

RESEARCH ARTICLE

Exercise training induces muscle remodeling independent of estrogen-related receptor (ERR) expression in adult zebrafish

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ABSTRACT

Locomotor capacity is a major determinant of fitness and is strongly modulated by exercise-induced physiological plasticity. In vertebrates, sustained submaximal exercise typically promotes a shift toward an aerobic muscle phenotype, characterized by enhanced oxidative capacity, angiogenesis and contractile remodeling. In mammals, these plastic responses are largely mediated by estrogen-related receptors (ERRs) and their coregulators; however, whether these regulatory mechanisms are conserved in fish remains unclear. Here, we tested the role of ERRs in exercise acclimation in adult zebrafish (*Danio rerio*), a laboratory model with multiple ERR paralogs following teleost-specific genome duplication events. Following 6 weeks of moderate intensity forced-swimming training, we assessed whole-animal performance (critical swimming speed) and metabolism alongside skeletal muscle contractile and metabolic phenotypes. We applied proteomic profiling and pathway enrichment analyses to evaluate changes in ERR expression and activity, and to identify molecular networks associated with muscle remodeling. Exercise training improved locomotor performance by increasing critical swimming speed and induced skeletal muscle remodeling consistent with a more oxidative and vascularized phenotype, without detectable changes in resting or maximal metabolic rates. Additionally, improvements in exercise training capacity were not accompanied by detectable changes in ERR expression as determined by proteomic analyses. Instead, it suggested alternative regulation distinct from canonical mammalian ERR signaling pathways. Our results demonstrate that zebrafish exhibit conserved exercise-induced phenotypic outcomes but rely on fundamentally different molecular mechanisms than mammals. These findings highlight the value and limitations of zebrafish as models for exercise physiology and underscore the evolutionary flexibility of signaling pathways governing conserved functional phenotypes.

KEY WORDS: Exercise, *Danio rerio*, Skeletal muscle, Plasticity, Estrogen-related receptor

INTRODUCTION

Locomotor capacity represents a major selection pressure in animal taxa, influencing fitness-adjacent behaviors including resource acquisition (Webb, 1984; Altmann, 1987), predator avoidance (Domenici, 2001; Clemente and Wilson, 2016), dispersal and novel

habitat colonization (Denton et al., 2017; Wilkinson et al., 2025), and social interactions (Seebacher and Krause, 2017; Jolles et al., 2020). Exercise itself is an energetic stressor that can induce plasticity in various tissues. Repeated bouts of exercise improve whole-animal performance and induce tissue-specific remodeling (i.e. acclimation) across vertebrate groups (Furrer et al., 2023). Often, when submaximal exercise is sustained over time, these repeated energetic challenges drive shifts toward more aerobic phenotypes (as reviewed in Holloszy and Coyle, 1984; Davison, 1997) characterized by improved cardiovascular function (Nystoriak and Bhatnagar, 2018; Furrer et al., 2023), enhanced skeletal muscle oxidative capacity and angiogenesis (Sänger, 1992; Hoppeler et al., 2007), and fiber type shifts (Andersen and Henriksson, 1977; Rockl et al., 2007). As such, physical activity is widely acknowledged for its therapeutic roles in health and the prevention of disease. Determining the pathways that underlie exercise acclimation is thereby important to understand lifestyle trade-offs, disease pathologies and potential therapeutics.

Fish represent an established model to explore exercise physiology from both ecological and biomedical angles. Zebrafish (*Danio rerio*), specifically, are a widespread laboratory model used in exercise physiology (McClelland et al., 2006; LeMoine et al., 2010a; Palstra et al., 2010; Gilbert et al., 2014), including in studies testing the effects of exercise on metabolic disease (Seebacher and James, 2019; Heinkele et al., 2021; Wang et al., 2022; Zou et al., 2023; Fang et al., 2025). In most teleosts, skeletal muscle is organized into distinct red (slow oxidative) and white (fast glycolytic) compartments separated by a layer of pink (intermediate) muscle with intermediate metabolic and contractile properties (as reviewed in Videler, 1993). Red muscle primarily supports sustained aerobic swimming, whereas white muscle contributes greatly to burst locomotion. Fish skeletal muscle is responsive to exercise and exhibits a high degree of cellular and tissue-level remodeling in response to energetic stress (Sänger, 1993; McClelland and Scott, 2014). This plasticity adjusts the machinery for both excitation–contraction coupling and energy metabolism to support efficiency and power of locomotion (Morash et al., 2014; Palstra et al., 2014; Sopariwala et al., 2023a). Accordingly, exercise-induced plasticity (i.e. the capacity to acclimate or train) is critical for maintaining performance under variable environmental conditions (McClelland and Scott, 2014; Burton et al., 2022), particularly with anthropogenic changes to natural waterways (e.g. hydroelectric dams).

The signaling pathways that link stressor cues with compensatory responses are not well characterized, particularly for non-mammalian models. Nuclear receptors represent good candidates because they modulate transcriptional programs in response to metabolic and environmental cues. Their position at the interface of small molecule signaling and large-scale transcriptional regulation thereby facilitates integration of environmental shifts with cellular and organismal-level responses. In mammals, the

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List of symbols and abbreviations

AP	alkaline phosphatase
AS	aerobic scope
BL	body length
COX	cytochrome oxidase
CS	citrate synthase
CV	coefficient of variation
DBD	DNA-binding domain
ERR	estrogen-related receptor
ERRE	estrogen-related receptor response element
GLM	general linear model
GO	gene ontology
HNF3	hepatocyte nuclear factor 3
ILR	isometric log-ratio
LDH	lactate dehydrogenase
LMM	linear mixed model
MEF2c	myocyte enhancer factor 2c
MMR	maximum metabolic rate
NRF	nuclear respiratory factor
OXPHOS	oxidative phosphorylation
PGC-1 α	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PPAR	peroxisome proliferator-activated receptor
RMR	resting metabolic rate
ROS	reactive oxygen species
SC	supercomplex
SDH	succinate dehydrogenase
SERCA	sarcoplasmic/endoplasmic reticulum calcium ATPase
SIRT1	sirtuin 1
U_{Cfast}	critical sustained swimming speed

estrogen-related receptors (ERRs) are orphan nuclear receptors that function as transcriptional regulators and coordinate a vast array of gene programs governing energy metabolism, angiogenesis and cellular remodeling (Giguère, 2008; Sopariwala et al., 2023a). ERR expression is relatively high in tissues with high metabolic demands such as skeletal muscle, brain, heart and brown adipose tissue (Bookout et al., 2006; Giguère, 2008; Gofflot et al., 2007).

ERR knockout models in mammalian vertebrates highlight the crucial role ERRs play in modulating plastic responses to changing energetic demands, including exercise training (Villena and Kralli, 2008; Perry et al., 2014; Sopariwala et al., 2023b; Watzel et al., 2023). In human and mouse skeletal muscle, ERR expression is upregulated directly in response to exercise training (Cartoni et al., 2005; Rangwala et al., 2010; Matsakas et al., 2012). ERRs, particularly ERR α and ERR γ , can promote oxidative phosphorylation (OXPHOS), vascularization and shifts in muscle fiber type (Sopariwala et al., 2023a). In mammals, changes in muscle phenotype are largely mediated through the interactions of ERRs with their main coactivator, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α), along with other transcriptional regulators of mitochondrial gene expression such as nuclear respiratory factors (NRFs) and peroxisome proliferator-activated receptor (PPARs) (Baar et al., 2002; LeMoine et al., 2010b; Masoumi-Ardakani et al., 2020). Through interactions with NRFs and myocyte enhancer factor 2c (MEF2c), PGC-1 α orchestrates gene programs that shape muscle metabolic and contractile properties (LeMoine et al., 2010b).

The adoption of fish as biomedical models relies on the principle that basic physiology is evolutionarily conserved, maintaining high molecular and functional homology despite more than 400 million years of divergence from their last common ancestor with humans.

However, conservation of higher-level traits does not always reflect conservation of underlying mechanisms and/or signaling pathways. For instance, whole-genome duplication events early in the radiation of bony fish may have contributed to the diversification and increased complexities of some regulatory networks (Kassahn et al., 2009; McClelland, 2012). There are five described ERR genes in zebrafish (α , β , γ -a, γ -b and δ) that correspond to three ERR paralogs in the mammalian genome (Bertrand et al., 2004; Tohmé et al., 2014). The amino acid sequence of the ERR DNA-binding domains (DBDs), which enables recognition of specific target gene sequences, is highly conserved between human and zebrafish paralogs, with 100% conservation in the α , β and γ -a isoforms, and approximately 90% conservation in γ -b. Despite this strong conservation, previous work has questioned whether the protein–protein interaction networks governing muscle phenotype in mammals and fish are truly homologous (McClelland et al., 2006; LeMoine et al., 2010a; McClelland, 2012), where pathways underlying exercise-induced remodeling may have diverged throughout vertebrate evolution. Interestingly, sequence analyses indicate that teleost PGC-1 α contains insertions in the NRF-1 and MEF2c binding domains and thereby lack the functional regions involved in metabolic remodeling (e.g. AMPK phosphorylation sites) (LeMoine et al., 2010b). By extension, this divergence suggests that ERR may regulate exercise remodeling through different channels in fish.

Here, we tested whether ERRs regulate exercise acclimation responses in zebrafish. We hypothesized that the primary functional role of ERRs in exercise acclimation is evolutionarily conserved, though the downstream signaling networks driving these responses may differ. Following a forced-swimming exercise training regime, we examined both contractile and metabolic phenotypes at the whole-animal and skeletal-muscle levels. We then used a global proteomic approach to quantify potential shifts in ERR protein expression levels and broader gene expression networks to gain insight into the signaling pathways underlying exercise-induced skeletal muscle remodeling in these fish. Although regulation via ERR is predominately regulated through expression in mammals (Cartoni et al., 2005; Rangwala et al., 2010; Matsakas et al., 2012), we implemented two complementary enrichment analyses to explore whether exercise remodeling may result from changes in ERR activity (e.g. through post-translational modifications or cofactor regulation), rather than expression profiles alone. Our findings suggest some marked differences that may be important when using this species as a model for exercise physiology. In a broader sense, our work provides insight into the flexibility and robustness of exercise signaling networks.

MATERIALS AND METHODS

Animals

All experimental procedures were approved by the McMaster University Animal Research Ethics Board (AREB) under animal use protocol 23-68. Adult AB strain wildtype zebrafish [*Danio rerio* (Hamilton 1822)] used for the experiments were bred in house at McMaster University (Hamilton, ON, Canada). All experimental zebrafish were housed at 28°C on an Aquatic Habitats for Accelerated Bioresearch (AHAB) zebrafish rearing system (Aquatic Habitats, Apopka, FL, USA) in 10-liter tanks of semi-recirculating dechlorinated water (pH=6.5–7.0, conductivity=300–400 μ S) before the start of exercise training regimes. Fish were fed Gemma (Skretting, Vancouver, BC, Canada) and live *Artemia* (INVE Aquaculture Inc., Salt Lake City, UT, USA) twice daily. Fish were maintained on a 14 h:10 h light:dark photoperiod.

Exercise training regime

Six- to eight-month-old AB strain wildtype zebrafish (*D. rerio*; $N=72$) of both sexes were randomly assigned to control (non-exercise) or exercise-trained groups. Mean body length was 3.27 ± 0.306 and 3.27 ± 0.312 cm, and mean body mass was 0.299 ± 0.0703 and 0.323 ± 0.0808 g, for the control and exercise groups, respectively. Exercise training experiments were replicated across three temporally separated batches ($n=12$ fish per group per batch). The exercise regime followed a previously established forced swimming protocol conducted (Simmonds and Seebacher, 2017) but extending it from 4 to 6 weeks in length. Control and exercise tanks ($615 \times 315 \times 305$ mm) were identical. Within each tank, fish were confined to an area 50 mm wide and 305 mm long in the center of the tank between two clear acrylic partitions fitted with mesh netting covering each end. A submersible wavemaker (DC24V-10W 2100 GPH, Boxtech) directed water flow through the enclosed swimming channel. A bundle of silicone tubing was used to promote laminar flow of water throughout the channel. Each tank contained submersible pumps (Mimouse centrifugal pump 82 GPH, Sicce, Italy) to ensure proper circulation and filtration of water. Water in tanks was continuously filtered and aerated throughout the 6-week training period to ensure optimal water quality. In short, fish swam against a water flow rate of 21 cm s^{-1} [$\sim 6\text{--}7$ body lengths (BL) s^{-1}] three times per day for 6 days per week. This training speed was chosen to represent moderate-intensity exercise ($\sim 30\text{--}35\%$ of critical swimming speed; McClelland et al., 2006; LeMoine et al., 2010a; Simmonds and Seebacher, 2017). One hour exercise training intervals were separated by 3-h recovery periods throughout the first week. Each subsequent week, the exercise interval increased by 0.5 h and the recovery period decreased by 0.5 h until week 4. Thereafter, the exercise and recovery intervals remained unchanged during weeks 5 and 6 of training.

Swim performance

Critical sustained swimming performance (U_{crit}) was used to assess swim performance following exercise acclimation, based on the original method (Brett, 1964) with subsequent modifications (Bamford and Seebacher, 2024). Individual fish were transferred into a clear acrylic swim flume (190×35 mm, length $\times$ diameter) connected to a submersible pump (24 V Bilge Pump 3000 GPH, Dpofirs). A bundle of plastic straws was positioned between the pump and flume to promote laminar flow throughout. The swim flume was submerged within a larger tank ($320 \times 610 \times 310$ mm) to ensure thermal stability (28°C) and provide a clear view of fish throughout trials. Fish were selected randomly and allowed to familiarize themselves within the flume for 10 min prior to the onset of the trial.

