exercise, there was an increase in total label recovery, but the proportion in the protein pool did not change, instead the proportion in the glucose and lactate pools increased (Fig. 2).

During the first 2h into recovery, there was a net efflux of lactate from the muscle into the blood, as shown by the drop in muscle lactate and the rise in blood lactate levels (Table 1). Despite this net efflux, there was a significant incorporation of labelled blood lactate into the muscle lactate pool; 0–2h post-exercise saw a near 10-fold increase in label incorporation above rest levels (Fig. 3A). During the remainder of the recovery period (i.e. from 2 to 8h), the amount of labelled blood lactate entering the muscle lactate pool declined though, even at these times, blood lactate concentration exceeded that in the muscle (Table 1).

Lactate incorporation into the lactate and glucose pools of heart muscle (Fig. 3C) paralleled the profile of the blood (Fig. 3B), indicating that label appearance was due to

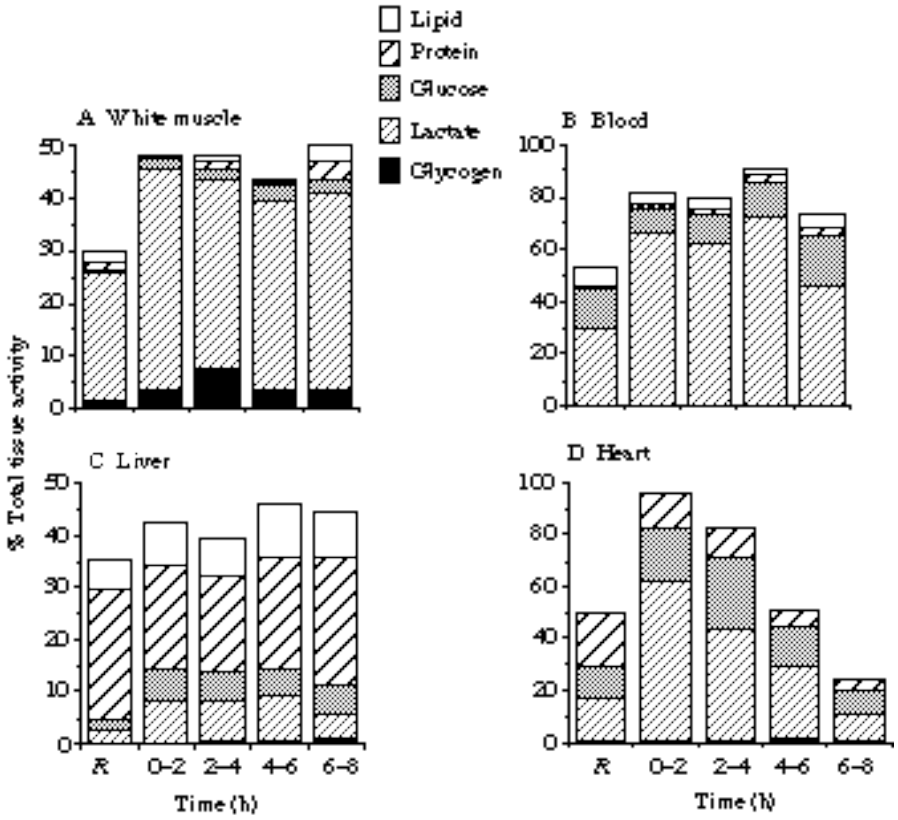

Fig. 2. Percentage of total tissue ^{14}C activity recovered in various metabolites at rest (*R*) and during recovery from exhaustive exercise. (A) White muscle; (B) blood; (C) liver; (D) heart. For the sake of clarity, only means are shown. *N* as given in Table 1.

uptake, rather than *in situ* metabolism. The main difference between heart and plasma, however, was in the amount of label incorporated into protein. There was very little label in plasma protein, but a large amount in heart protein, which appeared to increase as a result of exercise (Fig. 3C), even though there was no net change in protein content (Table 1).

Blood lactate met a variety of fates in liver (Fig. 3D). At rest, nearly four times as much blood lactate entered the protein pool as entered the lactate, glucose or lipid pools, and there was virtually no blood lactate entering the glycogen pool. After exercise, blood lactate incorporation into the lipid, protein, glucose and lactate pools increased by about three- to fourfold. However, only hepatic glucose and lactate contents showed a significant net increase during this period (Table 1).

