Recovery metabolism of skipjack tuna (*Katsuwonus pelamis*) white muscle: rapid and parallel changes in lactate and phosphocreatine after exercise

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Lactate, glycogen, and high-energy phosphate levels were measured in serial biopsies from tuna white muscle during recovery from 15 min of enforced swimming. Exercise caused glycogen and phosphocreatine levels to decrease sharply and lactate concentration to increase markedly (up to 150 $\mu mol \cdot g^{-1}$). Lactate was cleared from white muscle in less than 90 min, at rates comparable to those seen in mammals (about 1.3 $\mu mol \cdot g^{-1} \cdot min^{-1}$), and this was accompanied by nearly stoichiometric increases in white muscle glycogen (2 lactate : 1 glucosyl unit). The plasma lactate concentration remained elevated (35–40 mM) until lactate clearance from white muscle was completed, whereas the level of plasma glucose was constant (12–16 mM) for the entire 3-h recovery period. The exercise routine caused minimal changes in white muscle purine nucleotides apart from a slight, but significant, increase in IMP content. Transient changes in ATP appear to have resulted from short-term intense swimming activity noted during anesthetization. Unlike other teleosts, lactate clearance in tuna paralleled creatine rephosphorylation during recovery from exercise. We suggest that the postexercise adjustment of intracellular pH is responsible for this relationship. Lactate was seemingly metabolized within the white muscle mass, as indicated by *in situ* conservation of lactate carbon apparent from stiochiometric increases in white muscle glycogen levels. This prospect is discussed in view of low estimates of lactate utilization rates by other tissues and contrasted with expected high rates of whole-body lactate turnover during recovery.

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Le lactate, le glycogène et les phosphates utilisés pendant l'effort ont été mesurés dans des biopsies en séries prélevées dans le muscle blanc de Thonines à ventre rayé au cours de la période de récupération consécutive à une nage forcée de 15 min. L'exercice a entraîné une chute brusque du glycogène et de la phosphocréatine et une augmentation marquée de la concentration de lactate (jusqu'à 150 $\mu mol \cdot g^{-1}$). Le lactate est disparu du muscle blanc en moins de 90 min, à des taux comparables à ceux qui prévalent chez les mammifères (environ 1,3 $\mu mol \cdot g^{-1} min^{-1}$) et sa disparition s'accompagnait d'augmentations presque stoechiométriques du glycogène dans le muscle blanc (2 lactates : 1 unité de glucosyl). Le lactate est resté présent en concentration élevée dans le plasma (35–40 mM) jusqu'à la fin de la clearance du lactate dans le muscle blanc, alors que le glucose plasmatique est resté constant (12–16 mM) durant toute la période de récupération de 3 h. L'exercice n'a causé que des modifications minimes des nucléotides de purine dans le muscle, à l'exception d'une augmentation légère, mais significative, du contenu en IMP. Des modifications temporaires du contenu en ATP semblent avoir résulté de la courte période d'activité de nage intense observée au moment de l'anesthésie. Contrairement à ce qui a été enregistré chez d'autres téléostéens, la clearance du lactate s'accompagnait d'une rephosphorylation parallèle de la créatine au cours de la récupération qui a suivi l'exercice. Nous croyons que l'ajustement du pH intracellulaire à la suite d'un exercice est responsable de cette relation. Le lactate semble être métabolisé dans la masse même du muscle blanc, puisque le carbone du lactate est conservé *in situ*, comme l'indiquent les augmentations stoechiométriques du glycogène dans le muscle blanc. Cette théorie est examinée à la lumière des valeurs peu élevées des taux d'utilisation du lactate dans les autres tissus et confrontée aux taux élevés de remplacement du lactate total auxquels on doit s'attendre au cours de la récupération.

[Traduit par la rédaction]

Introduction

Tuna can sustain relatively high speeds of 3–5 body lengths/s indefinitely, yet they are also capable of short-term speeds of up to 20 body lengths/s. This intriguing combination of sprint and endurance abilities is supported by a metabolism with several unusual features. For example, skipjack tuna (*Katsuwonus pelamis*) are able to fuel burst swimming through an exceptional glycolytic capacity in white muscle, which can produce lactate concentrations of about 100 $\mu mol \cdot g^{-1}$ (Guppy *et al.* 1979; Hulbert *et al.* 1979).

Removal of elevated plasma lactate and metabolically pro-

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duced protons following exhaustive exercise occurs very rapidly in tuna (Barrett and Connor 1964; Perry *et al.* 1985). In fact, rates of lactate turnover in postexercise tuna resemble those observed in mammals (Weber *et al.* 1986), suggesting that the circulatory translocation of lactate accounts for some portion of the white muscle lactate clearance in recovery. In contrast, other teleosts appear to retain most of the lactate formed during exhaustive exercise within the white muscle mass and require long periods for lactate clearance (Batty and Wardle 1979; Milligan and McDonald 1988).

The rate of recovery from exercise in muscle of tuna may also be more similar to that of mammals than to that of other teleosts. Barrett and Connor (1964) found that the glycogen content of tuna muscle recovers within, at most, 2 h following exercise, whereas recovery takes up to 12–24 h within trout muscle (Milligan and Wood 1986; Schulte *et al.* 1992). It is not known if the rate of recovery of other intracellular metabolites, such as lactate and phosphocreatine, is also rapid in recovering tuna muscle. In studies of recovery from exercise in other teleosts, such as trout, the fish is exercised and terminal samples are collected by freeze clamping at selected times after exercise. Muscular contractions during sampling can cause artefacts resulting from changes in the concentrations of intracellular metabolites such as phosphocreatine and ATP. Nevertheless, with care and rapid sampling, it has been possible to follow the changes in intracellular metabolites after exercise in trout (Pearson *et al.* 1990; Schulte *et al.* 1992). It is not feasible to use this approach for tuna since 'resting' skipjack typically swim at 1–2 body lengths/s to keep from sinking and to 'ram ventilate' the gills (Guppy *et al.* 1979). The capture of free swimming tuna is not compatible with the requirement to reliably collect tissue samples from tuna that have not struggled.

