J. exp. Biol. 143, 321–331 (1989)
Printed in Great Britain © The Company of Biologists Limited 1989

OXIDATIVE PROPERTIES OF CARP RED AND WHITE MUSCLE

BY C. D. MOYES, L. T. BUCK, P. W. HOCHACHKA AND R. K. SUAREZ
Department of Zoology, University of British Columbia, Vancouver, Canada

Accepted 19 December 1988

Summary

Substrate preferences of isolated mitochondria and maximal enzyme activities were used to assess the oxidative capacities of red muscle (RM) and white muscle (WM) of carp (*Cyprinus carpio*). A 14-fold higher activity of citrate synthase (CS) in RM reflects the higher mitochondrial density in this tissue. RM mitochondria oxidize pyruvate and fatty acyl carnitines (8:O, 12:O, 16:O) at similarly high rates. WM mitochondria oxidize these fatty acyl carnitines at 35–70 % the rate of pyruvate, depending on chain length. WM has only half the carnitine palmitoyl transferase/CS ratio of RM, but similar ratios of beta-hydroxyacyl CoA dehydrogenase/CS. Ketone bodies are poor substrates for mitochondria from both tissues. In both tissues mitochondrial alpha-glycerophosphate oxidation was minimal, and alpha-glycerophosphate dehydrogenase was present at low activities, suggesting the alpha-glycerophosphate shuttle is of minor significance in maintaining cytosolic redox balance in either tissue. The mitochondrial oxidation rates of other substrates relative to pyruvate are as follows: alpha-ketoglutarate 90 % (RM and WM); glutamate 45 % (WM) and 70 % (RM); proline 20 % (WM) and 45 % (RM). Oxidation of neutral amino acids (serine, glycine, alanine, beta-alanine) was not consistently detectable. These data suggest that RM and WM differ in mitochondrial properties as well as mitochondrial abundance. Whereas RM mitochondria appear to be able to utilize a wide range of metabolic fuels (fatty acids, pyruvate, amino acids but not ketone bodies), WM mitochondria appear to be specialized to use pyruvate.

Introduction

Electrophysiological studies suggest that red muscle fibres (RM) are specialized for long-term, low-intensity exercise whereas white muscle fibres (WM) are involved in burst, high-intensity exercise (Bone, 1966; Rayner & Keenan, 1967). Steady-state exercise by RM is fuelled by the mitochondrial oxidation of pyruvate (derived from lactate, glucose or glycogen), fatty acids and ketone bodies. Although oxidative metabolism is inherently more efficient than anaerobic pathways [i.e. more adenosine triphosphate (ATP) obtained per mole of substrate], the high rates of ATP turnover required for burst-type exercise cannot be

Key words: pyruvate, lactate, fatty acids, mitochondria.

met by mitochondrial oxidative phosphorylation, probably because of limitations in both oxygen delivery and mitochondrial density (Hochachka, 1987). Consequently burst-type exercise by WM is fuelled by non-oxidative pathways such as phosphagen hydrolysis and anaerobic glycolysis.

Although oxidative metabolism in RM has a role in providing ATP for steady-state work, mitochondrial metabolism in WM may have an important role in rest and recovery. Basal metabolism of WM is probably supported aerobically, as the resting rate of oxygen consumption is similar in mammalian RM and WM (Beatty *et al.* 1974; Wardlaw & Kaplan, 1984). Mitochondrial oxidation of glucose, ketone bodies, lactate and fatty acids spares glycogen reserves and replenishes phosphagens, fuels which are required for anaerobic, high-intensity exercise. In fish, the lactate produced in WM during burst exercise is retained and apparently metabolized *in situ* (Turner *et al.* 1983*a*,*b*). WM mitochondria must play a critical role in lactate metabolism after burst exercise. Oxidation of lactate demands mitochondrial catabolism of pyruvate; WM mitochondrial oxidative metabolism must also provide the ATP necessary to fuel endogenous glycogen resynthesis from lactate.

