Endurance exercise differentially stimulates heart and axial muscle development in zebrafish (Danio rerio)

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Van der Meulen, T., H. Schipper, J. G. M. van den Boogaart, M. O. Huising, S. Kranenbarg, and J. L. van Leeuwen. Endurance exercise differentially stimulates heart and axial muscle development in zebrafish (Danio rerio). Am J Physiol Regul Integr Comp Physiol 291: R1040–R1048, 2006; doi:10.1152/ajpregu.00116.2006.—Mechanical load is an important factor in the differentiation of cells and tissues. To investigate the effects of increased mechanical load on development of muscle and bone, zebrafish were subjected to endurance swim training for 6 h/day for 10 wk starting at 14 days after fertilization. During the first 3 wk of training, trained fish showed transiently increased growth compared with untrained (control) fish. Increased expression of proliferating cell nuclear antigen suggests that this growth is realized in part through increased cell proliferation. Red and white axial muscle fiber diameter was not affected. Total cross-sectional area of red fibers, however, was increased. An improvement in aerobic muscle performance was supported by an increase in myoglobin expression. At the end of 10 wk of training, heart and axial muscle showed increased expression of the muscle growth factor myogenin and proliferating cell nuclear antigen, but there were major differences between cardiac and axial muscle. In axial muscle, expression of the “slow” types of myosin and troponin C was increased, together with expression of erythropoietin and myoglobin, which enhance oxygen transport, indicating a shift toward a slow aerobic phenotype. In contrast, the heart muscle shifts to a faster phenotype but does not become more aerobic. This suggests that endurance training differentially affects heart and axial muscle.

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MATERIALS AND METHODS

Fish

Embryos were generated from the gol-1 strain, characterized by reduced body pigmentation (32) and high fecundity, by natural mating and maintained at 28.5°C. When the larvae were free swimming, they were fed Paramecium three times a day. From 7 dpf, increasing amounts of live brine shrimp were added to the feed, and the amount of Paramecium was reduced proportionally. At 14 dpf, the larvae were transferred to the training and control tanks. From this age, the larvae were fed only brine shrimp twice daily (15 min before and immediately after training). From 35 dpf, the pretraining feed was supplemented with CypriCo Crumble Excellent Pellets (Coppens International).

Training Setup

A training setup for zebrafish was custom-built (Fig. 1A). To linearize the flow, water in the flow tank was led through a compact stack of straws and a tapering width of the channel. Grids placed in the tank kept the fish in a partition of the 1-m-long, 0.15-m-wide, 0.28-m-high flow tank. Copper-free tap water, which was filtered biologically, was used. Salts were added to the water to a conductivity of ~650 μS/cm. The temperature, 26.8°C (SD 0.5), was measured daily. Nitrate [21.0 mg/l (SD 6.8)], nitrite [0.0 mg/l (SD 0.0)], ammonia [0.0 mg/l (SD 0.1)], and pH [8.25 (SD 0.07)] were measured weekly. The water speed in the flow chamber could be varied gradually between 0 and 17 cm/s.

Training Regimen

Fish from six clutches were randomly assigned to the control or the trained group. Each day, starting at 14 dpf, larvae were trained continuously for 6 h by enforcement of a flow rate of three total body lengths per second (TL/s). Flow rate was adjusted after every sampling day. After 1 wk of training (at 21 dpf), flow rate was increased to 5 TL/s and increased daily on the basis of the extrapolated growth data from previous samples to closely match 5 TL/s. This training regimen represents about twice the normal activity of developing zebrafish (14). At 50 dpf, the maximum flow rate of the system was reached at 17 cm/s; thus the speed gradually declined toward 4.2 TL/s at 84 dpf because of growth of the fish. At the beginning of each training session, the flow rate was gradually increased over the course of 2–3 min to minimize stress. Three fish were removed from the experiment: two trained fish that stopped swimming during training and one control fish that was found dead early in the experiment. The training protocol was approved by the Dierexperimenten Commissie (Experimental Animal Committee) of Wageningen University (under number 2004020.d) and in accordance with the Dutch Wet op de Dierproeven (Law on Experiments With Animals).