The U_{Cfast} trial was conducted as reported in Bamford and Seebacher (2024), where the initial flow rate of the water was 16 cm s^{-1} ($\sim 4\text{--}6 \text{ BL s}^{-1}$) for 1 min. The speed was then increased by 6.0 cm s^{-1} in 1-min increments. The trial continued until the fish fatigued, which was defined as the point at which the fish could no longer maintain its position in the water column or after contacting the back of the flume three consecutive times. The critical swimming speed (U_{Cfast}) was calculated according to Brett (1964):

$$U_{\text{Cfast}} = U_i + \left(\frac{t_f}{t_i} \times U_{ii} \right), \quad (1)$$

where U_i is the penultimate velocity maintained for the full time interval, t_f is the time the fish swam at the final incomplete velocity,

t_i is the full interval for each increment and U_{ii} is the velocity increment. U_{Cfast} is reported as BL (total length) per second (s). The analysis includes three temporally separated experimental batches of fish, with 10–12 fish per treatment group in each batch (for exercise group, $n=30$ and for control group, $n=29$, with a random assortment of both sexes in each group).

Aerobic scope

Following completion of the 6-week exercise acclimation, metabolic performance was evaluated using intermittent-flow respirometry. Individual fish were placed into glass respirometry chambers submerged within a temperature-controlled (28°C), aerated water bath. Each respirometer was fitted with a continuous recirculation loop and an intermittent flush system to regulate oxygen concentration. Water used in respirometry trials was system water that is controlled for pH, conductivity and temperature. Recirculation pumps (Eheim Universal Pump 300, EHEIM, Germany) provided constant mixing of the chamber water within the glass respirometers (volume 156 ml) and allowed for continuous measurement of oxygen concentration using fiber-optic oxygen sensors (FireStingO₂, PyroScience, Germany). Flush pumps (Mimouse centrifugal pump 82 GPH) were turned on intermittently to replenish oxygenated water within the respirometer to ensure fish were not exposed to hypoxia stress. Resting metabolic rate (RMR) was measured overnight in intermittent cycles of 40 min of constant flushing and 15 min without flushing in a 156 ml volume chamber. All fish were tested within 4 days of training completion, with treatments split evenly across timepoints to ensure equal time from training completion to respirometry trials between groups. New water was provided at the start of every RMR trial. Oxygen consumption data from the first hour were excluded to minimize handling effects. Blank respirometry trials (i.e. chambers without fish) were conducted in parallel under identical conditions to correct for background microbial respiration. Maximum metabolic rate (MMR) was determined immediately after U_{Cfast} swimming trials. Fish were transferred directly into the glass respirometry chamber (volume 118 ml) post-exercise, and oxygen consumption was recorded continuously during the recovery period. Peristaltic pumps (IPC High Precision Multichannel Pump, Ismatec, Switzerland) were used to ensure proper mixing of water throughout trial. Fresh water was provided for every individual MMR measurement. This measurement reflects the combined oxygen demand of the fish's routine metabolism and the excess post-exercise oxygen consumption (Roche et al., 2013; Little et al., 2020). Blank respirometry trials were implemented before and after each MMR trial. Lastly, aerobic scope (AS) was calculated as the difference between maximum and resting metabolic rates for each individual, representing the metabolic capacity available for aerobic activity beyond maintenance. Sample sizes were 8–10 fish per group with a random assortment of each sex per treatment group.

Oxygen consumption data were analyzed using the R package 'respR' (Harianto et al., 2019) in RStudio (v4.5.1). Oxygen traces were first inspected visually to identify and exclude periods of noise or non-linear signal due to spontaneous fish activity. The rate of oxygen decline ($\text{mg O}_2 \text{ l}^{-1} \text{ h}^{-1}$) was calculated for a 100–200 s time period during each closed respirometry period, with the steepest, most linear segment ($R^2 > 0.9$) selected for each fish. A sliding window size of 30 s was used for all fish. These rates were then corrected for background respiration and converted to mass-specific metabolic rates ($\text{mg O}_2 \text{ g}^{-1} \text{ h}^{-1}$) based on individual body mass of each fish. Similar to MMR, for each fish, the slope of the

closed respirometry phase was measured for seven different cycles throughout the night using a sliding window size of 1500 s and a measurement time frame of 2000 s. The lowest rate from each cycle was selected, provided $R^2 > 0.9$. The RMR for each individual was then calculated as the average of the three lowest rates across the seven cycles to ensure value represented the true resting state. For RMR measurements, four fish (2 control and 2 exercise) were excluded from analysis due to leaking respirometry chambers. Similarly, four fish (1 control and 3 exercise) were excluded from MMR analysis due to leaking chambers. In addition, for AS measurements, there are a fewer number of individual fish included due to missing RMR measurements for corresponding individuals.

Tissue sampling

Upon completion of exercise training and swimming performance assessments, fish were weighed and standard length was measured to determine body condition (Fulton's K) as previously reported (Fulton, 1902; McClelland et al., 2006). Fish were then euthanized using a 400 mg l⁻¹ MS-222 (Sigma-Aldrich, Oakville, ON, Canada) solution buffered with 800 mg l⁻¹ Na₂HCO₃. A transverse section of muscle tissue obtained from immediately posterior to the dorsal fin was dissected from each fish and stored at -80°C before being used for subsequent kinetic enzyme assays, histological analysis and proteomic profiling.

Enzyme assays

Enzyme assays were performed on a transverse section of axial muscle ($n=9-10$ per group) to examine the maximal activity of key metabolic enzymes [lactate dehydrogenase (LDH), citrate synthase (CS) and cytochrome oxidase (COX)]. In addition, assaying sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) activity gave insight into the contractile function of the muscle. The metabolic enzyme assays were performed accordingly to previously published protocols (Dalziel et al., 2012). In short, whole muscle tissue used for the assays was weighed and freshly homogenized on ice prior to conducting the assay. The homogenization buffer was composed of 0.1% Triton X-100, 50 mmol l⁻¹ Hepes and 1 mmol l⁻¹ EDTA (pH 7.4). All assays were conducted at a test temperature of 28°C by measuring the rate of change in absorbance at 340 nm (LDH, SERCA), 412 nm (CS) and 550 nm (COX) for 10 min. The LDH assay was performed using a reaction mixture of 50 mmol l⁻¹ Tris (pH 7.4), 5 mmol l⁻¹ NADH and 25 mmol l⁻¹ sodium pyruvate, and LDH activity was coupled to the oxidation of NADH. CS activity was determined by measuring the disappearance of DTNB, using a final reaction mixture composed of 0.15 mmol l⁻¹ DTNB, 0.15 mmol l⁻¹ acetyl-CoA, 0.5 mmol l⁻¹ oxaloacetate and 50 mmol l⁻¹ Tris (pH 8.0). Lastly, COX activity was measured on freshly homogenized tissue using a reaction mixture containing 50 µmol l⁻¹ reduced cytochrome *c*, 0.5% Tween 20 and 50 mmol l⁻¹ Tris (pH 8.0). Maximal activity of SERCA in the zebrafish muscle was measured according to a previously published protocol (McCormick, 1993). Briefly, zebrafish muscle was freshly homogenized in a buffer containing 150 mmol l⁻¹ sucrose, 10 mmol l⁻¹ EDTA, 50 mmol l⁻¹ imidazole and 12 mmol l⁻¹ sodium-deoxycholate (pH 7.3). The final reaction mixture contained a 3:1 ratio of assay buffer [4 U lactate dehydrogenase, 5 U pyruvate kinase, 2.8 mmol l⁻¹ phosphoenolpyruvate, 0.7 mmol l⁻¹ adenosine triphosphate, 0.22 mmol l⁻¹ NADH and 50 mmol l⁻¹ imidazole (pH 7.5)] to salt solution [189 mmol l⁻¹ NaCl, 10.5 mmol l⁻¹ MgCl₂, 42 mmol l⁻¹ KCl and 50 mmol l⁻¹ imidazole (pH 7.5)]. SERCA activity was determined as the difference in NADH

disappearance at 340 nm in the presence and absence of a specific inhibitor (10 µmol l⁻¹ thapsigargin) for SERCA. Thapsigargin concentration was chosen based on a previously conducted study in zebrafish (Little and Seebacher, 2014). All biochemical reagents used for enzyme assays were purchased from Sigma-Aldrich. All assays were run in triplicate on a 96-well plate using a microplate spectrophotometer (BioTek Epoch, Agilent Technologies, CA, USA). To calculate enzyme activity, we used extinction coefficients of 6.22 (LDH, SERCA), 13.6 (CS) and 28.5 mM⁻¹ cm⁻¹ (COX).

Muscle histology

To characterize the skeletal muscle phenotype, a transverse section immediately posterior to the dorsal fin (~3 mm) was dissected following completion of the exercise training regime. The muscle section was embedded in resin medium (Cryomatrix™, Eppredia™, Fisher Scientific Company) and frozen immediately in liquid-nitrogen-cooled isopentane before storage at -80°C. Cryosections (9 µm thick) were cut at -20°C using a cryostat (CM1860, Leica Microsystems, Germany) and mounted onto Superfrost Plus microscope slides (Fisher Scientific Company). Slides were stored at -80°C until staining was performed. Muscle sections were stained for succinate dehydrogenase (SDH) and alkaline phosphatase (AP) activity following methods previously described (Egginton, 1990; Scott and Johnston, 2012; Borowiec et al., 2015), as a proxy for oxidative capacity and capillarization of the muscle, respectively. In short, SDH activity was visualized using an incubation medium containing 0.1% NBT, 41.7 mmol l⁻¹ Na₂HPO₄, 8.3 mmol l⁻¹ NaH₂PO₄ and 80 mmol l⁻¹ sodium succinate (pH 7.6). AP activity was visualized using 28 mmol l⁻¹ NaBO₂, 7 mmol l⁻¹ MgSO₄, 1 mmol l⁻¹ NBT and 0.5 mmol l⁻¹ BCIP (pH 9.3). Lastly, to identify the oxidative (slow-twitch) muscle fibers, sections were immunostained using a mouse IgA primary antibody (S58; 1:10; Developmental Studies Hybridoma Bank, Iowa City, IA, USA) and Alexa Fluor™ Plus 488 goat anti-mouse IgG secondary antibody (1:400; Cat. No. A32723TR, Invitrogen, Carlsbad, CA, USA) were used to stain for slow myosin heavy chain as previously described (Johnston et al., 2004; Rossi et al., 2018).

Quantitative image analysis was performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Slides were visualized and imaged using the OPTIKA IM-300 Series microscope. For SDH-stained sections, imaging was executed immediately after staining, and mean gray values were measured in four random squares (only red muscle, only pink muscle, only white muscle and background) using a 10,000 pixels² grid overlaid on the image. The background was subtracted from the mean gray value for each muscle type. Mean staining intensity was measured separately in the red (oxidative), pink (intermediate) and white (glycolytic) muscle regions on one lateral side of each fish to assess relative oxidative capacity. For AP-stained sections, the cross-sectional area of the red muscle was manually delineated on one lateral side of each section, and all visible capillaries within that region were counted to determine capillary density [capillaries counted per unit area (µm²)]. Analyses of SDH and AP stains were conducted on up to nine sections per fish, with 10–12 fish analyzed per treatment group. Fluorescent sections stained for slow myosin were mounted with Eppredia™ Immu-Mount™ (Fisher Scientific), visualized and imaged using the BioTek Cytation 5 Cell Imaging Multimode Reader (Agilent Technologies, CA, USA). For analysis of the slow myosin stain, a randomized grid was overlaid on each image, and the cross-sectional area of 50 random S58-positive fibers intersecting the grid were measured for each fish

to quantify cross-sectional area of individual oxidative fibers. A total of 11 control and 12 exercise-trained fish were analyzed.

Global proteomics

The global proteome was investigated in adult zebrafish from control ($n=6$) and exercise-trained ($n=6$) groups. Mixed skeletal tail muscle comprising red (oxidative), pink (intermediate) and white (glycolytic) muscle fibers was collected and flash frozen in liquid nitrogen before being stored at -80°C . Protein extraction was performed using a lysis buffer composed of 10 mmol l^{-1} Tris (2-carboxyethyl) phosphine (TCEP) and 40 mmol l^{-1} chloroacetamide to reduce and alkylate cysteine residues. Samples were sonicated at 4 W for 15 s to aid in tissue disruption. Proteins were then isolated using a chloroform/methanol precipitation protocol to remove contaminants and concentrate the sample. The resulting proteins were digested with sequencing-grade modified trypsin (Pierce) at a ratio of enzyme to protein ratio of 1:50 to 1:100, using $3.0\text{ }\mu\text{g}$ of trypsin per sample, and incubated overnight at 37°C . Digested peptides were lyophilized using a SpeedVac concentrator and desalted using Millipore C18 ZipTips, which was then followed by a second lyophilization step. Peptides were resuspended in 2% acetonitrile with 0.1% formic acid prior to mass spectrometry analysis. Peptides were separated using an Evosep One Liquid Chromatography (LC) system and analyzed with a Bruker timsTOF Pro mass spectrometer. Chromatographic separation was conducted using an IonOpticks Aurora Elite Column (15 cm length, $75\text{ }\mu\text{m}$ ID, with $1.79\text{ }\mu\text{m}$ beads) maintained at 50°C . LC buffer A consisted of 0.1% formic acid in water and buffer B consisted of 0.1% formic acid in acetonitrile. Data-independent acquisition (DIA) was performed using a pre-optimized DIA method file and data were processed and analyzed using Spectronaut (v19.7.250203.62635). All sample preparation and mass spectrometry analyses were carried out at the SickKids Proteomics, Analytics, Robotics and Chemical Biology Centre (SPARC) BioCentre, The Hospital for Sick Children (Toronto, ON, Canada).

