Mitochondrial metabolism of cardiac and skeletal muscles from a fast (*Katsuwonus pelamis*) and a slow (*Cyprinus carpio*) fish

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Tuna cardiac (atrium, compact and spongy ventricle) and skeletal muscle (red and white) were compared with carp tissues to determine the importance of mitochondrial differences in supporting the high aerobic capacities in tuna. Mitochondria isolated from red muscle of both species oxidized each of the physiological fuels at similar rates per milligram of mitochondrial protein, when differences in assay temperature are considered. The highest rate of oxygen consumption by ventricle mitochondria was 2 times greater in tuna than carp. The maximal oxidation rates were 3–4 times higher in ventricle than red muscle in both species. Tuna tissues had as much as 30–80% more mitochondrial protein per gram of tissue than carp. Morphometrically this was manifested as extremely densely packed mitochondrial cristae, rather than increased mitochondrial volume densities. In general, higher aerobic capacities of tuna ventricle and red muscle are primarily attributable to greater tissue mass and, to a lesser extent, differences in the nature or quantity of mitochondria per gram of tissue. Unlike ventricle and red muscle, tissues with relatively low mitochondrial contents in carp (white muscle, atrium) demonstrated several-fold higher mitochondrial contents in tuna. Enzyme analyses of tissue and isolated mitochondria suggest a greater dependence of tuna tissues on fatty acids as fuels. Activities of carnitine palmitoyl transferase (CPT) per milligram of protein were 2–2.5 times higher in tuna red muscle and ventricle mitochondria than in carp mitochondria from the same tissues. Whole tissue activity ratios of hexokinase/CPT, which indicate the relative importance of glucose and fatty acid metabolism, were 5 times higher in carp spongy ventricle and 12 times higher in carp compact ventricle. These data suggest that muscle aerobic capacity can be increased at several levels: tissue mass, mitochondrial volume density, cristae surface density, and mitochondrial specific activity. Large differences observed between carp and tuna muscles are due to cumulative effects of several of these factors.

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Les muscles cardiaques (muscle de l'oreillette, muscles compacts et spongieux du ventricule) et les muscles squelettiques (rouges et blanc) du Thon à ventre rayé ont été comparés à des tissus de la Carpe afin de déterminer l'importance des différences mitochondriales qui donnent lieu aux capacités aérobies élevées chez les thons. Les mitochondries extraites de muscles rouges des deux espèces oxydent chacun des combustibles physiologiques aux mêmes taux par milligramme de protéine mitochondriale lorsqu'on tient compte des différences de température expérimentale. Le plus haut taux de consommation d'oxygène enregistré dans les mitochondries du ventricule était 2 fois plus élevé chez le thon que chez la carpe. Les taux d'oxydation maximaux étaient de 3 à 4 fois plus élevés dans le ventricule que dans le muscle rouge chez les deux espèces. Les tissus du thon contenaient jusqu'à 30–80% plus de protéines mitochondriales/g de tissu que ceux de la carpe. Morphométriquement, cela se manifestait par la présence de crêtes mitochondriales extrêmement serrées plutôt que par une augmentation de la densité du volume mitochondrial. En général, la capacité aérobie plus élevée du ventricule et du muscle rouge du thon est surtout attribuable à une masse tissulaire plus grande et, à un degré moindre, à des différences dans la nature ou la quantité de mitochondries/g de tissu. Contrairement aux muscles du ventricule et au muscle rouge, le muscle blanc et le muscle de l'oreillette ont un contenu mitochondrial relativement faible chez la carpe, alors qu'il ont un contenu plusieurs fois plus élevé de mitochondries chez le thon. Des analyses enzymatiques des tissus et des mitochondries isolées ont démontré l'existence d'une dépendance plus grande à l'égard des acides gras comme combustibles dans les tissus du thon. L'activité de la carnitine palmitoyl transférase (CPT)/mg protéine était de 2 à 2,5 fois plus élevée dans les mitochondries du muscle rouge et du ventricule du thon que dans ceux des mitochondries des mêmes tissus chez la carpe. Le rapport entre l'activité de l'hexokinase et celle de la CPT dans les tissus entiers, rapport qui indique l'importance relative du métabolisme du glucose et du métabolisme des acides gras, était 5 fois plus élevé dans le muscle spongieux du ventricule et 12 fois plus élevé dans le muscle compact du ventricule chez la carpe. Ces résultats indiquent que la capacité aérobie du muscle peut être augmentée par plusieurs facteurs: la masse tissulaire, la densité du volume mitochondrial, la densité de la surface des crêtes et l'activité mitochondriale spécifique. Les différences observées entre les muscles de la carpe et ceux du thon sont attribuables aux effets cumulatifs de plusieurs de ces facteurs.

