Changes in muscle cell ultrastructure following exercise in *Salmo trutta*

W. Davison

Department of Zoology, University of Canterbury, Christchurch (New Zealand), October 25, 1982

Summary. One month of swimming at low speed produced very little change in the composition of red and white muscle fibers of the brown trout. This indicates that fish are unable to compensate for increased exercise levels by increasing the aerobic capacity of the muscle cells.

Teleost fish which are subjected to continuous exercise show many generalized changes, such as hypertrophy of the muscle fibers, with the degree of change being dependent upon the severity of the exercise[1-4]. However, fish do respond to exercise in different ways and this is related to the natural environment of the animal. For example, goldfish are normally found in still water and do not fare well in flowing water[1], whilst trout, normally found in fast flowing streams, react well to flowing water and grow quickly[2,3]. Swimming at low speed produces marked changes in red muscle but not in white[2,4], presumably because only the red muscle is actively contracting[5,6]. Trout which were exercised at low speed[2] showed marked increases in size, number and glycogen and lipid levels of the red muscle fibers. This study[2] also noted an increase in the size of red muscle mitochondria although this was merely a qualitative assessment. The present work is a description of quantitative assessments made on these muscle fibers.

Material and methods. A detailed description of the exercise regime has already been given[2]. Trout *(Salmo trutta)* were exercised for 1 month at a swimming speed of 1.5 body lengths per sec (bl sec^{-1}). This exercise produced a 40% increase in the size of the red muscle fibers but no change in the white fibers. Red and white muscle was dissected from control and experimental animals and processed for electron microscopy. Tissue was taken from a point immediately posterior to the body cavity (termed anterior muscle) and also from a region just anterior to the caudal peduncle (termed caudal muscle). Thick sections were stained with toluidene blue to allow determination of capillary density while ultrathin sections were used to acquire low power electron micrographs of the 2 fiber types. The composition of the cells was then determined gravimetrically.

Results and discussion. White muscle was composed mainly of contractile material (75%) with few, small mitochondria located just beneath the cell membrane (table, fig. a). Lipid was very rarely observed. There was no difference between white muscle fibers from the 2 regions and also between control and experimental animals. Differences in the chemical composition of fish muscle have been observed along the length of the body[7], but this study indicates that this is not obvious at the organelle level. Also, the lack of any change in the white muscle indicates that at 1.5 bl sec^{-1}, white fibers are not being used[5,6].

Red muscle was characterized by large numbers of mitochondria and high levels of lipid, found as lipid droplets associated with the mitochondria (table, fig. b). Differences were seen between the 2 regions of the control fish red muscle but not between the 2 regions of the experimental animals. The caudal red muscle of the control fish had more myofibrillar material and a correspondingly smaller amount of cytoplasm than the anterior region. There was also less lipid. This suggests that trout kept in still water move using a relatively small part of the myotome near to the tail, the rest of the myotome being underutilized. Lack of differences between the 2 regions of the experimental fish suggests that fish swimming against a water current use all of the myotome posterior to the body cavity, i.e., they use typical carangiform locomotion.

After 1 month of swimming, changes in the red muscle were relatively minor. Experimental animals grew at a much greater rate than the controls, and consumed much greater quantities of food[2]. Consequently, lipid was ingested at a rate in excess of that needed for maintenance and growth and was thus deposited in the red muscle cells. The increased lipid levels in the anterior red muscle were at the expense of the general cell cytoplasm, while in the caudal region the increase in lipid appeared to be at the expense of the myofibrillar material, a somewhat unlikely situation in actively contracting muscle. What seems to have happened is that the caudal red muscle was already 'trained' and so the cell constituents were at optimum levels. After a month of swimming, the cytoplasm and mitochondria were still at optimum levels when estimated as a percentage of the total cell. Thus the reduction of myofibrillar material was only apparent as it merely echoed the increase in lipid.

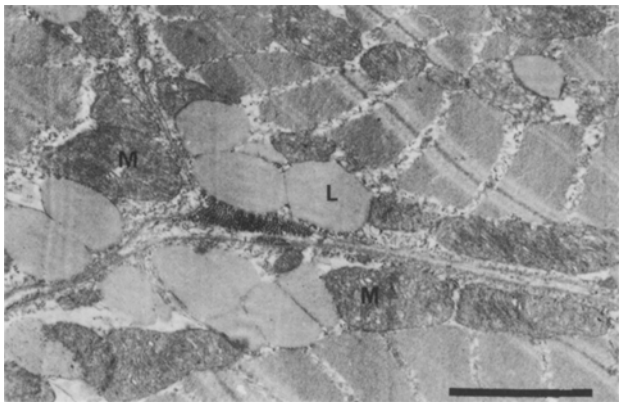
There was no change in the percentage of the cells occupied by mitochondria, nor was there a change in the blood supply to the cells. In fact, as the red muscle cells were larger[2], there was actually a decrease in the blood supply. This is in agreement with other workers[4,8] who found little or no change in the oxidative capacity of red muscle after exercise in fish, although similar work on mammals has shown that training does result in a increased oxidative capacity[9,10].

Thus it appears that fish have very little scope for altering the aerobic capacity of the swimming muscles in response to exercise training in marked contrast to mammalian tissues. Anaerobic capacity is, however, able to respond[4,8].