Consequences of functional hepatectomy to exercise recovery

Ligation of the hepatic portal circulation did not block blood flow to the liver

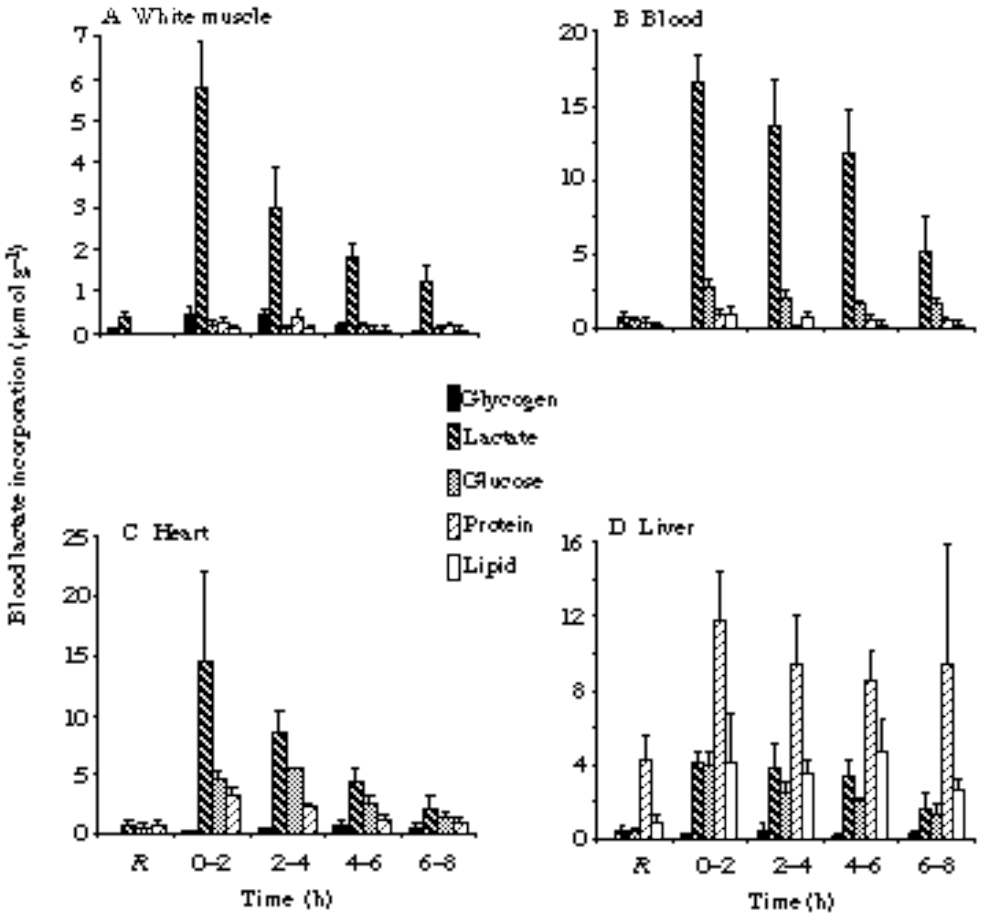

Fig. 3. Incorporation of blood lactate into various metabolites in (A) white muscle, (B) blood, (C) heart, (D) liver at rest (*R*) and following exhaustive exercise. Means ±1 S.E.M. *N* as given in Table 1.

completely, but was successful in severely compromising blood flow (see Materials and methods). Functional hepatectomy did not appear to alter the resting metabolite status of the fish as there were no significant differences between controls, shams or hepatectomized fish (Table 3). There were no apparent differences in the exercise ability of hepatectomized fish, since muscle glycogen depletion and lactate accumulation in the hepatectomized fish were the same as in controls and shams (Table 3). Immediately after exercise, blood lactate levels were significantly higher in the hepatectomized fish than in the other groups. The most striking effect of hepatectomy was an enhanced metabolic recovery from exercise. In hepatectomized fish, muscle glycogen and lactate levels returned to resting values by 6h, though blood lactate levels remained elevated (Table 3). In both control and sham groups, muscle glycogen and lactate levels were still significantly elevated above resting levels at 6h (Table 3).