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Introduction

An important determinant of the maximal aerobic power output of a muscle is the rate at which its mitochondria can produce ATP. Modest increases in aerobic capacity are observed in individual muscles with endurance training. Much greater differences in muscle aerobic capacity are observed when comparisons are made between muscle types (i.e., red, white, cardiac) or between species. Such comparisons suggest that there are two general mechanisms by which increased aerobic ATP production capacities are achieved in muscle: (*i*) more tissue (i.e., increased mass of muscle per kilogram of body weight), and (*ii*) higher mitochondrial capacity per gram of tissue. This can theoretically be achieved both quantitatively and qualitatively. Higher mitochondrial volume density (V_v(mt,f)) packs more mitochondria into a given tissue volume. Qualitative differences in mitochondria make more effective use of the intracellular volume devoted to mitochondria. One type of qualitative difference is more closely packed inner membrane cristae, measured as square metres of cristae surface area per cubic centimetre of mitochondrial volume (S_v(im,m)). Because the protein content of mitochondrial inner membranes is extremely high (Srere 1985), more closely packed cristae allow more mitochondrial membrane protein per cubic centimetre of mitochondrial volume. A second type of qualitative difference involves changes in the nature or efficiency of the mitochondrial enzymes, which would be manifested as an increased ATP production per milligram of mitochondrial protein.

Qualitative differences in mitochondria between high and low-performance tissues and species are not well established. It has been suggested as part of the symmorphosis hypothesis that individual mitochondria from mammalian skeletal muscles are fundamentally similar regardless of tissue aerobic capacity (Hoppeler and Lindstedt 1985). Similar oxygen consumption rates are indeed observed with in vitro mitochondrial preparations from red and white muscles of mammals (Pande and Blanchaer 1971; Schwerzmann et al. 1989). There is evidence for relatively minor changes in the nature of the mitochondria in response to endurance training, in which not all mitochondrial enzymes increase to the same extent (see Holloszy and Coyle 1984). However, when isolated mitochondria from trained and control rats are compared, rates of oxidation of physiological substrates (per milligram of mitochondrial protein) are not affected by training (Davies et al. 1981).

Qualitative differences in mitochondria are more obvious when comparisons are broadened to include nonmammalian vertebrates. A series of studies by Else and Hulbert (Else and Hulbert 1981, 1983; Hulbert and Else 1989) demonstrated that mammalian mitochondria have more densely packed cristae than mitochondria from the same tissues of similarly sized reptiles. Differences in muscle mitochondrial capacities per milligram of mitochondrial protein have also been observed. Red muscle mitochondria from elasmobranchs (Moyes et al. 1990) demonstrate rates of oxygen consumption that are 2 times higher than mitochondria from red or white muscle of teleosts (Moyes et al. 1989). Heart mitochondria tend to show higher rates of oxygen consumption per milligram of protein than do skeletal muscle mitochondria (e.g., Krieger et al. 1980 vs. Palmer et al. 1977). Furthermore, two intracellular populations of mitochondria (subsarcolemmal, interfibrillar) appear to have different oxidative capacities in both skeletal muscle (Kreiger et al. 1980) and heart (Palmer et al. 1977).

Apart from questions of oxidative capacity, increased aerobic performance appears to be accompanied by a change in tissue fuel preference. Part of the transition to higher aerobic work capacities induced by endurance training (Holloszy et al. 1985) or natural selection (Driedzic et al. 1987) appears to be a greater reliance on fatty acids. The extent to which these changes are facilitated by specialization of the individual mitochondria has not been thoroughly assessed. Relative rates of oxidation of pyruvate and palmitoyl carnitine do not change in mitochondria from endurance-trained rats (Davies et al. 1981). Training in fish increases tissue activities of β-hydroxyacyl-CoA dehydrogenase (HOAD) in both red and white muscle, with little change in other mitochondrial enzymes, suggesting a greater reliance on fatty acids (Johnston and Moon 1980*a*, 1980*b*).

In the present study we compare the oxidative properties of heart (atrium, spongy and compact ventricle) and skeletal muscle (red – slow oxidative and white – fast glycolytic) from the high performance species skipjack tuna (*Katsuwonus pelamis*) and a low performance species, the common carp (*Cyprinus carpio*). Our goal was to assess the relative importance of mitochondrial differences in determining the aerobic capacity of fish muscle.

Materials and methods

Animals

Skipjack tuna (*Katsuwonus pelamis*), were purchased from local commercial fishermen. Fish weighing from 1 to 3 kg and of undetermined sex were held for up to 4 days in 10 m diameter tanks supplied with continuously flowing seawater (25 ± 1°C) at the Kewalo Research Facility, National Marine Fisheries Service, Honolulu. Carp (*Cyprinus carpio*) weighing 1–2 kg and of both sexes were netted locally and held for up to 3 months in outdoor tanks supplied with continuously flowing fresh water (10–15°C) at the Department of Zoology, University of British Columbia. Carp were fed trout chow twice weekly.