Enrichment analyses

Differential abundance candidates

Protein expression data were examined using Spectronaut (v.20.1.250624, Biognosys AG) to identify differentially abundant proteins between treatment groups. Differential protein abundance was analyzed using an unpaired t -test to compare treatment groups. Fold changes were expressed as $\log_2$ -transformed ratios of averaged replicates (AVG $\log_2$ ratio). Proteins were considered differentially abundant if they met the default Spectronaut significance thresholds of $q < 0.05$ (multiple-testing corrected P -value) and an absolute $\log_2$ ratio ≥ 0.58 , corresponding to an approximately 1.5-fold change in expression. These criteria were applied to generate the candidate protein lists used for downstream analyses.

GO enrichment

Gene ontology (GO) enrichment analysis was conducted using the clusterProfiler package (v4.16.0; Wu et al., 2021; Xu et al., 2024) in RStudio (v4.5.1). Lists of differentially expressed proteins ('significant' set) and all identified proteins ('background' set) were generated for skeletal muscle proteomes. Each list contained UniProt accession identifiers, which were mapped to zebrafish (*D. rerio*) gene symbols and Entrez Gene IDs using the org.Dr.e.g.db organism-specific annotation database (v3.21.0; doi:10.18129/B9.bioc.org.Dr.e.g.db) and the AnnotationDbi interface (v1.70.0; doi:10.18129/B9.bioc.AnnotationDbi). Only proteins successfully annotated with an Entrez ID were retained for downstream analysis.

GO enrichment was performed, specifying org.Dr.e.g.db as the reference database and the background proteome as the universe. Analyses were conducted for the cellular component ontology, molecular function and biological processes. P -values were adjusted for multiple testing using the Benjamini–Hochberg (BH) false discovery rate (FDR) method, with significance thresholds set at $P < 0.05$ and $q < 0.2$. Enriched terms were visualized using enrichplot (v1.28.4; doi:10.18129/B9.bioc.enrichplot) and ggplot (v4.0.0; Wickham, 2016) R packages. To complement GO-based enrichment, transcription factor inference was performed using the g:Profiler g:GOST interface as it integrates curated transcription factor target associations from the TRANSFAC database, highlighting potential upstream regulators. Enrichment significance was assessed using a Bonferroni-adjusted multiple testing correction. To further assess the biological relevance and tissue specificity of the dataset, GO enrichment analysis was performed on the full detected proteome in our samples and compared with the zebrafish reference genome as our background. This gave us insight into the resolution of our protein extraction and mass spectrometry protocol to detect proteins compared with all those annotated in the zebrafish genome.

Promoter motif analysis

To identify potential ERR binding sites, all proteins detected in the muscle proteome (including both the total identified proteins and the candidate lists) were first mapped from UniProt_AC-ID to UniProt_KB identifiers using the UniProt ID mapping tool. The corresponding gene identifiers were then queried in Ensembl BioMart (Kinsella et al., 2011; Ensembl Release 113) to retrieve promoter regions extending 10 kb upstream of the transcription start site for each gene. Promoter sequences were compiled and analyzed in RStudio (v4.5.1) using various packages [Biostrings (v2.76.0; <https://bioconductor.org/packages/Biostrings>), TFBSTools (v1.46.0; Tan and Lenhard, 2016) and tidyverse (v2.0.0; Wickham, 2016)] to scan those sequences for transcription factor binding motifs. The canonical ERR response element (ERRE) half site motif (TNAAGGTCA; Sladek et al., 1997) was scanned across all promoter sequences, and all motif matches were reported. The resulting data were used to quantify and compare the proportion of genes containing at least one ERRE motif between the total protein set and the candidate protein subset in the muscle to assess potential enrichment of ERR-responsive genes. Chi-square tests of independence were performed to test for significant differences in motif occurrence between groups, with significance assessed at $P < 0.05$.

Statistical analyses

All statistical analyses were performed in RStudio (v4.5.1). All data were checked for homogeneity of variance (Levene's test) and normality (visual inspection of Q–Q plot). For swim performance (U_{Cfast}), a linear mixed model (LMM) was used with treatment and sex as fixed effects and batch included as a random effect to account for temporal variation among experimental replicates. A LMM was used to account for this temporal variation in RMR data collection. We generated ANOVA tables containing F - and P -values for fixed effects. For other whole-animal metabolic rate (MMR and AS) and histology measurements, data were analyzed using general linear models (GLMs) with treatment, sex and body mass (covariate) as fixed effects. Absolute oxygen consumption measures were used for statistical analyses, but the data are reported relative to body mass. For enzyme activity assays, data were analyzed using LMMs with treatment, sex and body mass (covariate) as fixed effects, and batch

as a random effect. CS data violated the assumption of homogeneity of variance; therefore, data were log-transformed prior to fitting the LMM. Because all assay samples were run in triplicate, outlier replicates were removed if the coefficient of variation (CV) was above a value of 20. If the CV of remaining duplicates remained >20, the sample was excluded from the dataset. Mixed models were fitted using the lme4 package (v1.1-37; Bates et al., 2015), and *P*-values for fixed effects were obtained using lmerTest (v3.1-3; Kuznetsova et al., 2017) with Satterthwaite's approximation for degrees of freedom. In addition, we treated the proportions of each muscle type as compositional data because the proportion of one muscle type depends on the other two types. This allowed us to assess whether exercise training influenced the relative proportions of red, pink and white muscle. We used methods for compositional data as previously outlined (Rossi et al., 2020; van den Boogaart and Tolosana-Delgado, 2013). In short, the proportions of each muscle type for each fish were isometric log-ratio (ILR) transformed and each ILR coordinate was analyzed using an ordinary least squares regression model with treatment as a fixed effect. Predicted ILR values were then back-transformed to obtained estimated muscle type proportions for each group. We then examined specific log-ratio contrasts by fitting linear models to pairwise log-ratios among muscle types in order to make biological meaningful conclusions about the specific contrasts between fiber types. All results were considered statistically significant at $P < 0.05$. All figures and graphs were generated using GraphPad Prism (v10.6.1) and Spectronaut (v.20.1.250624). Data are presented as means $\pm$ s.e.m (unless otherwise stated), with individual values overlaid or as box and whisker plots.

RESULTS

Exercise training enhanced locomotor performance

Following 6 weeks of forced-swim training, exercised zebrafish exhibited approximately 18% greater ($20.449 \pm 3.776 \text{ BL s}^{-1}$) critical swimming speed compared with control fish ($17.398 \pm 4.558 \text{ BL s}^{-1}$; $F = 10.546$, d.f.=54.027, $P = 0.002$; Fig. 1). Sex did not have a significant effect on critical swimming speed ($F = 0.486$, $P = 0.489$; Table 1). There was no significant difference in condition factor after training (control: 0.862 ± 0.035 ; exercise: 0.919 ± 0.027 ; $P = 0.209$). This improvement in swimming performance validated

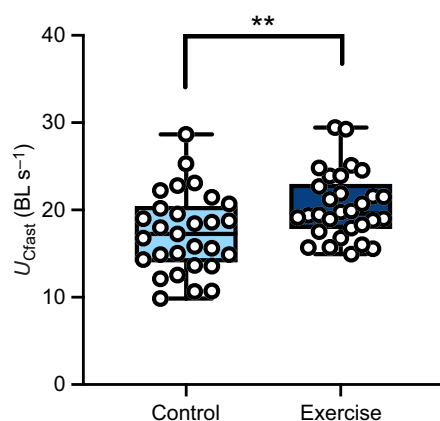


Fig. 1. The effects of exercise training on locomotor performance. Critical swimming speed (U_{Cfast}) following 6 weeks of exercise training ($n = 30$) compared with untrained controls ($n = 29$). Data are presented as box-and-whisker plots with whiskers representing minimum and maximum values (individual values overlaid). Asterisks denote a significance difference between groups (** $P < 0.01$).

Table 1. Results of linear mixed models (LMMs) testing the effects of exercise training on critical swimming speed (U_{Cfast}), lactate dehydrogenase (LDH), citrate synthase (CS), cytochrome oxidase (COX) activities, sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) activities, and resting metabolic rate (RMR)

Parameter	Estimate $\pm$ s.e.	<i>F</i>	<i>P</i>
U_{Cfast}			
Treatment	-1.4253 ± 0.4389	10.546	0.002**
Sex	-0.3195 ± 0.4582	0.486	0.48867
LDH activity			
Treatment	-2.5993 ± 2.3501	1.2234	0.2873
Sex	0.9993 ± 2.2919	0.1901	0.6694
Mass	-0.02007 ± 0.03689	0.2959	0.5946
CS activity			
Treatment	-0.1109 ± 0.04369	6.439	0.0232*
Sex	0.04531 ± 0.04259	1.1318	0.3047
Mass	-0.0000684 ± 0.000674	0.0103	0.9205
COX activity			
Treatment	-1.3715 ± 0.7295	3.5348	0.0789
Sex	-1.0396 ± 0.7728	1.8096	0.1976
Mass	0.005070 ± 0.008183	0.3838	0.5449
SERCA activity			
Treatment	-0.0428 ± 0.0451	0.9006	0.3577
Sex	0.0538 ± 0.03759	2.0483	0.1729
Mass	0.0002191 ± 0.000693	0.1030	0.7527
RMR			
Treatment	205.54 ± 323.331	0.4041	0.5356
Sex	-139.417 ± 339.619	0.1685	0.6872
Mass	-7.541 ± 5.271	2.0471	0.1745

Asterisks indicate level of significance (* $P < 0.05$, ** $P < 0.01$).

that our training protocol was sufficient for acclimation to sustained submaximal exercise.

Aerobic scope did not change with 6 weeks of training

RMR and MMR did not differ significantly between control and exercise groups (RMR, $F = 0.4041$, d.f.=13.479, $P = 0.536$; MMR, $F = 1.652$, d.f.=15, $P = 0.398$; Fig. 2A,B). Neither sex nor body mass demonstrated a significant effect on RMR or MMR ($P > 0.05$; Tables 1, 2). Consequently, aerobic scope, calculated as the difference between MMR and RMR, was not significantly affected by exercise training ($F = 2.4$, d.f.=12, $P = 0.789$; Fig. 2C). Body mass contributed significantly to aerobic scope ($P = 0.0211$; Table 2). Although aerobic scope is often used as an indicator of an organism's capacity to increase metabolic rate above resting levels, our results indicate that 6 weeks of submaximal exercise training did not improve this capacity in adult zebrafish (Fig. 2C).

Exercise increased maximal citrate synthase activity

There were no significant differences in LDH ($F = 1.2234$, d.f.=14.067, $P > 0.05$; Fig. 3A) or COX ($F = 3.5348$, d.f.=15.617, $P = 0.0789$; Fig. 3B) activities demonstrated in mixed muscle samples. In contrast, maximal activity of CS, a marker of mitochondrial density, was 26% higher in exercise-trained fish relative to controls ($F = 6.439$, d.f.=14.5, $P = 0.0232$; Fig. 3C). In addition to key metabolic enzymes, we focused on the contractile function of the muscle, and thus quantified SERCA activity as an indicator of calcium cycling capacity. Our data showed no significant increase in SERCA activity in exercise trained fish ($F = -0.949$, d.f.=15, $P > 0.05$; Fig. 4).

Exercise training induced hypertrophy and increased vascularization of oxidative muscle fibers

Muscle histology revealed significant structural remodeling in exercise-trained zebrafish. The SDH staining intensity, an indicator

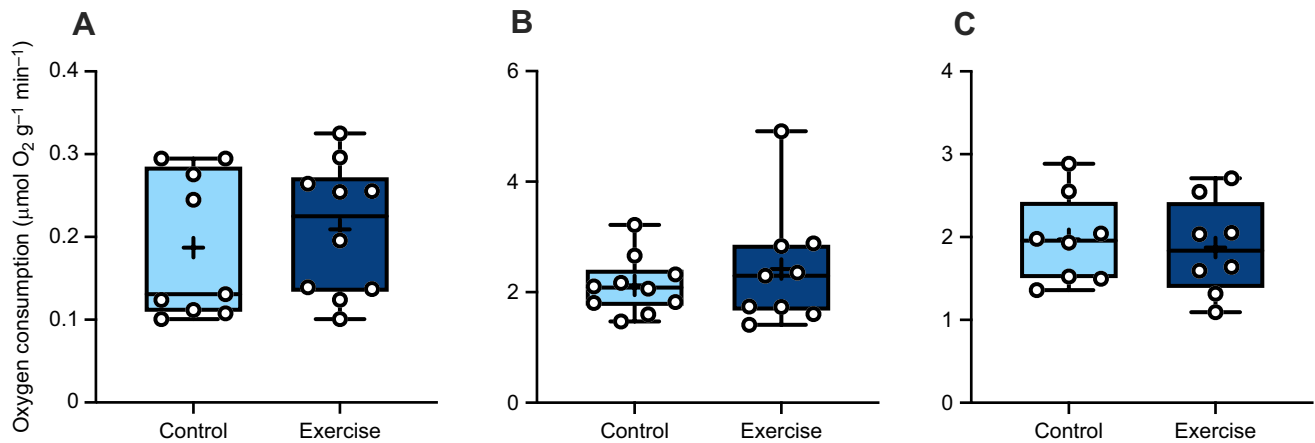


Fig. 2. The effects of exercise training on whole-animal metabolic rate. (A) Resting metabolic rates in trained ($n=10$) and control fish ($n=9$), (B) maximum metabolic rates in trained ($n=9$) and control fish ($n=10$), and (C) aerobic scope in trained and control groups ($n=8$ per group). Data are presented as box-and-whisker plots with the minimum and maximum values indicated by the whiskers, with individual data points overlaid. Crosses ('+') represent mean values.

of oxidative capacity, was significantly altered by training in the pink intermediate muscle ($F=5.01$, d.f.=19, $P=0.00857$), with a 20.3% increase in exercised fish (77.796 ± 8.808 a.u.) relative to

