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Capillary–fiber geometrical relationships in tuna red muscle

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The aim of this study was to examine the size and geometry of the capillary network in tuna red muscle, one of the most aerobic muscles in fish. Deep red muscle of 1.5- to 2-kg skipjack tuna, *Katsuwonus pelamis*, was perfusion fixed *in situ*, processed for electron microscopy, and analyzed by morphometry. Fiber cross-sectional area was 560 ± 30 (SE) μm^2 in the samples. Capillary length per fiber volume was 4143 ± 242 (SE) mm^{-2} and mitochondrial volume density 28.5 ± 1.0 (SE) %. Indexes of capillarity such as average number of capillaries around a fiber, capillary length and surface per fiber volume, and capillary surface per fiber surface were high for a fish muscle. In fact, the size of the capillary–fiber interface (i.e., capillary to fiber surface) at a given mitochondrial volume per fiber was not significantly different in tuna red muscle compared with rat soleus muscle. However, calculation of mitochondrial respiratory rates in tuna red muscle yielded a substantially lower value (approximately 1/20th) compared with muscles of mammals. Besides the possible effect of differences in operating temperatures and (or) mitochondrial function(s) in fish compared with mammals, this suggests that the large capillary–fiber interface in tuna may be related to functions other than oxygen delivery per se, such as substrate and (or) heat transfer between capillaries and muscle fiber.

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Le but de cette étude était de déterminer la taille et la géométrie du réseau de capillaires dans le muscle rouge du thon, l'un des muscles les plus aérobiques rencontrés chez un poisson. Des muscles rouges profonds de Thons à ventre rayé, *Katsuwonus pelamis*, de 1,5 à 2 kg ont été fixés par perfusion *in situ*, préparés pour examen au microscope électronique et analysés par morphométrie. La surface d'une coupe transversale de fibre était de 560 ± 30 (erreur standard) μm^2 dans les échantillons. La longueur de capillaire par volume de fibres a été évaluée à 4143 ± 242 (erreur standard) mm^{-2} et la densité volumique mitochondriale, à 28,5 ± 1,0 (erreur standard) %. Les indices de la capillarité, notamment le nombre moyen de capillaires autour d'une fibre, la longueur et la surface de capillaire par volume de fibre et la surface de capillaire par surface de fibre étaient élevés pour un muscle de poisson. En fait, la taille de l'interface capillaire–fibre (i.e., surface capillaire par rapport à celle des fibres) pour un volume donné de mitochondries par fibre dans le muscle rouge de thon était semblable à celle du muscle soleus de rat. Cependant, le calcul des taux respiratoires mitochondriaux dans le muscle rouge du thon a donné des valeurs beaucoup plus faibles (environ 1/20) que celles obtenues dans des muscles de mammifères. En plus des effets dus aux différences de températures ambiantes ou bien de fonctionnement des mitochondries entre les poissons et les mammifères, il est possible que l'importante interface capillaire–fibre chez le thon soit reliée à des fonctions autres que celle du transport d'oxygène per se, par exemple le transfert de substrat ou bien de chaleur des capillaires aux fibres musculaires.
[Traduit par la rédaction]

Introduction

With more and more attention being devoted to the capillary – muscle fiber interface, understanding the three-dimensional arrangement of capillary networks has taken on new importance in probing blood–tissue exchange kinetics. Most current quantitative studies of capillary geometry have focussed on mammalian and avian muscles (Mathieu-Costello *et al.* 1988; Mathieu-Costello 1991*a*, 1991*b*). They led to the following generalizations: (*i*) the use of capillary number per sectional area of fibers in transverse sections only ('capillary density') can underestimate capillary length by as much as 50–70% in shortened muscles; capillarity estimates must take into account the three-dimensional arrangement of the capillary network; (*ii*) capillary tortuosity within a spectrum of mammalian species (large to small) is a function of sarcomere length, not aerobic capacity; (*iii*) however, there was less tortuosity and a smaller effect of fiber shortening in the pectoralis muscle of the pigeon than in mammalian muscles; and finally (*iv*) in pigeon flight muscle, venular portions of the capillaries were arranged perpendicularly to muscle fibers, forming capillary manifolds around groups of fibers (Potter *et al.* 1991). Besides facilitating gas exchange and increasing O_2 extraction at the venular end of the network, the capillary manifolds could be involved in other functions such as heat transfer, and (or) the suggested blood-pumping action of the muscle during flight.

To gain further insight into the geometry of blood–tissue exchange in relation to mitochondrial metabolic capacity, the data base was extended to the flight muscles of bat and hummingbird (Mathieu-Costello *et al.* 1992*a*, 1992*b*). The main difference between bat and hummingbird flight muscles was the lack of capillary manifolds in the former and a lesser tor-

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TABLE 1. Sarcomere length, l_o, mean fiber cross-sectional area ($\bar{a}$(f), capillary number per fiber cross-sectional area ($Q_A(0)$), and capillary diameter ($\bar{d}$(c)) in skipjack tuna muscle

Animal No.	Muscle No.	Site No.	l_o (μm)	$\bar{a}$(f) (μm^2)	$Q_A(0)$ mm^{-2}	$\bar{d}$(c) (μm)
			Red muscle			
1	1	1	1.78±0.01	570±40	3055±147	3.79±0.05
1	2	2	1.74±0.01	644±32	2686±161	3.56±0.04
2	3	1	1.87±0.01	578±20	2513±72	5.11±0.04
2	4	3	1.77±0.01	709±28	2421±79	5.39±0.06
3	5	1	1.77±0.01	465±8	3712±112	3.87±0.04
3	6	4	1.85±0.02	458±26	3476±176	3.56±0.04
4	7	5	1.72±0.02	542±20	2653±119	3.35±0.04
4	8	2	1.77±0.02	513±12	2524±94	3.47±0.04
$\bar{x}(n = 8)$			1.78±0.02	560±30	2880±171	4.01±0.28
			White muscle			
2	9	6	1.83±0.01	4191±168	543±31	3.60±0.11

NOTE: Values are given as means ± SE. For sample site identification, see Fig. 1.

tuosity in the latter. In addition, compared with skeletal and cardiac muscle in other mammalian species, both muscle types displayed twice the capillary length density (capillary length per fiber volume at a given mitochondrial volume density). Similarly, capillary surface per fiber surface at a given mitochondrial volume per fiber was also some 2 times larger than in other mammalian muscles. These observations are consistent with the suggestion of an important functional role for the capillary–fiber interface in blood–tissue O_2 fluxes (Honig *et al.* 1991), and with measurements of O_2 uptake per millilitre of mitochondria in flying hummingbirds that are about twice as high as those in locomotory muscles of mammals running at $\dot{V}O_2$MAX (Suarez *et al.* 1991).

The purpose of this study was to further investigate capillary–fiber geometry in highly aerobic fish muscle and to compare the results with the above findings in mammalian and avian muscles. Tuna were chosen because they are capable of high swimming velocities and metabolic rates. Fish red cells are nucleated (as in bird) and can be larger than bird red cells (Altman and Dittmer 1961; Schmidt-Nielsen 1984). One might therefore anticipate both similarities and differences in rheological constraints on O_2 delivery to the muscle fibers in fish and bird muscle compared with mammalian muscle. In this study, we specifically addressed the question of whether or not the tuna red muscle (*i*) shows a similar capillary arrangement to that of pigeon, bat pectoralis muscle, or other mammalian muscles; (*ii*) has as high a capillary length density per volume density of mitochondria as that of bat and hummingbird muscles; and (*iii*) has as high a capillary surface per fiber surface, at a given mitochondrial volume per fiber, as that of bat and hummingbird flight muscles.