Sampling

Fish were sampled at 14 (before training), 17, 21, 24, 28, 35, 45, 63, and 84 dpf. Sampling of trained fish started immediately after the end of 6-h training session of that day. The control fish were sampled immediately before the trained fish. Both groups of fish were irreversibly anesthetized by 1.0% tricaine methane sulfonate buffered with 1.5% NaHCO₃. The animals to be used for analysis of gene
expression or determination of individual muscle fiber area \((n = 8)\) were immediately snap frozen in liquid nitrogen and stored at \(-80^\circ\mathrm{C}\) until RNA isolation. The other animals \((n = 14)\) were photographed in lateral view with a digital camera (model DP50; Olympus) mounted on a microscope (Stemi SV11; Zeiss) with AnalySIS software (Soft Imaging System). Because \(>45\text{-dpf}\) larvae were too large for this approach, a camera (model D-100; Nikon) with a 105-mm 1:2.8 lens (AF NIKKOR) and 0.5-cm graph paper were used for image calibration. Four of these fish were fixed overnight at \(4^\circ\mathrm{C}\) in Bouin’s fixative (125 ml of 40% neutral formaldehyde, 375 ml of saturated picric acid, and 50 ml of acetic acid, filtered before use) for histological analysis. The remaining 10 fish were weighed individually on a scale (model PE 360; Mettler-Toledo) after careful removal of excess liquid. After 10 wk of training, at 84 dpf and in addition to the normal sampling, individual organs were isolated from eight fish from each group.

Length Measurements

Length measurements were taken from the calibrated photos. TL is measured from the tip of the snout to a point halfway between the dorsal and ventral tips of the tail. Data were plotted over time as means with SD. A sigmoidal curve \(y = 1/[b_1 + b_2 \times \exp(-x/b_3)]\) was fitted through the length data in Matlab 7.0 (Mathworks) using least squares. The interval is a 95% nonsimultaneous confidence interval around the regression line for the true value of the function at the specified input values.

Determination of Total Red Muscle Fiber Area

For determination of total red muscle fiber area, the fish were dehydrated and embedded in paraffin (Paraclean) according to standard histological protocols. Sections (5 \(\mu\)m thick) were cut on a microtome (model 2040; Reichert-Jung) and collected on protein- 

Determination of Individual Muscle Fiber Area

To accurately determine individual muscle fiber diameter, we used 7-\(\mu\)m cryostat cross sections of fish (45 and 84 dpf) that were flash frozen in liquid nitrogen. The sections were collected on polylysine slides (Menzel-Gläser, Braunschweig, Germany) and stained without prior fixation for the presence of slow muscle fibers with an antibody against zebrafish slow muscle [S58 supernatant, a generous gift of Dr. Frank E. Stockdale (6)]. Fast muscle fibers were visualized with standard Crossmon staining. Red muscle fibers were photographed near the horizontal septum. White muscle fibers were photographed in an epaxial area at equal distances from the horizontal septum, midline, and skin. Individual muscle fibers were manually traced on the photographs, and their cross-sectional areas were measured using AnalySIS software (version 3.1). Differences in average fiber size between control and trained fish were evaluated with an independent-samples \(t\)-test (SPSS version 12.0.1). \(P < 0.05\) was accepted as significant.

RNA Isolation and cDNA Synthesis

RNA was isolated essentially as described elsewhere (33) with some minor modifications. Briefly, RNA was isolated with an RNaseasy kit (Qiagen) according to the manufacturer’s instructions. After elution in milliQ water, RNA was concentrated (but not dried) in a SpeedVac. Up to 1,000 ng of total RNA were used as the starting material for random hexamer-primed single-strand cDNA synthesis (Invitrogen). A non-RT control was incorporated for each sample. Samples were filled with milliQ water to a final concentration corresponding to 1 ng of total RNA used as starting material for cDNA synthesis per microliter and stored at \(-20^\circ\mathrm{C}\).