Table 2. Results of general linear models (GLMs) testing the effects of exercise training on succinate dehydrogenase (SDH), alkaline phosphatase, and slow myosin expression, maximum metabolic rate and aerobic scope

Parameter	<i>F/t</i>	<i>P</i>
SDH (red)		
Model	$F_{3,19}=2.728$	0.0727
Treatment	$t_{19}=-1.794$	0.0887
Sex	$t_{19}=-1.810$	0.0861
Mass	$t_{19}=0.842$	0.4101
SDH (pink)		
Model	$F_{3,19}=5.01$	0.01*
Treatment	$t_{19}=-2.931$	0.00857**
Sex	$t_{19}=-2.287$	0.03382*
Mass	$t_{19}=0.198$	0.84526
SDH (white)		
Model	$F_{3,19}=1.25$	0.3195
Treatment	$t_{19}=0.864$	0.3985
Sex	$t_{19}=-0.196$	0.8463
Mass	$t_{19}=1.833$	0.08246
Alkaline phosphatase		
Model	$F_{3,18}=9.348$	<0.001***
Treatment	$t_{18}=-5.073$	<0.001***
Sex	$t_{18}=-1.016$	0.323
Mass	$t_{18}=-1.079$	0.295
Slow myosin		
Model	$F_{3,19}=23.43$	<0.001***
Treatment	$t_{19}=-4.703$	<0.001***
Sex	$t_{19}=-3.968$	<0.001***
Mass	$t_{19}=4.681$	<0.001***
Maximum metabolic rate		
Model	$F_{3,15}=1.652$	0.2197
Treatment	$t_{15}=0.870$	0.3980
Sex	$t_{15}=-0.793$	0.4401
Mass	$t_{15}=-2.032$	0.0603
Aerobic scope		
Model	$F_{3,12}=2.40$	0.1187
Treatment	$t_{12}=-0.274$	0.7890
Sex	$t_{12}=-0.157$	0.8775
Mass	$t_{12}=-2.652$	0.0211*

Asterisks indicate level of significance (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).

controls (64.675 ± 12.674 a.u.) (Fig. 5A). There was a significant effect of sex on SDH intensity in the pink muscle, with males having greater staining intensity ($P=0.034$; Table 2). SDH staining intensity was not significantly altered by training in either red oxidative ($F=2.728$, d.f.=19, $P=0.0887$; Fig. 5A) or white glycolytic muscle ($F=1.25$, d.f.=19, $P>0.05$; Fig. S1). In addition, slow oxidative muscle fibers had approximately 34% greater cross-sectional area in exercise trained fish compared with control fish ($F=23.43$, d.f.=19; $P<0.001$; Fig. 5B). Body mass and sex significantly affected muscle fiber area ($P<0.001$; Table 2), with greater body mass having greater mean fiber area. Although there was not a significant difference between the body size of male and female fish ($F=0.00527$, d.f.=1, $P=0.943$), male fish also had significantly larger fiber area. Positive AP staining occurred in the walls of arterioles and endomysial capillaries despite the muscle fiber itself showing negative staining (Fig. 5C). Capillary density (capillaries per unit area of muscle, μm^2) within red muscle was significantly higher in exercised fish ($F=9.348$, d.f.=18, $P<0.001$) corresponding to a 75.9% increase ($0.5814 \pm 0.0932 \mu\text{m}^2$) relative to control fish ($0.3305 \pm 0.1362 \mu\text{m}^2$) (Fig. 5C).

Muscle fiber composition shifted with exercise training

Exercised fish had a significantly different overall muscle composition compared with controls, as indicated by the second ILR coordinate ($P=0.003$; Fig. 6). Back-transformed proportions showed that exercise fish had higher proportional contribution of red (8.7% versus 6.6%; $F=11.85$, d.f.=19, $P=0.0027$) and pink (12.6% versus 9.0%; $F=9.195$, d.f.=19, $P=0.0069$; Fig. 6) muscle relative to the proportion of white muscle (78.8% versus 84.3%) when compared with control fish. Consistent with these results, log-ratio analysis showed a significant increase in oxidative (red+pink) to white muscle ratio ($F=10.98$, d.f.=19, $P=0.0037$), whereas the red-to-pink ratio remained unchanged ($F=0.6919$, d.f.=19, $P=0.416$). Together, these results highlight that endurance exercise induced a shift towards a more oxidative muscle phenotype, increasing the relative contribution of red and pink fibers at the expense of white fibers.

Proteomic profiling of skeletal muscle

Quantitative proteomic analysis was conducted on skeletal muscle tissue collected from six fish from each group. Protein

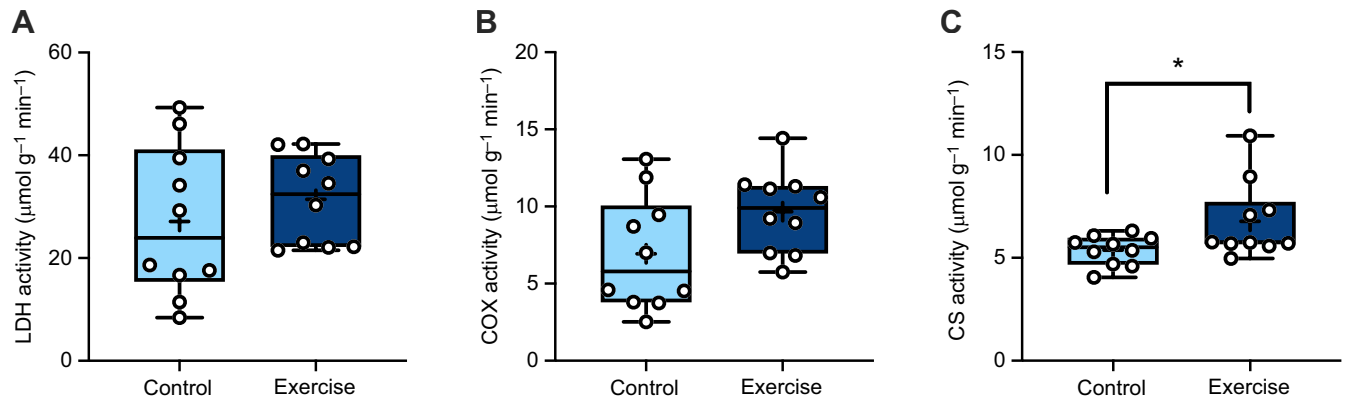


Fig. 3. The effects of exercise training on maximal metabolic enzyme activities of swim muscle. Activities of lactate dehydrogenase (LDH; A), cytochrome oxidase (COX; B) and citrate synthase (CS; C) in mixed skeletal muscle from exercise-trained and control zebrafish ($n=10$ per group for each enzyme). Data are presented with individual values overlaid, with minimum and maximum values indicated by whiskers. Asterisk indicates a significant difference between groups ($*P<0.05$). Crosses ('+') represent mean values.

identification yielded 4488 proteins across all samples at an FDR of $<1\%$. Principal component analysis (PCA) revealed clustering of samples by treatment group, indicating distinct protein expression profiles following exercise training (Fig. S2). The first principal component (PC1) accounted for 40.25% of the total variance, whereas the second principal component (PC2) explained another 16.38% (Fig. S2).

Differential expression analysis identified 96 proteins significantly altered by exercise ($|\log_2FC| \geq 1.5$, $q < 0.05$; Fig. 7; Table S1). Of these, 46 proteins were upregulated and 50 were downregulated in exercised fish relative to untrained controls. Among these, proteins associated with sarcomeric organization, including myosin binding protein Ha (mybpha), telethonin (tcap) and filamin C (flnca), were significantly upregulated in exercised fish (Table S1). In contrast, the slow myosin heavy chain paralog, smyhc3, was significantly downregulated following exercise training (Table S1). Exercise training was also associated with increased expression of several mitochondrial complex I proteins, including ndufa2, ndufa6, ndufa10, ndufs4 and mt-nd5 (Table S1). A complete list of differentially expressed proteins is provided in Table S1. Notably, ERR α was detected in the muscle tissue; however, protein expression levels did not differ significantly

between exercised and control fish (AVG $\log_2$ ratio = 0.09826, $q = 0.1962$; Fig. 7).

Functional enrichment analysis

To characterize the functional composition of the muscle proteome detected in the present study and evaluate the coverage of our protein extraction protocol, GO enrichment analysis was performed on all of the identified proteins in our muscle sample in reference to the whole annotated zebrafish genome. Within the biological process category, the most strongly enriched terms include translation (GO:0006412) and mitochondrial energy metabolism, including generation of precursor metabolites and energy (GO:0006091), energy derivation by oxidation of organic compounds (GO:0015980), cellular respiration (GO:0045333) and aerobic respiration (GO:0009060). These terms reflect extensive coverage of ribosomal proteins, OXPHOS components and tricarboxylic acid cycle components. In addition to metabolic processes, several muscle-specific GO terms were also significantly enriched, including muscle structure development (GO:0061061), striated muscle contraction (GO:0006941), actin filament-based process and organization (GO:0030029 and GO:0007015), and myofibril assembly (GO:0030239). Together, these results demonstrate that our proteomic approach effectively detected proteins spanning metabolic and mitochondrial functional categories relevant to exercise adaptations in muscle. At the level of molecular function, enriched categories were dominated by activities related to structural constituents of the ribosome, oxidoreductase activity and electron transfer. Complementing these findings, cellular component enrichment revealed prominent detected proteins localized to the ribosome, mitochondria and mitochondrial inner membrane, including components of the respiratory chain and ATP synthase complexes. These findings provided reinforce our resolution to detect muscle-related targets relative to the whole zebrafish genome.

Of those identified proteins, we investigated which were enriched (i.e. differentially expressed muscle proteins) in our exercise-trained fish. GO enrichment analysis was performed on the set of differentially expressed muscle proteins, using all detected proteins as the background, which revealed significant clustering/overrepresentation of cellular components associated with systemic and mitochondrial adaptations to exercise. Cellular component enrichment highlighted significant representation of proteins localized to the extracellular region (GO:0005576) and extracellular space (GO:0005615), suggesting that extracellularly

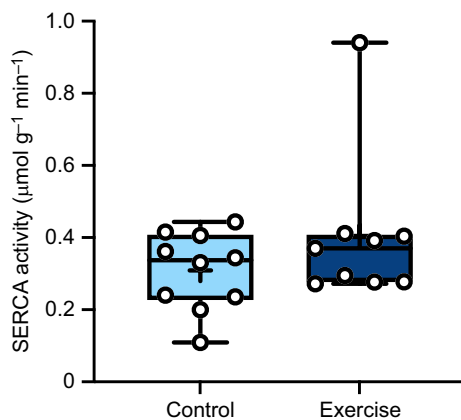


Fig. 4. The effects of exercise training on sarcoplasmic calcium reuptake in swim muscle. Maximal SERCA activity in control ($n=10$) and trained fish ($n=9$). Data are presented with individual values overlaid, with minimum and maximum values indicated by whiskers. Crosses ('+') represent mean values.

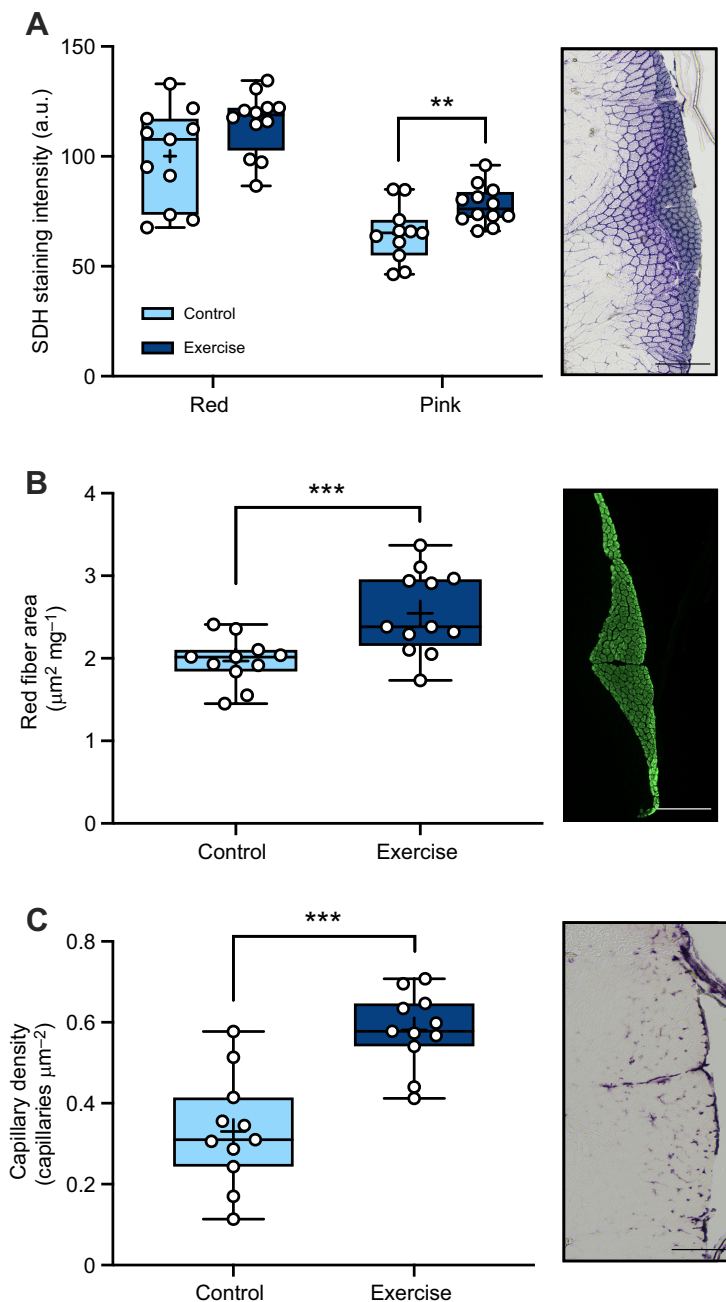


Fig. 5. The effects of exercise training on structural and vascular remodeling of oxidative muscle. (A) Succinate dehydrogenase (SDH) staining intensity in red and pink muscle from control ($n=11$) and trained fish ($n=12$). (B) Cross-sectional area of S58-positive fibers in control ($n=11$) and trained fish ($n=12$). (C) Muscle sections stained for alkaline phosphatase (capillary density) in oxidative muscle of control and trained fish ($n=11$ per group). Data are presented with individual values overlaid, with minimum and maximum values indicated by whiskers. Asterisks indicate a significant difference between groups (** $P<0.01$, *** $P<0.001$). Representative images of each muscle stain from one lateral side are shown on the right of each panel. Crosses ('+') represent mean values. Scale bar indicates 100 μm .

active proteins contribute significantly to the exercise-induced proteome in fish muscle. In addition, mitochondrial cellular components were also significantly enriched, such as respiratory chain complex I (including GO:0030964 and GO:0098803). These components included multiple complex I subunits (ndufa2, ndufa6, ndufa10, ndufs4 and mt-nd5). However, no biological processes or molecular function GO terms were significant. Subsequent KEGG pathway analysis demonstrated significant enrichment of glutathione metabolism, as well as drug metabolism, reflecting enhanced redox handling and metabolic detoxification capacity in the exercised muscle. In addition, transcription factor enrichment analysis identified hepatocyte nuclear factor 3 (HNF3) as the only significantly enriched transcription factor among proteins differentially expressed with exercise. These findings highlight the proteomic changes of zebrafish skeletal muscle with repeated bouts of submaximal exercise.