Fish are useful models for studying muscle metabolism owing to the homogeneity of fibre types (Bone, 1966). This is reflected in the marked differences in mitochondrial abundance in RM and WM, as indicated by differences in mitochondrial volume density [e.g. 15-fold in *Anguilla anguilla* (Jankowski, 1968), 20-fold in *Pollachius virens* (Patterson & Goldspink, 1972)] and the activity of mitochondrial marker enzymes. In the present study we examine the oxidative properties of WM and RM of carp. *Cyprinus carpio* possesses a substantial amount of RM, as well as WM which is largely uncontaminated by red fibres (Driedzic & Hochachka, 1978). We compare the capacities of mitochondria isolated from each tissue to oxidize pyruvate, fatty acids (short, medium and long chain), ketone bodies, amino acids and substrates involved in hydride shuttles. In parallel, the maximal activities of key enzymes of aerobic energy metabolism are also compared.

Materials and methods

Mitochondrial isolation

Adult carp, *Cyprinus carpio*, (1–2 kg) were obtained from a local fish farmer (Latek Enterprises) and held at 12°C for 5–10 days. Fish were killed by concussion. Mitochondria from RM and WM were prepared using a method modified from Moyes *et al.* (1988). RM samples were collected from the lateral line region near the dorsal fin. WM samples were collected from the epaxial region between the lateral line and dorsal fin. Carp possess substantial amounts of pink muscle fibres, which are recruited at intermediate swimming speeds and positioned between RM and WM (Johnston *et al.* 1977). Care was taken to dissect intermediate fibres from RM and WM samples. The tissue was diced and

homogenized in 9 volumes of ice-cold isolation medium (140 mmol l^{-1} KCl, 10 mmol l^{-1} EDTA, 5 mmol l^{-1} $MgCl_2$, 0·5 % bovine serum albumin, 20 mmol l^{-1} Hepes, pH 7·1 at 20 °C). Diced tissue was gently homogenized using a Potter–Elvejhem tissue grinder (1–2 passes with a loose pestle followed by three passes with a tight pestle). The homogenate was centrifuged for 10 min at 1000 *g*. The supernatant was collected and centrifuged for 10 min at 9000 *g*. The pellet was resuspended in isolation medium minus bovine serum albumin and recentrifuged for 10 min at 9000 *g*. The resultant pellet was resuspended in the same medium to approximately 4 mg mitochondrial protein ml^{-1}. Mitochondrial protein was determined by the Biuret method, using 10 % deoxycholate to solubilize the membrane proteins (Gornall *et al.* 1949).

Approximately 0·2 ml of mitochondrial suspension was added to 9 volumes of assay medium (140 mmol l^{-1} KCl, 5 mmol l^{-1} Na_2HPO_4, 20 mmol l^{-1} Hepes, pH 7·1 at 20 °C). Oxygen consumption was measured at 15 °C using a Clark-type electrode. Mitochondria were given saturating amounts of substrate followed by 0·3–0·4 mmol l^{-1} ADP. Mitochondrial quality was assessed as the respiratory control ratio (RCR), which is the ratio of the rate of oxygen consumption in the presence of ADP (state 3) to that after all the ADP has been phosphorylated (state 4). When mitochondria were given ADP in the absence of exogenous substrates, a brief burst of oxygen consumption was observed, representing oxidation of endogenous substrates, followed by a decrease to a low linear rate. At this point, saturating amounts of substrate were added to the cell and the resulting rate of oxygen consumption determined.

Enzyme assays

Tissue samples for enzyme assays were homogenized in 5 volumes of either solution A (50 mmol l^{-1} Tris, 1 mmol l^{-1} EDTA, 0·1 % Triton X-100, pH 7·6 at 20 °C) or solution B (50 mmol l^{-1} imidazole, 1 mmol l^{-1} EDTA, 5 mmol l^{-1} dithiothreitol, 0·1 % Triton X-100, pH 7·4 at 20 °C). The tissue was homogenized by three high-speed bursts of a Brinkman polytron, followed by three high-intensity bursts of sonication (Branson Sonifier with microtip). The homogenate was centrifuged for 10 min at 10 000 *g* to remove particulate material. Enzyme assays were performed on the supernatant fractions. Mitochondrial enzymes were assayed in the pellets resuspended in solution A and sonicated for 10 s at high intensity. All assays were performed using saturating amounts of substrates at 25 °C as described by Suarez *et al.* (1986). Citrate synthase (CS) and carnitine palmitoyl transferase (CPT) were assayed with homogenates prepared in solution A. Malate dehydrogenase (MDH), lactate dehydrogenase (LDH), glutamate dehydrogenase (GDH), glutamate–oxaloacetate transaminase (GOT), glutamate–pyruvate transaminase (GPT), beta-hydroxyacyl CoA dehydrogenase (HOAD) and alpha-glycerophosphate dehydrogenase (GPDH) were assayed in homogenates prepared in solution B.