Table 3. *Metabolite levels in various tissues prior to and following exercise in sham-operated and hepatectomized rainbow trout*

	Control			Sham-operated			Hepatectomized		
	Rest (*N*=5)	0 h (*N*=5)	6 h (*N*=5)	Rest (*N*=4)	0 h (*N*=4)	6 h (*N*=4)	Rest (*N*=3)	0 h (*N*=4)	6 h (*N*=4)
Blood									
Lactate	1.30± 0.52	5.94± 2.20*	4.74± 1.76*	1.18± 0.29	5.84± 1.53*	8.94± 5.27*	2.29± 1.76	8.91± 2.40*,†	5.99± 2.27*
Glucose	6.02± 3.28	8.33± 3.52	9.46± 3.49	6.95± 1.76	8.06± 2.800	9.91± 3.70*	4.88± 3.02	7.11± 4.08	4.62± 4.36
White muscle									
Glycogen	13.19± 1.96	0.97± 0.68*	4.22± 1.18*	8.90± 1.15	1.05± 0.29*	3.52± 1.02*	14.49± 1.72	1.24± 0.16*	9.22± 3.762†
Lactate	7.67± 1.01	27.36± 2.55*	10.23± 2.42*	5.28± 0.47	25.65± 3.19*	11.62± 2.02*	6.40± 0.24	22.93± 3.92*	6.23 0.66†
Glucose	1.62± 0.49	1.60± 0.29	2.57± 0.34	0.88± 0.35	1.82± 0.24	1.88± 0.29	1.94± 0.10	1.98± 0.36	2.47± 0.46
Liver									
Glycogen	75.47± 7.37	39.68± 15.96	49.64± 17.42	66.54± 11.82	69.23± 11.01	70.68± 8.56	59.41± 28.18	25.33± 21.67	54.22± 11.28
Lactate	1.53± 0.14	2.65± 0.12	1.04± 0.40	1.62± 0.48	2.17± 0.08	3.34± 0.35	2.90± 0.65	4.66± 1.53	4.78± 2.07
Glucose	5.22± 0.48	13.53± 1.95*	9.11± 2.43	6.11± 0.53	10.28± 0.63*	10.02± 0.48*	12.63± 4.85	7.65± 1.98	8.44± 1.06

Means ± 1 S.E.M.
All values are expressed as μmol g^{-1}, except for glycogen which is expressed as μmol glucose g^{-1}.
* indicates a significant (P<0.05) difference from the corresponding value in resting fish.
† indicates a significant (P<0.05) difference from the corresponding value in control and sham-operated fish.

Discussion

Methodology

The multiplicity of metabolic fates of lactate complicates the quantitative interpretation of isotopic distribution data (Katz, 1986). Of the many fates of lactate, oxidative and gluconeogenic processes dominate during the post-exercise recovery period in vertebrates (Withers *et al.* 1988; Gleeson and Dalessio, 1989; Pagnotta and Milligan, 1991; Girard and Milligan, 1992). There are two major problems associated with assessing the oxidative and gluconeogenic fates of labelled lactate: carbon recycling and carbon–carbon exchange.

Carbon recycling occurs when an end-product of lactate metabolism is utilized to form a second, common, end-product, such as occurs with the Cori cycle: blood lactate used for hepatic gluconeogenesis can re-enter the blood glucose pool. Since Cori cycle activity in fish is very low (Milligan and McDonald, 1988; Walsh, 1989; Pagnotta and Milligan, 1991), any error due to carbon recycling is probably minimal.

Carbon–carbon exchange reactions occur as labelled lactate carbons pass through the oxaloacetate pool and unlabelled carbons replace labelled carbons as pyruvate is converted to phosphoenolpyruvate (Katz, 1986). As a consequence, unlabelled carbon can replace labelled carbon in lactate in gluco- and glyconeogenic end-products, resulting in an underestimation of the contribution of lactate to glucose and glycogen synthesis.

The contribution of lactate to oxidative CO_2 production can be overestimated because of gluconeogenic CO_2 production produced by the decarboxylation reaction catalyzed by phosphoenolpyruvate carboxykinase (PEPCK). A related problem is the relative flux of lactate and glucose towards the Krebs cycle: if the flux from glucose exceeds that from lactate, then lactate oxidation tends to be overestimated. This is not likely to present a serious problem in the present study because, during recovery from exercise in trout, the net flux of lactate through the pyruvate pool is 4–5 times that of glucose (Pagnotta and Milligan, 1991).

