METABOLIC BIOCHEMISTRY

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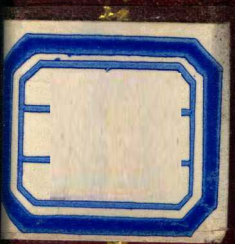

BIOCHEMISTRY AND MOLECULAR BIOLOGY OF FISHES, 4

METABOLIC BIOCHEMISTRY

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Preface

The idea of editing a series of volumes on The Biochemistry and Molecular Biology of Fishes was born out of the present-day lack of a forum for state-of-the-art review articles in this rapidly expanding field of research. On the one hand, researchers and students in this area always find themselves combing the literature on general (rat-dominated) biochemistry before discovering short and usually incomplete and disappointing coverage of the situation in the piscine setting. On the other hand, the rapidly expanding volume and quality of the primary literature in fish biochemistry and molecular biology supply convincing evidence for a maturing field. This discipline is no longer the younger sibling of rat or human biochemistry but has recently led to a number of major conceptual breakthroughs; for this reason, and because its activity domain is sometimes nonoverlapping with 'mainstream' biochemistry, the field is certainly ripe and ready for a review series of its own.

Comparative biochemistry and molecular biology and comparative physiology as disciplines by definition use organisms as a special kind of experimental parameter for probing general mechanisms and principles of function. In theory this approach is relatively blind to phylogenetic boundaries, but in practise the realities of funding and availability of experimental material greatly narrow the field of play. As a result, two phylogenetic groups — the insects and the fishes — have over the last several decades provided the bulk of the experimental data base in these disciplines. Interestingly, although comparative biochemistry in many ways grew out of comparative physiology, the growth and development of these two activities in the insect field have to major extent proceeded along independent paths. By contrast, the comparative physiology and biochemistry of fishes have not been so independent of one another and the tendency has been for the former to envelope the latter. We believe that the current conceptual developments in the fields as well as the simple logistics of dealing with massive data bases make this the right time for the reality of independence to match the perception of independence, which we feel is another important rationale for this review series.

Our goal is to provide researchers and students with a pertinent information source from theoretical and experimental angles. To be useful to students, theoreticians, and experimentalists alike, contributing authors are urged to emphasize concepts as well as to relate experimental results to the biology of the animals, to point out controversial issues, and to delineate as much as is possible directions for future research.