We have used an alternative method. Recently Bushnell *et al.* (1990) described a protocol for spinally blocking tuna, which permitted continuous measurement of cardiovascular parameters under controlled conditions. By using this procedure, and sampling muscle with a high-speed biopsy technique, we are able to separately collect and rapidly freeze muscle samples from the same fish at different times after exercise. The advantage of this approach is that we can analyze detailed muscle metabolite changes from individual fish and consequently use fewer fish overall to elucidate general patterns of recovery metabolism than would be necessary if using a terminal sampling procedure.

In the present study, the metabolism of white muscle carbohydrate and high-energy phosphate was investigated in tuna during recovery from exercise. The rate of lactate clearance from white muscle closely matched the rate of glycogen replenishment and, interestingly, paralleled the rapid rephosphorylation of creatine seen in exercise recovery. The rate of recovery is comparable to that seen in mammals, and we suggest that tuna conserve lactate to facilitate rapid recovery from exercise.

Materials and methods

Experimental animals

Live skipjack tuna, *Katsuwonus pelamis* (1–2 kg), were purchased from a local fisherman and maintained in outdoor holding tanks at the Kewalo Research Facility (Southwest Fisheries Center Honolulu Laboratory, National Marine Fisheries Service, National Oceanic and Atmospheric Administration). The tanks were supplied continuously with aerated sea water at 25 ± 2°C. Fish were not fed and were used for experiments within 3 days of capture.

Exercise and biopsy protocol

In preliminary studies, terminal muscle samples were taken to determine resting and postexercise levels of white muscle metabolites. Eleven unexercised fish were netted from the holding tank and killed with a sharp blow to the head. A sample of epaxial white muscle was dissected from behind the dorsal fin of each fish, freeze-clamped between Wollenberger tongs and immersed in liquid nitrogen as in earlier studies (Guppy *et al.* 1979). Very little struggling occurred during this procedure, and less than 20 s elapsed from the time of capture to the point of tissue immersion in liquid nitrogen. Three other fish were sampled in the same manner after first being isolated individually in another holding tank and chased with capture nets for 15 min. All dissected muscles were stored frozen over dry ice prior to homogenization and assay procedures. The two groups were designated unexercised freeze-clamped and exercised freeze-clamped, respectively.

A serial biopsy technique was developed to assess *in vivo* metabolite changes in tuna white muscle during recovery from exercise. Each fish was first exercised as described above, then captured by net and guided into a plastic bag containing buffered MS-222 (tricaine methane sulfonate, 1.0 $g \cdot L^{-1}$ with 1.0 $g \cdot L^{-1}$ $NaHCO_3$) dissolved in oxygen-saturated seawater. This group is referred to as exercised biopsied. Tuna taken from the holding tank and anesthetized without being chased served as unexercised controls and are referred to as unexercised biopsied. Immediately following anesthesia (about 2 min), each fish was placed on an operating table and the gills were irrigated with recirculated water containing a low level of anesthetic (MS-222, 0.1 $g \cdot L^{-1}$). At this time, initial biopsy samples were obtained from the epaxial muscle, using a 3-mm bore at 10–15 mm depth (ALKO Diagnostic Corporation). The tissue sample was drawn from the fish by suction into a chamber cooled with liquid nitrogen, and frozen almost instantly. Bleeding was minimal and was stopped easily by inserting a cotton applicator tip into the puncture. Skin was separated from the white muscle biopsy under liquid nitrogen and the sample stored over dry ice.

After the first sample was taken for biopsy, the fish was turned ventral side up on the operating table and the ventral aorta was cannulated following procedures described previously (Jones *et al.* 1986). The fish was then righted and a 20-gauge needle, 6 cm long, was inserted through the dorsal musculature (just lateral to the second spine of the dorsal fin) to the level of the spinal cord. Lidocaine hydrochloride (0.3 mL, 2% w/v) was then administered to establish a spinal block and arrest contractions of the swimming muscles. The needle was left in place for periodic re-injections throughout the experiment. Once secured in a foam-lined brace that was supported in a Perspex holding box (see Bushnell *et al.* 1990), the fish was presented with continuously flowing seawater (35 $L \cdot min^{-1}$) and allowed to recover from anesthesia.

All subsequent samples for biopsy were taken from the tuna while in this restrained, spinally blocked position. To do this, the water level in the holding chamber was lowered to expose the back of the fish. The gills remained submerged during this time. Samples for biopsy were taken from an area directly lateral to the dorsal midline, 10–20 cm posterior to the spinal needle insertion site. Samples of white muscle and blood (0.25 mL) were taken at 20, 40, 60, 100, 120, 140, and 180 min into the recovery period. Plasma was extracted immediately from red cells by centrifugation, then deproteinized with one volume of 0.6 M perchloric acid and stored on dry ice. A terminal muscle sample was also taken after the fish was killed with a blow to the head at the end of the experiment.

Tissue preparation and analysis

Biopsy samples were homogenized with an Ultra-turrax tissue homogenizer in 2 mL of 8% perchloric acid in 40% ethanol that had been precooled over dry ice. Homogenization temperature was maintained below −20°C. A 50-μL aliquot of homogenate was removed for measurement of muscle glycogen, and the remainder was centrifuged for 10 min (7500 × *g*, 2°C) in a Jouan table-top centrifuge. Potassium perchlorate was precipitated from the supernatant with 1 M KOH and the neutralized sample was stored on dry ice.