The extent to which carbon recycling, carbon–carbon exchange reactions and non-oxidative CO_2 production affect the quantitative interpretation of the results depends upon the exercise state of the animal. The consequent limitations imposed upon the interpretation of the results of our study are addressed in the discussion which follows.

Lactate metabolism in vivo

Total label recovery in the whole animal was unaffected by exercise, averaging 60–70% of the injected activity both at rest and during recovery from exercise. The major fate of blood lactate was oxidation, with 20–30% of the label being recovered as CO_2 in water and blood. Although the proportion of label recovered as CO_2 did not change with exercise and recovery, the amount of blood lactate incorporated into CO_2 increased significantly during the first 2h into recovery, indicating there was an increase in blood lactate oxidation. The role of various tissues in lactate oxidation and the contribution of lactate oxidation to whole-animal metabolic rate at rest and during the first 2h into recovery, when oxygen consumption is significantly elevated, are modelled for a 100 g trout in Table 4. Under resting conditions, lactate oxidation can potentially account for up

Table 4. *Estimation of lactate oxidation in various tissues in a 100g rainbow trout at rest and during the first 2h of recovery from exhaustive exercise*

Tissue[a]	Lactate oxidation rate[b] (μmol g^{-1} h^{-1})	Lactate oxidized (μmol)	% $\dot{M}_{O_2}$
Rest, $\dot{M}_{O_2}$=2.34 μmol g^{-1} h^{-1}*			
Heart	6	0.96	0.62
Liver	1.2	3.4	2.2
Gills	0.14	1.09	0.70
Red muscle	6	12	7.6
White muscle	1.2	180	38.5
Total		197.5	42.2
0–2h post-exercise, $\dot{M}_{O_2}$=3.78 μmol g^{-1} h^{-1}*			
Heart	180	28.8	11.4
Liver	2.8	7.8	3.1
Gills	0.24	1.87	0.7
Red muscle	72	144	57.1
White muscle	13.1[c]	1310	520
	1.2[d]	60	7.9
Total		1493	592
		242.5	96

[a]Relative tissue mass: heart, 0.08g; liver, 1.4g; white muscle, 50g (Pagnotta and Milligan, 1991); gills, 3.9g; red muscle, 1g (Stevens, 1968).

[b]Lactate oxidation rates were determined under the following conditions. *At rest*: heart, *in situ* perfused heart at low workload and 1mmol l^{-1} lactate (Milligan and Farrell, 1991); liver, *in vitro* isolated hepatocytes incubated in medium containing 1mmol l^{-1} lactate (Mommsen *et al.* 1988); gills, *in vitro* isolated gill filaments incubated in medium containing 2mmol l^{-1} lactate (Mommsen, 1984); red muscle, assuming similar to heart at low workload; white muscle, assuming 20% of red muscle value (Bilinski and Jonas, 1972). *0–2h post-exercise*: heart, as in note b, except at 10mmol l^{-1} lactate and a high workload (Milligan and Farrell, 1991); liver, as above, except at 5mmol l^{-1} lactate (French *et al.* 1983); gills, as above, except at 10mmol l^{-1} lactate (Mommsen, 1984); red muscle, value for heart at 10mmol l^{-1} lactate and low workload, since the fish were not swimming during recovery (Milligan and Farrell, 1991).

[c]Assuming 20% of red muscle value at 10mmol l^{-1} lactate.

[d]Assuming 20% of red muscle value at 1mmol l^{-1} lactate.

*Measured in present study.

to 40% of resting metabolic rate. This estimate is in good agreement with that reported by Weber (1991) for resting trout. Though on a per unit mass basis, the oxidative potential of white muscle is considerably less than that of red muscle, it has the potential to play a major role in lactate oxidation by virtue of its sheer bulk (approximately 50% of body mass). The observation that at rest the bulk of the radioactivity in white muscle is in lactate lends support to the notion that white muscle has the potential to be a lactate-oxidizing tissue. During recovery from exhaustive exercise, the contribution of the various tissues to lactate oxidation and that of lactate to supporting metabolic rate increase, reflecting a simple increase in substrate availability. Note that, after exercise,

the potential of white muscle for lactate oxidation far exceeds the observed metabolic rate. It is unlikely that during the recovery period white muscle is oxidizing lactate at this very high rate because the extra- and intracellular acid–base disturbance associated with exercise (Milligan and Wood, 1986*b*) may depress oxidative metabolism. Although the influence of acid–base status on muscle metabolism has not been investigated, studies on trout hepatocytes indicate that acid–base disturbances similar to that observed after exercise inhibit lactate oxidation (Walsh *et al.* 1988).