Materials and methods

Five skipjack tuna (*Katsuwonus pelamis*; body mass, M_b, 1.5–2 kg; fork length, 43–44 cm) of undetermined sex were used in this study. They were purchased from local commercial fishermen and held for up to 4 days in outdoor 10-m diameter tanks supplied with continuously flowing seawater (25 ± 1°C) at the Kewalo Research Facility, National Marine Fisheries Service, Honolulu, Hawaii. After being netted and anesthetized with sodium pentobarbital (40 mg/kg i.p.), they were suspended ventral side up at the surface of a small aquarium. The gills were irrigated with seawater during the entire procedure. A midline incision was made to expose the heart. Heparin (2000 U) was injected directly into the ventricle, followed by papaverine hydrochloride (30 mg). A cannula was inserted into the ventral aorta through the bulbus arteriosus, and the sinus venosus was cut open to secure outflow. Vascular perfusion fixation of the muscles was performed in four animals and infusion of casting material in one animal.

Muscle fixation by vascular perfusion

We followed the same procedure as described elsewhere in detail (Mathieu-Costello 1987). Briefly, the entire vasculature was perfused with saline (11.06 g NaCl/L; 350 mosM; 20 000 USP units heparin/L). Perfusion fixation followed with a 6.25% solution of glutaraldehyde in 0.1 M sodium cacodylate buffer (total osmolarity of the fixative, 1100 mosM; pH 7.4). All perfusions were performed at a nonpulsatile pressure of 80–90 mmHg (1 mmHg = 133 Pa). Two mucle samples (approximately 1 cm × 4 mm × 1 mm) of the red muscle were analyzed in each fish, as well as one white muscle sample (Table 1). All samples were taken from transverse sections of the fish at the level of the 8–10th spine of the first dorsal fin (Fig. 1). They were cut into thin longitudinal strips, stored in glutaraldehyde fixative (total osmolarity, 1100 mosmol; pH 7.4), and processed for electron microscopy as previously described (Mathieu-Costello 1987).

Tissue sectioning

One-micrometre sections were cut on an LKB Ultrotome III and stained with 0.1% aqueous toluidine blue solution. From each muscle sample, 4–8 blocks were cut into four transverse sections (angle between normal to section and fiber axis, $\alpha = 0°$) and four longitudinal sections ($\alpha = \pi/2$), following procedures described elsewhere (Mathieu-Costello 1987). Sarcomere length, l_o, was measured in each longitudinal section with a magnification of ×630; 10 measurements of groups of consecutive sarcomeres were systematically sampled over the entire area of each section, then averaged.

Ultrathin sections (50–70 nm) were cut transversely to the muscle fiber axis in each sample. They were contrasted with uranyl acetate and bismuth subnitrate (Riva 1974). Electron micrographs for morphometry were taken on 70-mm films with a Zeiss 10 transmission electron microscope. Micrographs of a carbon grating replica (E. F. Fullam Inc., Schenectady, N.Y.) were recorded for calibration on each film.

Microcorrosion casts

We used the same procedures as in the previous study of pigeon flight muscle (Potter *et al.* 1991). Briefly, a modified Batson's No. 17 casting compound (Nopanitaya *et al.* 1979) was infused into the ventral aorta at a constant flow rate of 9 mL/min using a Harvard

FIG. 1. Example of a macroscopic view of a transverse section of skipjack tuna (animal 2, Tables 1–4) examined at the level of the 10th spine of the first dorsal fin. The specific sites (1, 3, 6) where samples were taken from this fish, as well as those examined in other animals (Table 1), are indicated. Note that even after the blood was washed out of capillaries during the fixation by perfusion, the red muscle is still very dark because of its high myoglobin content. See text for details on the relative area of red and white muscle in this section.

infusion pump. Following infusion of approximately 80 mL of casting material, the fish was left undisturbed for 30 min to allow polymerization to occur. Samples (approximately 1–3 cm) of the red muscle were taken in a transverse section of the fish at the level of the 7th spine of the first dorsal fin. They were digested away by 40% potassium hydroxide at 60°C for a few days, washed in distilled water, and air dried. Then they were mounted on stubs with conductive carbon cement, sputter coated with gold in a Technics Hummer II apparatus, and examined with a Cambridge Instrument Stereoscan 360 electron microscope.

Morphometry

Capillary counts per fiber sectional area in transverse and longitudinal sections, $Q_A(0)$ and $Q_A(\pi/2)$, respectively, were collected by point counting, using an eyepiece square grid test A 100 (see Weibel 1979 Appendix 3) on 1-μm sections of plastic-embedded perfusion-fixed tissue examined at a magnification of ×400 with a Leitz Ortholux microscope. On average, 17 ± 1 (transverse sections) and 24 ± 2 fields (longitudinal sections) were examined per sample, yielding totals of 1832 ± 119 and 293 ± 26 fiber profiles in transverse and longitudinal sections, respectively, per red muscle sample. In the white muscle, we examined a total of 15 and 18 fields in transverse and longitudinal sections, respectively. This yielded a total of about 200 (transverse) and 110 fiber profiles (longitudinal) in the sample. The point counts of capillary profile numerical density were collected, stored, and processed using an Apple computer. As in previous studies (Mathieu-Costello 1987), capillary density estimates were related to the muscle fibers as a reference space in all samples to avoid variation due to the unreliable preservation of the intercellular spaces by the preparation procedures.

The method used to estimate the degree of orientation, i.e., anisotropy of capillaries in each sample has been described elsewhere in detail (Mathieu *et al.* 1983). We first estimated the ratio between capillary counts per sectional area of transverse and longitudinal sections of muscle fibers, $Q_A(0)$ and $Q_A(\pi/2)$, respectively. This ratio was then used to calculate, via a table or graph of known coefficients, (*i*) the capillary orientation concentration parameter, K, and (*ii*) the coefficient $c(K,0)$ relating capillary counts per unit area of fiber in transverse sections, $Q_A(0)$, and capillary length per volume of muscle fiber, $J_V(\text{c,f})$. Knowing $c(K,0)$, an estimate of $J_V(\text{c,f})$ can be obtained by using the following equation:

$$[1] \quad J_V(\text{c,f}) = c(K,0) \cdot Q_A(0)$$

which is based on the Fisher axial distribution of capillary segment orientation, relative to the axis of anisotropy (Mathieu *et al.* 1983). As described elsewhere (Mathieu *et al.* 1983; Mathieu-Costello 1987), the coefficient $c(K,0)$ is a direct indication of the amount of capillary length added by tortuosity and branching. For straight and unbranched capillaries, all parallel to the muscle fiber axis (perfect anisotropy), $K = \infty$ and $c(K,0) = 1$. For capillaries with no preferred orientation (isotropy), $K = 0$ and $c(K,0) = 2$, indicating that capillary length density is actually twice that given by a simple estimate of 'capillary density' in transverse sections, $Q_A(0)$.

Capillary diameter, $\bar{d}(\text{c})$, fiber cross-sectional area, $\bar{a}(\text{f})$, fiber cross-sectional perimeter, $\bar{b}(\text{f})$, and capillary number around a fiber, N_{CAF}, were measured with an image analyzer (Videometric 150, American Innovision Inc.). On average, 154 ± 17 (SE) fibers were measured per sample. They were randomly selected on the same transverse sections used to estimate $Q_A(0)$. Capillary diameter, $\bar{d}(\text{c})$, was obtained from circular profiles only (difference between smaller and longer diameters less than 15%). On average, 147 ± 5 (SE) capillary profiles were measured per sample. The selection of circular profiles to estimate $\bar{d}(\text{c})$ assumed capillary cross-sectional circularity, based on our previous findings in rat muscles (Mathieu-Costello 1987).