Composition of muscle fibers

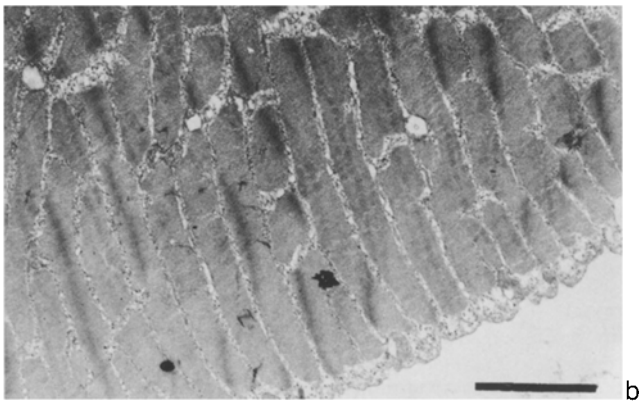
	Myofibrils	Mitochondria	Lipid	Cytoplasm	Capillary: fiber ratio
Control					
Anterior red	43.9 ± 2.50***	24.4 ± 2.04	6.7 ± 0.89***	24.8 ± 1.22***	0.85 ± 0.022
Caudal red	55.6 ± 1.91**	23.1 ± 1.59	3.5 ± 1.32***	17.9 ± 1.37	0.85 ± 0.060
Anterior white	73.6 ± 1.64	3.4 ± 0.53	-	23.0 ± 1.46	0.44 ± 0.039
Caudal white	76.3 ± 1.48	2.8 ± 0.57	-	20.9 ± 1.24	0.39 ± 0.049
Experimental					
Anterior red	45.8 ± 1.27	23.1 ± 1.31	14.8 ± 2.23	15.8 ± 1.82	0.94 ± 0.074
Caudal red	46.9 ± 2.26	24.0 ± 1.39	13.7 ± 2.28	15.4 ± 1.16	0.74 ± 0.030
Anterior white	74.1 ± 1.80	2.9 ± 0.60	-	22.7 ± 1.47	0.42 ± 0.046
Caudal white	70.4 ± 3.70	4.1 ± 1.09	-	25.6 ± 4.79	0.39 ± 0.048

Values are percentages ± SE. The values for cytoplasm include the sarcoplasmic reticulum. The asterisks denote the level of significance (t-test); *0.05, **0.01, ***0.001. Upper, anterior vs caudal; lower, control vs experimental.



a

a White muscle. Note the few, small mitochondria and the lack of lipid. × 3070. Bar represents 5 μm.



b

b Red muscle. There are many large mitochondria (M) and lipid droplets (L). × 3700. Bar represents 5 μm.

This inability to improve the aerobic capacity is undoubtedly related to the poor blood supply of fish muscle, and this is probably a function of the low arterial blood pressure produced by passage of blood through the gills.

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Localization of substance P-immunoreactive nerve fibers in the human digital skin

C.-J. Dalsgaard, C.-E. Jonsson, T. Hökfelt and A.C. Cuello[1]

Departments of Anatomy and Histology, Karolinska Institutet, Department of Plastic Surgery, Karolinska Hospital, S-10401 Stockholm (Sweden), and Department of Pharmacology and Human Anatomy, University of Oxford, Oxford (England), December 7, 1982

Summary. Substance P-immunoreactive nerve endings were localized in human digital skin by the use of indirect immunohistochemical technique. It was found that substance P-like immunoreactivity was present in free nerve endings in the dermal papillae and in the epidermis. Some Meissner's corpuscles also contained substance P positive nerve endings. Furthermore, substance P-immunoreactive nerves were localized in close connection to sweat gland ducts and blood vessels. The functional significance of these findings was discussed with regard to pain mediation and inflammatory response.

Substance P (SP), originally discovered by von Euler and Gaddum[2] and identified as an undecapeptide by Chang and Leeman[3] has a widespread distribution in the central and peripheral nervous system, as revealed by biochemical[4,5] and immunohistochemical[6,7] techniques. The higher concentration of SP in the dorsal roots compared to the ventral roots led Lembeck[4] to suggest that SP was a transmitter substance in the primary afferent neurons. This was strongly supported by the elegant biochemical and electrophysiological experiments of Otsuka and colleagues[8]. Immunohistochemical studies have shown SP-like immunoreactivity in a population of predominantly small sized sensory neurons with central axons terminating in the superficial laminae of the dorsal horn and spinal trigeminal nucleus and the peripheral branches innervating the skin[7,9]. Furthermore, electrophysiological studies have demonstrated an action of SP on nociceptive units in the spinal cord[10,11]. SP has also been shown to play an important role in the peripheral tissues in neurogenic inflammation, causing vasodilation and plasma extravasation by antidromic activation via axon collaterals[12].

In the present study the occurrence of SP immunoreactive fibers and nerve endings in human digital skin has been investigated, by the use of an indirect immunohistochemical technique.

Blocks of human digital skin were taken with a 3-mm punch from the palmar part of the distal phalanges of healthy volunteers (the authors). The specimens were immediately fixed in a fresh solution of 0.2% parabenzoquinone and 2% paraformaldehyde for 2 h[13,14], stored overnight in 0.1 M phosphate buffer solution with 5% sucrose added, cut perpendiculary on a cryostat at 14 μm and then processed for indirect immunohistochemistry according to Coons and collaborators[15]. Briefly, the sections were incubated in a humid atmosphere at 4 °C for 18–24 h with monoclonal antibodies to SP (dilution 1:200)[16], rinsed and incubated with FITC-conjugated rabbit anti-rat antibodies (dilution 1:10, Miles, U.K.) at 37 °C for 30 min, rinsed,