TABLE 1. Metabolites in white muscle of skipjack tuna

	Freeze-clamped unexercised (N = 11)	Biopsies from spinally blocked tuna				Freeze-clamped exercised (N = 3)
		Initial		Final (180 min)		
		Unexercised (N = 5)	Exercised (N = 4)	Unexercised (N = 5)	Exercised (N = 4)	
PCr (% total Cr)	74.6±3.4	54.5±9.6[a]	20.1±8.7[a,b]	75.7±3.2	76.5±8.5	11.5±4.7[a,b]
Lactate (μmol · g^{-1})	7.3±3.8	38.5±10.4[a]	107.5±36.7[a,b]	11.9±8.1	14.9±15.7	75.8±4.8[a,b]
Glycogen (μmol glucosyl units · g^{-1})	145.0±34.4	127.3±56.9	88.6±32.4[c]	145.6±40.1	139.3±53.6	74.6±25.1[d]
Pyruvate (μmol · g^{-1})	0.05±0.01	0.39±0.14[a]	0.79±0.30[a]	0.05±0.01	0.19±0.09	0.14±0.04[e]

NOTE: Values are given as means ± SD. Freeze-clamped unexercised fish were taken directly from the holding tank, whereas freeze-clamped exercised fish were chased for 15 min and then killed immediately. Samples for biopsy were taken from both exercised and unexercised spinally blocked tuna. Differences were significant at $p < 0.05$.

[a]Significantly different from freeze-clamped controls and final biopsy (unexercised and exercised).

[b]Significantly different from initial biopsy, unexercised.

[c]$p = 0.09$ for comparison with freeze-clamped controls, and $p < 0.05$ for comparison with final biopsy, exercised.

[d]Significantly different from freeze-clamped controls and final biopsy, unexercised.

[e]Significantly different from all means except final biopsy, exercised.

Total creatine (creatine (Cr) + phosphocreatine (PCr)) content remains constant for a given tissue (Connett 1988) and was used as an initial reference for all muscle metabolites measured. This is a reliable normalization procedure (Sabina *et al.* 1983) that avoids possible inaccuracies associated with weighing small, deep-frozen tissue samples. Conversion to micromoles per gram, for comparison with other studies, was made after determining total creatine content in tuna white muscle samples of known weight. A factor of 39 (μmol total creatine · g white muscle^{-1}) was used for these conversions.

Muscle lactate, glucose, Cr and PCr were measured according to Bergmeyer (1985), modified for use with microtitration plates (0.3 mL) and a Titertek Multiskan plate spectrophotometer. Pyruvate was determined fluorimetrically following Lowry and Passonneau (1972). Glycogen was measured in aliquots of white muscle homogenate and is presented as micromoles of glucosyl units per gram of tissue. Muscle glycogen was digested by incubating the homogenates for 3 h at 40°C with amyloglucosidase (Boehringer Mannheim, 2 mg · mL^{-1}) in acetate buffer, pH 4.8 (Bergmeyer 1985). Perchloric acid (25 μL, 70%) was used to halt the incubation, and glucose was determined in the extract after neutralization. Assays modified for the plate spectrophotometer were also used to measure glucose (Sigma Diagnostics assay) and lactate (Bergmeyer 1985) in the neutralized plasma samples.

The purine nucleotides adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), and inosine monophosphate (IMP) were measured by high-performance liquid chromatography using an LKB 2152 controller and 2150 titanium pump coupled to a 2220 recording integrator. Column preparation and elution conditions were as described by Schulte *et al.* 1992. Briefly, an Aquapore AX-300 7-μm weak anion exchanger (Brownlee Labs) was used for the separation process, and detection was made using a BIO-RAD flow-through uv monitor (254 nm). An isocratic elution was established initially (60 nM KH_2PO_4, pH 3.2) for 5 min, followed by a linear gradient (60 mM KH_2PO_4, pH 3.2, to 750 nM KH_2PO_4, pH 3.5) over 10 min. The final concentration and pH were maintained for 12 min. Between runs the column was re-equilibrated for 6 min with the starting buffer. The column temperature was 55°C and the flow rate was 2 mL · min^{-1} throughout.

Data analysis

Linear regression was used to determine break points in the curves describing the recovery of white muscle lactate and PCr. Break points were used as estimates of recovery time for these metabolites, and means from exercised and nonexercised tuna were compared using the two-sample t-test. Comparisons of all other metabolite concentrations in the white muscle of the various groups of tuna were assessed with Tukey's multiple comparison test. Dependent t-tests were used to compare metabolite levels in biopsies taken at different times throughout the recovery period.

Results

Muscle metabolites after exercise

Terminal muscle samples from the exercised freeze-clamped tuna show significantly elevated lactate and pyruvate levels and depressed glycogen and PCr levels (Table 1), consistent with the utilization of anaerobic glycolysis to fuel intense white muscle activity. Similarly, the initial biopsy sample from the exercised biopsied fish had significantly elevated lactate and pyruvate and depleted PCr relative to that from the unexercised freeze-clamped tuna (Table 1). Glycogen in the first biopsy sample was reduced, but not significantly, compared with that in the unexercised freeze-clamped fish (p = 0.09), which likely reflects the large variation associated with these values.

An intermediate metabolite profile was observed in the initial biopsy samples of the unexercised biopsied tuna (Table 1), with lactate and PCr levels significantly different from those in both unexercised and exercised freeze-clamped fish. These changes were probably the consequence of the fish struggling prior to complete anesthesia, perhaps combined with a direct effect of the MS-222 anesthetic on muscle metabolism (see Van den Tillart *et al.* 1989).

It should be noted that the relative amount of Cr in the phosphorylated state is very high in tuna white muscle. In mammals and other teleosts, PCr levels of 30–50% of total creatine are typically measured. Van Waarde *et al.* (1990) suggested that, based on measurements with NMR, *in vivo* Cr was 80% phosphorylated in teleost white muscle, and they asserted that lower levels arise because of handling stress and (or) less than instantaneous freezing of tissue samples. The comparable values of 75% in white muscle of resting and recovered tuna presented in this study (Table 1) attest to the reliability of the freezing methods used.