Capillary–fiber ratio, $N_N(\text{c,f})$ was computed as the product of capillary counts per fiber cross-sectional area, $Q_A(0)$, and fiber cross-sectional area, $\bar{a}(\text{f})$.

Capillary–fiber perimeter ratio, in transverse section, $B_B(0)$ was estimated by intersection counting using the equation

$$[2] \quad B_B(0) = I_c/I_f$$

where I_c and I_f are the intersections of test lines of a test grid with the boundaries of capillaries and fibers, respectively, in transverse sections. We used a 100-point square grid eyepiece system on the same sections used to estimate capillary number per fiber cross-sectional area, $Q_A(0)$. On average, 63 ± 4 (SE) fields were measured per sample examined at a magnification of ×1000. Intersections were counted with both the horizontal and the vertical lines of the grid.

Capillary surface per fiber volume, $S_V(\text{c,f})$, and capillary surface per fiber surface, $S_S(\text{c,f})$, were estimated as (Mathieu-Costello *et al.* 1991)

$$[3] \quad S_V(\text{c,f}) = J_V(\text{c,f}) \cdot \pi \cdot \bar{d}(\text{c})$$

and

$$[4] \quad S_S(\text{c,f}) = B_B(0) \cdot c'(K',0)$$

where $c'(K',0)$ is an anistropy coefficient relating capillary perimeter per fiber cross-sectional area and capillary surface per volume of fiber. Except for muscles with a large degree of tortuosity of capil-

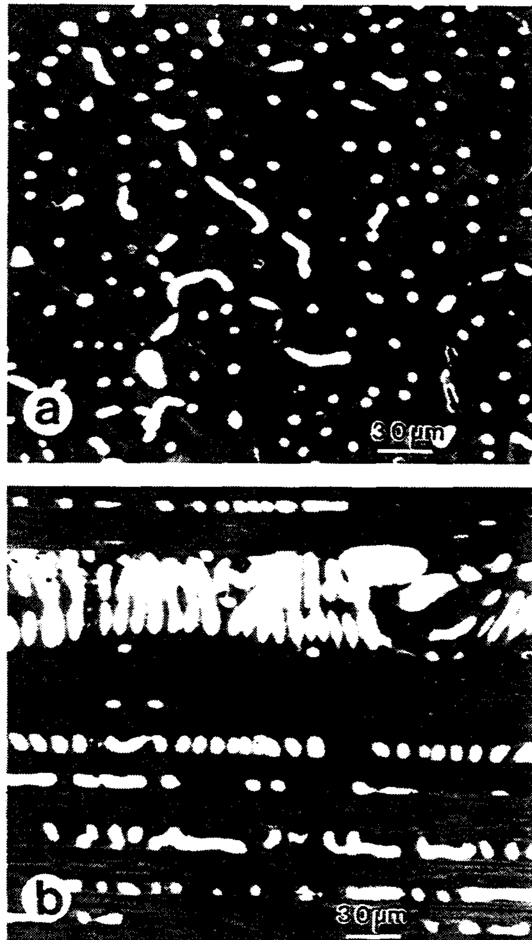

FIG. 2. Light micrographs of portions of muscle bundles in transverse (*a*) and longitudinal sections (*b*) of tuna red muscle. Capillaries are empty after the fixation by vascular perfusion. Note the large number of capillary branches running perpendicularly to the muscle fiber axis (*b*).

laries (coefficient $c(K,0) > 1.53$) the value of $c'(K',0)$ is 1. The maximal value of $c'(K',0)$ is 1.27 (for $c(K,0) = 2$; see Mathieu-Costello *et al.* 1991).

The volume density of mitochondria, myofibrils, and lipid droplets per volume of muscle fiber was estimated by standard point-counting procedure at a final magnification of × 22 500. Fourteen to sixteen micrographs were obtained by systematic sampling in one ultrathin transverse section from two randomly chosen blocks per sample in red muscle (total, 28–32 micrographs/sample) and four in the white muscle (total, 80 micrographs). Contact prints of the electron micrographic (EM) films were projected on a square grid test A 144 (see Weibel 1979 Appendix 3), using a microfilm reader (Documator DL 2, Jenoptic, Jena).

Mitochondrial volume per micrometre fiber length, $V_N(\text{mt,f})$, was calculated as

$$[5] \quad V_N(\text{mt,f}) = V_V(\text{mt,f}) \cdot \bar{a}(\text{f})$$

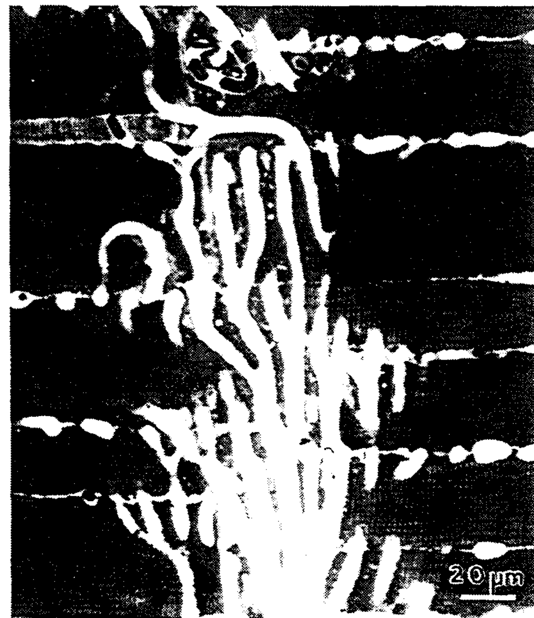

FIG. 3. Light micrograph of a section of a capillary manifold in tuna red muscle (compare with Fig. 4 in Potter *et al.* 1991).

Statistical analysis

Data are expressed as means ± SE. The standard errors of the estimates of capillary and mitochondrial density, as well as fiber cross-sectional area and perimeter, were calculated by pooling the data of the pictures from one sample (2–4 randomly chosen blocks cut transversely and (or) longitudinally) and applying formulas for the standard error of ratios. Therefore, the standard error indicates the variability between pictures at the sampling location analyzed in each sample. The standard error of the estimates of capillary diameter, $\bar{d}(\text{c})$, and number around a fiber, N_{CAF}, indicates the variability between capillaries and fibers, respectively. Sample and group means were compared using the Student's *t*-test. Differences were taken as significant for $p < 0.05$.