All biochemicals were purchased from Sigma Chemical Co, St Louis, MO.

Results

Table 1 summarizes the maximal enzyme activities found in carp RM and WM. The difference in mitochondrial density between the tissues was reflected in the higher activity of enzymes located exclusively (CS, GDH) or primarily (HOAD, CPT) in the mitochondrial compartment. CS and GDH activities in RM were 14-fold and fourfold higher than those in WM, whereas HOAD and CPT activities were 12- and 36-fold higher in RM than WM, respectively. Although there were clear differences in mitochondrial abundance between the tissues, it was also important to establish whether the mitochondria themselves differed between tissues. Table 1 also expresses the maximal activities of certain mitochondrial enzymes as enzyme activity per milligram of mitochondrial protein as well as activity ratios (enzyme activity per unit of CS activity). The CPT activity per milligram of mitochondrial protein was approximately fourfold higher in RM than WM. GDH activity per milligram of mitochondrial protein was slightly lower in RM. Activities of HOAD and CS per milligram of mitochondrial protein were slightly higher in RM than WM. Whole tissue activities of MDH were similar in RM and WM. Activities of GOT and GPT were sevenfold and 2·5-fold higher in RM than in WM. The maximal activities of GPDH, the cytoplasmic portion of the alpha-glycerophosphate shuttle, were similar in both RM and WM.

Table 1. *Maximal activities of enzymes involved in aerobic metabolism of red and white muscle of carp (25°C)*

	Red muscle	White muscle
Enzyme units g^{-1} tissue		
CS	49·0 ± 4·4	3·5 ± 0·2
CPT	0·71 ± 0·06	0·016 ± 0·0058
HOAD	10·6 ± 1·76	0·87 ± 0·045
GDH	3·9 ± 0·21	0·79 ± 0·11
MDH	263 ± 21	294 ± 20
GOT	213 ± 20	29 ± 2·4
GPT	3·8 ± 0·19	1·55 ± 0·21
LDH	756 ± 132	1050 ± 84
GPDH	1·7 ± 0·31	2·58 ± 0·22
Enzyme units $unit^{-1}$ mitochondria		
CS/mg protein	1·80 ± 0·16	1·13 ± 0·08
CPT/CS	8·3 ± 0·74	3·6 ± 0·6
CPT/mg protein	15	4
HOAD/CS	0·21 ± 0·02	0·25 ± 0·02
HOAD/mg protein	0·38	0·28
GDH/CS	0·079 ± 0·005	0·23 ± 0·037
GDH/mg protein	0·14	0·25

Activities are μmol substrate converted min^{-1} g wet $mass^{-1}$, μmol min^{-1} unit citrate $synthase^{-1}$ or μmol min^{-1} mg mitochondrial $protein^{-1}$. Mean ± S.E., $N = 6$.

See text for explanation of abbreviations.

Table 2. *Respiratory control ratios (RCR) and oxygen consumption of mitochondria isolated from carp red and white muscle (15°C)*

	Red muscle	*N*	White muscle	*N*
RCR values				
Pyruvate	15·1 ± 1·5	7	7·8 ± 0·64	8
Lauroyl carnitine	13·3 ± 1·5	7	–	
State 3 substrate ($mmol\,l^{-1}$)				
Malate (0·1)	6·0 ± 0·98	8	5·4 ± 0·7	9
Pyruvate (2·5) + malate (0·1)	54·6 ± 6·8	6	51·6 ± 7·3	7
Acetoacetate (5) + malate (0·1)	8·9 ± 1·3	5	7·7 ± 1·4	5
Beta-hydroxybutyrate (5) + malate (0·1)	8·8 ± 1·5	5	6·2 ± 0·7	5
Octanoyl-DL-carnitine (0·4) + malate (0·1)	45·4 ± 8·5	7	18·4 ± 1·6	6
Lauroyl-DL-carnitine (0·1) + malate (0·1)	55·9 ± 7·6	7	36·0 ± 4·2	7
Palmitoyl-DL-carnitine (0·025) + malate (0·1)	54·1 ± 5·9	7	31·4 ± 4·4	7
Alpha-ketoglutarate (2·5) + malate (0·1)	43·2 ± 5·0	6	37·5 ± 1·9	5
ADP only	4·7 ± 1·69	7	4·1 ± 0·6	7
Glutamate (5)	33·3 ± 5·79	6	19·7 ± 4·2	6
Proline (5)	25·2 ± 6·5	6	11·0 ± 2·0	6
Malate (5)	18·2 ± 2·0	6	22·4 ± 7·3	5
Serine (5)	6·4 ± 0·8	5	ND	5
Alpha-glycerophosphate (10)	ND	5	ND	5

State 3 rates (in the presence of ADP) are expressed as nmol O consumed min^{-1} mg mitochondrial $protein^{-1}$, mean ± S.E.
ND, not detectable.