Real-Time Quantitative PCR

Primer express software (Applied Biosystems) was used to design primers (Table 1). Five microliters of cDNA and forward and reverse primer (300 nM each) were added to 12.5 \(\mu\)l of Quantitect Sybr Green

Table 1. Primers used in RT-PCR experiments

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Forward</th>
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<th>Reverse</th>
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<td>Epo</td>
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Epo, erythropoietin; Gdf8, growth and differentiation factor 8; IGF, insulin-like growth factor; myhz2, fast myosin heavy chain; myhz5, slow myosin heavy chain; NADHd, NADH dehydrogenase; PCNA, proliferating cell nuclear antigen; PFK-m, muscle phosphofructokinase; SDHα, succinate dehydrogenase subunit A; TropC, troponin C.

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PCR Master Mix (Qiagen), with addition of milliQ to a final volume of 25 μL. Real-time quantitative PCR (95°C for 15 min, 45 cycles at 94°C for 15 s, 60°C for 30 s, and 72°C for 30 s, followed by 60°C for 1 min) was carried out on a Rotorgene 2000 real-time cycler (Corbett Research, Sydney, Australia). After each run, melt curves were collected for detection of fluorescence from 60°C to 90°C at 1°C intervals. The level of expression was determined at a threshold of 0.04. Target gene expression was corrected for reference gene expression and for primer efficiency and plotted relative to the control group as described previously (19). Dual internal reference genes (40S and β-actin) were used in all real-time quantitative PCR experiments, and results were confirmed to be very similar after standardization to either gene. Only results standardized for β-actin expression are shown. Differences were evaluated with a Mann-Whitney U-test with SPSS (version 12.0.1) and were considered significant when P < 0.05.

RESULTS

Behavior

In the control tank, zebrafish covered the entire aquarium in their exploration and foraging behavior (Fig. 1B). In the training tank, on initiation of water flow, the fish momentarily swam more actively in all directions before they began to swim against the direction of the current (Fig. 1C). At all water speeds, the fish retained an intermittent swimming pattern: after small bursts of activity, the fish coasted. Coasting is evident from a lack of tail movement and slacking of the dorsal, pelvic, and tail fins. The fish actively pursued particles and continued this behavior during training. When the water speed was returned to normal at the end of a training session, the trained fish immediately assumed the swimming behavior of the control fish.

Size and Mass

For determination of the effects of training on growth, the length and mass of the fish were determined. At the start of the experiment, TL of the fish was 8.3 mm (SD 1.2). At the end of the experiment TL was 40.3 mm (SD 2.2) and 40.7 mm (SD 1.7) for control and trained fish, respectively (Fig. 2A). Although trained fish were similar in size to the control fish at the end of the experiment, they were larger during much of the training period. The difference is significant (P < 0.05) from 21 to 45 dpf. The trained fish initially grew faster than the control fish, but after 35 dpf, the control fish grew faster (Fig. 2A, inset) until, at 63 dpf, both groups are again indistinguishable in terms of size. Mass of the control fish increased from 0.0055 g (SD 0.0021) at 14 dpf to 0.68 g (SD 0.19) at 84 dpf (Fig. 2B). The trained fish tended to be heavier at all time points after 14 dpf; however, this difference in mass reached statistical significance (P < 0.05) only at 21 and 24 dpf.

Muscle Fiber Cross-Sectional Area

To establish whether training increases the red muscle fiber mass, the total cross-sectional area of all red muscle fibers was determined over time. When the trained and control fish were compared over the entire training period using a Wilcoxon signed-rank test over time, the trained fish had a larger (P < 0.05) total cross-sectional red muscle fiber area (Fig. 3). In addition to a larger total cross-sectional red muscle fiber area, the control and trained fish showed ample fat deposition.