Results

Figure 1 shows the macroscopic appearance of a transverse section of skipjack tuna after vascular perfusion fixation. The red muscle appeared very dark in spite of the washout of the red blood cells during the perfusion procedure (see Materials and methods and Fig. 2). This was due to the large myoglobin content of the muscle fibers. The cross-sectional areas of the red and white muscle (Fig. 1) were about 8 and 55 cm^2, respectively. The red muscle represented about 10% of the fish total cross-sectional area. Low-power light micrographs of transverse and longitudinal sections of perfused red muscle are shown in Figs. 2*a* and 2*b*. Transverse sections revealed a small fiber size and large capillary density (Fig. 2*a*) in all samples. As in pigeon flight muscle (Mathieu-Costello 1988 and 1991*b*), longitudinal sections of tuna red muscle showed straight capillaries running parallel to the muscle fibers, as well as capillaries oriented perpendicularly to the muscle fiber axis (Figs. 2*b* and 3). This suggested the presence of capillary manifolds in the muscles (compare Fig. 3 in this study with

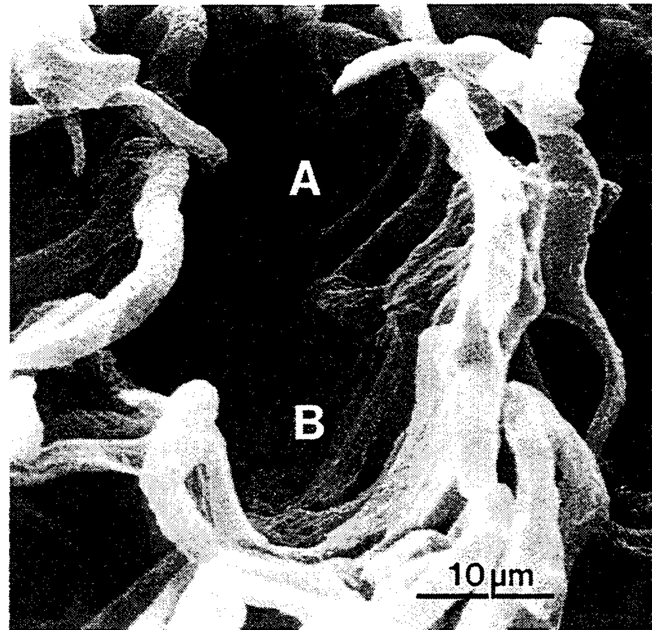

FIG. 4. Scanning electron micrograph of a cross-sectional view of a corrosion cast from tuna red muscle. Note the dense envelope formed by capillaries. Based on fiber dimensions (see Results), two muscle fibers (A and B) could be contained in the empty space. Micrograph taken by M. L. Costello.

TABLE 2. Morphometric estimates of degree of capillary geometry: counts in transverse ($Q_A(0)$) and longitudinal sections ($Q_A(\pi/2)$), orientation concentration parameter (K), anisotropy coefficient ($c(K,0)$), length density ($J_V(c,f)$), and surface per fiber volume ($S_V(c,f)$) in skipjack tuna muscle

Muscle No.	$Q_A(0)$ mm^{-2}	$Q_A(\pi/2)$ mm^{-2}	K	$c(K,0)$	$J_V(c,f)$ mm^{-2}	$S_V(c,f)$ mm^{-1}
			Red muscle			
1	3055±147	1791±76	1.03±0.13	1.47±0.05	4491±265	53.5±3.2
2	2686±161	1941±175	0.63±0.20	1.64±0.10	4405±377	49.3±4.3
3	2513±72	1495±85	1.00±0.13	1.48±0.05	3719±165	59.7±2.7
4	2421±79	1104±40	1.56±0.11	1.31±0.02	3172±114	53.7±2.0
5	3712±112	1669±112	1.59±0.17	1.31±0.04	4863±209	59.1±2.6
6	3476±176	2087±115	0.99±0.14	1.48±0.05	5144±313	57.5±3.6
7	2653±119	1454±67	1.17±0.13	1.42±0.04	3767±200	39.6±2.2
8	2524±94	1375±48	1.18±0.10	1.42±0.03	3584±153	39.1±1.7
$\bar{x}(n = 8)$	2880±171	1615±114	1.14±0.11	1.44±0.04	4143±242	51.4±2.9
			White muscle			
9	543±31	170±18	2.57±0.4	1.16±0.03	630±39	7.1±0.5

NOTE: Values are given as means ± SE. For muscle identification, see Table 1.

Fig. 4 in Potter *et al.* 1991). The examination of vascular casts by scanning electron microscopy showed capillaries forming a dense envelope of blood around portions of muscle fibers in tuna red muscle (Fig. 4).

Sarcomere length, fiber cross-sectional area, capillary number per fiber cross-sectional area, and capillary diameter are given in Table 1. Sarcomere length ranged from 1.72 to 1.87 μm in the samples. The average fiber cross-sectional area of 560 ± 30 μm^2 yielded an equivalent circular fiber diameter of 27 ± 1 μm in red muscle. Capillary number per fiber cross-sectional area was 2880 ± 171 mm^{-2}. In white muscle, fiber size was about 8 times greater than, and capillary density was about 5 times lower than, that of red muscle (Table 1). Mean capillary diameter ranged from 3.35 to 5.39 μm (Table 1) in red muscle. It was 3.6 μm in the white muscle sample.

Morphometric estimates of capillary orientation coefficients, K and $c(K,0)$, and capillary length and surface per fiber volume, $J_V(c,f)$ and $S_V(c,f)$, respectively, are given in Table 2. The

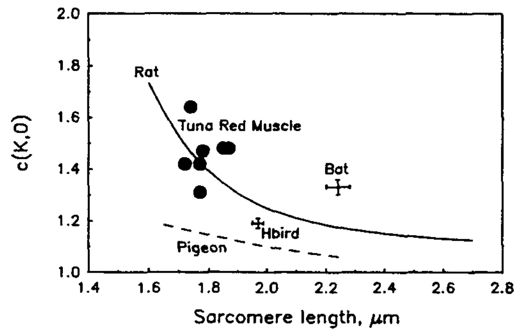

FIG. 5. Plot of anisotropy coefficient $c(K,0)$, relating $Q_A(0)$ to $J_V(c,f)$, against sarcomere length in tuna red muscle, and group mean values in bat (Mathieu-Costello *et al.* 1992*b*) and hummingbird (Hbird) flight muscles (Mathieu-Costello *et al.* 1992*a*). Relationships between $c(K,0)$ and sarcomere length in rat hindlimb (n = 42) (Mathieu-Costello *et al.* 1991) and pigeon pectoralis muscle (Mathieu-Costello 1991*b*) are shown by the solid and broken lines, respectively.

narrow range of sarcomere length, l_o, in the samples did not allow us to examine the relationship(s) between the orientation coefficients and fiber shortening, i.e., reduction in sarcomere length. Comparison with data from previous studies showed that the values of $c(K,0)$ in tuna red muscle were similar to those previously found in rat hindlimb muscles examined at similar sarcomere length (Fig. 5, solid line). For comparison, the curvilinear relationship between $c(K,0)$ and l_o in pigeon and mean group values in bat pectoralis and hummingbird pectoralis and supracoracoideus muscles are also shown in Fig. 5. As mentioned in the Materials and methods section, the coefficient $c(K,0)$ is an index of the amount of capillary length added by tortuosity and branching. In red muscle, capillary length per volume of muscle fiber ($\bar{x}$ = 4143 ± 242 mm^{-2}) was 44% larger than a simple estimate of 'capillary density' in transverse sections, $Q_A(0)$, would indicate (the group mean value of the $c(K,0)$ was 1.44 ± 0.04, Table 2; see eq. 1). In the white muscle sample, $c(K,0)$ was 1.16 ± 0.03 and $J_V(c,f)$ was 630 mm^{-2}. $S_V(c,f)$ ranged from 39.1 to 59.7 mm^{-1} in red muscle. It was 6–9 times smaller in white muscle (Table 2).