Table 2 summarizes the results of the studies using mitochondria isolated from RM and WM. The RCR, determined for the preferred substrates of WM (pyruvate) and RM (pyruvate, lauroyl carnitine), was used as an index of mitochondrial quality. Although the maximal RCR values were higher for mitochondria isolated from RM than for those isolated from WM, the consistently high RCRs indicate that both preparations were well coupled.

In WM mitochondria pyruvate was oxidized at the highest rate. Fatty acyl carnitines were oxidized at significantly lower rates than pyruvate (LSD, $P = 0{\cdot}05$), 35–70 % the rate of pyruvate depending upon chain length (C8 < C12, C16). In RM mitochondria, pyruvate and fatty acyl carnitines (8:O, 12:O, 16:O) were oxidized at similarly high rates. Octanoate oxidation was not detectable in WM mitochondria but was detectable at low rates in about half the RM

preparations. Acetoacetate and beta-hydroxybutyrate were generally poor substrates compared with pyruvate in both RM and WM, but the ability to oxidize ketone bodies was highly variable.

Alpha-ketoglutarate was oxidized nearly as fast as pyruvate in both RM and WM. Glutamate was oxidized at about 75 % the rate of alpha-ketoglutarate in RM (LSD, not significant) and 50 % the rate in WM (LSD, $P = 0{\cdot}05$). Proline was oxidized at about 30 % the rate of alpha-ketoglutarate in WM (LSD, $P = 0{\cdot}05$), and about 60 % the rate of alpha-ketoglutarate in RM (LSD, $P = 0{\cdot}05$). Oxidation of serine was not consistently detectable. No oxidation of 5 mmol l^{-1} glycine, 5 mmol l^{-1} alanine or 5 mmol l^{-1} beta-alanine ($\pm 0{\cdot}1$ mmol l^{-1} malate $\pm$ $0{\cdot}1$ mmol l^{-1} alpha-ketoglutarate) was detected in mitochondria from either tissue. Oxidation of alpha-glycerophosphate (10 mmol l^{-1}) was not detectable in either RM or WM mitochondria.

Maximal rates of oxygen consumption (see pyruvate oxidation in Table 2) were similar for WM and RM mitochondria, suggesting that the maximal capacity of the electron transport system is similar in both tissues.

Discussion

The oxidative properties of carp muscle found in this study suggest that mitochondria from different fibre types are specialized for oxidation of different sets of substrates. Also, important differences between teleosts and mammalian muscle mitochondria are evident.

Carp mitochondria from RM and WM were unable to oxidize ketone bodies at high rates. Mammalian muscle homogenates oxidize acetoacetate at 30–60 % of the rate obtained with pyruvate, depending on fibre type (Hooker & Baldwin, 1979). Acetoacetate, when available in the circulation, is oxidized at high rates by perfused rat skeletal muscle (Ruderman *et al.* 1971). Thus, the rate of ketone body oxidation in mammalian skeletal muscle is primarily dependent on substrate availability, i.e. the rate of hepatic ketogenesis. In contrast, even if ketone bodies were available to muscle in teleosts, they appear not to be effectively utilized. Isolated perfused teleost hearts loose contractility when given only acetoacetate as respiratory substrate (Driedzic & Hart, 1984). Since pyruvate and ketone bodies typically enter mitochondria on the same carrier at similar maximal velocities (LaNoue & Schoolwerth, 1979), the low capacity for ketone body oxidation of teleost muscle mitochondria probably reflects low levels of ketolytic enzymes rather than depressed mitochondrial transport. Teleosts are thought not to rely on ketone bodies as fuel, even after extended periods of food deprivation (Zammit & Newsholme, 1979).