There was a significant, yet transient, decline in ATP concentration in the exercised biopsied fish, but exercised freeze-

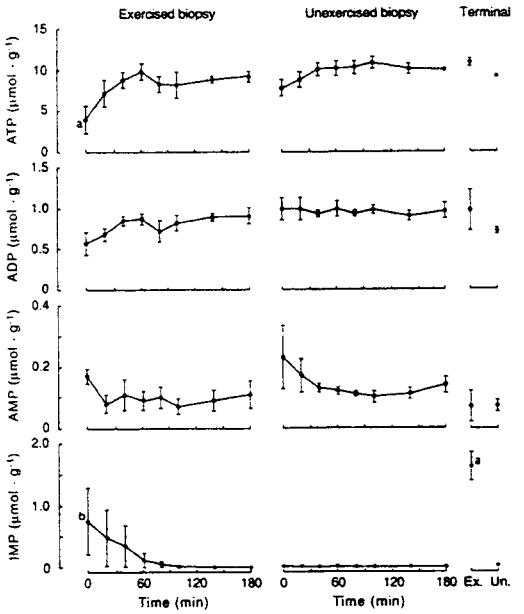

FIG. 1. Purine nucleotide levels (mean ± SE) in white muscle biopsies of unexercised ($N = 5$) and exercised ($N = 4$) tuna. Purine levels in muscle from terminally sampled, unexercised (Un., $N = 11$) and exercised (Ex., $N = 3$) fish are also shown. *a*, significantly different from Un., $p < 0.001$; *b*, significantly different from Un., $p < 0.05$.

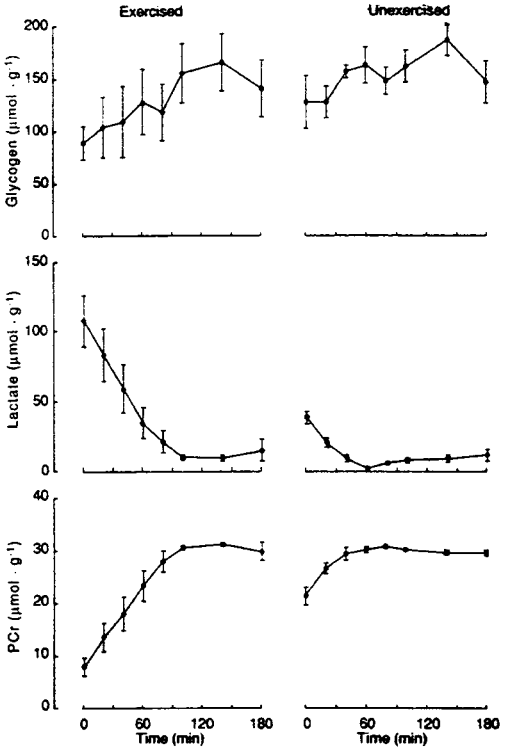

FIG. 2. Glycogen, lactate, and PCr levels (mean ± SE) in white muscle biopsy samples from exercised ($N = 4$) and unexercised ($N = 5$) skipjack tuna. See Table 1 for statistical analysis.

clamped fish showed no changes in white muscle ATP relative to resting controls (Fig. 1). IMP increased significantly in both groups of exercised fish, whereas there were no changes evident in either of the ADP or AMP pools. No significant changes in the purine nucleotides were evident among the unexercised biopsied fish. Levels of white muscle purine nucleotides in all biopsied fish were indistinguishable from resting concentrations within 20–40 min after exercise.

Lactate, glucose, and glycogen in recovery

Changes in white muscle lactate and glycogen during 180 min of recovery from exercise are illustrated in Fig. 2. In both groups of biopsied fish the lactate recovery curves declined initially in a linear fashion and after 80–100 min remained constant at the pre-exercise level. Lactate concentrations in the final biopsy sample taken from exercised and unexercised biopsied fish were not significantly different from the resting level (Table 1). Biopsy glycogen concentrations were more variable than lactate concentrations in recovery, and significant differences between initial and final tissue levels were detected in only the exercised group (Table 1). However, most of the variation in recovering fish appeared to result from differences between individual fish. Consequently, *t*-tests on paired data indicate that glycogen concentrations in the white muscle of exercised biopsied fish were significantly higher in the final biopsy sample than at the start of the recovery period. Glycogen concentration in the final biopsy sample was not different from that of unexercised freeze-clamped fish at rest (Table 1) and, in fact, complete replenishment was evident at 80–100 min (glycogen concentration at 100 min was 154 ± 55.5 $\mu mol \cdot g^{-1}$, not significantly different from the final biopsy glycogen content).

The plasma lactate concentration in the exercised biopsied tuna was essentially steady, between 35–40 mM, during the first half of the recovery period (Fig. 3) and only began to decline after muscle lactate had reached the resting level. In the unexercised biopsied fish, plasma lactate content dropped steadily between 20 and 100 min recovery and remained constant thereafter at about 10 mM. Plasma was not obtained from unexercised freeze-clamped fish, but the apparent resting steady state achieved in recovery by the unexercised biopsied fish is comparable to previous measurements of plasma lactate in resting skipjack tuna (Perry *et al.* 1986).

The plasma concentration of glucose remained in a constant steady state throughout the recovery period in biopsied fish (Fig. 3). The glucose concentration averaged 12.9 ± 2.9 and 13.6 ± 3.5 mM (mean ± SD) for the control and exercised biopsied groups, respectively.