The low-power electron micrograph shown in Fig. 6 illustrates the abundance of mitochondria in the muscle fibers of tuna red muscle. Table 3 gives morphometric data on fiber ultrastructure. In red muscle, mitochondria occupied from 24 to 32% of the muscle fiber volume. About one-sixth (muscle 7) to more than one-third (muscles 1–3) of the total volume of mitochondria was subsarcolemmal. The fractional volume of subsarcolemmal mitochondria varied greatly among sampling sites in 2 out of 4 animals (Table 3; muscles 3–4 and 7–8). Mitochondrial volume density was about 10 times lower in the white muscle (Table 3). The volume of lipid droplets per volume of muscle fiber ranged from 0 to 2% and also varied greatly between samples in the same animal (Table 3; muscles 1–2, 3–4, and 5–6); in several red muscle samples (muscles 1, 6–8), it was as low as in the white muscle sample (Table 3). Figure 7 shows the plot of $J_V(c,f)$, against mitochondrial volume density, $V_V(mt,f)$, in the samples. For comparison, the linear regression between $J_V(c,f)$ and $V_V(mt,f)$ in bat and rat muscles, as well as mean group values in hummingbird, bat, and rat muscles are shown in Fig. 7.

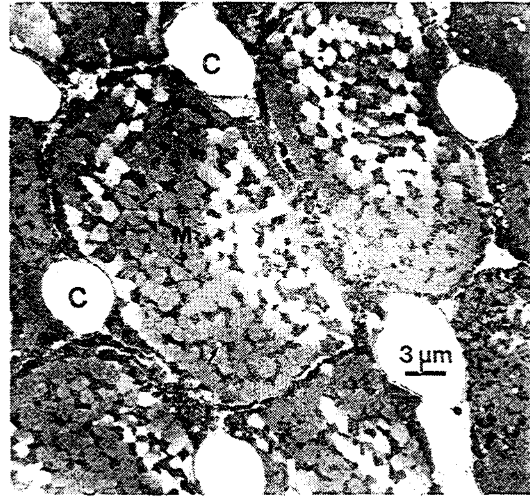

FIG. 6. Electron micrograph of transverse section of muscle fibers and adjacent capillaries (C) and skipjack tuna red muscle. Note the large density of mitochondria (M) and relatively few lipid droplets (arrows) in the muscle fibers.

Capillary surface per volume of mitochondria, i.e., the ratio between $S_V(c,f)$ (Table 2) and $V_V(mt,f)$ (Table 3) was 180 ± 7 mm^2/mm^3 in red muscle, and 254 ± 33 mm^2/mm^3 in white muscle. The surface of inner mitochondrial membrane per unit volume of mitochondria was 63 000 (subsarcolemmal) and 71 000 mm^2/mm^3 (interfibrillar mitochondria) in red muscle and was 31 000 mm^2/mm^3 in white muscle (Moyes *et al.* 1992). This yields a capillary surface per unit surface area of inner mitochondrial membrane of about 2.6 × 10^{-3} (red muscle) and 8.2 × 10^{-3} (white muscle). Thus, the aggregate surface of *inner* mitochondrial membrane was about 400 times (red muscle) and 125 times (white muscle) greater than capillary surface area. The surface areas of mitochondrial outer membrane were 7500 and 4700 mm^2/mm^3 in red and white muscle, respectively (O. Mathieu-Costello, unpublished data). Thus, capillary surface per unit surface area of outer mitochondrial membrane was 0.024 (red muscle) and 0.054 (white muscle). In red muscle the aggregate surface area of *outer* mitochondrial membrane was about 10 times smaller than inner mitochondrial membrane surface area, and about 40 times greater than capillary surface area. In white muscle the aggregate surface area of outer mitochondrial membrane was about 7 times smaller than inner mitochondrial membrane surface area and about 20 times greater than capillary surface area.

Estimates of capillary–fiber morphometrics and mitochondrial volume per fiber in each sample are given in Table 4. Capillary–fiber perimeter ratio ranged from 0.26 to 0.34 in red muscle. It was about one-fourth these values in white muscle. The number of capillaries around each fiber, N_{CAF}, was similar in red (range 4.43–5.36) and white muscle (4.77) in spite of the 8-fold difference in fiber size between the muscles (Table 1). The capillary surface anisotropy coefficient, $c'(K',0)$, had a value of 1 (i.e., capillary–fiber perimeter ratio, $B_B(0)$, was a direct estimate of capillary surface per fiber surface, $S_S(c,f)$) in all samples except one. In muscle 2,

TABLE 3. Morphometric estimates of fiber ultrastructure: volume density of total (V_V(mt,f)), subsarcolemmal (V_V(ms,f)), and interfibrillar (V_V(mi,f)) mitochondria, and volume density of intracellular lipids (V_V(li,f)) and myofibrils, (V_V(my,f))

Muscle No.	V_V(mt,f) (%)	V_V(ms,f) (%)	V_V(mi,f) (%)	V_V(li,f) (%)	V_V(my,f) (%)
			Red muscle		
1	29.9±1.6	11.4±1.5	18.5±0.9	0.1±0.0	66.0±1.9
2	30.5±1.6	12.3±2.1	18.2±0.9	1.1±0.3	61.4±1.5
3	32.4±1.9	12.3±2.4	20.1±1.0	0.9±0.3	64.6±2.1
4	26.0±1.5	5.4±1.5	20.6±0.9	2.0±0.5	69.0±1.6
5	29.8±1.1	7.9±1.3	21.8±0.9	1.7±0.4	67.4±1.5
6	29.6±1.4	8.5±1.7	21.1±0.7	0.0±0.0	66.4±1.5
7	24.4±0.6	4.2±0.8	20.2±0.8	0.0±0.0	68.9±1.2
8	25.1±1.1	8.1±1.2	17.1±0.6	0.0±0.0	65.2±1.5
$\bar{x}(n = 8)$	28.5±1.0	8.8±1.1	19.7±0.6	0.7±0.3	66.1±0.9
			White muscle		
9	2.8±0.3	0.7±0.2	2.1±0.2	0.1±0.0	69.3±1.0

NOTE: Values are given as means ± SE. For muscle identification, see Table 1.

TABLE 4. Data relating capillary–fiber morphometrics to mitochondrial amount per micrometre length of fiber (V_N(mt,f)) in skipjack tuna muscle: fiber perimeter ($\bar{b}$(f)), capillary–fiber perimeter ratio in transverse sections (B_B(0)), capillary–fiber number ratio (N_N(c,f)), and capillary number around a fiber (N_{CAF})

Muscle No.	$\bar{b}$(f) (μm)	B_B(0)	V_N(mt,f) (μm^3)	N_N(c,f)	N_{CAF}
			Red muscle		
1	108±3	0.26±0.01	170±15	1.74±0.15	5.32±0.16
2	113±4	0.27±0.01	196±14	1.73±0.13	5.36±0.18
3	114±3	0.34±0.01	187±13	1.45±0.07	4.43±0.10
4	124±3	0.34±0.01	184±13	1.72±0.09	4.72±0.10
5	100±2	0.31±0.01	139±6	1.73±0.06	5.12±0.13
6	98±3	0.31±0.01	136±10	1.59±0.12	5.16±0.12
7	102±3	0.29±0.01	132±6	1.44±0.08	5.16±0.15
8	100±3	0.30±0.01	129±6	1.29±0.06	4.47±0.12
$\bar{x}(n = 8)$	107±3	0.30±0.01	159±10	1.59±0.06	4.97±0.13
			White muscle		
9	253±1.0	0.07±0.01	117±13	2.28±0.16	4.77±0.21

NOTE: Values are given as means ± SE. For muscle identification, see Table 1.

the value of $c(K,0)$ was 1.64 (Table 2). This yielded a $c'(K',0)$ of 1.05 in that sample (see Mathieu-Costello *et al.* 1991 for the estimation of $c'(K',0)$). Thus, S_S(c,f) was 5% larger than B_B(0) in the sample (see eq. 4). The plot of S_S(c,f) against mitochondrial volume per micrometre length of fiber, V_N(mt,f) is shown in Fig. 8. For comparison, the linear regression between S_S(c,f) and V_N(mt,f) in rat soleus muscle is also shown (broken line), as well as group averages in bat muscles and hummingbird flight muscle.