Mitochondrial isolation

Fish were netted, quickly stunned by a blow to the head, and decapitated. A 2-cm steak was taken from the tuna immediately posterior to the anus. Red and white muscle were collected from both sides of the vertebral column. Whole ventricles from a single tuna or 2–3 carp were used for isolated mitochondrial studies. Mitochondria were prepared as described previously (Moyes et al. 1989) with the following modifications. Initial centrifugations of 5 min at 1400 × *g* (4°C) were used to sediment undispersed tissue. Mitochondria were collected and washed by 7-min centrifugations at 9000 × *g* (4°C). Aliquots of resuspended pellet (4–8 mg protein/mL) were frozen immediately in liquid nitrogen for enzyme assays. Mitochondria were used within 1.5 h for the oxygen consumption experiments.

Mitochondrial oxygen consumption

Incubations of tuna and carp mitochondria were performed at 25 and 15°C, respectively, which correspond to acclimation temperature. Small volumes of mitochondrial suspensions (25 μL of tuna heart, 50 μL of tuna red muscle, 100 μL of carp heart) were added to 2 mL of incubation medium of the following composition: 140 mM KCl, 20 mM HEPES, 5 mM Na_2HPO_4, and 0.5% bovine serum albumin, pH 7.3, at 20°C. Oxygen consumption rates were monitored by a YSI Clark-type electrode. Respiratory control ratios (RCR) were determined for each preparation by adding carbon substrates followed 4–6 min later by 0.5 mM ADP. The RCR is the ratio of the state 3 rate (+ADP) to the state 4 rate determined after all the ADP is phosphorylated. To determine oxidative rates, mitochondria were given 0.1 mM malate and 0.5 mM ADP, then incubated 1–3 min until oxygen consumption reached a low, linear rate. At this point, saturating amounts of substrates were added to the cuvettes to elicit the state 3 oxygen consumption rates[2]

[2]Rates of mitochondrial respiration are expressed per unit atomic oxygen, the substrate for the electron transport system. Tissue and animal respiration rates are expressed per unit molecular oxygen, by convention.

TABLE 1. Oxidation of various substrates by mitochondria from heart and skeletal muscle

	Tuna		Carp		
	Heart	Red muscle	Heart	Red muscle[a]	White muscle[a]
RCR	14.2±2.8 (6)	14.9±2.2 (6)	>20 (6)		
Malate	20±2 (6)	8±2 (7)	24±3 (6)	6±1	5±1
Pyruvate	403±41 (6)	106±12 (7)	146±5 (6)	55±7	52±7
Palmitoyl carnitine	178±29 (6)	104±12 (7)	103±4 (6)	54±6	31±4
Lauroyl carnitine	150±14 (6)	87±11 (6)	89±4 (6)	56±8	36±4
Octanoyl carnitine	83±4 (6)	50±6 (6)	49±4 (6)	45±9	18±2
Acetyl carnitine	87±10 (6)	36±4 (6)	91±3 (6)		
Acetoacetate	58±5 (5)	16±2 (3)		9±1	8±1
Glutamate	53±5 (4)	23±4 (4)		33±6	20±4

NOTE: Values are given as means ± SE, in nmol O/(min · mg mitochondrial protein). Values in parentheses are number of preparations. Tuna data were collected at 25°C, carp at 15°C. RCR, respiratory control ratio.
[a]Data are from Moyes et al. (1989).

Enzyme assays

Tissues for enzyme analyses were quickly collected, frozen in liquid nitrogen, and stored on dry ice. Atrium, compact ventricle, and spongy ventricle were separated prior to freezing. All assays were performed within 7 days of tissue collection. Tissue samples were weighed and added to 6 vol. (w/v) ice-cold homogenization medium (20 mM HEPES, pH 7.4, 1 mM EDTA, 0.1% Triton X-100). Homogenates were prepared by dicing, sonicating (1 burst of 10 s on Kontes sonifier), homogenizing (3 bursts of 10 s on Ultra-Turrax homogenizer), and sonicating (3 bursts of 10 s) the tissue. Particulate matter was sedimented by centrifugation (5 min at 9000 × *g*, 4°C). Mitochondrial suspensions were diluted with the same homogenization medium (1/9 v/v) and sonicated (3 bursts of 10 s). Activities lost in the sedimented pellet were not determined but are assumed to be minimal, as this extraction procedure routinely yields measurements of enzyme activity comparable to, or as much as 3 times higher than, values previously reported for these species (e.g., Guppy et al. 1979; Sidell et al. 1987).

Maximal enzyme activities were determined using a Perkin–Elmer Lambda 2 spectrophotometer interfaced with an IBM computer using PECSS (Perkin–Elmer Computerized Spectroscopy Software). The cuvete holder was thermostatted to 25°C for both species. All assays were performed in duplicate, as follows:

Citrate synthase (CS): 20 mM Tris-HCl (pH 8.0), 0.2 mM 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), 0.3 mM acetyl-CoA, and 0.5 mM oxaloacetate. Oxaloacetate was omitted for the control assays. Control rates were typically <5% of the CS rate.

Carnitine palmitoyltransferase (CPT): 20 mM Tris-HCl (pH 8.0), 0.2 mM DTNB, 0.1 mM palmitoyl-CoA, and 5 mM L-carnitine. Carnitine was omitted for the control assays. CPT rates were typically 2–3 times greater than control rates.