Estimates of recovery time (lactate and phosphocreatine)

Figure 4 depicts lactate and PCr concentrations in tuna white muscle from representative exercised and unexercised biopsied fish during recovery. Every fish showed the same general recovery pattern consisting of an initial phase of rapid change in lactate and PCr levels followed by a period in which the levels of these metabolites changed much more slowly. Both

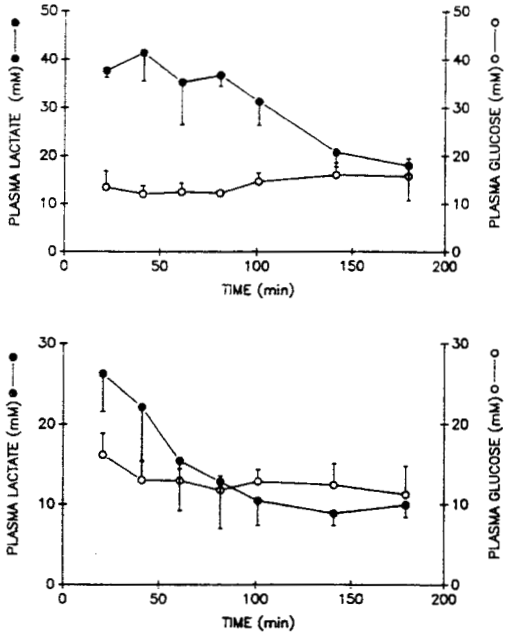

FIG. 3. Lactate and glucose levels (means ± SE) in plasma of spinally blocked tuna during recovery from exercise. Upper panel, fish that were exercised for 15 min. Lower panel, unexercised controls.

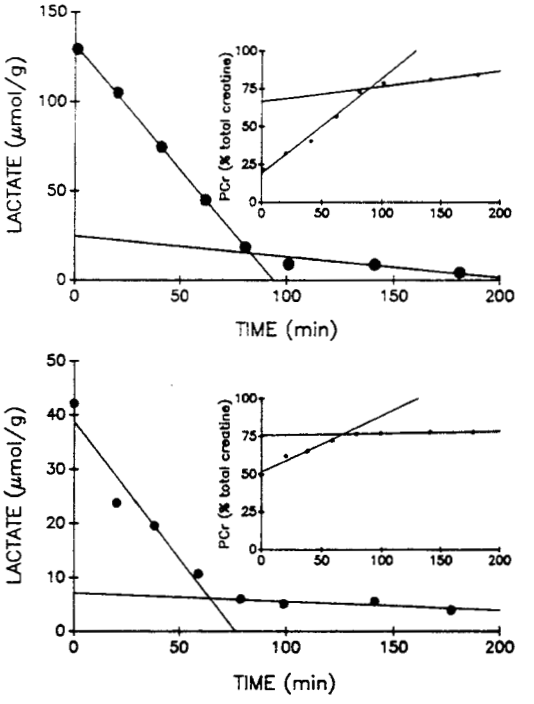

FIG. 4. Lactate and PCr in white muscle of representative spinally blocked tuna, showing regression lines for determining break points in metabolite recovery. Upper panel, a fish that was exercised for 15 min. Lower panel, an unexercised spinally blocked fish.

stages were linear, which permitted the estimation of recovery time by solving a set of regression equations for each of the fish biopsied. This analysis assumes a homogeneous distribution of muscle metabolites, which appears to be valid on the basis of the consistent changes in lactate and PCr levels observed during recovery for each fish despite random selection of biopsy sites. The variability in muscle glycogen content during recovery prevented the use of the breakpoint determination procedure on these data.

It became apparent that the experimental protocol produced a wide range of recovery times, rather than two discrete groupings, which reflected the degree of muscle lactate accumulation or PCr depletion. This essentially agrees with the earlier observation that the unexercised biopsied fish exhibited a muscle metabolite profile that was intermediate to that of the resting and exercised freeze-clamped tuna, probably resulting from the uncontrolled activity during anesthetization. The recovery times overlapped somewhat between exercised and unexercised biopsied fish, and therefore it seemed more appropriate to consider recovery time as a continuum with respect to exercise intensity. This is emphasized in Fig. 5, in which recovery time is displayed as a linear function of the concentration of both lactate and PCr in the first biopsy sample obtained from individual fish. As a result of this overlap, the average lactate (and PCr) recovery time for the exercised biopsied fish was only marginally different from the lactate (and PCr) recovery time for the unexercised biopsied fish (Table 2).

Another interesting outcome of the comparisons in Table 2 was that the time necessary for lactate recovery, for either the exercised or nonexercised biopsied fish, was not significantly different from the respective PCr recovery time. Furthermore, if individual biopsied fish were once again considered, then there was a striking synchrony between the rates of lactate disappearance and PCr repletion (Fig. 6 inset). There was also a close relationship between the concentrations of lactate and PCr in the individual biopsy samples (Fig. 6). This suggests that, unlike other teleosts that have been studied, some regulatory mechanism seemingly operates to tightly couple major adjustments of these metabolites in tuna white muscle.

Discussion

Muscle lactate and glycogen recovery

The disappearance of white muscle lactate levels at a rate of 1.3 $\mu mol \cdot g^{-1} \cdot min^{-1}$ is up to 20 times faster than has been measured for trout (0.055–0.12 $\mu mol \cdot g^{-1} \cdot min^{-1}$). This unusually rapid clearance of lactate is intermediate between the rate of clearance for humans (0.66 $\mu mol \cdot g^{-1} \cdot min^{-1}$) and rats (about 2.5 $\mu mol \cdot g^{-1} \cdot min^{-1}$) following maximal exercise (Hermansen and Vagge 1977; Meyer and Terjung 1979).

Weber *et al.* (1986) noted that despite high rates of whole-body lactate turnover in tuna, lactate recovery apparently occurs too quickly in white muscle to result from turnover alone, suggesting that some portion of the lactate remained in the white muscle as a substrate for postexercise, *in situ* metab-

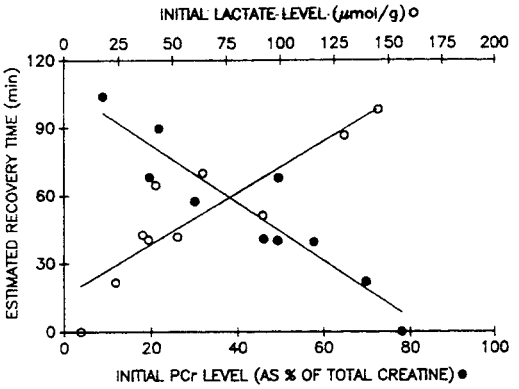

FIG. 5. Estimated recovery time in relation to lactate and PCr concentration determined in the initial biopsy of individual fish. Points shown on the lower x-axis are mean lactate and PCr levels determined in 11 resting tuna (Table 1). Regressions: $y = -1.28$ (PCr) + 107.9, $R^2 = 0.93$, and $y = 0.57$ (lactate) + 15.8, $R^2 = 0.88$.