Given that high concentrations of exogenous lactate ($10\,mmol\,l^{-1}$) can serve as the sole oxidative substrate for cardiac muscle over a range of power outputs (Milligan and Farrell, 1991), the conclusion that cardiac muscle can act as a significant sink for blood lactate is not unreasonable. On the basis of *in vitro* estimates of cardiac muscle oxygen consumption at various workloads (Farrell and Steffensen, 1987), it is estimated that blood lactate oxidation can fuel approximately 20% of cardiac aerobic metabolism at rest and easily 100% after exercise. This conclusion is consistent with the high proportion of radioactivity found as lactate in the heart (Fig. 2) and the notion that, when plentiful, blood lactate is a preferred substrate for cardiac muscle metabolism (Lanctin *et al.* 1980; Milligan and Farrell, 1991). Likewise, the estimate that cardiac lactate oxidation can account for 11% of total metabolic rate is not unreasonable as it is about twice that predicted from *in vitro* estimates of cardiac $\dot{M}_{O_2}$ at U_{crit} (Farrell and Steffensen, 1987), but within the limit of predicted maximal cardiac $\dot{M}_{O_2}$ of 4–5 $\mu mol\,min^{-1}\,g^{-1}$ (assuming a power output of 7–8 $mW\,g^{-1}$ and a cardiac efficiency of 20–25%; Farrell and Steffensen, 1987; Milligan and Farrell, 1991). This analysis indicates that, although it is a relatively small tissue (only half the mass of liver, for example), cardiac muscle has the potential to play a major role in the clearance of blood lactate.

The other major site of blood lactate clearance is the red muscle. Under resting conditions, assuming red muscle has a metabolic rate comparable to that of cardiac muscle (Bilinski and Jonas, 1972), blood lactate can fuel approximately 20% of aerobic metabolism. Estimating the potential of blood lactate as a fuel after exercise is more problematic, because there are no estimates of red muscle metabolic rate during recovery from burst-type exercise. However, assuming either (1) no change from rest or (2) an increased metabolic rate comparable to that of cardiac muscle, lactate can fuel 78–240% of red muscle metabolism. Again, this analysis illustrates the potential of muscle to act as an oxidative sink for lactate. Studies on red muscle metabolism under conditions that simulate a resting, swimming and exhaustively exercised fish are required to test this hypothesis adequately.

One of the more striking findings of our study is the accumulation of labelled lactate by the white muscle in both fish at rest and during recovery from exercise. This probably represents lactate uptake rather than carbon recycling *via* the Cori cycle (i.e. uptake of glucose which is then converted to lactate), because the amount of lactate incorporated into the muscle lactate pool was 240 times that of blood glucose incorporation into the muscle lactate pool (Pagnotta and Milligan, 1991). Lactate uptake occurred despite an electrochemical gradient favouring lactate release at rest and during the first 2h of recovery, which indicates some type of active uptake by the muscle (Turner and Wood, 1983).

Table 5. *Contribution of blood glucose and lactate to muscle glycogen resynthesis during recovery from exhaustive exercise in rainbow trout*

Hours after exercise	ΔGlycogen[a] (μmol g^{-1})	^{14}C[lactate] incorporated[b] (μmol g^{-1})	% Lactate[c]	% Glucose[d]
0–2	2.23	0.28	6.2	
2–4	0.51	0.49	47.8	0.2
4–6	1.62	0.05	3.7	0.4

[a]Difference in glycogen concentration between start and end of time interval indicated.
[b]Amount of blood ^{14}C[lactate] incorporated into glycogen, from Fig. 3.
[c]From present study.
[d]From Pagnotta and Milligan (1991).