Hydride shuttles (malate/aspartate, alpha-glycerophosphate shuttles) are responsible for transport of reducing equivalents, generated primarily in aerobic glycolysis, into the mitochondria for oxidation. These serve as a mechanism for maintaining cytoplasmic redox balance under conditions of high aerobic glycolytic flux. The physiological relevance of possessing one shuttle over another i

vertebrate tissues is not well established. Although mammalian muscles have GPDH activities, it is the enzymes of the malate/aspartate shuttle, not GPDH, which increase in response to endurance training, suggesting this shuttle is most important in mammalian skeletal muscle (Holloszy, 1977; Holloszy & Booth, 1976). In fish, the relative importance of the two shuttles is less clear. White muscle of skipjack tuna is thought to use the alpha-glycerophosphate shuttle, based primarily on high GPDH levels (Guppy *et al.* 1979). In other fish species the levels of GPDH correlate with the activity of the species (tuna > trout > eel) (Hochachka & Guppy, 1977). Carp RM and WM possess low levels of cytoplasmic GPDH (Table 1) and mitochondria from neither tissue were able to oxidize alpha-glycerophosphate at detectable rates (Table 2), suggesting the alpha-glycerophosphate shuttle is not a major hydride shuttle in carp skeletal muscle. Both RM and WM mitochondria oxidize malate and alpha-ketoglutarate at high rates and possess considerable MDH and GOT activities, consistent with an operational malate/aspartate shuttle in this tissue.

When comparing carp RM and WM mitochondria several differences are evident. Despite a greater GDH activity per milligram of mitochondrial protein, WM mitochondria are less able to oxidize glutamate (LSD, $P = 0{\cdot}05$) and proline (LSD, $P = 0{\cdot}05$), amino acids which enter the Krebs cycle at alpha-ketoglutarate *via* GDH. Separate transporters are thought to operate for glutamate (glutamate/OH^-, glutamate/aspartate exchangers) and proline (LaNoue & Schoolwerth, 1979). As alpha-ketoglutarate oxidation is similar in both tissues, it is possible that the lower rates of oxidation of glutamate and proline in WM are the result of limitation at the level of their transporters.

The most profound difference in oxidative properties of WM and RM mitochondria is in their ability to use fatty acids. RM mitochondria are able to use pyruvate and fatty acyl carnitines equally well but in WM the rate of pyruvate oxidation is 1·5- to threefold greater than that of fatty acyl carnitine oxidation. This difference correlates with a lower activity of CPT per milligram of mitochondrial protein in WM (25 % that in RM), suggesting that activity of CPT may limit the rate of fatty acid oxidation in this tissue. This preference of WM mitochondria for oxidation of pyruvate over fatty acids is not unique to fish. Rabbit WM, but not RM, mitochondria are less able to oxidize fatty acids than pyruvate, possessing 50 % of the CPT per milligram of mitochondrial protein of RM mitochondria (Pande & Blanchaer, 1971).

The differences in mitochondrial properties reflect the differences in metabolic organization of WM and RM. Red and white muscle fibres of rats exhibit similar resting $\dot{V}_{O_2}$ (Beatty *et al.* 1974; Wardlaw & Kaplan, 1984). If we assume the basal metabolic rates are similar in different fibre types, then the resting rates of ATP production in WM are met aerobically, as in RM. In some fish species, WM is recruited at sustainable swimming speeds (*Pollachius virens*, Johnston & Moon, 1980), although this has not been shown for carp (Johnston *et al.* 1977). Furthermore, hypertrophy of WM, as well as RM, follows training at sustainable swimming speeds, implying WM has an aerobic capacity (Johnston & Moon,

1980). Endurance training in mammals also leads to an increase in oxidative capacity of red, white and mixed fibre types (Baldwin *et al.* 1972; Jansson & Kaijser, 1977). If WM can function aerobically during rest and recovery, and even during low-intensity exercise, the fuel preference of the WM mitochondria may provide some insights regarding the energy metabolism of this tissue.

Unlike those in RM, WM mitochondria are less able to utilize fatty acyl carnitines than pyruvate, apparently due to lower CPT activities/unit mitochondria (CS or mg mitochondrial protein). This, coupled with the lower levels of triglyceride (rat, Reitman *et al.* 1973; rainbow trout, Robinson & Mead, 1973) and acyl CoA synthetase (Pande & Blanchaer, 1971), suggests that lipid is a poor substrate for WM. Potential pyruvate-generating substrates include endogenous glycogen, exogenous glucose and endogenous and exogenous lactate. Of these potential substrates, glucose and lactate are probably the most significant; oxidation of endogenous glycogen at rest would be detrimental as it is the only substrate available for phosphogen synthesis during burst exercise. Rates of glucose uptake and phosphorylation at rest are similar in different mammalian fibre types (Ferre *et al.* 1985; Challiss *et al.* 1986), indicating that the rates of pyruvate production *via* glycolysis may be similar in both tissues at rest.