Hexokinase (HK): 20 mM imidazole (pH 7.4), 1 m ATP, 0.5 mM $NADP^+$, 5 mM glucose, 5 mM $MgCl_2$, 5 mM dithiothreitol, and excess glucose-6-phosphate dehydrogenase. Glucose was omitted in control assays. The HK rate was typically 2–3 times greater than the control rate.

Mitochondrial ultrastructure

Morphometric measurements of mitochondrial volume density (V_v(mt,f)) and cristae surface density (S_v(im,m)) were done as described previously (Hoppeler et al. 1981; Mathieu et al. 1981). Details on tissue preparation are found in Mathieu-Costello et al. (1992). We analyzed two randomly chosen blocks in red muscle and compact and spongy ventricle, and four blocks in white muscle. A total of 32 pictures (each sample of heart and red muscle) and 80 pictures (white muscle) were examined at a magnification of × 24 000 to estimate V_v(mt,f). The S_v(im,m) was estimated from pictures of mitochondria taken at random from 10 (red muscle, heart) to 40 (white muscle) fibers examined at a final magnification of × 220 000. We measured a total of 20 subsarcolemmal and 40 interfibrillar mitochondria in red muscle, 40 interfibrillar mitochondria in white muscle, and 20 and 40 interfibrillar mitochondria in spongy and compact portions of the ventricle, respectively.

Results

Mitochondrial oxidation

The results of the isolated mitochondrial studies are summarized in Table 1. In both species maximal oxygen consumption rates were several times higher in mitochondria from heart than in those from muscle: 4 times in tuna, 3 times in carp. In each of the tissues examined, pyruvate was oxidized at the highest rates among all substrates tested. Tuna red muscle mitochondria oxidized palmitoyl carnitine and pyruvate at similar rates, as did carp red muscle mitochondria. Heart mitochondria of both species oxidized palmitoyl carnitine at lower rates than pyruvate (55% lower in tuna, 30% lower in carp). This pattern of pyruvate preference over fatty acids also occurred in carp white muscle.

Comparisons of mitochondrial respiration rates between species are complicated by the differences in assay temperatures (25°C for tuna, 15°C for carp), which were chosen to mimic acclimation temperatures. Carp red muscle mitochondria demonstrate a Q_{10} of 1.5, using lauroyl carnitine as substrate (Moyes et al. 1988). It is not known if a similar Q_{10} is applicable for all substrates or if the rates would change in such a manner if the acclimation temperature was 25°C. For the purposes of this study a Q_{10} of 1.5 is assumed for all substrates, with the recognition that this may be an oversimplification. With these reservations in mind, it appears that carp and tuna mitochondria are similar in most respects within tissue types. Maximal oxygen consumption (pyruvate) by cardiac mitochondria is almost 2 times higher in tuna than carp, whereas red muscle mitochondria respire maximally at similar rates in each species. Acetyl carnitine oxidation by carp heart mitochondria is about 150% that of tuna. Glutamate oxidation by carp red muscle mitochondria is about twice that of tuna.

Tissue enzymes

A summary of tissue enzyme activities is presented in Table 2. Citrate synthase activity per gram wet weight is used as an index of mitochondrial capacity. Of all tissues examined, the highest activities were observed in tuna atrium. Within tuna

TABLE 2. Tissue activities of citrate synthase (CS), carnitine palmitoyltransferase (CPT), and hexokinase (HK)

	CS (U/g)	CPT (U/g)	CPT/CS (mU/U)	HK (U/g)	HK/CPT (U/U)
Tuna					
Atrium (7)	87.88±2.45	1.26±0.18	14±2	13.55±0.42	10.8±1.4
Compact (7)	63.37±4.15	2.52±0.26	40±3	6.66±0.68	2.6±0.20
Spongy (7)	74.58±5.76	2.51±0.25	35±6	8.10±0.78	3.2±0.29
Red muscle (7)	79.8±2.15	2.40±0.05	30±0.4	2.03±0.16	0.85±0.05
White muscle (7)	16.1±0.95	nd	—	1.06±0.1	—
Carp					
Atrium (6)	18.12±1.27	0.30±0.022	17±1	6.97±1.24	23.2±3.6
Compact (5)	29.60±1.88	0.34±0.012	12±1	10.45±1.12	30.7±4.0
Spongy (5)	43.32±6.35	0.78±0.042	21±5	11.07±0.54	14.2±1.3
Red muscle[a] (6)	49.00±4.4	0.71±0.06	14.5	0.55±0.06	0.77
White muscle[a] (5)	3.50±0.2	nd	—	nd	—

NOTE: Values are given as means ± SE, where 1 U/g is 1 μmol substrate used/(min · g). All enzymes were assayed at 25°C. nd, not detected; —, not determined.
[a]Data are from Moyes et al. (1989), except the HK data.