To discover whether training affects hypertrophic muscle fiber growth, the cross-sectional area of individual white and red muscle fibers was determined at 45 and 84 dpf. At 45 dpf, the mean cross-sectional area of the individual white muscle fibers was 1,571 μm² (SD 839) and 1,801 μm² (SD 744) in control (n = 4 fish, 374 fibers) and trained (n = 4 fish, 566 fibers) fish, respectively (Fig. 4A). Fibers from trained fish were not significantly larger than fibers from control fish (P = 0.602). At 84 dpf, the mean cross-sectional area of the white fibers was 3,331 μm² (SD 1,231) and 3,228 μm² (SD 1,250) in control (n = 4 fish, 489 fibers) and trained (n = 4 fish, 455 fibers) fish, respectively (Fig. 4B), which is not statistically significant (P = 0.553).

At 45 dpf, cross-sectional area of the individual red muscle fibers was 732 μm² (SD 264) and 730 μm² (SD 246) in trained (n = 4 fish, 277 fibers) and control (n = 4 fish, 288 fibers) fish, respectively (Fig. 4C), which is not statistically significant (P = 0.372). At 84 dpf, cross-sectional area of the red fibers was 1,298 μm² (SD 478) and 1,308 μm² (SD 393) in control (n = 4 fish, 313 fibers) and trained (n = 4 fish, 249 fibers) fish, respectively (Fig. 4D), which is not significantly different (P = 0.935).

Kinetics of Muscle Gene Expression

The expression of muscle-specific genes is regulated over time as a result of growth and development and was predicted
to be regulated by endurance training. We selected a panel of representative muscle metabolism processes for investigation: sarcomere construction, energy generation, growth, cell proliferation, and oxygen transport. The expression kinetics of the selected genes were established in trained and control fish over the initial 3 wk of training and compared. Because of age-related changes in gene expression, all expression values are given relative to the initial samples at 14 dpf, so that control and trained values may be compared.

*Genes involved in sarcomere construction.* A decrease ($P < 0.05$) was observed in the expression of fast myosin heavy chain 2 (myhz2) after 21 dpf (Fig. 5A) and in slow myosin heavy chain 5 (myhz5) after 14 dpf (Fig. 5B). Fast muscle-specific troponin C showed a slightly elevated expression at 21 dpf, and slow muscle-specific troponin C remained constant (not shown).

*Genes involved in energy generation.* Muscle phosphofructokinase (PFK-m) is involved in glycolysis. Its expression remained constant over time. NADH dehydrogenase (NADHd) and succinate dehydrogenase complex subunit A (SDHa) are involved in mitochondrial metabolism. NADHd increased ($P < 0.05$) after 14 dpf (Fig. 5C). SDHa expression remained constant (not shown).

*Genes involved in growth.* Growth and differentiation factor 8 (gdf8 and myostatin), an inhibitor of muscle growth, was elevated after 14 dpf (Fig. 5D). Expression of myogenin, a muscle growth promoter, showed much variation but appeared to increase as well (Fig. 5E). Insulin-like growth factor (IGF), which also regulates muscle growth, was upregulated after 14 dpf (Fig. 5F), whereas expression of IGF receptors a and b was not changed (not shown).

*Genes involved in cell proliferation.* Proliferating cell nuclear antigen (PCNA), an indicator of cell division activity, showed elevated expression levels after 14 dpf (Fig. 5G).

*Genes involved in oxygen transport.* Expression of myoglobin, which is important in the uptake and delivery of oxygen to muscle, was upregulated after 14 dpf (Fig. 5H). Expression of erythropoietin (Epo), which regulates red blood cell production and hemoglobin synthesis, was also upregulated over time (Fig. 5I).

Fig. 3. Total red muscle fiber area transiently increases as a result of endurance training. Cross-sectional area of the 4 quarters of total red muscle fiber area were measured and averaged to obtain values for individual fish.