TABLE 2. Recovery times of PCr and lactate in tuna white muscle

	Recovery time (min)	
	Exercised fish ($N = 4$)	Nonexercised fish ($N = 5$)
PCr	$79.9 \pm 20.9^{a,b}$	42.4 ± 16.4^{b}
Lactate	76.4 ± 20.3^{a}	42.3 ± 15.2

NOTE: Values are given as means ± SD. Within each test group, PCr recovery time closely matches that of lactate (see also Fig. 5), whereas differences between exercised and unexercised fish were only marginally different.

[a] $0.07 < p < 0.08$ for comparison with nonexercised fish.

[b] $p > 0.05$ for comparison with lactate recovery time.

olism. In some teleosts, in which rates of lactate turnover are greatly mismatched with actual clearance rates from white muscle during recovery from exercise (Milligan and McDonald 1988), retention and utilization of lactate within the white muscle mass is likely the dominant means of lactate disposal. Such a proposal of *in situ* metabolism of muscle lactate is prevalent throughout the area of exercise-recovery metabolism of both mammals and ectotherms. Some evidence comes from Hermansen and Vaage (1977) who biopsied human quadriceps muscle following maximal exercise and point out that the closely matched rates of lactate and glycogen change in muscle, combined with a small arterio-venous difference for lactate, suggest direct reconversion of lactate to glycogen. The enzymic steps involved in the process are not understood well (see Bonen *et al.* 1989 for a partial review), but numerous studies on vertebrate muscle propose varying degrees of *in situ* conversion of lactate to glycogen and demonstrate the dependence of the pathway on muscle fiber type, pH, arterial lactate concentration, and an extramitochondrial pathway of glycogen resynthesis (Bonen *et al.* 1990; Johnson and Bagby 1988; Pagliassotti and Donovan 1990). One proposed pathway involves malic enzyme and phosphoenolpyruvate carboxykinase (Connett 1979). Neither of these enzymes has been

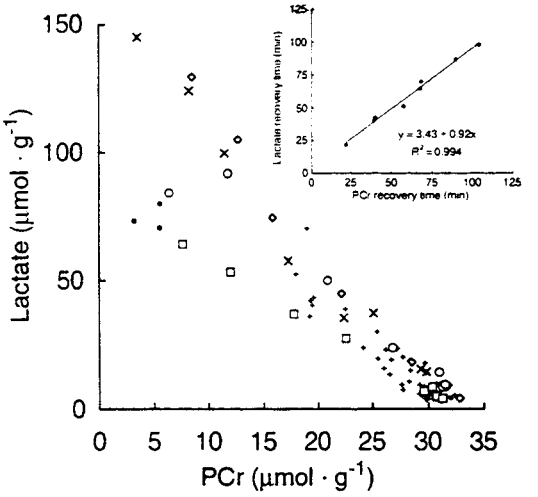

FIG. 6. White muscle lactate in relation to PCr in biopsy samples from exercised (large symbols; each symbol type represents a different fish) and unexercised (small crosses; individuals not depicted) spinally blocked tuna. Also shown are measurements from muscle taken from three fish immediately following 15 min exercise (small ●). Inset: Estimated lactate recovery time in relation to estimated PCr recovery time for individual fish.

looked for in the white muscle of tuna, but they are present in the white muscle of marlin (Suarez *et al.* 1986).

Results of the present study support and extend the suggestion that tuna white muscle clears lactate *in situ*, since the nearly stoichiometric changes in the lactate and glycogen recovery profiles (i.e., 2 lactate removed : 1 glucosyl unit reformed) indicate mainly a glyconeogenic fate for most of the lactate produced during burst activity. The extent to which this result approaches a quantitative representation of lactate incorporation into white muscle glycogen is reinforced by estimates of minimal glycogenesis from plasma glucose and low capacities for other tuna tissues to utilize lactate (discussed below).

Plasma lactate and glucose: utilization in tuna tissues

Plasma lactate concentration in the exercised tuna remained essentially constant for the period during which muscle lactate declined sharply. In terms of the kinetics of lactate, the restrictive condition of a plasma steady state means that lactate had to enter the plasma pool at the same rate as that at which it was removed. Furthermore, a 35–40 mM level of plasma lactate suggests, indirectly, a high rate of flux through the plasma pool in tuna (see Weber *et al.* 1986). In the postexercise state, white muscle is the probable source of plasma lactate that could be utilized either oxidatively or for gluconeogenesis in other tissues. In mammals, the primarily oxidative disposal of lactate observed at rest and during sustained, aerobic activity has led to the formation of the 'lactate shuttle' hypothesis (Brooks 1986), which proposes the translocation of lactate from producing to consuming muscle fibres.