Another potential pyruvate-generating substrate is lactate. It is produced under resting conditions (Weber *et al.* 1986) but during burst exercise lactate is produced at high rates and can accumulate to very high levels, 90 mmol l^{-1} in tuna WM (Guppy *et al.* 1979). Fish, particularly those species with high proportions of WM, retain a large fraction of the lactate generated during burst exercise (Turner *et al.* 1983*a*,*b*). In the flathead sole there is minimal washout of lactate into the blood, yet tissue lactate levels approach resting levels after 12 h of recovery (Turner *et al.* 1983*b*). It has been suggested that WM lactate is metabolized *in situ* (Wardle, 1978; Milligan & McDonald, 1988) or undergoes a noncirculatory transfer to RM (Wittenberger, 1973) and is used for either gluconeogenesis/glycogenesis or oxidation. Glycogen synthesis from lactate in rat skeletal muscle may be significant in recovery where concentrations of glucose are low and those of lactate high (Shiota *et al.* 1984). There is considerable uncertainty as to the route of gluconeogenesis in skeletal muscle, but it appears to be unlike the liver pathway (see Connett, 1979). Hepatic gluconeogenesis requires pyruvate transport into the mitochondria where pyruvate carboxylase catalyses production of oxaloacetate. Pyruvate carboxylase is absent from mammalian (Crabtree *et al.* 1972) and fish muscle (Johnston & Moon, 1979). Two alternative routes of phosphoenolpyruvate production have been suggested: reversal of pyruvate kinase (Dyson *et al.* 1975; McLane & Holloszy, 1979) and malic enzyme/MDH/phosphoenolpyruvate carboxykinase (Bendall & Taylor, 1970; Connett, 1979). Neither of these processes necessarily involves mitochondrial enzymes, but either pathway would require mitochondrial (i.e. non-glycolytic) ATP to fuel net reversal of glycolytic flux.

The maximal capacity for lactate oxidation by carp WM can be calculated from the mitochondrial rates of pyruvate oxidation (Table 2) and the activity of mitochondrial enzymes (Table 1). The maximal rate of pyruvate oxidation i

approximately 50 nmol O min^{-1} mg protein^{-1}. This represents 10 nmol pyruvate oxidized min^{-1} mg protein^{-1}, assuming complete oxidation of pyruvate generates reducing equivalents at pyruvate dehydrogenase, isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase, succinate dehydrogenase and malate dehydrogenase. Extrapolation to the whole tissue (3·5 units CS g wet mass^{-1} and 1·1 units CS mg mitochondrial protein^{-1}) gives a rate of approximately 30 nmol pyruvate oxidized min^{-1} g wet mass^{-1}. Thus, a lactate load of 20 μmol g^{-1} could be completely oxidized in approximately 10 h, which is about the time required to reduce lactate concentrations to rest levels *in vivo* (e.g. 8–12 h in rainbow trout, Turner *et al.* 1983*a*; 12 h in flathead sole, Turner *et al.* 1983*b*). Of course, the actual rate of lactate oxidation *in vivo* would depend on several factors including LDH activity, and the rates of pyruvate transport and mitochondrial oxidation.

In conclusion, we have shown that mitochondria from RM and WM differ in oxidative properties as well as relative enzyme levels. Whereas RM appears able to utilize a wide range of fuels (fatty acids, pyruvate, amino acids), WM appears specialized for oxidation of pyruvate, perhaps derived from lactate generated during high-intensity exercise.

We wish to thank Mr Mike Latek for supplying carp. This research was supported by the Natural Sciences and Engineering Research Council of Canada.

References

BALDWIN, K. M., KLINKERFUSS, G. H., TERJUNG, R. L., MOLE, P. A. & HOLLOSZY, J. O. (1972). Respiratory capacity of white, red and intermediate muscle: adaptive response to exercise. *Am. J. Physiol.* **222**, 373–378.

BEATTY, C. H., CURTIS, S., YOUNG, M. K. & BOCEK, R. M. (1974). Oxidation of amino acids by red and white muscle fibre groups. *Am. J. Physiol.* **227**, 268–272.