TABLE 3. Mitochondrial enzymes assayed in isolated mitochondria

	CS (U/mg)	CPT (U/mg)	Mitochondrial protein (mg/g)	$\dot{V}_{O_2MAX}$ (μmol O_2/(min · g))
Tuna				
Heart (6)	1.94±0.16	0.030±0.002	35.0[a]	7.0
Red muscle (6)	1.64±0.06	0.039±0.003	48.7	2.6
Carp				
Heart (6)	1.34±0.11	0.016±0.001	24.4	1.8[b]
Red muscle[c]	1.80±0.16	0.015	27.2	0.75[b]
White muscle[c]	1.13±0.08	0.004	3.09	0.081[b]

NOTE: Values are given as means ± SE, where 1 U/mg is 1 μmol substrate converted/(min · mg mitochondrial protein). Enzymes were assayed at 25°C.
[a]Calculated as (CS/g)/(CS/mg), weighted for the relative amounts of compact and spongy myocardium (carp, compact, 37% of total mass, Bass et al. 1973; skipjack tuna, 65.6%, Farrell et al. 1992).
[b]Estimates of oxygen consumption rates for carp are at 15°C.
[c]Enzyme data are from Moyes et al. (1989).

heart, CS activity is ranked atrium : spongy myocardium : compact myocardium (100:85:72). Within carp heart, CS activity is ranked spongy myocardium : compact myocardium : atrium (100:67:42). Comparing the two species, tuna CS activity is higher in all tissues examined (2.1× higher in compact myocardium, 1.8× in spongy myocardium, and 1.6× in red muscle but particularly in atrium and white muscle (4.5−5×).

CS activities, expressed as units per gram wet weight (Table 2) and units per milligram of mitochondrial protein (Table 3), allow the calculation of milligrams of mitochondrial protein per gram of tissue (Table 3). Tuna tissues have a higher mitochondrial protein content than those of comparable carp tissue (43% higher in heart, 80% in red muscle). The maximal ventricular oxygen consumption can be estimated from mitochondrial protein content (Table 3) and oxygen consumption per milligram of protein (Table 1). When the differences in assay temperature are considered, tuna tissues have 130−140% higher maximal oxygen consumption per gram than the corresponding carp tissues.

CPT activities were also higher in tuna tissues: atrium, 4.2×; compact, 7.4×; spongy, 3.2×; red muscle, 3.4×. Within tuna, CPT activities were similar in red muscle and compact and spongy ventricle, each being approximately twice that of atrium. CPT activities in carp atrium and compact ventricle were each about half those in spongy ventricle and red muscle.

In oxidative muscles, HK/CPT ratios may reflect the fuel preferences of the tissue. Red muscle HK activities are several times lower than those in the heart tissues. In tuna and carp red muscle, HK activity is slightly lower than CPT activity, resulting in HK/CPT ratios less than unity. HK activity per gram is similar in the heart tissues of each species but, unlike red muscle, it is several times higher than CPT activity. HK/CPT in tuna ventricle is approximately 3, but in carp this ratio is 14 in spongy ventricle and 30 in compact ventricle.

Mitochondrial enzymes

Direct comparisons of mitochondrial enzymes (activity per milligram of mitochondrial protein) between species (Table 3) are possible because all enzymes were assayed at 25°C. No clear pattern emerges in CS activity per milligram of protein between tissues or species. Activities are marginally higher in heart than red muscle in tuna, but the reverse is seen in carp. Activities in tuna heart are slightly higher than those in carp heart. Activities are similar in red muscles of each species.

CPT activity is often used as an index of the capacity for fatty

TABLE 4. Tuna muscle mitochondrial ultrastructure

	Volume of mitochondria/volume of fiber (%)		Surface area of inner mitochondrial membrane/volume of mitochondria (m^2/cm^3)		Surface area of inner mitochondrial membrane/volume of fiber (m^2/cm^3)
	Subsarcolemmal mitochondria	Total mitochondria	Subsarcolemmal mitochondria	Interfibrillar mitochondria	
Heart					
Compact	1.7±0.1	25.4±1.0	—	57.88±1.57	14.70
Spongy	1.7±0.5	25.3±0.7	—	56.63±1.36	14.33
Red muscle	12.3±2.4	32.4±1.9	63.20±1.60	70.61±1.67	21.89
White muscle	0.3±0.2	2.5±0.3	—	31.32±1.66	0.78

acid utilization by a tissue. This study demonstrates that differences in CPT activity are not necessarily accompanied by differences in the maximal mitochondrial capacity to utilize fatty acids. CPT activity per milligram of protein is 2−2.5 times higher in tuna than in the same tissues in carp, but carp and tuna have similar tissue-specific oxidation rates for mitochondrial palmitoyl carnitine, when differences in assay temperature are considered. Although CPT activities (U per milligram of protein) were 30% higher in tuna red muscle than tuna ventricle (Table 3), mitochondria from heart oxidized palmitoyl carnitine at 70% greater rates than red muscle (Table 1). A similar situation occurs in carp, in which 2-fold greater palmitoyl carnitine oxidation rates in heart (Table 1) occurred despite similar CPT activities per milligram of protein (Table 3).