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Abbreviations

AA	Amino acid(s)
AChR	Acetylcholine receptor
ACTH	Adrenocorticotropic hormone
AlaAT	Alanine aminotransferase
ALD	Aldolase
AQ	Ammonia quotient
AS	Atlantic salmon cell line
AspAT	Aspartate aminotransferase
BBMV	Brushborder membrane vesicles
BCAAT	Branched-chain amino acid aminotransferase
BCKAD	Branched-chain α-ketoacid dehydrogenase
BF-2	Bluegill fry cell line
BiP	Immunoglobulin binding protein
BLMV	Basolateral membrane vesicles
cAMP	3′,5′-cyclic adenosine-monophosphate
CCO	Cytochrome C oxidase
CHSE-214	Chinook salmon epithelium cell line
CPK	Creatine phosphokinase
CPT	Carnitine palmitoyl transferase
CS	Citrate synthase
DAG	Diacylglycerol
DHT	5α-Dihydrotestosterone
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
E2	17β-Estradiol
EAA	Essential amino acid(s)
EDTA	Ethylenediaminetetraacetic acid
EO	Electric organ
EOD	Electric organ discharge
EPO	Erythropoietin
FAA	Free amino acid(s)
FABP	Fatty acid binding protein
FBPase	Fructose 1,6-bisphosphatase
FFA	Free fatty acid(s)
FG	Fast glycolytic (muscle fiber)
FHM	Fathead minnow cell line
FOG	Fast oxidative glycolytic (muscle fiber)
G6Pase	Glucose 6-phosphatase
G6PDH	Glucose 6-phosphate dehydrogenase
GABA	Gamma-aminobutyrate
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GDH	Glutamate dehydrogenase
GLP	Glucagon-like peptide
GPase	Glycogen phosphorylase
αGPDH	α-Glycerophosphate dehydrogenase
GSase	Glycogen synthase
HDL	High density lipoproteins
HEPE	12-Hydroxyeicosapentaenoate
HETE	12-Hydroxyeicosatetraenoate
HK	Hexokinase
HPLC	High performance liquid chromatography
HSP	Heat-shock protein
IDL	Intermediate density lipoproteins
LCAT	Lecithin:cholesterol acyl transferase
LDH	Lactate dehydrogenase
LDL	Low density lipoproteins
LT	Leukotrienes
LX	Lipoxins
ME	Malic enzyme
MT	17α-Methyltestosterone
NEAA	Non-essential amino acids
NMJ	Neuromuscular junction
NMR	Nuclear magnetic resonance
ODC	Ornithine decarboxylase
PAF	Platelet activating factor
PC	Pyruvate carboxylase
PCA	Perchloric acid
PCr	Phosphocreatine
PDG	Phosphate-dependent glutaminase
6PGDH	6-Phosphogluconate dehydrogenase
PEPCK	Phosph*enol*pyruvate carboxykinase
PFK-1	Phosphofructokinase-1
PG	Prostaglandins
PGI	Phosphoglucose isomerase
PGK	Phosphoglycerate kinase
PK	Pyruvate kinase
PKA	Protein kinase A
PKC	Protein kinase C
PMN	Pacemaker nucleus
PtdA	Phosphatidic acid
PtdCho	Phosphatidylcholine
PtdEtn	Phosphatidylethanolamine
PtdIns	Phosphatidylinositol
PtdSer	Phosphatidylserine
PUFA	Polyunsaturated fatty acids
RQ	Respiratory quotient
RT-2	Rainbow trout germ cell line
RTG	Rainbow trout gonad cell line
SDA	Specific dynamic action
SO	Slow oxidative (muscle fiber)
T3	3,5,3′-Triiodo-L-thyronine
TAG	Triacylglycerol
TF	Turbot fin cell line
TPI	Triosephosphate isomerase
TRH	thyrotropin releasing hormone
TX	Thromboxanes
VHDL	Very high density lipoproteins
VLDL	Very low-density lipoprotein
XDH	Xanthine dehydrogenase
XO	Xanthine oxidase

Contents

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CHAPTER 1

Design for a high speed path for oxygen: tuna red muscle ultrastructure and vascularization

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I. Introduction

Because it is one of the most aerobic muscles in fish, the red muscle of tuna is of particular interest to study strategies and constraints in structural designs for high O_2 flux from capillary to muscle fiber mitochondria. Tuna can maintain extremely high aerobic metabolic rates and reach high swimming speeds[4]. The tuna red muscle is well known to operate at higher than ambient water temperature by conserving heat *via* the central counter-current heat exchange (for review, see ref. 36), and white muscle lactate turnover rates after exercise are known to be closer to those found in mammals than in other fish[1,39]. In this chapter, we summarize our morphometric findings on the three-dimensional arrangement of the capillary network and its relationships with fiber ultrastructure in red muscle of skipjack tuna, *Katsuwonus pelamis*, in comparison to highly aerobic skeletal muscles of birds and mammals. Muscles designed for high sustainable activity (hummingbird and bat flight muscles as well as the red muscle of tuna) are all composed of only one population of very highly aerobic fibers, instead of the mosaic of fiber types with different metabolic pattern found in the vast majority of skeletal muscles. This homogeneity allows one to specifically examine capillary-fiber geometrical relationships across species, in particular vascular supply in relation to muscle

fiber aerobic capacity in cases of very high demand for O_2 flux. As summarized further in this chapter, previous studies showed striking similarities in structural design for high O_2 flux in hummingbird and bat flight muscles despite several differences in capillary-fiber geometry[28,29]. In fish as in birds, red blood cells are nucleated and less deformable than mammalian red cells, but they can be larger than bird red cells, and fishes operate at different body temperature than both birds and mammals. Thus, it is of particular interest: (1) to examine capillary-fiber structural arrangement in the red muscle of one of the most athletic fishes known; and (2) to compare it with that in highly aerobic skeletal muscles of birds and mammals.

II. Materials and methods

While the details of methods used here have been described elsewhere[22], it is important to briefly highlight aspects that are relevant to properly explain the results.

1. Animals

Five Skipjack tuna (*Katsuwonus pelamis*); body mass 1.5–2 kg; fork length 43–44 cm) were purchased from local commercial fishermen and held in outdoor 10 m diameter holding tanks supplied with continuously flowing seawater (25 ± 1°C) at the Kewalo Research Facility (National Marine Fisheries Service, Honolulu, Hawaii).