BENDALL, J. R. & TAYLOR, A. A. (1970). The Meyerhoff quotient and the synthesis of glycogen from lactate in frog and rabbit muscle. A reinvestigation. *Biochem. J.* **118**, 887–893.

BONE, Q. (1966). On the function of the two types of myotomal muscle fiber in elasmobranch fish. *J. mar. biol. Ass. U.K.* **46**, 321–349.

CHALLISS, R. A. J., HAYES, D. J. & RADDA, G. K. (1986). An investigation of arterial insufficiency in the rat hindlimb: correlation of skeletal muscle bloodflow and glucose utilization *in vivo*. *Biochem. J.* **240**, 395–401.

CONNETT, R. J. (1979). Glycogenesis from lactate in frog striated muscle. *Am. J. Physiol.* **237**, C231–C236.

CRABTREE, B., HIGGINS, S. J. & NEWSHOLME, E. A. (1972). The activities of pyruvate carboxylase, phophoenolpyruvate carboxykinase and fructose diphosphatase in muscle from vertebrates and invertebrates. *Biochem. J.* **130**, 391–396.

DRIEDZIC, W. R. & HART, T. (1984). Relationship between exogenous fuel availability and performance by teleost and elasmobranch hearts. *J. comp. Physiol.* **154**, 593–599.

DREIDZIC, W. R. & HOCHACHKA, P. W. (1978). Metabolism in fish during exercise. In *Fish Physiology*, vol. VII (ed. W. S. Hoar & D. J. Randall), pp. 503–543. New York: Academic Press.

DYSON, R. D., CARDENAS, J. M. & BARSOTTI, R. J. (1975). The reversibility of skeletal muscle pyruvate kinase and an assessment of its capacity to support glycogenesis. *J. biol. Chem.* **250**, 3316–3321.

FERRE, P., LETURQUE, A., BURNOL, A.-E., PENICAUD, L. & GIRARD, J. (1985). A method to quantify glucose utilization *in vivo* in skeletal muscle and white adipose tissue of the anaesthetized rat. *Biochem. J.* **228**, 103–110.

GORNALL, A. G., BARDAWILL, C. J. & DAVID, M. M. (1949). Determination of serum proteins by the biuret reaction. *J. biol. Chem.* **177**, 751–766.

GUPPY, M., HULBERT, W. C. & HOCHACHKA, P. W. (1979). Metabolic sources of heat and power in tuna muscles. II. Enzyme and metabolite profiles. *J. exp. Biol.* **82**, 303–320.

HOCHACHKA, P. W. (1987). Limits. How fast and how slow muscle metabolism can go. In *Advances in Myochemistry, Proceedings of the Second Congress of Myochemistry* (ed. G. Benzi), pp. 3–12. London: John Libby Eurotext Ltd.

HOCHACHKA, P. W. & GUPPY, M. (1977). Variation on a theme by Embden, Meyerhof, and Parnas. In *Oxygen and Physiological Function* (ed. F. F. Jobsis), pp. 292–310. Dallas: Professional Information Library.

HOLLOSZY, J. O. (1977). Adaptations of muscular tissue to training. In *Exercise and Heart Disease* (ed. E. H. Sonnenblick & M. Weson), pp. 25–38. New York: Grune & Stratten.

HOLLOSZY, J. O. & BOOTH, F. W. (1976). Biochemical adaptation to endurance exercise in muscle. *A. Rev. Biochem.* **46**, 273–291.

HOOKER, A. M. & BALDWIN, K. M. (1979). Substrate oxidation specificity in different types of mammalian muscle. *Am. J. Physiol.* **236**, C66–C69.

JANKOWSKI, H. D. (1968). On the structure and metabolic rate of the skeletal muscle of the eel (*Anguilla vulgaris* L.). In *Quantitative Biology of Metabolism* (ed. A. Locker), pp. 91–94. Berlin: Springer-Verlag.

JANSSON, E. & KAIJSER, L. (1977). Muscle adaptation to extreme endurance training in man. *Acta physiol. Scand.* **100**, 315–324.

JOHNSTON, I. A., DAVISON, W. & GOLDSPINK, G. (1977). Energy metabolism of carp swimming muscles. *J. comp. Physiol.* **114**, 203–216.

