Endurance Exercise Training Affects Fast White Axial Muscle in the Cyprinid Species *Chalcalburnus Chalcoides Mento* (Agassiz, 1832), Cyprinidae, Teleostei

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Abstract

This study undertakes an investigation on the effects of an endurance exercise training programme on axial muscle in the cyprinid species *Chalcalburnus chalcoides mento* (Agassiz, 1832), the Danube bleak. Previous studies on this species revealed biochemical evidence that it is white axial muscle which responds to an endurance training. To determine whether this could be verified also on a structural basis, red, intermediate and white axial muscle were investigated by means of histochemistry, electronmicroscopy and morphometry. Compared to other cyprinid species, the Danube bleak responds to an endurance exercise training regime with adaptive changes of fast white muscle rather than slow red muscle. Qualitative and quantitative analyses revealed that training induced significant increases in fibre diameters of intermediate and white muscle fibres and capillary supply in red and white muscle as well as volume density of myofibrils in red muscle fibres. Volume densities of mitochondria and lipid, and mATPase- and SDH-activity were unaffected. It is concluded that this adaptive strategy enables this fish with its typical burst-like mode of swimming (which was maintained for the whole period of the training) to meet the specific demands of an endurance exercise training.

Key words: endurance exercise training, muscle fibre types, electronmicroscopy, morphometry, capillarization.

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Fish can be exercised rather easily by forcing them to swim against a water current in a flume or respirometer [10] making them an ideal subject for exercise training studies. With respect to endurance exercise training most fish respond with locomotor muscle changes that would promote slow muscle aerobic power production (for reviews see [4, 14, 16]). Besides growth rates, food conversion efficiencies, anaerobic recovery and systemic parameters of aerobic capacity endurance, training affects also muscle morphology and phenotype. Although data on the effect of fish exercising are sometimes contradictory [3, 4, 8, 9, 12, 16] there are some general responses.

Typical features which can be observed in endurance trained slow muscle tissue are an increased myoglobin content allowing better oxygen transport within muscle tissue, increased capillarization to allow sufficient oxygen supply matching the increased oxygen demand of slow muscle which is indicated by the increased mitochondrial content of the trained muscle fibres and increased lipid content which serves both as an energy source and intracellular oxygen store [15, 16]. Amongst others enzymes involved in lipid metabolism increase their activity, indicating that exercising fish use mainly lipid as an energy source and fuel [6, 11]. An increase in the proportion of slow muscle (fibre number and fibre size, respectively) is another more general training response in teleost fish [7, 12, 20], a feature which can be observed also with “natural training” [2].

In contrast to the above, the cyprinid species Danube bleak *Chalcalburnus chalcoides mento* (Agassiz, 1832), however, show biochemical changes suggesting increased power from white axial muscle. As previously shown, white muscle fibres respond with increased glycolytic potential and the lactate produced in white muscle during training is supposed to be oxidized in heart and red muscle due to the high activity of LDH in these tissues [7]. These observations now seek additional evidence on the ultrastructural level for training adaptations in this muscle.

The present study undertakes a combined morphological/morphometrical and histochemical investigation analysing fibre diameters, enzymatic activities, capillary to fibre ratio, capillary density and volume densities of appropriate cellular components like mitochondria and lipid of the three main axial muscle fibre types, namely red, intermediate and white.

Material and Methods

One year old Danube bleak were obtained from stocks raised from artificially inseminated eggs. Fish were maintained in a training flume [10] for 17 weeks. Experimental fish were forced to swim against a water flow rate of 25 cm s^{-1} corresponding to a speed of 2.5-3.5 body lengths per second. Water temperature (20°C) and 15/9 h day/night cycle were kept constant throughout the experiment. Fish were fed four times a day with *Artemia* and commercial fish food at a total daily ration of 4-5% of the fish mass. During the feeding periods the flow of the flume was automatically reduced for 20 minutes to the control level. Control fish were kept under same conditions but at a flow rate of only 3 cm s^{-1}.

After the training period fish were killed with an overdose of tricainemethanesulphonate (MS222) and samples of axial muscle were excised in the anal region and prepared for either electron microscopy-based morphometry or histochemistry (for further details see [12]). mATPase- and succinic dehydrogenase (SDH)-reactions were used to differentiate between red, intermediate and white fibres [17]. Video images of mATPase reacted sections together with the software programme „Optimas“ (Stemmer, Munich) served for estimation of fibre area, perimeter and mean fibre diameter. Stereological analysis was performed as described by [18] using a test system B36f [19] and a final magnification of 22330x. The following cellular components per muscle fibre were analysed: subsarcolemmal mitochondria, intermyofibrillar mitochondria, total amount of mitochondria, subsarcolemmal lipid, intermyofibrillar lipid, total amount of lipid, myofibrils, sarcotubular system and subsarcolemmal cytoplasm. Capillary to fibre ratio (number of capillaries per number of muscle fibres) and capillary density (number of capillaries per unit cross-sectional muscle fibre area) were estimated from electronmicrographs using a final magnification of 4930x.

Results

Regarding mATPase- and SDH-activity of muscle fibres no differences between trained and untrained fish could be observed.

In contrast to red fibre diameter, both intermediate (all following values are means ± S.E.; 34.25 ± 0.43 and 38.98 ± 0.51, untrained and trained, respectively) and white (41.80 ± 0.51 and 48.93 ± 0.55, untrained and trained, respectively) fibre diameters were significantly higher in trained fish ($p < 0.001$).