It is not likely that oxidative metabolism is responsible for the removal of white muscle lactate in tuna. The oxygen consumption of skipjack tuna swimming at 2–5 lengths/s is calculated to be 0.68 $\mu mol \cdot g^{-1} \cdot min^{-1}$ (Gooding *et al.* 1981),

which is 4 times the rate in a recovering trout (Milligan and McDonald 1988). This rate of oxygen consumption is still about 30 times too slow to account for the rate of lactate disappearance by oxidation. It is possible that tuna have higher oxygen consumptions during recovery. However, it is unlikely that red muscle or the heart of tuna even have the capacity to oxidize lactate at the rate required. Based on maximal estimates of mitochondrial oxidation of pyruvate, tuna red muscle and heart ventricle could maximally oxidize 0.9 and 2.5 μmol lactate $\cdot g^{-1} \cdot min^{-1}$, respectively (Moyes *et al.* 1992). Thus, in a 1-kg animal, red muscle (assume 70 g $\cdot kg^{-1}$) could oxidize about 5 mmol of lactate and the heart (4 g $\cdot kg^{-1}$, Farrell *et al.* 1992) could oxidize about 0.8 mmol of lactate in 80 min. Combined oxidation in these tissues would therefore account for the removal of less than 10% of an initial white muscle lactate load of 72 mmol (based on 108 μmol $\cdot g^{-1}$, Table 1). If the temperature of tuna red muscle was 10°C higher than the ambient temperature (Hochachka *et al.* 1978) then maximal oxidation would still account for less than 15% of the lactate clearance.

The low capacity of tuna red muscle to oxidize lactate may mean that red muscle is glyconeogenic to some degree, using lactate derived from white muscle in a manner similar to the version of a lactate shuttle proposed for reptiles (Gleeson and Dalessio 1990). The glycogen content of tuna red muscle is reduced following exhaustive swimming activity (Hulbert *et al.* 1979), but the quantitative importance of lactate incorporation into red muscle glycogen is expected to be low because of the relative masses of red and white muscle. Integration of a shuttling mechanism with the lactate and glycogen changes observed in tuna must accommodate the apparent conservation of lactate carbon within the white muscle mass.

Another way for simultaneous glycogen recovery and lactate disappearance to occur is through the delivery of plasma glucose to muscle for glycogenesis. However, the capacity for skipjack tuna liver to utilize lactate as either a gluconeogenic or an oxidizable substrate is low (Buck *et al.* 1992), and in the general context of postexercise lactate removal, contributes negligibly. Weber *et al.* (1986) came to a similar conclusion by noting that Cori cycle activity is probably minimal in tuna recovering from exercise. While liver glycogenolysis and gluconeogenesis from amino acids are also potential sources of plasma glucose in teleost fishes (Suarez and Mommsen 1987), it remains unlikely that turnover from all of these sources is high enough to supply glucose at a rate that matches the rapid glycogen recovery evident in tuna white muscle. It is generally difficult to predict glucose flux rates because turnover is often independent of plasma glucose concentration (see Bonen *et al.* 1989; Weber *et al.* 1986). However, turnover rate in the present study would have to have been more than an order of magnitude higher than rates measured in tuna by Weber *et al.* (1986), to reflect just 50% repletion of the white muscle glycogen by plasma glucose.

The implication remains that much of the lactate formed during exercise stays in the white muscle or is retrieved from the plasma, and is the principal substrate for glycogen resynthesis. It is interesting that plasma lactate in the exercised tuna declined markedly only after lactate and glycogen recovery in white muscle approached completion. High plasma lactate likely produces a concentration gradient favorable for lactate uptake and utilization by the tissues mentioned above, but may also help to attenuate diffusive efflux of lactate from white muscle. In this regard, tuna resemble those mammals in which prolonged elevation of plasma lactate seems necessary to sustain *in situ* muscle glyconeogenesis (Johnson and Bagby 1988; Stevenson *et al.* 1987). This is different from the situation in other teleosts, in which plasma lactate is elevated only transiently relative to the prolonged period required for white muscle lactate and glycogen recovery.

There is an apparent inconsistency between the predictably high whole-body lactate turnover rates measured with radiolabeled lactate (see Weber *et al.* 1986) and the proposed intramuscular reconversion of lactate to glycogen. In trout, although plasma lactate kinetics appear unrelated to white muscle glycogen replenishment, there is evidence that an initial outward flux of lactate from white muscle is followed by inward transport to favor metabolism *in situ* (Turner and Wood 1983). A similar kinetic pattern in tuna would mean that whole-body turnover reflects lactate uptake from plasma by the lactate-producing as well as lactate-consuming tissues. Simultaneous lactate extraction and removal has been observed in exercising human leg muscle, in which the extraction of tracer lactate from plasma occurs despite net lactate release by muscle (Stanley *et al.* 1986).

Tuna need to swim continuously and this may mean that muscular contractions in free swimming fish influence changes in lactate and other muscle metabolites, compared with spinally blocked fish. There are no studies of lactate removal from white muscle of free swimming tuna for comparison of methodologies, but the rate of glycogen replenishment in skipjack tuna measured by terminal sampling in recovery from exercise (Barrett and Connor 1964) is comparable to changes in lactate and glycogen measured in the present study. In addition, whereas swimming after intense exercise might change both the rate of lactate clearance from white muscle and whole-body turnover, it is still likely that the principal fate of lactate in tuna is as we have proposed, since the potential to oxidize lactate is low and the proportion of red muscle relative to total muscle mass is small.

The rate of lactate clearance in mammals and tuna is comparable (Weber *et al.* 1986). However, the disposal of lactate appears to be either oxidative (lactate shuttle) or gluconeogenic through the liver (Cori cycle) in mammals whereas neither mechanism seems adequate to explain lactate turnover in tuna. Rather, in tuna, there appears to be a requirement for rapid recovery and, in addition, rapid replenishment of glycogen. Disposal of lactate by oxidative means would require the regeneration of glycogen reserves from food sources, or gluconeogenesis from amino acid precursors. Both of these processes are incompatible with the requirement for rapid replenishment of glycogen. We suggest that glycogen reserves represent a short-term fuel reserved for burst swimming and that by conserving lactate it is possible to conserve glycogen. An analogy can be drawn with the PCr stores, which are available as a short-term energy store that can also be rapidly replenished after exercise. Indeed, the cycle of glycogen to lactate to glycogen is, in a sense, analogous to the cycle of PCr dephosphorylation during exercise and subsequent rapid rephosphorylation in recovery.