Fig. 4. Muscle fiber cross-sectional area increases over time, and white muscle cross-sectional area is transiently increased as a result of training. White and red muscle fiber areas were grouped in 400- and 200-mm² groups, respectively. dpf, Days postfertilization.
During the first 3 wk, training effects on gene expression included an increase in the expression of PCNA and myoglobin (Fig. 5, G and H). Expression of the other above-mentioned genes was not significantly affected by training.

Differential Effects of 10 Weeks of Training on Axial Muscle and Heart

The effects of prolonged training were investigated after 10 wk of training in axial muscle and heart. In axial muscle, training significantly elevated expression of slow myhz5 and slow troponin C, myogenin, Epo, myoglobin, and PCNA (Fig. 6A). Expression of fast myhz2, fast troponin C, and myostatin did not respond to training. In the heart, training significantly elevated expression of fast myhz2, slow myhz5, fast troponin C, myogenin, and PCNA (Fig. 6B). Expression of slow troponin C, myostatin, Epo, and myoglobin did not respond to training.

To put the observed changes in gene expression into perspective, we compared their relative expression levels in axial muscle and heart from control fish. Figure 7A shows the

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Fig. 5. Kinetics of muscle gene expression as obtained by quantitative RT-PCR. Expression was first normalized to β-actin expression. Then expression at 14 dpf, immediately before initiation of training, was set to 1 (dashed line) so that expression could be followed over time and differences induced by training could be observed. Muscle constituents fast and slow myosin heavy chain (myhz2 and myhz5, respectively; A and B) decreased over time, irrespective of training. NADH dehydrogenase (NADHd; C), a measure of mitochondrial activity, increased over time but was not affected by training. Muscle growth factors growth and differentiation factor 8 (gdf8, D (inhibitor)) and myogenin (E) and insulin-like growth factor (IGF)-Ea2 [F (activators)] increased over time but were unaffected by training. Proliferating cell nuclear antigen (PCNA; G), an indicator of cell proliferation, increased over time and was further enhanced by endurance training, as was myoglobin (H), which increased aerobic capacity of muscle. Expression of erythropoietin (Epo, I), which stimulates red blood cell formation, increased over time but was unaffected by training.

*Significantly different from 14 dpf (P < 0.05). #Significant difference between trained and control (P < 0.05).
relative abundance of messengers for the selected genes in axial muscle and heart of control fish, in which the expression in muscle was set to 1. The relative abundance of fast myhz2 [0.0072 (SD 0.0095)] and fast troponin C [0.0056 (SD 0.0077)] messengers was lower ($P < 0.0005$) in heart than in axial muscle, that of slow myhz2 [0.46 (SD 0.60)] was similar in heart and axial muscle, and that of slow troponin C {[39 (SD 8.6)] $\times 10^3$} was higher ($P < 0.0005$) in heart than in axial muscle. The muscle growth factors myostatin [0.54 (SD 0.56)] and myogenin [1.1 (SD 1.3)] were expressed at similar levels in heart and axial muscle. Epo [136 (SD 42)] and myoglobin [24 (SD 7)] were more abundant ($P < 0.0005$) in heart than in axial muscle. Basal proliferation levels appeared similar, as indicated by PCNA expression levels [2.3 (SD 2.7)]. In trained fish, the pattern was similar (Fig. 7B), but there were scale differences. The relative abundance of fast myhz2 [0.016 (SD 0.018)] and fast troponin C [0.012 (SD 0.007)], $P < 0.005$ and, in contrast to control fish, slow myhz5 [0.18 (SD 0.15)], was lower ($P < 0.001$) in heart than in axial muscle, whereas that of slow troponin C {[2.2 (SD 0.6)] $\times 10^3$} was higher ($P < 0.0005$) in heart. The muscle growth factors myostatin [1.3 (SD 0.5)] and myogenin [0.60 (SD 0.76)] were expressed at similar levels in heart and axial muscle. Epo [47 (SD 24)] and myoglobin [35 (SD 13)] were more abundant ($P < 0.0005$) in heart than in axial muscle. Basal proliferation levels also appeared similar in trained fish, as indicated by PCNA expression levels [3.2 (SD 3.6)].