Tuna mitochondrial ultrastructure

Mitochondrial volume density (V_v(mt,f)) and cristae surface density (S_v(im,m)) determinations are summarized in Table 4. Tuna red muscle has a high V_v(mt,f), approximately 30 times higher than that of white muscle. In eight muscle samples from four fish, V_v(mt,f) ranged from 24 to 32% (Mathieu-Costello et al. 1992). Heart V_v(mt,f) is approximately 25% in both compact and spongy myocardium. Subsarcolemmal mitochondria comprised 35% of the total in red muscle but only 7% in the heart. In other red muscle samples, subsarcolemmal mitochondria comprised 17−40% of the total mitochondrial volume (Mathieu-Costello et al. 1992). S_v(im,m) in red muscle is higher than in tuna heart. The total mitochondrial cristae surface area per cubic centimetre of muscle in heart was 65% of the value in red muscle. A representative electron micrograph of interfibrillar mitochondria in red muscle is shown in Fig. 1. It is taken from one of the micrographs used for morphometry, i.e., it represents mitochondria selected by systematic random sampling.

Discussion

Aerobic capacity

There are two potential strategies to increase tissue aerobic capacity: (*i*) more aerobic tissue per kilogram of body mass or (*ii*) higher mitochondrial capacity per gram of tissue (morphometrically as increased V_v(mt,f) or S_v(im,m), metabolically as increased oxidation per milligram of protein or milligrams of mitochondrial protein per gram). When tuna and carp are compared, each of these strategies is used to some extent, and the relative importance of each depends on the tissue. In general, it appears that highly aerobic tissues (heart, red muscle) differ between species primarily in functional mass and to a lesser extent in mitochondrial capacity per gram. In contrast, muscles that are mitochondria-poor in carp (white muscle, atrium) have mitochondrial capacities per gram that are several times higher.

The interspecies differences in red muscle mitochondrial properties are modest. Substrate oxidation rates per milligram of mitochondrial protein are similar between species, when differences in assay temperature are considered. Tuna red muscle, however, has about 80% more mitochondrial protein per gram of tissue than that of carp. As the V_v(mt,f) found in tuna red muscle (32%) is within the range found in other teleosts (25−38%, see Johnston 1981), the higher amount of mitochondrial protein per gram must be manifested as more densely packed cristae. The intracellular volume devoted to mitochondria in fish red muscle may approach the spatial limit allowed by cells that must also perform mechanical work (Weibel 1985, Hochachka 1987). Higher mitochondrial volume densities are observed in tissues such as the heater organ of billfish (Block 1986), which demonstrate high metabolic rates but do not perform mechanical work. If such a constraint exists in fish muscle, adaptations toward increased mitochondrial capacity per gram of tissue may necessarily involve increased S_v(im,m).

Cristae packing in tuna red muscle is exceptionally high for a vertebrate skeletal muscle. This avenue for increasing tissue aerobic capacity may also be limited in tuna red muscle. The value of 63−70 m^2/cm^3 (Table 4) reported for tuna red muscle S_v(im,m) is greater than the range reported for skeletal muscles of antarctic fish (25−37 m^2/cm^3, Archer and Johnston 1991) and a wide variety of mammals (20−40 m^2/cm^3, Hoppeller and Lindstedt 1985), hummingbird flight muscle (58 m^2/cm^3, Suarez et al. in press), and mammalian and reptilian hearts (38−66 m^2/cm^3, Else and Hulbert 1983). Srere (1985) calculated that a S_v(im,m) of 83 m^2/cm^3 is the maximum degree of cristae packing that will still allow enough matrix space for two average-sized Krebs cycle enzymes, each in contact with an opposing membrane. Thus it appears that the only structural strategy available to tuna to increase the total aerobic capacity of red muscle by several fold is to increase recruitable tissue mass.

Active, pelagic fish generally possess more red muscle than benthic or sluggish fish (Greer-Walker and Pull 1975). Higher aerobic capacity in fish skeletal muscle could also be achieved by utilizing white muscle during steady-state swimming. The limits to the use of white muscle at low swim speeds are based on innervation pattern and muscle ultrastructure (Johnston 1981). The fiber orientation necessary in white muscle to power burst exercise compromises its ability to function at lower

FIG. 1. Representative high-power electron micrograph of interfibrillar mitochondria in tuna red muscle, and used for morphometry. Note the high density of mitochondrial cristae. Mi, mitochondrion; My, myofibril.

swimming speeds (Rome et al. 1988). Electrophysiological studies suggest tuna white muscle is indeed recruited at sustainable swim speeds (Brill and Dizon 1979) as in several other fish species (Johnston 1981) but not in carp (Johnston et al. 1977). Previous metabolic and ultrastructural studies support an aerobic role for tuna white muscle (Guppy et al. 1979; Hulbert et al. 1979). It is highly vascularized and contains significant stores of intracellular lipid, a fuel that can only be used aerobically. In the present study, the mitochondrial content of tuna white muscle, based on CS activity per gram, was found to be 5 times higher than in carp, supporting the suggestion that tuna white muscle is used aerobically.