Purine nucleotide recovery

Typically, exhaustive exercise in teleosts causes nearly a total depletion of the ATP pool and a stoichiometric increase in IMP (Mommsen and Hochachka 1988; Schulte *et al.* 1992). Recovery patterns vary somewhat, but, as with lactate clearance, a prolonged period is generally required for recovery to

pre-exercise levels. The results presented here for tuna are different in that 15 min of intense swimming caused very little change in the white muscle purine nucleotides of exercised freeze-clamped fish. However, the fish were not exhausted, in the sense of fuel depletion, by the exercise routine employed, and glycolysis and PCr hydrolysis seemed sufficient to protect the ATP concentration. A 50% decrease in ATP content occurred in biopsied fish that were exercised and then anesthetized, but this change was transient and was not evident in freeze-clamped fish that were sampled immediately after exercise. These changes were seemingly caused by the additive effects of the exercise protocol combined with a short-term, more violent struggling action noted upon exposure to the anesthetic. The extent to which the anaesthetic influenced purine nucleotide concentrations independently of exercise-induced changes is not clear, although some effect seems likely, given that MS-222 exposure can cause minor perturbations in white muscle energy status (Van den Thillart *et al.* 1989).

The decline in ATP concentration in the exercised, spinally blocked tuna did not result in a stoichiometric adjustment in the remaining purine nucleotide pool, suggesting that other products of purine metabolism (e.g., adenosine, adenylosuccinate, etc.) probably accumulated. Some purine accumulation in IMP was evident in only the exercised fish, but this was minor considering the size of the phosphorylated adenylate pool. ATP recovered to normal resting levels within 20–40 min after removal of the anesthetic, indicating that the precursors were readily available for adenylate resynthesis. In comparison, trout take up to 24 h to restore ATP concentrations to resting levels. The rapid recovery in tuna ATP levels is consistent with the rapid recovery observed for other metabolites.

Lactate and phosphocreatine

The extraordinary inverse correlation between the concentration of PCr and the concentration of lactate has not, to our knowledge, been previously reported in muscle. Meyer and Terjung (1979) appeared to find a similar relationship between concentrations of PCr and lactate in the recovering gastrocnemius of rats. However, they suggested that the recovery of phosphocreatine was biphasic, whereas the recovery of lactate was fitted best by a single exponential. A comparable relationship between PCr and lactate has also been noted in hypoxic turtle brains (Lutz *et al.* 1984).

We suggest that recovery of intracellular pH (pH_i), dependent on the clearance of lactate, causes the apparent linkage of phosphocreatine to lactate during recovery from exercise in tuna. If, as proposed, lactate is a glyconeogenic substrate, then there would be simultaneous consumption of protons within the white muscle. This suggestion is supported by the work of Tang and Boutilier (1991) who found that protons produced in rainbow trout during intense exercise were most likely cleared by metabolic processes within the white muscle compartment.

A discussed earlier, net efflux seems to account minimally for clearance of lactate from tuna white muscle. Thus, an imbalance, if any, between the clearance of lactate and protons would have only a minimal effect on the relationship between pH_i and lactate. Nevertheless, the efflux of lactate from tuna white muscle may also be linked with proton transport. Lactate and proton movements in muscle appear to be coupled in a carrier-mediated transport process (Mason and Thomas 1988; Juel and Wibrand 1989). Furthermore, lactate transport across the mammalian sarcolemma is highly sensitive to a pH gradient (Roth and Brooks 1990). It is not clear to what extent these findings can be generalized, as Wiseman *et al.* (1989) found that the efflux of proton and lactate equivalents was not tightly coupled in molluscan muscle tissue. In tuna there is an excess of blood lactate over blood metabolic protons after exercise, but Perry *et al.* (1985) noted that more efficient removal of protons from plasma, rather than differential release of lactate and protons from white muscle, probably accounted for this difference. Directly measuring pH_i and lactate in white muscle would resolve the relationship between the concentrations of protons and lactate. Nevertheless, the evidence is consistent with the suggestion that changes in pH_i are linked with the concentration of lactate.

Phosphocreatine is linked to pH_i through the near equilibrium reaction catalyzed by creatine kinase, where

$$PCr + ADP + H^+ \rightleftharpoons ATP + Cr$$

In addition to pH_i, the ratio of ATP/ADP will also affect the final concentration of PCr. Work on turtle brain and fish white muscle indicates a clear relationship between acidosis, or glycolysis, and PCr depletion induced by hypoxia (Lutz *et al.* 1984; Van Waarde *et al.* 1990). During recovery from anoxia the rephosphorylation of PCr in fish muscle becomes dissociated from recovery of pH_i and presumably lactate clearance. However, during recovery from anoxia there were substantial increases in the ratio of ATP/ADP, which would dissociate pH_i from PCr. Similarly, adenylate ratios seem to influence PCr in rainbow trout white muscle where, in recovery from exhaustive exercise, lactate clearance occurs at a much slower rate than PCr repletion (Schulte *et al.* 1992). The close relationship between lactate and PCr in tuna suggests that changes in PH_i determine the extent of phosphorylation of creatine during recovery. Furthermore, the PCr in tuna white muscle would contribute to the already substantial buffering capacity of this tissue (Castellini and Somero 1981).

Tuna have long been regarded as the elite athletes of the sea, and, in terms of exercise performance, appear to have more similarities with mammals than other teleosts. Certainly, the rates of recovery are more in line with mammalian rates than other teleosts. But there are differences, as tuna appear to have developed a mechanism by which they are not only able to recover from the exercise quickly but are also ready to engage in another burst of high-speed swimming. More direct evidence would be useful in establishing the importance of the proposed glycogen–lactate–glycogen cycle in the white muscle of tuna. In particular, a more extensive correlative analysis of tuna white muscle metabolites, including measurements of pH_i and extracellular pH, would obviously help to delineate the relationships between lactate, PCr, free adenylates, and pH_i during recovery from exercise.

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