2. Tissue preparation

After the tunas had been netted and anesthetized, muscle perfusion fixation with glutaraldehyde fixative (four animals) or infusion with Batson's casting material (one animal) were performed following procedures and subsequent tissue processing described elsewhere in detail[22]. Transverse and longitudinal sections (1 μm thick) of perfusion-fixed tissue were used for light microscopy morphometry of capillarity and fiber size. Ultrathin transverse sections (50–70 nm) were examined with a Zeiss 10 transmission electron microscope and sampled for morphometry of fiber ultrastructure. Samples injected with casting material were examined with a Stereoscan 360 scanning electron microscope (Cambridge Instrument).

3. Morphometry

Sarcomere length was measured on longitudinal sections, after careful control of the angle of each section[19]. Fiber cross-sectional area, capillary diameter and capillary number around a fiber were measured on transverse sections with an image analyzer. Capillary numbers per fiber sectional area in transverse and longitudinal sections were collected by point-counting, and the data were used to estimate

the degree of orientation of capillaries and capillary length per fiber volume[18]. Capillary-to-fiber ratio (i.e. capillary number per fiber number) was computed as the product of capillary density (i.e. number per fiber cross-sectional area) and mean fiber cross sectional area. Capillary surface per fiber volume was obtained by intersection-counting on vertical (i.e. longitudinal) sections using a cycloid grid[2]. Capillary-to-fiber perimeter ratio in transverse section, which is an index of the size of the capillary-fiber interface[25] was measured by intersection-counting in transverse sections[21], and capillary surface per fiber surface estimated as the product of capillary-to-fiber perimeter ratio and an orientation coefficient $c'(K',0)$ as described elsewhere[25].

The volume of mitochondria per volume of muscle fiber was estimated by standard point-counting[22], and mitochondrial volume per μm fiber length calculated as the product of mitochondrial volume density and fiber cross-sectional area. Where appropriate, data on fiber size and capillary density were normalized to sarcomere length, in order to compare morphological data between muscles, independent of the particular length at which each sample was fixed and therefore examined. A normalizing sarcomere length of 2.1 μm was chosen because it is in the mid-range of the sarcomere lengths where maximal tension is developed in skeletal muscles, and it is within the range of operating sarcomere lengths in hindlimb muscles of mammal during terrestrial locomotion (range, 1.7–2.7 μm)[6], wing muscles of bird during wing beat cycle (1.7–2.3 μm)[5] and red muscle in fish during swimming at slow speed (1.9–2.2 μm)[35].

III. Results and discussion

Figure 1a–c illustrates the high capillary density, small fiber size and high mitochondrial volume density previously reported in red muscle of tuna[3,10,16,22]. In longitudinal sections (Fig. 1b), we found a large number of capillaries cut in transverse or oblique section, as well as branches running perpendicular to the muscle fiber axis. This suggested the presence of capillary manifolds in tuna red muscle, as previously found in the highly aerobic pectoralis muscle of pigeon[20]. Figure 2a,b illustrate the remarkable similarity between the appearance of capillary manifolds in tuna red muscle (Fig. 2a) and pigeon pectoralis muscle (Fig. 2b). In that study, Potter and coworkers[34] showed that these capillary branches oriented perpendicular to the muscle fiber axis are venular capillaries which form dense manifolds around groups of muscle fibers. The examination of microcorrosion casts of tuna red muscle also showed that capillaries form a dense envelope of blood around muscle fibers (Fig. 2c).

The functional implications of the particular arrangement of venular capillaries in those muscles are not fully understood. Capillary manifolds could facilitate an increased vascular supply to and from the muscle fibers at the venular end of the network where substrates and O_2 content are lowest and metabolite concentration highest. They could also be related to other functional aspects such as heat dissipation and/or the blood pumping action of the muscle during flight in