**DISCUSSION**

We addressed the effects of increased mechanical load on developing zebrafish. In the present study, we increased mechanical loads by imposing an endurance-training regimen. In large salmonid fish species, training induces effects that are comparable to, although less profound than, those observed in humans (9, 10). These effects include an enhanced aerobic
potential of white and red muscle, a relative increase in the size of the red muscle compartment, increased fiber cross-sectional area, and improved heart performance (9, 10). In the small zebrafish, effects of short periods of training during development on aerobic and anaerobic metabolism have been reported (1, 27), but a comprehensive picture of the effects of endurance training is lacking. We therefore trained zebrafish for 10 wk and studied the effects of endurance training on muscle at macroscopic, histological, and molecular levels.

Bagatto et al. (1) reported that a training regimen of 2–5 TL/s for zebrafish larvae at 4 dpf for 24 h/day or at 21 dpf for 15 h/day resulted in high mortality. Mortality was higher among younger fish and increased with higher flow rate and longer duration of training (1). There is a risk that any differences in such a setup between trained and control fish are confounded by high mortality and are not necessarily the result of adaptation. Therefore, we designed a less severe training regimen for the present study. Training commenced at 14 dpf, when larvae had switched to a diet of artemia, and consisted of a daily period of 6 h at 5 TL/s, instead of 15 TL/s, and lasted for 10 wk; 5 TL/s represents about twice the normal average swimming speed (14). Zebrafish retained their intermittent swimming pattern, with bursts of activity alternating with short periods of coasting, at all times. The fish were able to keep up with the training regimen and actively pursued small particles that floated by. These results indicate that maximal sustainable swimming speed was not reached. We also did not experience more mortality in our trained fish than in controls. Together, these observations suggest that the imposed training regimen was sustainable. During training, the fish swam close to the front grid in a school. This type of behavior may serve to reduce the energy cost of swimming against a current, by exploiting the vortices that are generated by objects in the flow (25), such as the grid or, in this case, other animals. Nevertheless, the trained fish were more active than the control fish.

Zebrafish are slow-growing fish that reach a maximum TL of 40–50 mm over a 3-yr life span. The growth rate decreases when they are about one-third of adult size (30), which occurs at ~30 days of age. We observed this decrease in growth rate in our control fish (Fig. 2, inset). Trained fish initially showed a faster growth rate than control fish, but growth declined to a rate slower than that of control fish after 40 dpf. A transient difference in TL between trained and control fish disappeared at 60 dpf (Fig. 2). In larger fish species, training results in lasting differences in body size (9, 10); however, the maximum size in zebrafish is probably genetically determined. Alternatively, a forced swimming speed that is constant in TL per second might prove increasingly more intensive as body length increases (17), causing an energy deficit, along with a decrease in growth rate, in older stages. However, growth rate decreased in control and trained fish. In addition, both groups were fed ad libitum and retained fat reserves, indicating that the preferred energy reserves for endurance training (24) are still present. Therefore, this intensity hypothesis is implausible.