ERR activity did not appear to regulate exercise-induced remodeling

To assess whether ERR activity may contribute to the observed exercise-induced remodeling, we evaluated the prevalence of ERREs in the promoter regions of genes corresponding to identified proteins. Among all identified proteins in the muscle, 17.4% contained at least one ERRE, whereas 18.8% of candidate (differentially expressed) proteins contained the consensus sequence matching ERRE. The proportion of ERRE-containing genes was not significantly different in the candidate list compared with the background proteome ($\chi^2=0.0411$, d.f.=1, $P=0.839$), suggesting that ERR signaling may not directly account for the observed proteomics differences with exercise training. These results suggest there is no enrichment of potential ERR targets among proteins responsive to exercise training, in contrast to the role of ERR signaling in mediated exercise-induced transcriptional responses in mammals.

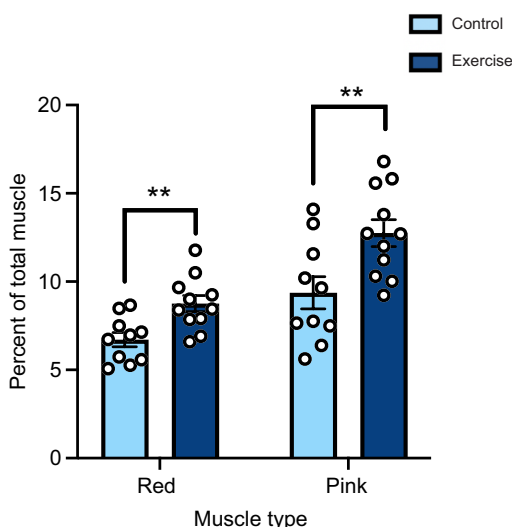


Fig. 6. The effects of exercise training on muscle type composition. Percentage of red and pink muscle in control ($n=10$) and trained fish ($n=11$). Data are presented as means $\pm$ s.e.m., with individual values overlaid. Asterisks indicate a significant difference between groups (** $P<0.01$).

DISCUSSION

We hypothesized that ERR regulates exercise acclimation in zebrafish, though its binding partners may differ from mammalian systems. Our exercise training regime was sufficient to enhance locomotor performance in these zebrafish (i.e. fish acclimated to exercise), but contrary to our hypothesis, we found no evidence that ERR possesses a regulatory role in exercise-induced remodeling, at the level of either protein expression or functional activation. This result establishes an important boundary condition for ERR signaling in fish and indicates that exercise-responsive metabolic remodeling occurs independently of ERR regulation. Despite

greater swimming performance in exercised fish, there was no evidence of plastic remodeling to whole-animal metabolism. However, forced exercise was associated with muscle remodeling, with subtle increases in oxidative enzyme activity and marked hypertrophy and capillarization of oxidative muscle fibers. Overall, we found that conservation of downstream physiological traits in fish is likely achieved through distinct upstream regulatory programs relative to mammals.

Exercise acclimation phenotype

Adult zebrafish mounted a clear acclimation response to our submaximal exercise training regime, as evidenced by improved locomotor performance (Fig. 1) and remodeling of the skeletal muscle. In our study, the exercise-induced increase in CS activity (Fig. 3C), muscle capillary density (Fig. 5C) and shift toward more oxidative fiber types (Fig. 6) represent hallmarks of an enhanced aerobic phenotype. Indeed, these findings align with previous work showing robust plasticity of skeletal muscle in fish subjected to prolonged swimming (Farrell et al., 1991; Pelster et al., 2003; McClelland et al., 2006; Morash et al., 2014; Palstra et al., 2014). To meet the energetic demands of exercise, the swimming muscle of fishes can coordinate oxygen and nutrient supply with ATP demand (Maxwell and Ratcliffe, 2002; Morash et al., 2014). Increased tissue capillarization reduces the diffusion distance between red blood cells and myofibrillar mitochondria, thereby enhancing oxygen delivery to sites of oxidative ATP production and facilitating the removal of metabolic waste products (Pelster et al., 2003; Rodnick and Planas, 2016). Previous studies in cyprinids (*Chondrostoma nasus* and *Leuciscus cephalus*) and larval zebrafish, for instance, report an increase in muscle vascularization and increases in red muscle fiber capillarization with endurance exercise training (Sanger, 1992; Pelster et al., 2003). Notably, enhanced muscle vascularization with training may be elicited by transient muscle-specific hypoxia (Maxwell and Ratcliffe, 2002).

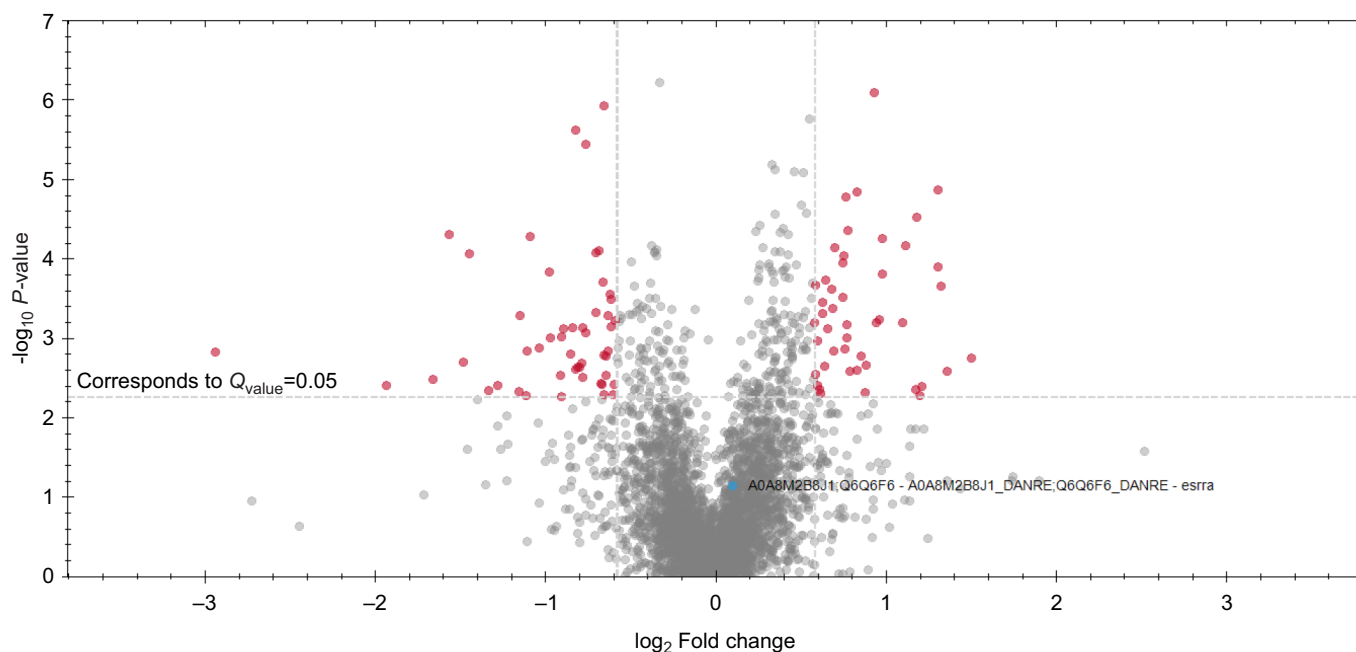


Fig. 7. The effects of exercise training on *ERRα* expression. Volcano plot depicts protein abundance across control and exercise groups ($n=6$ per group), with a random assortment of sexes (control=3 males, 3 females; exercise=4 males, 2 females), with differentially abundant protein candidates highlighted in red. A total of 4488 proteins were identified in the skeletal muscle (grey), of which 96 met criteria for differential abundance between treatments. *ERRα* expression and the respective accession number is labelled in blue.

This remodeling is consistent with our findings here, showing that exercise training induces angiogenesis and contributes to enhanced aerobic capacity of the muscle.

In addition to morphological and angiogenic changes in the muscle fibers, there was a significant increase in the maximal activity of CS, traditionally an indicator of mitochondrial content (Fig. 3C), and a trend towards increased COX activity as another indicator of mitochondrial capacity (Fig. 3B). Previous work shows variable effects of endurance training on CS expression and activity in fishes. For instance, 4 weeks of moderate training increased CS activity and mRNA expression in mixed muscle of adult zebrafish (McClelland et al., 2006), consistent with findings in other fishes (e.g. Johnston and Moon, 1980a,b; Magnoni et al., 2013). In contrast, 8 weeks of training induced significant upregulation of CS expression in zebrafish white muscle with no change in red muscle (LeMoine et al., 2010a). The increase in CS activity reported in our study may be driven by the hypertrophy of red and pink muscle observed in our histological analyses, rather than representing enhanced OXPHOS capacity at the cellular level. Importantly, exercise-induced improvements in muscle performance are not limited to structural or mitochondrial remodeling, but may also involve changes in calcium handling, as excitation–contraction coupling and fatigue resistance depend on efficient calcium release and reuptake across the sarcoplasmic reticulum (Seebacher and Walter, 2012). We found that SERCA activity remained unchanged following exercise training (Fig. 4), suggesting that maximal calcium re-uptake capacity of mixed muscle was not upregulated in parallel with exercise remodeling.

Local aerobic remodeling without elevated whole-animal metabolism

An unexpected finding was that the more aerobic muscle phenotype of exercise-trained fish was not accompanied by higher whole-animal maintenance costs (i.e. RMR) or enhanced maximum metabolic capacity (MMR) (Fig. 2). Several mechanistic explanations may help reconcile this finding. First, red muscle constitutes only a small fraction of total muscle mass in fish (~10%; Walker and Pull, 1973), and thus its contribution to whole-animal metabolism may have been too small to detect using our whole-animal respirometry setup. Consequently, high inter-individual variation in RMR may mask any modest metabolic effects of exercise-induced muscle remodeling. Second, remodeled muscle may have improved mitochondrial efficiency. In mammals, exercise training reduces proton leak and increases coupling efficiency, allowing mitochondria to produce more ATP per molecule of oxygen consumed (Conley, 2016; Zoladz et al., 2016). If similar adjustments occurred in exercise-trained zebrafish, remodeling could support higher ATP production without the assumed stoichiometric increases in oxygen consumption. This efficiency-based remodeling is consistent with broader physiological principles indicating that animals match ATP production with cellular energy demand, especially during sustained energetic challenges (Holloszy and Coyle, 1984; Hochachka and Somero, 2002). In addition to reducing leak, adjustments in COX function via isoform switching may further promote proton pumping efficiency (Kocha et al., 2015). Zebrafish possess two COX6a isoforms, and these subunits can modulate the number of protons pumped per electron transferred to enhance ATP production economics (Little et al., 2010). Mammalian COX4 subunits are oxygen responsive, and isoform switching from COX4-1 to COX4-2 improves OXPHOS in hypoxic conditions (Fukuda et al., 2007; Little et al., 2010; Kocha et al., 2015). Although the hypoxia

responsiveness of COX4 paralogs in fish has been questioned (Kocha et al., 2015), understanding how exercise acclimation affects mitochondrial efficiency may help reconcile this disconnect between aerobic fiber hypertrophy without subsequent signatures in whole-animal metabolism. Overall, improved tissue capillarization and mitochondrial efficiency likely offset oxygen demand in the aerobic fibers and can serve to explain how exercise-trained fish can possess larger, more oxidative muscle fibers without increasing basal energetic costs.

Proteomics

In development, zebrafish *ERRα* marks a group of early muscle precursor cells that will later develop into slow muscle (Bardet et al., 2004). Aside from developmental expression patterns, the tissue-specific patterns of ERRs in adult zebrafish are relatively unknown. In mammals, expression fold changes form the basis of ERR-mediated remodeling with exercise (Cartoni et al., 2005; Rangwala et al., 2010; Matsakas et al., 2012). In our study, *ERRα* was the only ERR isoform detected in the muscle, and its expression levels remained unchanged with exercise training (Fig. 7). Given that the various ERR isoforms likely possess similar physiochemical properties (similar in sequence and structure), their absence in our dataset likely suggests true absence or low abundance, as opposed to systematic bias in protein extraction methods or instrument settings.