No effect of training could be observed regarding cellular components in any of the three fibre types analysed with the exception of red muscle myofibrillar content being significantly higher in trained fish (40.92 ± 0.72 and 46.97 ± 1.30, untrained and trained, respectively; $p < 0.01$).

A remarkable training response was noteable regarding capillary supply (Fig.). Both, capillary density of red (806 ± 64 and 1657 ± 144, untrained and trained, respectively; $p < 0.005$) and white muscle (146 ± 6 and 252 ± 14, untrained and trained, respectively; $p < 0.001$) and capillary to fibre ratio of red (0.97 ± 0.06 and 1.8 ± 0.19, untrained and trained, respectively) and white muscle (0.49 ± 0.03 and 0.92 ± 0.11, untrained and trained, respectively) of trained fish were significantly higher ($p < 0.05$) than in untrained ones.

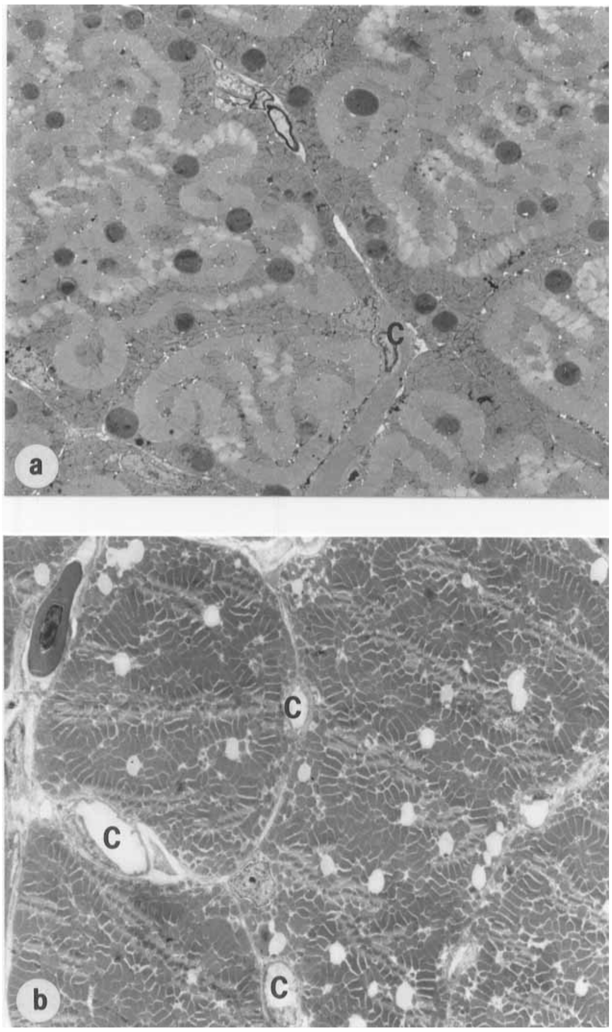

Figure. Electronmicrographs of transversely sectioned red muscle fibres in untrained (a) and trained (b) Chalcalburnus chalcoides mento*. Note the differences in capillarization (C). x2053.*

Discussion

As previously shown biochemically [7], *Chalcalburnus chalcoides mento* also morphologically responds to an endurance training programme differently compared to other cyprinid fish species [1, 10, 12]. Although slow (red) muscle capillarization is increased with training as has been shown also in a previous study for two other cyprinid species [12], it is mainly fast (white) muscle which responds to training with an increase in capillary supply and fibre diameter. Muscle fibre cross-sectional area is directly correlated with muscle force or tension. The training induced increase in fibre diameter can be seen as force improvement to meet the typical burst-like swimming activity of Danube bleak which is maintained during the training period.

Compared to other cyprinid species Danuble bleak shows the highest amounts of lipid and mitochondria in its muscle fibres [13] which indicates the high aerobic capacity of axial muscle. In contrast, capillary supply is rather low compared to other cyprinid species [15]. To further increase aerobic muscle capacity it seems a sensible way to improve the capillarization of muscle tissue which is shown by the increase of capillary to fibre ratio and capillary density of red and white muscle, a training response which this cyprinid species share with rainbow trout, *Oncorhynchus mykiss* [3]. Capillary density is, thereby, a more unbiased index of tissue oxygen supply as it takes into account fibre size [5].

Another advantage of a better capillary supply is the fact that capillaries transport not just oxygen but also lipid and glucose and, moreover, remove waste products like lactate and metabolic heat. As a consequence, the limiting factor in oxidative muscle fibres is oxygen supply whereas in glycolytic muscle fibres it is the removal of waste products.

The observed increased capillarization of trained Danube bleak red muscle improves oxygen supply necessary to meet the obviously high demand of oxygen due to the high volume density of mitochondria. It should be mentioned that capillary density is commonly used as a morphological indicator of oxygen supply [5], whereas the volume density of mitochondria can serve as an estimate of oxygen demand. Furthermore, the supposed lactate oxidation by red muscle [7] is certainly improved by a better capillary supply.

Increased capillarization of trained Danube bleak white muscle, on the other hand, meets the above mentioned limitation by improving performance through both improved glycogen synthesis pathways in response to the improved nutrient vasculature and enhanced removal of waste metabolic products such as lactate and heat and thus preventing local fatigue.

In conclusion, the adaptive changes in capillary supply of red and white muscle together with increased fibre diameters of intermediate and white fibres and increased myofibrillar content of red fibres enables the fish to meet the demands of an endurance exercise training without changing its typical burst-like swimming behaviour. It shows once more that training responses are species-specific relating to the swimming activity and life-style of the fish species and it is a further example of the wide range in adaptational changes of the highly plastic muscle tissue.

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