Whole body growth and hypertrophic growth in white and intermediate muscle are limited after zebrafish reach a TL of 20 mm. Red and heart muscle hyperplasia continues into adulthood, and hypertrophic growth can be found in all muscle fiber types (15, 34). Despite a potential for hypertrophic growth, endurance training did not increase the muscle fiber hypertrophy, as judged by cross-sectional area in white or red muscle (Fig. 4); whereas, this is a common response to training in larger fish (9, 10). The total area of red muscle fibers, however, did increase (Fig. 3). Because this process was not accompanied by red muscle fiber hypertrophy, a hyperplastic response, consistent with a continued hyperplastic capacity of red muscle fibers (34), is suggested. This is supported by the increased PCNA expression throughout the 10-wk training period (Figs. 5 and 6). After 10 wk of training, in the axial muscle, the increased PCNA expression is accompanied by an increased expression of slow myhz5 and slow troponin C. Furthermore, the aerobic capacity of the axial musculature was increased through increased expression of Epo and myoglobin. The increase in aerobic capacity is not necessarily limited to red muscle, as white muscle tissue may also show an increase in aerobic capacity, e.g., via an increase in capillarization after training (8, 27, 29). In conclusion, the axial muscle responds to endurance training by a shift toward a slow aerobic phenotype, which is accompanied by a hyperplastic increase in red muscle fibers.

The heart is a continuously active muscle and, as such, performs endurance exercise in control fish. In control fish, slow troponin C, Epo, and myoglobin are much more abundant in heart than in axial muscle at 84 dpf (Fig. 7). This suggests that, in control fish, heart muscle has a slow, red muscle-like phenotype, which is consistent with the obligatory aerobic metabolism of cardiac muscle fibers. Similar to red muscle fibers in fish, the heart muscle retains the ability to grow and fully regenerate through hyperplasia (15, 28). Although heart size may be increased by training (13, 16, 18), it generally is not (for review see Refs. 9 and 10). Furthermore, training for 11 days in zebrafish did not result in any postexercise changes in cardiac activity (27). In the present study, we compared the effect of 10 wk of training on gene expression levels in heart and axial muscle. Both muscles showed increased expression of PCNA and myogenin, indicating hyperplastic growth of heart and axial muscle after prolonged endurance training. However, training also differentially affected heart and axial muscle. Axial muscle shifted toward a slower, more aerobic phenotype, as described above. In heart muscle, expression of fast troponin C and fast and slow myosin heavy chain increased, whereas expression of Epo, myoglobin, and slow troponin C did not change (Fig. 6). This indicates that the heart shifts to a faster phenotype and does not increase aerobic capacity.

In control hearts, the above-mentioned high levels of myoglobin and Epo expression (Fig. 7) are necessary for extraction of oxygen from the oxygen-depleted venous blood that supplies the spongy myocardium (7, 12). In the pufferfish Fugu rubripes, the heart is the major Epo-producing organ (3), in contrast to mammals, in which the kidney performs this function (21, 23). The lack of increase in expression of these genes indicates that the existing capacity for oxygen extraction was large enough to manage the increased demand of endurance training. The increase in myogenin, PCNA, and, mainly, fast contractile proteins suggests, instead, that the heart responded by hyperplastic growth and shifted to a faster phenotype. This indicates that the heart becomes more powerful.

We compared basal transcription levels between heart and axial muscle in control fish. The relatively high transcription of slow troponin C, Epo, and myoglobin in the heart correlates with a lack of transcription modulation after training, whereas...
expression of genes that are expressed at a similar or lower level than in axial muscle, e.g., myogenin, fast myh2, and PCNA, may be increased. Vice versa, expression of genes with lower transcription in axial muscle was modulated as a result of training. This may be indicative of optimal transcription of the highly expressed genes, whereas genes with lower expression can still be upregulated in transcription.

In this study, fish were fed ad libitum, and we observed a transiently increased growth rate in the trained fish, which could have resulted from greater food intake by trained fish. This should also be regarded as a result of the training regimen. The differences in fish size may have had an effect on the size of the total red muscle fiber area; however, feeding is not expected to affect muscle composition with respect to fast-to-slow fibers ratios. In addition, because the RT-PCR analysis uses normalization to internal reference genes, the different sizes of trained and control fish are not relevant in the analysis of gene expression over the first 3 wk of training.

Collectively, our results indicate that endurance training enhances slow aerobic muscle development in the axial musculature and shifts the heart to a faster phenotype. Thus, although the different organs and tissues are still in the process of maturation and differentiation, they show profound plasticity and respond to increases in mechanical load.

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