Discussion

Small fiber size, high capillary numerical density, and high mitochondrial volume density are well established characteristics of tuna red muscle (Bone 1978; George and Stevens 1978; Hulbert *et al.* 1979; Dickson 1988). In this study, we used recently developed morphometric methods to quantify the geometry of blood–tissue exchange in tuna. We characterized the structural potential for O_2 delivery to the muscle fibers (capillary length and surface area) and examined the relationship(s) between capillarity and fiber mitochondrial volume. Comparison with highly aerobic muscles of birds and mammals revealed both similarities and striking differences in capillary–fiber structural relationships in tuna red muscle.

Fiber size

The average fiber circular diameters of 27 ± 1 (SE) μm (red muscle), and about 73 μm (white muscle) are smaller than or similar to those previously reported in tuna muscle after fixation by immersion in glutaraldehyde solution and processing for electron microscopy. Bone (1978) measured mean fiber diameters of 31 and 100 μm in skipjack red and white muscle, respectively. George and Stevens (1978) reported values of 34.58 ± 6.16 (SD) (red muscle) and 66.03 ± 11.59 (SD) μm (white muscle) in kawakawa, *Euthynnus affinis*. Looking at individual fiber dimensions, Hulbert *et al.* (1979) found a vast range of fiber diameters both in red (12–52) and white (20–110 μm) tuna muscle. Based on our previous comparisons with

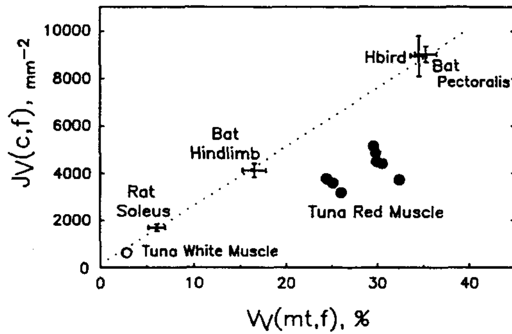

FIG. 7. Plot of capillary length per fiber volume, J_V(c,f), against mitochondrial volume per volume of muscle fiber, V_V(mt,f), in tuna red muscle (●) and white muscle (○). Linear regression in bat and rat muscles (broken line, $J_V(c,f) = 249 \cdot V_V(mt,f) + 142$; $r = 0.993$) and group mean values in bat hindlimb and pectoralis muscle and in rat soleus muscle are from Mathieu-Costello *et al.* (1992*b*); group mean value in hummingbird (Hbird) pectoralis and supracoracoideus muscles is from Mathieu-Costello *et al.* (1992*a*).

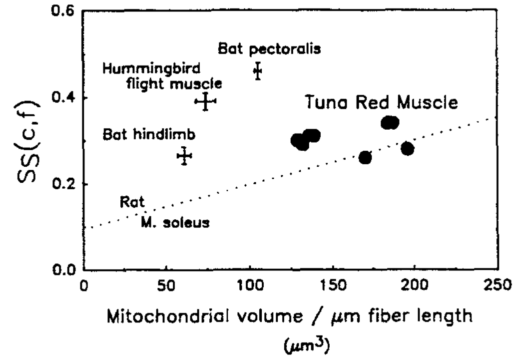

FIG. 8. Correlation between capillary surface per fiber surface, S_S(c,f), and mitochondrial volume per micrometre fiber length, V_N(mt,f), in tuna red muscle (●). Linear regression in rat soleus muscle (broken line, $S_S(c,f) = 0.0010 \cdot V_N(mt,f) + 0.1$; $r = 0.67$) and group mean values in bat hindlimb and pectoralis muscle are from Mathieu-Costello *et al.* (1992*b*); group mean value in hummingbird flight muscles (pectoralis and supracoracoideus) is from Mathieu-Costello *et al.* (1992*a*).

material prepared for histochemistry (see Mathieu-Costello *et al.* 1992*b*), we expect a minimal amount of tissue shrinkage during the fixation and preparation procedure used in this study compared with muscle *in situ*. The amount of tissue shrinkage in freshly frozen tissue processed for histochemistry is known to be minimal (Brodal *et al.* 1977). In various fish specimens, including *Katsuwonus* and *Euthynnus*, Dickson (1988) reported average fiber diameters of 30–35 and 65–70 μm in red and white muscle processed for histochemistry. This suggests that the above differences between studies are not likely to be related to tissue shrinkage during preparation. Besides interanimal and (or) interspecies variability, a source of variation in fiber cross-sectional area is the variable amount of fiber shortening during sample removal from intact muscles (see Mathieu-Costello *et al.* 1989). To our knowledge, this is the first study in which tuna muscles were fixed by vascular perfusion-fixation, i.e., *in situ* fiber length was preserved. The average sarcomere length values in the samples (Table 1) are within the range of *in situ* sarcomere lengths measured in red and white muscle of coalfish (Patterson and Goldspink 1972). We are not aware of any report of sarcomere length in excised muscles of tuna.

In axial musculature of African lungfish, Dunn *et al.* (1981) measured sarcomere lengths of 1.5 and 2.0 μm for red and white muscle, respectively. Average fiber diameter *at 2.1 μm sarcomere length* was 20 in red and 66 μm in white muscle (calculated from Dunn *et al.* 1981) compared with 25 (red muscle) and 68 μm (white muscle) in tuna (calculated from data in Table 1), suggesting that fiber size in tuna muscle is not the lowest among fish. Other reported values for fiber cross-sectional area in fish red muscle (no sarcomere length indicated) ranged from 471 μm^2 in trout (Johnston and Moon 1980) to 1115 μm^2 in highly aerobic fibers of anchovy (Johnston 1982*b*). Thus, fiber size in tuna red muscle was not particularly small compared with other fish, and a small fiber size is not necessarily a characteristic of highly aerobic fibers in fish (mitochondrial volume density in anchovy red muscle was very high; see below). In tuna red muscle, fiber cross-sectional area was similar to that of aerobic fibers in pigeon flight muscle (Mathieu-Costello 1991*b*), bat flight muscle, and bat hindlimb (Mathieu-Costello *et al.* 1992*b*) at a similar sarcomere length. The cross-sectional area of tuna red muscle fibers was about 4 times smaller than in rat soleus muscle (Mathieu-Costello 1987), 3–4 times smaller than in dog gastrocnemius muscle (Mathieu-Costello 1991*a*), and 2–4 times greater than in hummingbird pectoralis and supracoracoideus muscle (Mathieu-Costello *et al.* 1992*a*).

The length of thick and thin filaments muscle was 1.45 ± 0.02 and 1.75 ± 0.03 μm (red muscle) and 1.43 ± 0.03 and 1.67 ± 0.03 μm (white muscle), respectively. The values in red muscle are very close to those in hummingbird flight muscle similarly prepared (Mathieu-Costello *et al.* 1992*a*). The values for thick filament are consistent with those reported in fish (Akster 1981; Sosnicki *et al.* 1991), rat (Walker and Schrodt 1969; Mathieu-Costello 1987), and bat muscles (Mathieu-Costello *et al.* 1992*b*) after glutaraldehyde fixation. These data support the idea of a constant myosin filament length in muscles of vertebrates. The values for thin filaments are similar to that reported in white and pink fibers of perch (Akster 1981). They are smaller than in fixed red and white muscle of carp (Sosnicki *et al.* 1991), rat (Walker and Schrodt 1969), or bat (Mathieu-Costello *et al.* 1992*b*), and living rat muscles (Close 1972). Variations in the length of thin filaments have been reported in crustacean (Franzini-Armstrong 1970) and fish muscles (Akster 1981). To our knowledge, filament length in tuna has never been reported. Shorter filament lengths allow faster rates of contraction (Franzini-Armstrong 1970; Akster 1981; Otten 1987) and may have implications in contraction velocity rates in tuna. The relationship(s) between filament length, functional sarcomere length range, and contraction velocity in red and white muscle of tuna at different swimming speeds is (are) not known.