JOHNSTON, I. A. & MOON, T. W. (1979). Glycolytic and gluconeogenic enzyme activities in the skeletal muscles and liver of a teleost fish (*Pleuronectes platessa*). *Biochem. Soc. Trans.* **7**, 661–662.

JOHNSTON, I. A. & MOON, T. W. (1980). Endurance exercise training in the fast and slow muscles of a teleost fish (*Pollachius virens*). *J. comp. Physiol.* **135**, 147–156.

LANOUE, K. & SCHOOLWERTH, A. C. (1979). Metabolite transport in mitochondria. *A. Rev. Biochem.* **48**, 871–922.

MCLANE, J. A. & HOLLOSZY, J. O. (1979). Glycogen synthesis from lactate in the three types of skeletal muscle. *J. biol. Chem.* **254**, 6548–6553.

MILLIGAN, C. L. & MCDONALD, D. G. (1988). *In vivo* lactate kinetics at rest and during recovery from exhaustive exercise in coho salmon (*Oncorhynchus kisutch*) and starry flounder (*Platichthys stellatus*). *J. exp. Biol.* **135**, 119–131.

MOYES, C. D., BUCK, L. T. & HOCHACHKA, P. W. (1988). Temperature effects on mitochondria isolated from carp red muscle. *Am. J. Physiol.* **254**, R611–R615.

PANDE, S. V. & BLANCHAER, M. C. (1971). Carbohydrate and fat in energy metabolism of red and white muscle. *Am. J. Physiol.* **220**, 549–553.

PATTERSON, S. & GOLDSPINK, G. (1972). The fine structure of red and white myotomal muscle fibers of the coalfish (*Gadus virens*). *Z. Zellforsch. mikrosk. Anat.* **133**, 463–474.

RAYNER, M. D. & KEENAN, M. J. (1967). Role of red and white muscles in the swimming of the skipjack tuna. *Nature, Lond.* **214**, 392–393.

REITMAN, J., BALDWIN, K. M. & HOLLOSZY, J. O. (1973). Intramuscular triglyceride utilization by red, white and intermediate skeletal muscle and heart during exhausting exercise. *Soc. exp. Biol. Med.* **142**, 628–631.

ROBINSON, J. S. & MEAD, J. F. (1973). Lipid absorption and deposition in rainbow trout (*Salmo gairdneri*). *Can. J. Biochem.* **51**, 1050–1058.

RUDERMAN, N. B., HOUGHTON, C. R. S. & HEMS, R. (1971). Evaluation of the isolated rat hindquarter for the study of muscle metabolism. *Biochem. J.* **124**, 639–651.

SHIOTA, M., GOLDEN, S. & KATZ, J. (1984). Lactate metabolism in the perfused rat hindlimb. *Biochem. J.* **222**, 281–292.

SUAREZ, R. K., BROWN, G. S. & HOCHACHKA, P. W. (1986). Metabolic sources of energy for hummingbird flight. *Am. J. Physiol.* **251**, R537–R542.

TURNER, J. D., WOOD, C. M. & CLARK, D. (1983*a*). Lactate and proton dynamics in the rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **104**, 247–268.

TURNER, J. D., WOOD, C. M. & HÕBE, H. (1983*b*). Physiological consequences of sever

exercise in the inactive benthic flathead sole (*Hippoglossoides elassodon*): a comparison with the active pelagic rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **104**, 269–288.

WARDLAW, G. M. & KAPLAN, M. L. (1984). Oxygen consumption and oxidative capacity of muscles from young obese and nonobese Zucker rats. *Am. J. Physiol.* **247**, R911–R917.

WARDLE, C. S. (1978). Non-release of lactic acid from anaerobic swimming muscle of plaice (*Pleuronectes platessa*) L. A stress response. *J. exp. Biol.* **77**, 141–155.

WEBER, J.-M., BRILL, R. W. & HOCHACHKA, P. W. (1986). Mammalian metabolite flux rates in a teleost: lactate and glucose turnover in tuna. *Am. J. Physiol.* **250**, R452–R458.

WITTENBERGER, C. (1973). Metabolic interaction between isolated white and red carp muscles. *Rev. roum. Biol. Ser. Zool.* **18**, 71–76.

ZAMMIT, V. A. & NEWSHOLME, E. A. (1979). Activities of enzymes of fat and ketone-body metabolism and effects of starvation on blood concentration of glucose and fat fuels in teleost and elasmobranch fish. *Biochem. J.* **184**, 313–322.