Candidates

Proteomic profiling of the skeletal muscle revealed shifts that align with the transition towards a more aerobic phenotype, including changes in proteins associated with sarcomeric organization, contractile regulation and mitochondrial metabolism. Notably, there was an absence of any obvious upstream transcriptional regulators, including transcription factors, coactivators/corepressors or chromatin remodellers. Thus, although our data suggest alternative regulatory nodes upstream or parallel to canonical ERR-PGC-1α pathways, we are limited in our capacity to identify any non-ERR transcriptional or metabolic controllers of this response.

Increases in mybpha (myosin binding protein Ha), tcap (telethonin) and flnca (filamin C) suggests enhanced sarcomeric stability, and capacity for mechanotransduction in exercised muscle, supporting exercise-induced remodeling and improved mechanical efficiency. These proteins are components of mechanosensory complexes that transduce mechanical strain into intracellular signaling, providing a feedback mechanism for sustained muscle remodeling during repeated contractile loading during exercise.

Interestingly, smyhc3, a slow myosin heavy chain paralog, was significantly downregulated with exercise training, which contrasts histological findings that show greater proportions of red and pink muscle. This pattern may therefore reflect a shift in slow myosin isoform composition. Although no single isoform showed strong compensatory upregulation, combined increases in smyhc1 and smyhc2 suggest potential redistribution among slow-fiber isoforms that may be biologically meaningful, but individually fall below candidate filtering thresholds. In adult zebrafish trunk muscle, smyhc2 and smyhc3 define distinct slow-fiber subsets, whereas smyhc1 is typically associated with developing muscle (Elworthy et al., 2008; Nord et al., 2014). Consistent with our findings, Palstra et al. (2010) reported increased *smyhc1* gene expression in endurance trained zebrafish, potentially reflecting muscle hypertrophy with training.

Interestingly, we found that shifts in contractile protein expression were not accompanied by changes in calcium-handling proteins, including SERCA (atp2a1/2) and ryanodine receptor (ryr1a/b, ryr3), providing no evidence for increased calcium cycling capacity or excitation–contraction coupling capacity with training, consistent with unchanged MMR. In addition, multiple subunits of mitochondrial complex I were significantly upregulated in trained muscle (ndufa2, ndufa6, ndufa10, ndufs4 and mt-nd5), highlighting a potential remodeling of the electron transport chain at its primary site of NADH oxidation and proton pumping. Preferential upregulation of complex I is consistent with the metabolic demands of sustained exercise, which increases reliance on NADH-linked substrate oxidation to a greater extent than FADH₂-linked pathways, and may enhance ATP yield per oxygen molecule given that complex I contributes four protons per electron pair. Our findings mirror patterns reported in human skeletal muscle, where complex I is the most robustly upregulated respiratory complex in response to exercise training (Porter et al., 2015; Guaras et al., 2016; Greggio et al., 2017) and has been proposed to be primarily regulated at the level of protein expression.

Beyond energetic efficiency, complex I is also a major site of mitochondrial reactive oxygen species (ROS) generation, particularly during high electron flux conditions typical of aerobic exercise (Cocco et al., 2009; Maranzana et al., 2013; Goncalves et al., 2015). Accordingly, exercise has been shown to promote increased assembly of respiratory chain supercomplexes (SCs), particularly involving complex I (Greggio et al., 2017; Huertas et al., 2017). The remodeling of complex I into SCs may reduce ROS production during high electron transport flux during exercise, thereby protecting mitochondria from oxidative damage (Ghelli et al., 2013; Maranzana et al., 2013; Huertas et al., 2017; Greggio et al., 2017; Casuso et al., 2019). Notably, some ROS production is beneficial as it contributes to redox-sensitive signaling pathways; however, ROS production and the potential for oxidative damage depend on the type and intensity of exercise in both fish (Espírito-Santo et al., 2026) and mammals (Thirupathi et al., 2021).

Moreover, *cox7a2l* is a proposed regulator of SC formation (Lapiente-Brun et al., 2013; Williams et al., 2016), and we observed a 17.5% increase in *cox7a2l* protein abundance with exercise. Formation of these SCs may further improve respiratory efficiency by reducing diffusion distances between complexes (Genova and Lenaz, 2014; Moreno-Loshuertos and Enriquez, 2016), thereby supporting elevated oxidative flux during exercise without a proportional increase in oxidative damage. Finally, sustained exercise can induce cycles of tissue hypoxia and reoxygenation in the muscle, which are conditions known to exacerbate ROS generation and further favor SC assembly.

Changes in oxygen transport further highlight the trend towards a more oxidative phenotype. Increases in hemoglobin beta adult 2 protein, *hbba2*, suggest localized adjustments in oxygen handling, potentially via hypoxia-responsive pathways, which would complement improved mitochondrial function by supporting oxygen delivery to oxidative fibers. Together, these patterns indicate that exercise training promotes qualitative remodeling within existing slow muscle fibers, involving coordinated shifts in mitochondrial efficiency, oxygen transport and structural reinforcement, rather than large-scale fiber type replacement.

Proteomic trends suggestive of functional remodeling

Consistent with improved aerobic performance, we observed coordinated upregulation, albeit below candidate filtering

thresholds, of multiple electron transport chain and OXPHOS proteins, including COX subunits (mt-co1, mt-co2, *cox5a1*, *cox6a2/b2/c* and *cox7a2/b*), cytochrome *c* (*cyc*) and ATP synthase components (including assembly factors, catalytic F1 subunits and peripheral stalk components). Together, these patterns indicate a modest enhancement of mitochondrial ATP-producing capacity, consistent with the shift towards greater oxidative metabolic capacity in the muscle. Subtle increases in transcription factor A (*tfam*) and myoglobin further support subtle increases in mitochondrial content and improved oxygen handling. Proteins involved in lipid transport and β -oxidation, including carnitine palmitoyltransferase 2, hydroxyacyl-CoA dehydrogenase and medium-chain acyl-CoA dehydrogenase, also showed modest increases, consistent with greater reliance on aerobic lipid metabolism. Taken together, the dataset demonstrates robust and comprehensive coverage of skeletal muscle-specific proteins. Core components of OXPHOS, contractile machinery spanning both slow and fast fiber programs, and key calcium-handling were consistently detected. Notably, several red-muscle-specific proteins were recovered at high spectral abundance, indicating that mixed muscle samples were not excessively diluted by dominant white muscle mass as fiber-type specific signals were retained. However, isolation of distinct muscle types may provide greater sensitivity for examining fiber-type specific exercise acclimation responses.

Despite these metabolic shifts, canonical mammalian regulators of exercise-induced muscle remodeling (e.g. PGC-1 α , NRFs, PPARs, SIRT1, MEF2) were not detected. Together with prior work in zebrafish (McClelland et al., 2006, 2012; LeMoine et al., 2010a), our findings question the conserved role of PGC-1 α , suggesting that exercise-induced remodeling in fish muscle may rely on alternative regulatory pathways. In addition, the mechanism of muscle remodeling may also depend on the duration and type of exercise. However, absence of detection in the proteomics dataset cannot be equated with true biological absence in the muscle, as these proteins are often low in abundance and may have fallen below detection limits in our methodology. Importantly, detection of a broad range of muscle-specific and nuclear receptor proteins, including ERR α , reinforces our sensitivity to capture biologically meaningful variation in skeletal muscle composition and metabolism. However, several low-abundance regulatory proteins involved in exercise-induced remodeling, including PGC-1 α , NRF1/2, PPARs, SIRT1 and MEF2, may remain undetected owing to expression limitations or exhibit transient changes that are not captured by this approach. Overall, the proteomic profile supports refined enhancement of mitochondrial capacity to optimize efficiency and better support the oxidative muscle phenotype, sufficient to improve locomotor performance without large changes in whole-animal metabolic capacity.

Enrichment analysis for ERR activity

Apart from the lack of changes in ERR protein abundance, there was little evidence that changes in ERR activity directly regulated the exercise phenotypes observed in this study. GO enrichment analysis did not reveal overrepresentation of canonical transcriptional pathways or known direct ERR target gene networks. Instead, the only significant enrichments were restricted to specific cellular components, notably extracellular matrix-related terms and complex I of the electron transport chain – a trend that reinforces our enzymatic and histological findings. Transcription factor enrichment analysis identified HNF3 as the only significantly enriched regulator, however HNF3 is primarily recognized for its role in development, with no well-established

role in mediating exercise-induced plasticity. The absence of enrichment for well-established ERR-associated transcriptional programs should be interpreted cautiously, as it relies on the assumption that ERR binding partners and downstream targets are conserved between mammals and zebrafish. Moreover, there are relatively few differentially expressed candidates in our proteomic dataset, reflecting our conservative approach to candidate protein identification.

Our complementary enrichment analysis approach trades off the aforementioned assumptions with the assumption that the ERRE motifs are evolutionarily conserved between fish and mammals. In general, nuclear receptor response elements can be highly conserved, even across the entire animal kingdom (Reitzel et al., 2018). Based on sequence alignment, the DBD appears well conserved between zebrafish and human ERR paralogs, with 100% conservation in the alpha, beta and gamma-a isoforms, and approximately 90% conserved in gamma-b. If ERR were exerting regulatory effects via changes in activity, we would expect strong signatures of target gene upregulation in our candidate list. Although motif presence does not guarantee functional binding, lack of enrichment of ERRE motifs in promoter regions of differentially abundant proteins suggests that ERR-mediated transcriptional programs are not contributing to the observed proteomic changes. Taken together, this pattern (or lack thereof) suggests that ERR is unlikely to regulate exercise acclimation in zebrafish through its effects on activity or expression.

Conclusions

Overall, our findings suggest a distinct exercise acclimation pathway in fishes. As in other vertebrates, skeletal muscle becomes more oxidative, capillarized and better suited for sustained swimming. Given that zebrafish are prominent biomedical models, understanding how they respond to routine exercise may help identify pathways that protect against metabolic disease and reveal alternative mechanisms that could be mimicked pharmacologically to benefit human health. From an ecological perspective, our work also has potential implications for resilience in the face of anthropogenic changes to waterways, including hydroelectric dams, storm drains, culverts or canals. Finally, the evolution of locomotor capacity fundamentally shaped vertebrate physiology, as increased mobility offered organisms greater autonomy over the environmental conditions they experience. The recurrence of broadly similar exercise-induced phenotypes across diverse animal taxa may suggest that the molecular pathways regulating exercise training are conserved. Our study challenges such a view. We show that, despite similar apparent phenotypic responses to exercise, muscle remodeling in fishes is likely mediated by molecular pathways distinct from those in mammals. This finding provides important context for zebrafish as an exercise model for human health and disease.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: R.K.M., A.G.L.; Formal analysis: R.K.M., A.G.L.; Funding acquisition: A.G.L.; Investigation: R.K.M., S.K.G., G.S.R., A.G.L.; Methodology: R.K.M., A.G.L.; Resources: A.G.L.; Supervision: G.S.R., A.G.L.; Validation: R.K.M., S.K.G., G.S.R., A.G.L.; Visualization: R.K.M., A.G.L.; Writing – original draft: R.K.M.; Writing – review & editing: R.K.M., G.S.R., A.G.L.

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Data and resource availability

All relevant data and details of resources can be found within the article and its supplementary information.