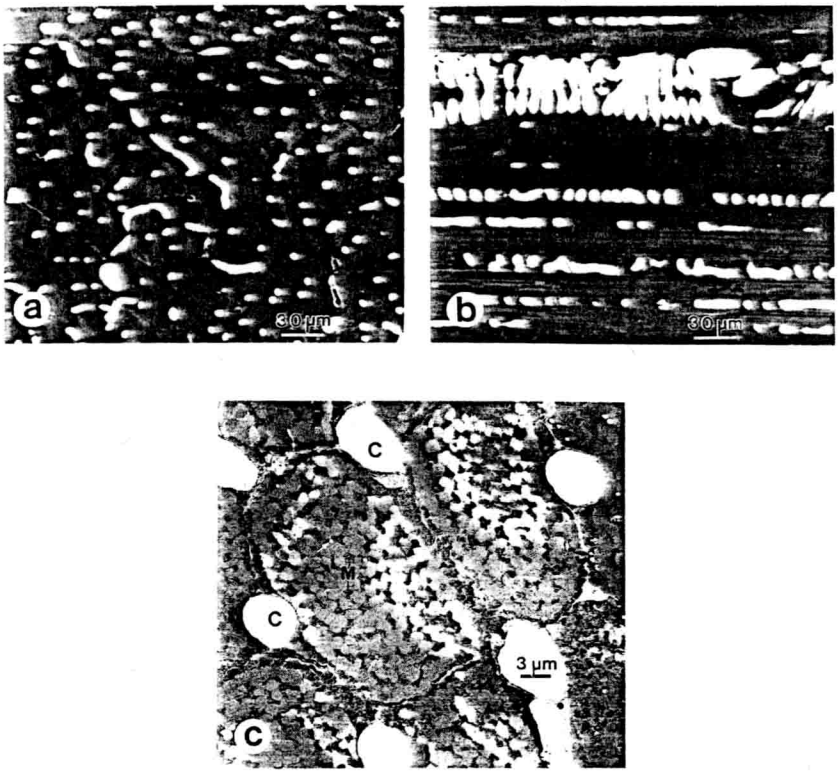

Fig. 1. Fine structure of tuna red muscle. a and b: light micrographs of portions of muscle bundles in transverse and longitudinal sections, respectively. c: electron micrograph of transverse section of muscle fibers and adjacent capillaries (c). Capillaries are empty after the fixation by vascular perfusion. Note large capillary density and small fiber size (a–c), large number of capillary branches running perpendicular to the muscle fiber axis (b) and high density of mitochondria, M (c). From ref. 22.

birds. Interestingly, however, capillary manifolds were found in flight muscle of hummingbird[28], but not in bat[24,29]. The fact that they were found in tuna red muscle also suggest possible rheological implications since in fish, as in bird, red blood cells are nucleated and less deformable than mammalian red cells. Another possibility in tuna is transfer of heat from the muscle at the venular end of the network, as it possibly favors heat removal in bird flight muscle[20,22].

Table 1 summarizes morphometric data on capillarity and fiber ultrastructure in red muscle of tuna compared with tuna white muscle, and aerobic muscles of birds and mammals with large differences in aerobic capacities. In tuna red muscle, fiber cross-sectional area was small (~500 μm^2) but not as small as in ultimate cases of high aerobic capacity in bird and mammal. In hummingbird and bat flight muscles, average fiber cross-sectional area was ~200 and 300 μm^2, respectively, in tissues similarly prepared. Note that the number of capillaries per number of fibers was similar in tuna red muscle and hummingbird flight muscle (~1.6). However,