The major differences between species in myocardial oxidative capacity per kilogram of body weight are attributable to muscle mass. Mass alone affords tuna a 5-fold advantage in oxidative capacity per kilogram of body mass relative to carp (4.0 vs. 0.76 g ventricle/kg). Skipjack tuna ventricular mass is higher than in most fish species (Poupa et al. 1981; Sidell et al. 1987), close to the range found in mammals (Poupa and Ostadal 1969; Driedzic et al. 1987). Higher mitochondrial capacity per gram of ventricle is also obvious in tuna, but this is of lesser quantitative importance than the increased ventricle mass. Tuna have more milligrams of mitochondrial protein per gram of ventricle (43% greater) and a 2-fold higher oxidative capacity per milligram of mitochondrial protein than carp. These three factors combine to give tuna a 14-fold greater myocardial oxidative capacity per kilogram of body mass, but much of this difference is due to ventricular mass.

Compared with tuna red muscle, ventricle has lower V_v(mt,f) and lower S_v(im,m). Although teleological, it is instructive to ask why tuna ventricle mitochondria are less packed than they could be, based on what is observed in red muscle. Large hearts are needed to obtain the high ventral aortic pressures observed in vivo (Brill and Bushnell 1991). However, power output per gram of ventricle in tuna is similar to that in other fish species (Brill and Bushnell 1992; Farrell et al. 1992), so selection for higher aerobic capacities per gram of tissue may have been minimal. As hemodynamic considerations probably determine atrial size, it is not surprising that the ratio of atrial mass to ventricular mass is similar in each species (15% in tuna, 18% in carp). As was the case with ventricle, the greater mass of tuna atrium (0.60 vs. 0.14 g atrium per kilogram of body mass in carp) provides a 4-fold higher aerobic capacity. However, tuna atrium also has an approximately 5-fold greater mitochondrial capacity per gram than carp atrium, based on CS activity per gram of tissue. The combined effect of higher mitochondrial capacity per gram and increased tissue mass provides tuna atrium with a 20-fold greater aerobic capacity per kilogram of body mass than carp atrium.

Studies using combined physiological, biochemical, and morphometric techniques suggest that mammalian skeletal muscle mitochondria at $\dot{V}_{O_2MAX}$ in vivo may operate close to the maximal rates obtained with isolated mitochondria in vitro (Schwertzman et al. 1989). In the case of tuna heart mitochondria, the maximal oxygen consumption ($\dot{V}_{O_2MAX}$) we predict from in vitro studies is close to that estimated to be required by the heart at whole animal $\dot{V}_{O_2MAX}$. Mycardial oxygen consumption of trout swam at maximal sustained speed has been estimated to be 80 μL oxygen/(min · kg body mass), corresponding to 95 μL (or 4.0 μmol) oxygen/(min · g ventricle) (based on 15°C trout with 0.87 g ventricle/kg working at 7.2 mW/g, Graham and Farrell 1990). The maximal cardiac power output observed in skipjack tuna in vivo is 25 mW/kg body mass, determined on spinally blocked fish (Brill and Bushnell 1991). Using trout data relating oxygen consumption and power output (Graham and Farrell 1990), this work rate would require a myocardial oxygen consumption of 3.3 μmol/(min · g ventricle) (based on 25°C tuna with 4 g ventricle/kg). Maximal mitochondrial capacity found in this study (7.0 μmol O_2/(min · g) is more than 2 times greater than the maximal myocardial oxygen consumption rate observed in vivo. It has been suggested that the maximal cardiac output may be 1.5–2 times greater than that observed with spinally blocked tuna (Brill and Bushnell 1991). If this were the case, tissue maximal work rates could only be met aerobically by utilizing the entire mitochondrial capacity. However, in vivo, oxygen or substrate delivery, NADH/NAD^+ ratio, or availability of ADP or phosphate could prevent ventricle mitochondria from reaching their maximal capacity. Given the high rates of lactate release

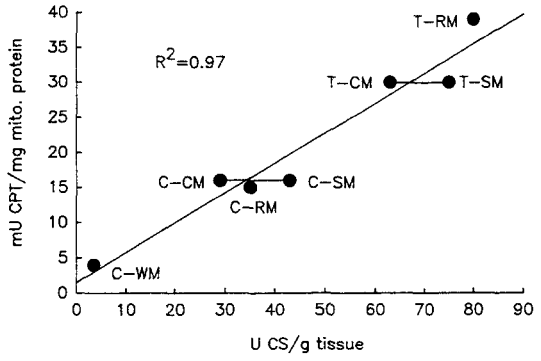

FIG. 2. Relationship between mitochondrial content (U citrate synthase per gram of tissue) and mitochondrial specific activity of carnitine palmitoyl transferase. A unit (U) represents the amount of enzyme required to convert 1 μmol of substrate to product in 1 min. C, carp; T, tuna; RM, red muscle; WM, white muscle; CM, compact myocardium; SM, spongy myocardium; mito., mitochondrial.

by isolated perfused tuna hearts (Farrell et al. 1992), it is likely that the heart's impressive glycolytic capacity would augment aerobic ATP production at such high power outputs.