There was an increase in blood lactate incorporation into the muscle glycogen pool after exercise. During the period of net glycogen synthesis, blood lactate is estimated to account for 4–6% of the total glycogen synthesized (Table 5). This represents direct *in situ* incorporation of blood lactate into the muscle glycogen pool given that blood glucose was found to account for less than 0.5% of the glycogen resynthesized (Table 5). These observations provide the basis for a very strong argument that lactate-based *in situ* glycogenesis is the means by which muscle glycogen is resynthesized after exercise. The biochemical pathway by which this is achieved remains elusive, but a very compelling argument is put forward by Moyes *et al.* (1992) that muscle glycogenesis proceeds by reversal of pyruvate kinase.

Role of the liver in post-exercise recovery

Given the conclusions drawn from the above discussion, it was of interest to determine the role of the liver in recovery from exhaustive exercise. We attempted to address this question by studying trout that had been functionally hepatectomized by restricting the blood flow to the liver. As pointed out in Materials and methods, we were partially successful, in that we were able to reduce blood flow to the liver by approximately 70%, thus compromising liver function. Two striking observations came out of this experiment: (1) hepatectomy did not interfere with the ability of trout to perform burst exercise and (2) muscle metabolic recovery was enhanced in hepatectomized fish.

The conclusion that hepatectomy did not interfere with the trout's ability for burst exercise is supported by the observation that, in controls, shams and hepatectomized fish, there were no differences in muscle glycogen or lactate content or blood glucose, either at rest or immediately after exercise. This suggests that, during exercise, white muscle is behaving as a self-contained unit. Muscle is not dependent upon blood-borne substrates (e.g. glucose derived from hepatic gluconeogenesis) to fuel its increased energy demand, instead it appears to rely exclusively on endogenous substrates, primarily glycogen. Limited exogenous fuel utilization by white muscle is consistent with the low hexokinase activity found in fish skeletal muscle (Crabtree and Newsholme, 1972; Moon and Johnston, 1980).

Enhanced muscle metabolic recovery in hepatectomized fish was the most surprising observation, which may be linked to the higher blood lactate levels seen in these fish immediately after exercise. During the exercise period, the liver may act as a sink for lactate released from the muscle. When this sink is plugged, by reducing blood flow, more lactate remains in the blood, causing the elevated blood levels relative to controls and shams. Isotopic studies demonstrated that there is a significant uptake of blood lactate and incorporation into the muscle glycogen pool during recovery. It would appear then, that white muscle in hepatectomized fish has greater access to blood lactate and utilizes this to replenish its glycogen store more rapidly. This implies that glycogen resynthesis in trout muscle may be retarded because of a non-recoverable loss of substrate (i.e. lactate) from the muscle, since the lactate released is utilized by the liver.

Metabolic recovery from exhaustive exercise in trout

The release of lactate from muscle represents 10–20% of the total produced. Of that released to the blood, 30–40% of the lactate serves as an oxidative fuel for such tissues as the heart and red muscle and, according the analysis in Table 4, has the potential to be a significant fuel. A substantial portion of the lactate released to the blood, some 20–30%, reappears in the muscle lactate pool, almost certainly as a consequence of direct uptake, and becomes incorporated into muscle glycogen. The mechanism(s) of lactate uptake by muscle and controlling factors are unknown. Nonetheless, these observations are very compelling evidence for lactate-based *in situ* glycogenesis as the mechanism for muscle glycogen restoration. Such a mechanism is not unique to trout white muscle, but appears to be the rule in ectotherms, since similar mechanisms operate in other fish species (Girard and Milligan, 1992), as well as amphibians (Withers *et al*. 1988; Fournier and Guderley, 1992) and reptiles (Gleeson and Dalessio, 1990; Gleeson, 1991).

It has been argued that the advantage of using muscle rather than the liver as a centre for lactate recycling may be related to a glycogen-sparing effect (Fournier and Guderley, 1992). *In situ* glycogenesis leads to a more quantitative recovery of lactate into glycogen than is possible with hepatic-based gluconeogenesis. The transfer of lactate from the muscle to the liver, *via* the blood, as required by the Cori cycle, means that a significant portion of the lactate could be channelled to other tissues and, thus, lost to muscle glycogenesis. Similarly, any glucose produced from muscle lactate may meet fates other than muscle glycogenesis. There is, then, a potential for lactate ‘leakage’, which would prevent quantitative recycling of lactate in the muscle. Certainly, the observation that the plugging of one of these ‘leaks’ by hepatectomy resulted in faster restoration of muscle glycogen lends support to this argument.

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