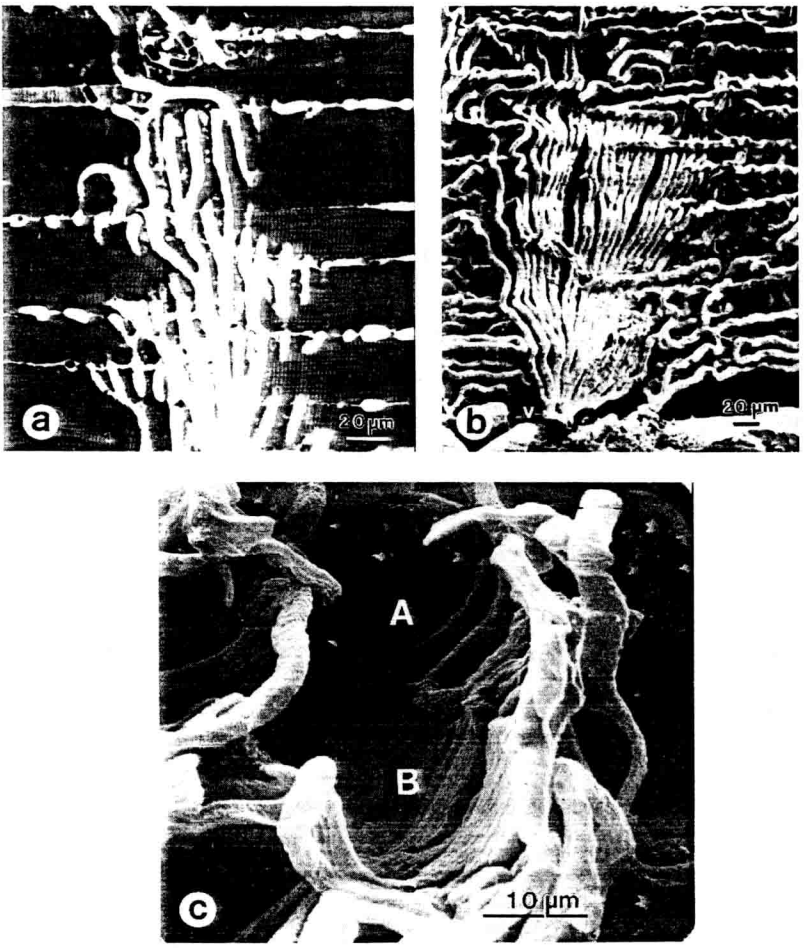

Fig. 2. Examples of capillary manifolds. a: light micrograph in a longitudinal section of tuna red muscle. b and c: scanning electron micrographs of vascular corrosion casts examined perpendicular to the surface of the manifold in pigeon flight muscle (b) and in cross-section in tuna red muscle (c). Note the remarkable similarity between the appearance in tuna (a) and pigeon (b) muscles, and the dense envelope formed by capillaries around muscle fibers (c). Based on fiber dimensions, two muscle fibers (A and B) could be contained in the empty space in c. From refs. 22 (a,c) and 34 (b).

because of the difference in fiber size, there was a huge difference in capillary numerical density between the muscles. The number of capillaries per mm^2 fiber cross-sectional area at 2.1 μm sarcomere length was 3400 in tuna red muscle and 8000 in flight muscle of hummingbird.

Capillary length density is an important estimate of capillarization which accounts for capillary geometry, and determines capillary volume and surface area available for exchange per unit volume of fiber and mitochondria. Figure 3 shows estimates of the degree of capillary orientation, expressed as the percentage added

TABLE 1

Morphometric data on fiber size, capillarity and ultrastructure in tuna red muscle, compared with white muscle (tuna) and highly aerobic skeletal muscles of birds and mammals

Animal Body weight (g)	Muscle (n)	$a(f)_{2.1}$ (μm^2)	$Q_A(0)_{2.1}$ (mm^{-2})	$J_V(c,f)$ (mm^{-2})	$N_N(c,f)$	N_{CAF}	$V_v(mt,f)$ (%)	$V_v(ms,f)$ (%)
Tuna								
1500–2000	Red muscle (8)	475 ± 25	3391 ± 197	4143 ± 242	1.59 ± 0.06	4.97 ± 0.13	28.5 ± 1.0	8.8 ± 1.1
	White muscle (1)	3652 ± 148	623 ± 36	630 ± 39	2.28 ± 0.16	4.77 ± 0.21	2.8 ± 0.3	0.7 ± 0.2
Hummingbird								
3–4	Flight muscle (7) (pectoralis, supracoracoideus)	201 ± 14	8001 ± 782	8947 ± 869	1.55 ± 0.06	4.82 ± 0.15	34.5 ± 0.9	14.2 ± 1.0
Pigeon								
550–620	Pectoralis (6)	363 ± 19	4096 ± 602	4140 ± 568	2.25 ± 0.21	5.41 ± 0.33	23.0 ± 2.0	6.8 ± 1.2
Bat								
15–16	Pectoralis (6)	318 ± 10	6394 ± 38	9025 ± 342	2.02 ± 0.10	5.63 ± 0.23	35.3 ± 1.2	16.8 ± 1.6
	Hindlimb (6) (quadriceps femoris, gluteus maximus)	447 ± 35	2865 ± 238	4111 ± 299	1.24 ± 0.06	3.92 ± 0.15	16.5 ± 1.3	4.3 ± 0.6
Rat								
340–745	Soleus (9)	2028 ± 125	1301 ± 129	1685 ± 152	2.59 ± 0.24	5.96 ± 0.35	6.1 ± 0.9	1.3 ± 0.3

Values are group mean ± SE.