Capillarity

The average number of capillaries around a fiber, N_{CAF}, in red and white muscle (Table 4) was very close to that previously reported in skipjack tuna red muscle (4.75; Bone 1978). Notice that there are approximately the same number of capil-

laries around each fiber in red and white muscle (Table 4) in spite of the large difference in fiber size (Table 1). Larger values for N_{CAF} have been reported in other fish (e.g., 5.3–6.6 in red muscle of flathead, *Platycephalous bassensis*, Mosse 1978). Compared with highly aerobic muscle of birds and mammals, the number of capillaries around a fiber was the same in tuna red muscle as in hummingbird flight muscle, in which fiber size was substantially smaller (Mathieu-Costello *et al.* 1992*a*). It was significantly smaller than in bat pectoralis muscle (5.63 ± 0.23) and rat soleus muscle (5.96 ± 0.35), i.e., in muscles with smaller (bat pectoralis muscle) or substantially larger fiber size (rat soleus muscle; Mathieu-Costello *et al.* 1992*b*).

We are not aware of any report of capillary density (e.g., capillary–fiber ratio or capillary number per fiber cross-sectional area) or geometry in tuna muscle. When compared with red muscle of various species of fish with similar fiber size (e.g., Mosse 1979), tuna capillary–fiber ratio was high (1.29–2.28; Table 4) but not the highest for fish. Similar or greater values have been found in fish red muscle with larger fiber size. For example, capillary–fiber ratios of 1.87–2.45 were reported in flathead red muscle (Moose 1978). In 28°C-acclimated carp it was 2.2 ± 0.1 and increased to 4.8 ± 0.2 with acclimation to 2°C (Johnston 1982*a*). For comparison, capillary–fiber ratio in hummingbird flight muscle was 1.55 ± 0.06. It was 2.02 ± 0.10 and 1.24 ± 0.06 in bat pectoralis muscle and hindlimb, respectively, and 2.59 ± 0.24 in rat soleus muscle.

Except for slow muscle of anchovy, in which substantially higher capillary density has been reported (Johnston 1982*b*), capillary number per fiber cross-sectional area in tuna red muscle (Table 1) was among the highest in fish muscles (for review see Egginton and Johnston 1983). In slow muscle of Conger eel fixed at an average sarcomere length of 2.3 μm, Egginton and Johnston (1983) measured an anisotropy coefficient, $c(K,0)$, of 1.016. Such a high degree of capillary orientation was possibly related to the large number of anastomoses in the muscle (Egginton and Johnston 1983). Tuna red muscle also showed a high number of capillary branches running perpendicularly to the muscle fiber axis (Figs. 2*b*, 3, and 4). However, the capillary orientation coefficient ($c(K,0)$; Fig. 5 and Table 2) was substantially higher than previously reported in fish muscle. Besides a direct effect of sarcomere length on capillary anisotropy (Fig. 5), it is possible that differences in capillary branching between Conger eel and tuna muscles account for the difference in $c(K,0)$ values. In pigeon pectoralis muscle, the degree of anisotropy of capillaries was much lower than in tuna or rat muscle fixed at similar sarcomere length (Fig. 5). The large difference in $c(K,0)$ values between muscles shows the importance of considering capillary geometry when comparing the size of the capillary network in muscles. Londraville and Sidell (1990) also measured high $c(K,0)$ values (1.73 and 1.45) in red muscle samples of two antarctic fish, *Trematomus newnesi* and *Notothemia gibberifrons*, respectively. In tuna red muscle (this study), capillary tortuosity and branching added an average of 1267 mm^{-2} to capillary length per fiber volume compared with straight capillaries oriented strictly parallel to the muscle fibers. In pigeon pectoralis muscle fixed at approximately the same sarcomere length (muscle 1 in Mathieu-Costello 1991*b*), the product of $Q_A(0)$ and $(c(K,0) - 1)$ was 520 mm^{-2}.

Mitochondrial density

There are numerous reports of mitochondrial volume density in fish muscle (for review see Dunn *et al.* 1981). The value of 35% reported by Hulbert *et al.* (1979) for tuna red muscle is greater than the range of mitochondrial volume densities we found in this study. In white muscle, mitochondrial volume density was 2.8% (this study) compared with 2% reported by Hulbert *et al.* 1979. High mitochondrial densities (20–30%) have been reported in red muscle of a number of fish species, including shark (Kryvi 1977), eel (Hulbert and Moon 1978), trout, and carp (Johnston and Moon 1981). The highest mitochondrial volume density for fish (45.5%) was found in anchovy red muscle (Johnston 1982*b*).

Capillarity and O_2 demand

It is interesting that in tuna red muscle, the linear regression of $J_V(c,f)$ and $V_V(mt,f)$, $r = 0.52$ ($J_V(c,f) = 123 \cdot V_V(mt,f) + 619$), yielded a capillary length per fiber volume of 4309 mm^{-2} at 30% mitochondrial density. This value of $J_V(c,f)$ is about one-half that in bat muscles at the same mitochondrial volume density (Fig. 7). Comparison with data obtained for capillary and mitochondrial density in a number of species of fish (for review see Egginton and Johnston 1983) is not possible because sarcomere length and capillary orientation were not reported. Both capillary number per fiber cross-sectional area, $Q_A(0)$, and $c(K,0)$ depend on sarcomere length, and the estimation of $J_V(c,f)$ requires knowledge of capillary orientation (see eq. 1). In two studies in which the degree of orientation of capillaries was estimated, capillary length per fiber volume at a given mitochondrial volume density in the muscle fibers was substantially smaller than in tuna red muscle. In red muscle of Conger eel, $J_V(c,f)$ was 625 mm^{-2} for a fiber mitochondrial volume density of 22.7% (Egginton and Johnston 1983). In antarctic fish in which mitochondrial volume density distributions in the muscle fibers ranged from 20 to 40%, $J_V(c,f)$ averaged 505–639 mm^{-2} (Londraville and Sidell 1990).

At a given mitochondrial volume per fiber, the average number of capillaries around a fiber, (N_{CAF}), was also smaller in tuna red muscle than in highy aerobic muscles of birds and mammals. The linear regression between N_{CAF} and mitochondrial volume per micrometre length of fiber, $V_N(mt,f)$, in bat pectoralis muscle, bat hindlimb, and rat soleus muscle yields mean group values for N_{CAF} of 6.58 ± 0.20 (bat pectoralis muscle), 5.64 ± 0.15 (bat hindlimb) and 6.45 ± 0.28 (rat soleus muscle) at 159 μm^3 mitochondrial volume per fiber (calculated from Mathieu-Costello *et al.* 1992*b*). The mean group value for tuna red muscle at a similar mitochondrial volume per fiber (Table 4) was significantly smaller. As for bat and rat muscles,, the average number of capillaries around a fiber in hummingbird pectoralis and supracoracoideus muscles at a given mitochondrial volume per fiber was also significantly higher than in tuna.