Fuel preference

One of the consequences of training in mammals is greater reliance on fatty acids as fuels. Although a considerable part of this shift is related to fatty acid supply to the mitochondria, clear metabolic changes also occur at the muscle. Mitochondrial density in mammals increases with endurance training, and fatty acid catabolic enzymes increase in parallel, resulting in greater enzyme activity per gram of tissue (Holloszy et al. 1985). Endurance training of coalfish and brook trout causes increases in HOAD activities in red and particularly white muscle, suggesting that an increased capacity for fat oxidation also occurs in fish (Johnston and Moon 1980*a*, 1980*b*). Analagous to the intratissue changes that occur with endurance training is the increasing dependence on fatty acids as fuel in hearts of species with higher cardiac power outputs. When resting power output was plotted against enzyme activity, Driedzic et al. (1987) found that glycolytic enzymes (e.g., hexokinase) plateaued at low power demands, whereas the activity of enzymes catabolizing fatty acids (e.g., CPT) continued to increase. Along this line, the HK/CPT ratios may indicate the relative importance of exogenous glucose and fatty acids as fuels. The data from Driedzic et al. 1987 show that heart HK/CPT ratios of birds (pigeon 1.7) and mammals (rat 1.1) are considerably lower than those of reptiles (fence lizard 7.3, turtle 33), amphibians (grass frog 7.8, mudpuppy 21) and fish (trout 10.9, ocean pout 12, sea raven 22, hagfish 28). In this study, the HK/CPT ratio of carp ventricular tissue is as high as in other poikilotherms (14–30) but in tuna, the ratios are closer to those found in homeotherms (approximately 3), due primarily to higher CPT rates. Part of the differences in CPT activity per gram of tissue are due to a higher mitochondrial abundance in tuna, but there is also a difference in CPT activity per milligram of mitochondrial protein. Comparison of tissues with widely different aerobic capacities, from carp white muscle through to tuna ventricle, reveals a 10-fold difference in CPT activity per milligram of mitochondrial protein that correlates with the mitochondrial content of the tissue (Fig. 2). Higher CPT specific activity does not necessarily result in greater rates of fatty acyl carnitine oxidation with isolated mitochondria in vitro (see the Mitochondrial enzymes section in the Results and also Palmer et al. 1977). The maximal mitochondrial capacities for palmitoyl carnitine oxidation rates utilize only a fraction of the measured CPT activity. For instance, in situ activity of 0.01 U CPT/mg mitochondrial protein can provide enough fatty acid substrate to result in an oxygen consumption of 460 nmol O (min · mg mitochondrial protein), well beyond the maximal rates observed in vitro. Even this may be an underestimate, as the activity of CPT in the direction required for palmitoyl carnitine oxidation is approximately 9 times greater than that in the direction which is assayed by the DTNB method (Palmer et al. 1977). It is likely that the higher CPT activities confer kinetic advantages to favour fatty acid utilization. Taken together, these data support the general hypothesis that more aerobic tissues rely more on fatty acids than glucose as fuel.

Previous calculations suggest that tuna cardiac mitochondrial capacity is similar to that which would be required at whole animal $\dot{V}_{O_2MAX}$. If this mitochondrial capacity were realized in vivo, ventricular aerobic power output could reach 53 mW/kg body mass (14 mW/g ventricle) with pyruvate (from lactate, glucose) as substrate. However, a maximum of only 24 mW/kg (6 mW/g ventricle) could be generated with fatty acids as primary fuel. It should be noted that this fat-oxidizing capacity is sufficient to support the highest cardiac outputs measured in vivo (Brill and Bushnell 1991). This difference is due to the 2-fold mitochondrial fuel preference of pyruvate over fatty acids. As the enzyme analyses suggest fatty acids are relatively more important in tuna ventricle, the fuel preference of these mitochondria for pyruvate over fatty acids may reflect the capacity to use lactate as a fuel for heart metabolism. Lactate produced in tuna white muscle during exercise can reach 80–100 μmol/g (Guppy and Hochachka 1978) and appears to be partly released from the tissue postexercise (Arthur et al. 1992). Lactate release following exercise may occur in channel catfish (Cameron and Cech 1990) but not in salmon or flounder (Milligan and McDonald 1988). Arthur et al. (1992) found that lactate concentration in tuna blood did not fall below 10 mM even when the white muscle lactate appeared to have recovered. Given the high rate of mitochondrial pyruvate oxidation relative to fat, it is tempting to speculate that fat may be used to fuel myocardial metabolism at low cruising speeds, whereas lactate may be used during or following high-intensity exercise, when the metabolic rate is high, blood lactate is elevated, and increased cardiac outputs are required.

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