$a(f)_{2.1}$ and $Q_A(0)_{2.1}$, mean fiber cross-sectional area and capillary numerical density at 2.1 μm sarcomere length, respectively; $J_V(c,f)$, capillary length per fiber volume; $N_N(c,f)$, capillary-to-fiber number ratio; N_{CAF}, capillary number around a fiber; $V_v(mt,f)$ and $V_v(ms,f)$, volume density of mitochondria (total and subsarcolemmal, respectively). In pigeon, $a(f)_{2.1}$, N_{CAF}, $V_v(mt,f)$ and $V_v(ms,f)$ are values in aerobic fibers only. Species: skipjack tuna, *Katsuwonus pelamis*[22], rufous hummingbird, *Selaphorus rufus*[28], bat, *Eptesicus fuscus*[29], pigeon (White King), *Columbia livia*[20,23], Sprague Dawley rat[19,29].

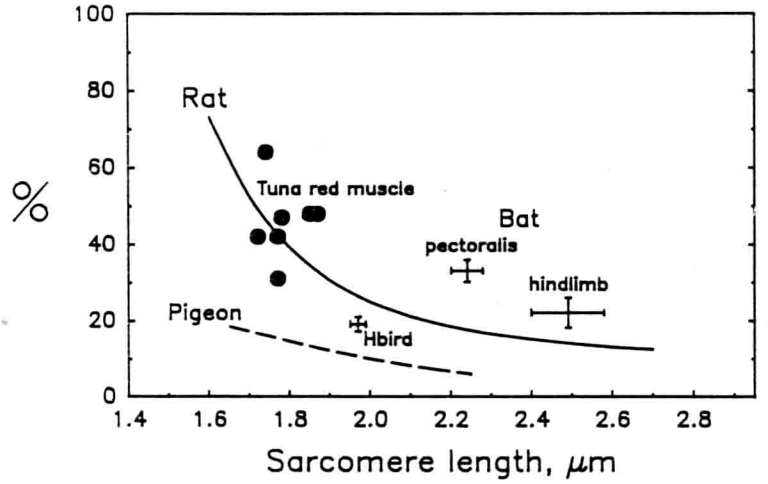

Fig. 3. Plot of the degree of orientation of capillaries (expressed as the percentage added to capillary length by tortuosity and branching, compared with straight, unbranched capillaries oriented parallel to the muscle fiber axis) against sarcomere length in red muscle of tuna (solid circle) compared with group mean values (±SE) in highly aerobic muscles of birds and mammals. From refs. 22 (tuna), 28 (hummingbird) and 29 (bat). Relationships in rat hindlimb (solid line) and pigeon pectoralis muscles (broken line) are from refs. 25 and 20, respectively.

to capillary length by tortuosity and branching, against sarcomere length in the muscles listed in Table 1. It is now well established that both fiber size[7,11,26] and the degree of orientation of capillaries[19,27,33] are functions of sarcomere length. As a consequence, capillary density in transverse sections can underestimate capillary length per fiber volume by a different percentage (e.g. 10–70% in rat muscles; see solid line in Fig. 3) depending on the sarcomere length at which samples are fixed and therefore examined. Figure 3 also shows that the degree of orientation of capillaries can vary between muscles and/or animals, for example bat flight muscle compared with rat *soleus*, or bird compared with mammal. Therefore, it may not be appropriate to compare muscle capillarity based on capillary densities in transverse sections alone even if all samples are fixed at the same sarcomere length[20,22,28,29,32].

It is interesting to note that in tuna red muscle, the contribution of tortuosity and branching to capillary length was similar to that in rat muscles (Fig. 3) in spite of the different geometry. In contrast to tuna and bird muscles[22,28] skeletal muscles of mammals showed no evidence of capillary manifolds[29]. The different contribution of capillary tortuosity and branching to capillary length in tuna red muscle compared with bird muscles (Fig. 3) may be related to differences in fiber size, yielding differences in length and/or number of branches in manifolds between the muscles. In addition, capillary length density in tuna red muscle and pigeon pectoralis muscle were remarkably similar (4100 mm/mm^3) in spite of the different degree of capillary orientation, capillary-to-fiber ratio and fiber size. It was less than half the capillary length density seen in ultimate cases such as the flight muscles of hummingbird and small bats (Table 1).