In tuna red muscle, capillary surface per fiber surface (0.30 ± 0.01 at 159 μm^3 mitochondrial volume per fiber, Table 4) was not significantly different from that in rat soleus (0.32 ± 0.02) at the same mitochondrial volume per fiber of 159 μm^3 (calculated from Mathieu-Costello *et al.* 1992*b*). In bat pectoralis muscle, capillary surface per fiber surface was about twice that found in tuna red muscle at a similar mitochondrial volume per fiber (Fig. 8). There have been several reports of capillary contact length, fiber perimeter, fiber cross-sectional area, and mitochondrial volume density in fish muscles, including anchovy red muscle, which has the highest reported mitochondrial volume density for fish (Johnston 1982*b*). Percent capillary–fiber contact, i.e., the ratio between capillary contact length and fiber perimeter is an equivalent measurement to the stereological estimate of capillary–fiber perimeter

ratio, $B_B(0)$; see Mathieu-Costello *et al.* 1991. Capillary–fiber percent contact length was 31% in hagfish (Flood 1979), 23% in shark (Totland *et al.* 1981), and 51% in anchovy (Johnston 1982*b*). Thus, the 30% value in tuna red muscle (Table 4) was not the highest for fish muscle. Capillary surface (μm^2) supplying 1 μm^3 of mitochondria was 0.06 in shark (Totland *et al.* 1981) and 0.18 in anchovy (Johnston 1982*b*). In tuna red muscle, the value of this variable (i.e., $B_B(0) \cdot \bar{b}(f)/V_N(mt,f)$ was 0.20 ± 0.02. This underscores the importance of considering fiber size when comparing the relationship(s) between capillarity and mitochondrial volume in muscles. In anchovy, mitochondrial volume density (45%) and capillary–fiber surface ratio (51%; Johnston 1982*b*) were almost 2 times higher than in tuna red muscles. Capillary contact length per fiber in anchovy (91.6 ± 5.1 μm) was 3 times greater than in tuna ($B_B(0) \cdot \bar{b}(f) = 32.1 \pm 1.4$ μm). Besides differences in capillary–fiber surface ratio, the difference in capillary contact length was due to the different fiber size in the muscle. Fiber cross-sectional area was about 2 times greater in anchovy (Johnston 1982*b*) than in tuna red muscle. As a result, mitochondrial volume per micrometre length of fiber, $V_N(mt,f)$, was substantially greater in anchovy (507 μm^3; calculated from Johnston 1982*b*) than in tuna (159 μm^3). However, capillary surface per unit volume of mitochondria in the muscle fibers was very close in the two muscles.

Functional implications

Obviously, a number of functional variables including operating temperature, blood flow, functional hematocrit, and blood O_2-carrying capacity affect O_2 delivery to the muscle fibers. From a structural point of view, the arrangement of the capillary network in tuna red muscle resemble that of highly aerobic muscles of bird (pigeon pectoralis, and hummingbird pectoralis and supracoracoideus muscles) in that evidence of capillary manifolds was found. Such an arrangement that increases capillary surface at the venular end of the network where O_2 tension is lower will enhance O_2 delivery to the muscle fibers (Potter *et al.* 1991). Gaehtgens *et al.* (1981*a*, 1981*b*) suggested that a different capillary architecture in bird muscles may compensate for the less favorable rheological properties of nucleated red cells. Hughes *et al.* (1982) found red blood cells of a teleost fish, the yellowtail *Seriola quinqueradiata*, less deformable than human red cells when compared at their normal environmental temperature. To our knowledge there are no available data on tuna red cell deformability. In skipjack tuna, red cells are nucleated as in other fish and they are rather large (short axis: 7.40 ± 0.07 μm; long axis, 9.61 ± 0.05 μm). Thus, the presence of capillary manifolds in bird and tuna muscles could be related to the larger size and lesser deformability of their red blood cells compared with those of mammals. It could also be implicated in the transfer of heat. It is well known that tuna red muscle operates at higher than ambient water temperature by conserving heat in the counter-current system of the central rete (Stevens and Neill 1978). Cold arterial blood coming from the gills is warmed up by venous blood returning from the muscle. It is possible that the particular arrangement of capillaries in tuna red muscle favours heat recovery from the muscle at the venular end of the network, as it possibly favors heat removal in bird flight muscle.

Capillary surface per fiber surface at given mitochondrial volume per muscle fiber was approximately 50% lower than that of bat and hummingbird flight muscle, and it was not significantly different from that of rat soleus muscle. Moyes *et al.* (1992) measured maximal respiratory rates of 2.6 μmol O_2/(min · g of muscle) in tuna red muscle mitochondria at 25°C *in vitro*. If we consider that 1 g of tuna red muscle consists of about 0.80 cm^3 of fibers (see Suarez *et al.* 1991), we find that it contains $0.80 \cdot 0.285 = 0.23$ cm^3 of mitochondria. These values and the above mitochondrial respiratory rate per gram of tissue yield a maximal mitochondrial respiratory rate in tuna muscle *in situ* of 0.22 mL O_2/(min · mL mitochondria). This value is considerably smaller than that estimated in locomotory muscles of mammals running at $\dot{V}O_{2MAX}$ (3–5 mL O_2/(min · mL mitochondria); Schwerzmann *et al.* 1989) and in flying hummingbird (7–10 mL O_2/(min · mL mitochondria); Suarez *et al.* 1991). Mitochondrial maximal respiratory rates *in vitro* are known to be lower than those estimated *in vivo* (see Suarez *et al.* 1991). However, we do not expect the above differences in maximal respiratory rates between tuna, mammals, and birds to be solely due to differences between *in vitro* and *in vivo* measurements. Maximal *in vitro* rates for hummingbird flight muscle mitochondria were 39% of those estimated *in vivo* during hovering flight (Suarez *et al.* 1991). *In vitro* maximal rates of cat muscle mitochondria were 62% of the highest rates estimated *in vivo* (Schwerzmann *et al.* 1989). A number of unknowns, including tuna maximal O_2 consumption and red muscle contribution to O_2 uptake at $\dot{V}O_{2MAX}$ preclude the estimation of red muscle mitochondria maximal respiratory rates *in vivo*. If we assume *in vitro* measurements to be as low as 25% of those achievable *in vivo*, maximal respiratory rate of red muscle mitochondria in tuna (0.9 mL O_2/(min · mL of mitochondria) is about 3–5 times lower than in mammals and 7–10 times lower than in hummingbird. Why the maximum (state 3) respiratory capacities of tuna mitochondria are lower than those found with mammalian or avian muscle preparations is not clear. One possible explanation is that the muscles operate at a different temperature. While it is believed that wild skipjack tuna have excess core temperatures of 1–2°C/kg$^{0.6}$ during normal swimming, the amount by which core temperature increases during burst activity is not clear (Stevens and Neill 1978). Assuming Q_{10} values are about 2.5–3 for mitochondrial respiratory rates (Hochachka and Somero 1984), a 10°C increase in operating temperature would yield values for maximal mitochondrial respiratory rates in tuna close to those found in mammals. Interestingly, excess core temperatures of 8.6°C (white muscle) and 9.1°C (red muscle) have been observed in one group of skipjack tuna (Stevens and Neill 1978). Another implication is that fish muscle mitochondria may be designed for functions other than simply oxidative phosphorylation. For example, protein and amino acid metabolism is up-regulated in fish muscle compared with other vertebrates (Hochachka and Somero 1984). Possibly, the high mitochondrial volume densities observed may be required for the enzymes of amino acid and protein turnover. The great size of the capillary–fiber interface in tuna may also be related to functions other than O_2 per se, for example, substrate and (or) heat transfer between capillaries and muscle fibers.

Acknowledgments

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