References

- Altmann, S. A. (1987). The impact of locomotor energetics on mammalian foraging. *J. Zool.* **211**, 215–225. doi:10.1111/j.1469-7998.1987.tb01530.x
- Andersen, P. and Henriksson, J. (1977). Training induced changes in the subgroups of human type II skeletal muscle fibres. *Acta Physiol. Scand.* **99**, 123–125. doi:10.1111/j.1748-1716.1977.tb10361.x
- Baar, K., Wende, A. R., Jones, T. E., Marison, M., Nolte, L. A., Chen, M. A. Y., Kelly, D. P. and Holloszy, J. O. (2002). Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. *FASEB J.* **16**, 1879–1886. doi:10.1096/fj.02-0367com
- Bamford, S. M. and Seebacher, F. (2024). A fast fish swimming protocol that provides similar insights to critical swimming speed. *Biol. Open.* **13**, bio060543. doi:10.1242/bio.060543
- Bardet, P. L., Obrecht-Pflumio, S., Thisse, C., Laudet, V., Thisse, B. and Vanacker, J. M. (2004). Cloning and developmental expression of five estrogen-receptor related genes in the zebrafish. *Dev. Genes Evol.* **214**, 240–249. doi:10.1007/s00427-004-0404-6
- Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48. doi:10.18637/jss.v067.i01
- Bertrand, S., Brunet, F. G., Escriva, H., Parmentier, G., Laudet, V. and Robinson-Rechavi, M. (2004). Evolutionary genomics of nuclear receptors: from twenty-five ancestral genes to derived endocrine systems. *Mol. Biol. Evol.* **21**, 1923–1937. doi:10.1093/molbev/msh200
- Bookout, A. L., Jeong, Y., Downes, M., Yu, R. T., Evans, R. M. and Mangelsdorf, D. J. (2006). Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network. *Cell.* **126**, 789–799. doi:10.1016/j.cell.2006.06.049
- Borowiec, B. G., Darcy, K. L., Gillette, D. M. and Scott, G. R. (2015). Distinct physiological strategies are used to cope with constant hypoxia and intermittent hypoxia in killifish (*Fundulus heteroclitus*). *J. Exp. Biol.* **218**, 1198–1211. doi:10.1242/jeb.114579
- Brett, J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Board Can.* **21**, 1183–1226. doi:10.1139/f64-103
- Burton, T., Ratikainen, I. I. and Einum, S. (2022). Environmental change and the rate of phenotypic plasticity. *Glob. Change Biol.* **28**, 5337–5345. doi:10.1111/gcb.16291
- Cartoni, R., Léger, B., Hock, M. B., Praz, M., Crettenand, A., Pich, S., Ziltener, J. L., Luthi, F., Dériaz, O., Zorzano, A. et al. (2005). Mitofusins 1/2 and ERRα expression are increased in human skeletal muscle after physical exercise. *J. Physiol.* **567**, 349–358. doi:10.1113/jphysiol.2005.092031
- Casuso, R. A., Al-Fazazi, S., Hidalgo-Gutiérrez, A., López, L. C., Plaza-Díaz, J., Rueda-Robles, A. and Huertas, J. R. (2019). Hydroxytyrosol influences exercise-induced mitochondrial respiratory complex assembly into supercomplexes in rats. *Free Radic. Biol. Med.* **134**, 304–310. doi:10.1016/j.freeradbiomed.2019.01.027
- Clemente, C. J. and Wilson, R. S. (2016). Speed and maneuverability jointly determine escape success: exploring the functional bases of escape performance using simulated games. *Behav. Ecol.* **27**, 45–54. doi:10.1093/beheco/arv080
- Cocco, T., Pacelli, C., Sgobbo, P. and Villani, G. (2009). Control of OXPHOS efficiency by complex I in brain mitochondria. *Neurobiol. Aging.* **30**, 622–629. doi:10.1016/j.neurobiolaging.2007.08.002
- Conley, K. E. (2016). Mitochondria to motion: optimizing oxidative phosphorylation to improve exercise performance. *J. Exp. Biol.* **219**, 243–249. doi:10.1242/jeb.126623
- Dalziel, A. C., Ou, M. and Schulte, P. M. (2012). Mechanisms underlying parallel reductions in aerobic capacity in non-migratory threespine stickleback (*Gasterosteus aculeatus*) populations. *J. Exp. Biol.* **215**, 746–759. doi:10.1242/jeb.065425
- Davison, W. (1997). The effects of exercise training on teleost fish, a review of recent literature. *Comp. Biochem. Physiol. A.* **117**, 67–75. doi:10.1016/S0300-9629(96)00284-8
- Denton, R. D., Greenwald, K. R. and Gibbs, H. L. (2017). Locomotor endurance predicts differences in realized dispersal between sympatric sexual and unisexual salamanders. *Funct. Ecol.* **31**, 915–926. doi:10.1111/1365-2435.12813
- Domenici, P. (2001). The scaling of locomotor performance in predator–prey encounters: from fish to killer whales. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **131**, 169–182. doi:10.1016/S1095-6433(01)00465-2

- Egginton, S. (1990). Numerical and areal density estimates of fibre type composition in a skeletal muscle (rat extensor digitorum longus). *J. Anat.* **168**, 73–80.
- Elworthy, S., Hargrave, M., Knight, R., Mebus, K. and Ingham, P. W. (2008). Expression of multiple slow myosin heavy chain genes reveals a diversity of zebrafish slow twitch muscle fibres with differing requirements for Hedgehog and Prdm1 activity. *Development* **135**, 2115–2126. doi:10.1242/dev.015719
- Espírito-Santo, C., Guardiola, F. A., Ozório, R. O. and Magnoni, L. J. (2026). Swimming in fish: integrating redox homeostasis and immune responses. *J. Exp. Biol.* **229**, jeb251212. doi:10.1242/jeb.251212
- Fang, Y., Qu, J., Zhao, J., Qu, L., Wang, L., Luo, C., Yang, Q., Wu, W., Sun, D. and He, D. (2025). Swimming training in a T2DM zebrafish model restores mitochondrial function to alleviate anxiety-like behaviors and metabolic dysregulation. *Front. Biosci.* **30**, 37100. doi:10.31083/FBL37100
- Farrell, A. P., Johansen, J. A. and Suarez, R. K. (1991). Effects of exercise-training on cardiac performance and muscle enzymes in rainbow trout, *Oncorhynchus mykiss*. *Fish Physiol. Biochem.* **9**, 303–312. doi:10.1007/BF02265151
- Fukuda, R., Zhang, H., Kim, J. W., Shimoda, L., Dang, C. V. and Semenza, G. L. (2007). HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. *Cell* **129**, 111–122. doi:10.1016/j.cell.2007.01.047
- Fulton, T. W. (1902). *Rate of Growth of Sea Fishes*. Edinburgh: Neill & Company.
- Furrer, R., Hawley, J. A. and Handschin, C. (2023). The molecular athlete: exercise physiology from mechanisms to medals. *Physiol. Rev.* **103**, 1693–1787. doi:10.1152/physrev.00017.2022
- Genova, M. L. and Lenaz, G. (2014). Functional role of mitochondrial respiratory supercomplexes. *Biochim. Biophys. Acta Bioenerg.* **1837**, 427–443. doi:10.1016/j.bbabi.2013.11.002
- Ghelli, A., Tropeano, C. V., Calvaruso, M. A., Marchesini, A., Iommarini, L., Porcelli, A. M., Zanna, C., De Nardo, V., Martinuzzi, A., Wibbrand, F. et al. (2013). The cytochrome *b* p.278Y>C mutation causative of a multisystem disorder enhances superoxide production and alters supramolecular interactions of respiratory chain complexes. *Hum. Mol. Genet.* **22**, 2141–2151. doi:10.1093/hmg/ddt067
- Giguère, V. (2008). Transcriptional control of energy homeostasis by the estrogen-related receptors. *Endocr. Rev.* **29**, 677–696. doi:10.1210/er.2008-0017
- Gilbert, M. J., Zerulla, T. C. and Tierney, K. B. (2014). Zebrafish (*Danio rerio*) as a model for the study of aging and exercise: physical ability and trainability decrease with age. *Exp. Gerontol.* **50**, 106–113. doi:10.1016/j.exger.2013.11.013
- Gofflot, F., Chartoire, N., Vasseur, L., Heikkinen, S., Dembele, D., Le Merer, J. and Auwerx, J. (2007). Systematic gene expression mapping clusters nuclear receptors according to their function in the brain. *Cell* **131**, 405–418. doi:10.1016/j.cell.2007.09.012
- Goncalves, R. L., Quinlan, C. L., Perevoshchikova, I. V., Hey-Mogensen, M. and Brand, M. D. (2015). Sites of superoxide and hydrogen peroxide production by muscle mitochondria assessed ex vivo under conditions mimicking rest and exercise. *J. Biol. Chem.* **290**, 209–227. doi:10.1074/jbc.M114.619072
- Greggio, C., Jha, P., Kulkarni, S. S., Lagarrigue, S., Broskey, N. T., Boutant, M., Wang, X., Alonso, S. C., Ofori, E., Auwerx, J. et al. (2017). Enhanced respiratory chain supercomplex formation in response to exercise in human skeletal muscle. *Cell. Metab.* **25**, 301–311. doi:10.1016/j.cmet.2016.11.004
- Guaras, A., Perales-Clemente, E., Calvo, E., Acín-Pérez, R., Loureiro-Lopez, M., Pujol, C., Martínez-Carrascoso, I., Nuñez, E., García-Marqués, F., Rodríguez-Hernández, M. A. et al. (2016). The CoQH2/CoQ ratio serves as a sensor of respiratory chain efficiency. *Cell Rep.* **15**, 197–209. doi:10.1016/j.celrep.2016.03.009
- Harianto, J., Carey, N. and Byrne, M. (2019). respR – An R package for the manipulation and analysis of respirometry data. *Methods Ecol. Evol.* **10**, 912–920. doi:10.1111/2041-210X.13162
- Heinkele, F. J., Lou, B., Erben, V., Bennewitz, K., Poschet, G., Sticht, C. and Kroll, J. (2021). Metabolic and transcriptional adaptations improve physical performance of zebrafish. *Antioxidants* **10**, 1581. doi:10.3390/antiox10101581
- Hochachka, P. W. and Somero, G. N. (2002). *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. Oxford: Oxford University Press.
- Holloszy, J. O. and Coyle, E. F. (1984). Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J. Appl. Physiol.* **56**, 831–838. doi:10.1152/jappl.1984.56.4.831
- Hoppeler, H., Klossner, S. and Flück, M. (2007). Gene expression in working skeletal muscle. In *Hypoxia and the Circulation* (ed. R. C. Roach, P. D. Wagner and P. H. Hackett), pp. 245–254. New York: Springer.
- Huertas, J. R., Al Fazazi, S., Hidalgo-Gutiérrez, A., López, L. C. and Casuso, R. A. (2017). Antioxidant effect of exercise: exploring the role of the mitochondrial complex I superassembly. *Redox Biol.* **13**, 477–481. doi:10.1016/j.redox.2017.07.009
- Johnston, I. A. and Moon, T. W. (1980a). Endurance exercise training in the fast and slow muscles of a teleost fish (*Pollachius virens*). *J. Comp. Physiol. B.* **135**, 147–156. doi:10.1007/BF00691204
- Johnston, I. A. and Moon, T. W. (1980b). Exercise training in skeletal muscle of brook trout (*Salvelinus fontinalis*). *J. Exp. Biol.* **87**, 177–194. doi:10.1242/jeb.87.1.177
- Johnston, I. A., Abercromby, M., Vieira, V. L., Sigursteindóttir, R. J., Kristjánsson, B. K., Sibthorpe, D. and Skúlason, S. (2004). Rapid evolution of muscle fibre number in post-glacial populations of Arctic charr *Salvelinus alpinus*. *J. Exp. Biol.* **207**, 4343–4360. doi:10.1242/jeb.01292
- Jolles, J. W., King, A. J. and Killen, S. S. (2020). The role of individual heterogeneity in collective animal behaviour. *Trends Ecol. Evol.* **35**, 278–291. doi:10.1016/j.tree.2019.11.001
- Kassahn, K. S., Dang, V. T., Wilkins, S. J., Perkins, A. C. and Ragan, M. A. (2009). Evolution of gene function and regulatory control after whole-genome duplication: comparative analyses in vertebrates. *Genome Res.* **19**, 1404–1418. doi:10.1101/gr.086827.108
- Kinsella, R. J., Kähäri, A., Haider, S., Zamora, J., Proctor, G., Spudich, G., Almeida-King, J., Staines, D., Derwent, P., Kerhornou, A. et al. (2011). Ensembl BioMarts: a hub for data retrieval across taxonomic space. *Database* **2011**, bar030. doi:10.1093/database/bar030
- Kocha, K. M., Reilly, K., Porplycia, D. S., McDonald, J., Snider, T. and Moyes, C. D. (2015). Evolution of the oxygen sensitivity of cytochrome c oxidase subunit 4. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **308**, R305–R320. doi:10.1152/ajpregu.00281.2014
- Kuznetsova, A., Brockhoff, P. B. and Christensen, R. H. (2017). lmerTest package: tests in linear mixed effects models. *J. Stat. Softw.* **82**, 1–26. doi:10.18637/jss.v082.i13
- Lapiente-Brun, E., Moreno-Loshuertos, R., Acín-Pérez, R., Latorre-Pellicer, A., Colás, C., Balsa, E., Perales-Clemente, E., Quirós, P. M., Calvo, E., Rodríguez-Hernández, M. A. et al. (2013). Supercomplex assembly determines electron flux in the mitochondrial electron transport chain. *Science* **340**, 1567–1570. doi:10.1126/science.1230381
- LeMoine, C. M., Loughheed, S. C. and Moyes, C. D. (2010a). Modular evolution of PGC-1α in vertebrates. *J. Mol. Evol.* **70**, 492–505. doi:10.1007/s00239-010-9347-x
- LeMoine, C. M., Craig, P. M., Dhekney, K., Kim, J. J. and McClelland, G. B. (2010b). Temporal and spatial patterns of gene expression in skeletal muscles in response to swim training in adult zebrafish (*Danio rerio*). *J. Comp. Physiol. B.* **180**, 151–160. doi:10.1007/s00360-009-0398-5
- Little, A. G. and Seebacher, F. (2014). Thyroid hormone regulates cardiac performance during cold acclimation in zebrafish (*Danio rerio*). *J. Exp. Biol.* **217**, 718–725. doi:10.1242/jeb.096602
- Little, A. G., Kocha, K. M., Loughheed, S. C. and Moyes, C. D. (2010). Evolution of the nuclear-encoded cytochrome oxidase subunits in vertebrates. *Physiol. Genomics* **42**, 76–84. doi:10.1152/physiolgenomics.00015.2010
- Little, A. G., Dressler, T., Kraskura, K., Hardison, E., Hendriks, B., Prystay, T., Farrell, A. P., Cooke, S. J., Patterson, D. A., Hinch, S. G. et al. (2020). Maxed out: optimizing accuracy, precision, and power for field measures of maximum metabolic rate in fishes. *Physiol. Biochem. Zool.* **93**, 243–254. doi:10.1086/708673
- Nord, H., Burguiere, A. C., Muck, J., Nord, C., Ahlgren, U. and von Hofsten, J. (2014). Differential regulation of myosin heavy chains defines new muscle domains in zebrafish. *Mol. Biol. Cell.* **25**, 1384–1395. doi:10.1091/mbc.e13-08-0486
- Nystoriak, M. A. and Bhatnagar, A. (2018). Cardiovascular effects and benefits of exercise. *Front. Cardiovasc. Med.* **5**, 408204. doi:10.3389/fcvm.2018.00135
- Magnoni, L. J., Crespo, D., Ibarz, A., Blasco, J., Fernandez-Borras, J. and Planas, J. V. (2013). Effects of sustained swimming on the red and white muscle transcriptome of rainbow trout (*Oncorhynchus mykiss*) fed a carbohydrate-rich diet. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **166**, 510–521. doi:10.1016/j.cbpa.2013.08.005
- Maranzana, E., Barbero, G., Falasca, A. I., Lenaz, G. and Genova, M. L. (2013). Mitochondrial respiratory supercomplex association limits production of reactive oxygen species from complex I. *Antioxid. Redox Signal.* **19**, 1469–1480. doi:10.1089/ars.2012.4845
- Masoumi-Ardakani, Y., Aminizadeh, S. and Shahouzehi, B. (2020). The adaptations induced by Estrogen Related Receptor alpha (ERRα) disruption and exercise training on healthy and diabetic rat's liver. *Biologia* **75**, 2341–2350. doi:10.2478/s11756-020-00532-9
- Matsakas, A., Macharia, R., Otto, A., Elashry, M. I., Mouisel, E., Romanello, V., Sartori, R., Anthor, H., Sandri, M., Narkar, V. et al. (2012). Exercise training attenuates the hypermuscular phenotype and restores skeletal muscle function in the myostatin null mouse. *Exp. Physiol.* **97**, 125–140. doi:10.1113/expphysiol.2011.063008
- Maxwell, P. H. and Ratcliffe, P. J. (2002). Oxygen sensors and angiogenesis. *Semin. Cell Dev. Biol.* **13**, 29–37. doi:10.1006/scdb.2001.0287
- McClelland, G. B. (2012). Muscle remodeling and the exercise physiology of fish. *Exerc. Sport Sci. Rev.* **40**, 165–173.
- McClelland, G. B. and Scott, G. R. (2014). Muscle plasticity. In *The Physiology of Fishes* (ed. D. H. Evans, J. B. Claiborne and S. Currie), pp. 1–31. Boca Raton, FL: CRC Press.
- McClelland, G. B., Craig, P. M., Dhekney, K. and Dipardo, S. (2006). Temperature- and exercise-induced gene expression and metabolic enzyme changes in skeletal muscle of adult zebrafish (*Danio rerio*). *J. Physiol.* **577**, 739–751. doi:10.1113/jphysiol.2006.119032

- McCormick, S. D.** (1993). Methods for nonlethal gill biopsy and measurement of Na^+ , K^+ -ATPase activity. *Can. J. Fish. Aquat. Sci.* **50**, 656–658. doi:10.1139/f93-075
- Morash, A. J., Vanderveken, M. and McClelland, G. B.** (2014). Muscle metabolic remodeling in response to endurance exercise in salmonids. *Front. Physiol.* **5**, 452. doi:10.3389/fphys.2014.00452
- Moreno-Loshuertos, R. and Enríquez, J. A.** (2016). Respiratory supercomplexes and the functional segmentation of the CoQ pool. *Free Radic. Biol. Med.* **100**, 5–13. doi:10.1016/j.freeradbiomed.2016.04.018
- Palstra, A. P., Tudorache, C., Rovira, M., Brittijn, S. A., Burgerhout, E., Van den Thillart, G. E., Spaik, H. P. and Planas, J. V.** (2010). Establishing zebrafish as a novel exercise model: swimming economy, swimming-enhanced growth and muscle growth marker gene expression. *PLoS ONE* **5**, e14483. doi:10.1371/journal.pone.0014483
- Palstra, A. P., Rovira, M., Rizo-Roca, D., Torrella, J. R., Spaik, H. P. and Planas, J. V.** (2014). Swimming-induced exercise promotes hypertrophy and vascularization of fast skeletal muscle fibres and activation of myogenic and angiogenic transcriptional programs in adult zebrafish. *BMC Genomics* **15**, 1136. doi:10.1186/1471-2164-15-1136
- Pelster, B., Sanger, A. M., Siegle, M. and Schwerte, T.** (2003). Influence of swim training on cardiac activity, tissue capillarization, and mitochondrial density in muscle tissue of zebrafish larvae. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **285**, R339–R347. doi:10.1152/ajpregu.00110.2003
- Perry, M. C., Dufour, C. R., Tam, I. S., B'chir, W. and Giguère, V.** (2014). Estrogen-related receptor- α coordinates transcriptional programs essential for exercise tolerance and muscle fitness. *Mol. Endocrinol.* **28**, 2060–2071. doi:10.1210/me.2014-1281
- Porter, C., Reidy, P. T., Bhattarai, N., Sidossis, L. S. and Rasmussen, B. B.** (2015). Resistance exercise training alters mitochondrial function in human skeletal muscle. *Med. Sci. Sports Exerc.* **47**, 1922. doi:10.1249/MSS.0000000000000605
- Rangwala, S. M., Wang, X., Calvo, J. A., Lindsley, L., Zhang, Y., Deyneko, G., Beaulieu, V., Gao, J., Turner, G. and Markovits, J.** (2010). Estrogen-related receptor γ is a key regulator of muscle mitochondrial activity and oxidative capacity. *J. Biol. Chem.* **285**, 22619–22629. doi:10.1074/jbc.M110.125401
- Reitzel, A. M., Macrander, J., Mane-Padros, D., Fang, B., Sladek, F. M. and Tarrant, A. M.** (2018). Conservation of DNA and ligand binding properties of retinoid X receptor from the placozoan *Trichoplax adhaerens* to human. *J. Steroid Biochem. Mol. Biol.* **184**, 3–10. doi:10.1016/j.jsbmb.2018.02.010
- Roche, D. G., Binning, S. A., Bosiger, Y., Johansen, J. L. and Rummer, J. L.** (2013). Finding the best estimates of metabolic rates in a coral reef fish. *J. Exp. Biol.* **216**, 2103–2110. doi:10.1242/jeb.082925
- Rockl, K. S., Hirshman, M. F., Brandauer, J., Fujii, N., Witters, L. A. and Goodyear, L. J.** (2007). Skeletal muscle adaptation to exercise training: AMP-activated protein kinase mediates muscle fiber type shift. *Diabetes* **56**, 2062–2069. doi:10.2337/db07-0255
- Rodnick, K. J. and Planas, J. V.** (2016). The stress and stress mitigation effects of exercise: cardiovascular, metabolic, and skeletal muscle adjustments. In *Fish Physiology* (ed. A. P. Farrell and J. G. Richards), pp. 251–294. London: Academic Press.
- Rossi, G. S., Turko, A. J. and Wright, P. A.** (2018). Oxygen drives skeletal muscle remodeling in an amphibious fish out of water. *J. Exp. Biol.* **221**, jeb180257. doi:10.1242/jeb.180257
- Rossi, G. S., Cramp, R. L., Wright, P. A. and Franklin, C. E.** (2020). Frogs seek hypoxic microhabitats that accentuate metabolic depression during dormancy. *J. Exp. Biol.* **223**, jeb218743. doi:10.1242/jeb.218743
- Sänger, A. M.** (1992). Effects of training on axial muscle of two cyprinid species: *Chondrostoma nasus* (L.) and *Leuciscus cephalus* (L.). *J. Fish Biol.* **40**, 637–646. doi:10.1111/j.1095-8649.1992.tb02611.x
- Sänger, A. M.** (1993). Limits to the acclimation of fish muscle. *Rev. Fish Biol. Fish.* **3**, 1–15. doi:10.1007/BF00043295
- Scott, G. R. and Johnston, I. A.** (2012). Temperature during embryonic development has persistent effects on thermal acclimation capacity in zebrafish. *Proc. Natl. Acad. Sci. USA* **109**, 14247–14252. doi:10.1073/pnas.1205012109
- Seebacher, F. and James, R. S.** (2019). Increased physical activity does not improve obesity-induced decreases in muscle quality in zebrafish (*Danio rerio*). *J. Appl. Physiol.* **127**, 1802–1808. doi:10.1152/jappphysiol.00433.2019
- Seebacher, F. and Krause, J.** (2017). Physiological mechanisms underlying animal social behaviour. *Philos. Trans. R. Soc. B* **372**, 20160231. doi:10.1098/rstb.2016.0231
- Seebacher, F. and Walter, I.** (2012). Differences in locomotor performance between individuals: importance of parvalbumin, calcium handling and metabolism. *J. Exp. Biol.* **215**, 663–670. doi:10.1242/jeb.066712
- Simmonds, A. I. and Seebacher, F.** (2017). Histone deacetylase activity modulates exercise-induced skeletal muscle plasticity in zebrafish (*Danio rerio*). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **313**, R35–R43. doi:10.1152/ajpregu.00378.2016
- Sladek, R., Bader, J. A. and Giguère, V.** (1997). The orphan nuclear receptor estrogen-related receptor α is a transcriptional regulator of the human medium-chain acyl coenzyme A dehydrogenase gene. *Mol. Cell. Biol.* **17**, 5400–5409. doi:10.1128/MCB.17.9.5400
- Sopariwala, D., Nguyen, H. and Narkar, V.** (2023a). Estrogen-related receptor signaling in skeletal muscle fitness. *Int. J. Sports Med.* **44**, 609–617. doi:10.1055/a-2035-8192
- Sopariwala, D. H., Rios, A. S., Pei, G., Roy, A., Tomaz da Silva, M., Thi Thu Nguyen, H., Saley, A., Van Druenen, R., Kralli, A., Mahan, K. et al.** (2023b). Innately expressed estrogen-related receptors in the skeletal muscle are indispensable for exercise fitness. *FASEB. J.* **37**, e22727. doi:10.1096/fj.202201518R
- Tan, G. and Lenhard, B.** (2016). TFBSTools: an R/bioconductor package for transcription factor binding site analysis. *Bioinformatics* **32**, 1555–1556. doi:10.1093/bioinformatics/btw024
- Thirupathi, A., Wang, M., Lin, J. K., Fekete, G., István, B., Baker, J. S. and Gu, Y.** (2021). Effect of different exercise modalities on oxidative stress: a systematic review. *Biomed Res. Int.* **2021**, 1947928. doi:10.1155/2021/1947928
- Tohmé, M., Prud'homme, S. M., Boulahtouf, A., Samarut, E., Brunet, F., Bernard, L., Bourguet, W., Gibert, Y., Balaguer, P. and Laudet, V.** (2014). Estrogen-related receptor γ is an in vivo receptor of bisphenol A. *FASEB. J.* **28**, 3124–3133. doi:10.1096/fj.13-240465
- van den Boogaart, K. G. and Tolosana-Delgado, R.** (2013). *Analyzing Compositional Data with R*. Berlin: Springer.
- Videler, J. J.** (1993). *Fish Swimming*. London: Chapman and Hall.
- Villena, J. A. and Kralli, A.** (2008). ERR α : a metabolic function for the oldest orphan. *Trends Endocrinol. Metab.* **19**, 269–276. doi:10.1016/j.tem.2008.07.005
- Walker, M. G. and Pull, G.** (1973). Skeletal muscle function and sustained swimming speeds in the coalfish *Gadus virens* L. *Comp. Biochem. Physiol. A* **44**, 495–501. doi:10.1016/0300-9629(73)90502-1
- Wang, L., Ma, J., Wu, W., Fang, Y., Liu, F., Yang, Q., Hu, X., Gu, X., He, Z., Sun, D. et al.** (2022). Effect of aerobic exercise as a treatment on type 2 diabetes mellitus with depression-like behavior zebrafish. *Life. Sci.* **300**, 120578. doi:10.1016/j.lfs.2022.120578
- Wattez, J. S., Eury, E., Hazen, B. C., Wade, A., Chau, S., Ou, S. C., Russell, A. P., Cho, Y. and Kralli, A.** (2023). Loss of skeletal muscle estrogen-related receptors leads to severe exercise intolerance. *Mol. Metab.* **68**, 101670. doi:10.1016/j.molmet.2023.101670
- Webb, P. W.** (1984). Body form, locomotion and foraging in aquatic vertebrates. *Am. Zool.* **24**, 107–120. doi:10.1093/icb/24.1.107
- Wickham, H.** (2016). Data analysis. In *ggplot2: Elegant Graphics for Data Analysis* (ed. R. Gentleman, K. Hornik and G. Parmigiani), pp. 189–201. Cham: Springer.
- Wilkinson, C., Brose, U., Dyer, A., Hirt, M. R. and Ryser, R.** (2025). A mechanistic approach to animal dispersal – quantifying energetics and maximum distances. *Ecol. Lett.* **28**, e70085. doi:10.1111/ele.70085
- Williams, E. G., Wu, Y., Jha, P., Dubuis, S., Blattmann, P., Argmann, C. A., Houten, S. M., Amariuta, T., Wolski, W., Zamboni, N. et al.** (2016). Systems proteomics of liver mitochondria function. *Science* **352**, aad0189. doi:10.1126/science.aad0189
- Wu, T., Hu, E., Xu, S., Chen, M., Guo, P., Dai, Z., Feng, T., Zhou, L., Tang, W., Zhan, L. et al.** (2021). clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. *Innov.* **2**, 100141. doi:10.1016/j.xinn.2021.100141
- Xu, S., Hu, E., Cai, Y., Xie, Z., Luo, X., Zhan, L., Tang, W., Wang, Q., Liu, B., Wang, R. et al.** (2024). Using clusterProfiler to characterize multiomics data. *Nat. Protoc.* **19**, 3292–3320. doi:10.1038/s41596-024-01020-z
- Zoladz, J. A., Koziel, A., Woyda-Ploszczyc, A., Celichowski, J. and Jarmuszkiewicz, W.** (2016). Endurance training increases the efficiency of rat skeletal muscle mitochondria. *Pflügers Arch.* **468**, 1709–1724. doi:10.1007/s00424-016-1867-9
- Zou, Y. Y., Tang, X. B., Chen, Z. L., Liu, B., Zheng, L., Song, M. Y., Xiao, Q., Zhou, Z., Peng, X. and Tang, C. F.** (2023). Exercise intervention improves mitochondrial quality in non-alcoholic fatty liver disease zebrafish. *Front. Endocrinol.* **14**, 1162485. doi:10.3389/fendo.2023.1162485