Histone deacetylase activity modulates exercise-induced skeletal muscle plasticity in zebrafish (*Danio rerio*)

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Simmonds AI, Seebacher F. Histone deacetylase activity modulates exercise-induced skeletal muscle plasticity in zebrafish (*Danio rerio*). *Am J Physiol Regul Integr Comp Physiol* 313: R35–R43, 2017. First published April 12, 2017; doi:10.1152/ajpregu.00378.2016.—Aerobic exercise has a positive impact on animals by enhancing skeletal muscle function and locomotor performance. Responses of skeletal muscle to exercise involve changes in energy metabolism, calcium handling, and the composition of contractile protein isoforms, which together influence contractile properties. Histone deacetylases (HDAC) can cause short-term changes in gene expression and may thereby mediate plasticity in contractile properties of skeletal muscle in response to exercise. The aim of this project was to determine (in zebrafish, *Danio rerio*) the traits that mediate interindividual differences in sustained and sprint performance and to determine whether inhibiting class I and II HDACs mediates exercise-induced changes in these traits. High sustained performers had greater aerobic metabolic capacity [citrate synthase (CS) activity], calcium handling capacity [sarcoplasmic reticulum ATPase (SERCA) activity], and slow contractile protein concentration [slow myosin heavy chain (MHC)] compared with low performers. High sprint performers had lower CS activity and slow MHC concentrations compared with low performers, but there were no significant differences in lactate dehydrogenase activity or fast MHC concentrations. Four weeks of aerobic exercise training increased sustained performance, CS activity, SERCA activity, and slow MHC concentration. Inhibiting class I and II HDACs increased slow MHC concentration in untrained fish but not in trained fish. However, inhibiting HDACs reduced SERCA activity, which was paralleled by a reduction in sustained and sprint performance. The regulation of muscle phenotypes by HDACs could be a mechanism underlying the adaptation of sustained locomotor performance to different environmental conditions, and may therefore be of therapeutic and ecological significance.

myosin heavy chain; SERCA; metabolism; locomotion; sprint performance; sustained performance

Skeletal muscle provides the force that powers locomotion. In humans, regular locomotor activity is essential to preserve aerobic performance and muscle strength, and thereby prevent numerous chronic diseases (3, 41). In other species, locomotor performance is closely linked to Darwinian fitness, because it facilitates escaping predators (19), catching prey (5), and reproductive success (18, 24).

Increases in locomotor activity (exercise) induce physiological changes in skeletal muscle that include calcium handling capacity, metabolic capacity, and composition of contractile MHC isoforms (11, 14, 27). In particular, aerobic exercise can result in increases in the concentration of sarcoplasmic reticulum calcium ATPase (SERCA), which is responsible for the sequestration of calcium into the sarcoplasmic reticulum (SR) to facilitate muscle relaxation (1) and supporting subsequent contractions and fatigue resistance (37). Aerobic exercise also increases mitochondrial capacities which are reflected in the activities of enzymes such as citrate synthase and cytochrome c oxidase (9, 27). These changes in metabolism may be paralleled by increases in slow MHC I mRNA expression (40) and protein concentration (27). Additionally, aerobic exercise can also increase the mitochondrial capacity of fast fibers (17).

Modifications to muscle phenotypes result from repression or activation of pathways that regulate transcription, particularly those mediated by AMP-activated protein kinase (AMPK) (20, 26, 32). The increase in AMP-to-ATP ratio that is associated with exercise activates AMPK in an intensity-dependent manner (8). At the same time, the amplitude and duration of calcium flux between the SR and cytosol, which are proportional to the force output, activate calmodulin-dependent protein kinase (CaMK) via phosphorylation in an intensity-dependent manner (4). Once activated, both AMPK and CaMK phosphorylate HDACs, thereby initiating their nuclear export (28) and relieving their repressive effects on transcription (13).

HDACs are a family of enzymes that catalyze the removal of an acetyl group from the lysine side chains of histones. Histone acetyltransferases (HATs) reverse this reaction. In an acetylated state histones have looser contact with DNA and result in increased transcription (31). The repressive activity of class IIa HDACs (HDAC 4, 5, 7, and 9) requires a co-repressor complex with HDAC3 and SMRT/N-Cor (10). Class IIa HDAC corepressor complexes bind to the myocyte enhancer factor-2 (MEF2), a transcription factor that regulates the expression of slow skeletal muscle proteins, and inhibit MEF2-dependent transcription (29). Aerobic exercise causes nuclear export of HDAC4 and HDAC5 via the AMPK and CaMK pathways, which increases MEF2 activity and histone 3 acetylation (28, 29). Histone 3 acetylation activates the enhancer and promoter regions such as the MEF2 binding sites in the promoter regions of slow muscle fiber genes (7, 30). Class IIa HDACs may thereby regulate the aerobic exercise response. It was our aim to determine whether inhibiting class I and II HDACs mediates the aerobic exercise-induced changes in skeletal muscle proteins, enzyme activities, and locomotor performance.

We approached this aim in two steps. First, we determined whether differences in metabolic capacities (aerobic: citrate synthase activity, CS; anaerobic: lactate dehydrogenase activ-
ity, LDH), calcium handling capacities (SERCA activity), and muscle fiber compositions (slow and fast MHCs), could explain differences in locomotor performance between high and low performing individuals (experiment 1). We tested the hypotheses that sustained performance is facilitated by higher CS and SERCA activities, and higher slow MHC isoform concentration, whereas sprint performance is facilitated by higher LDH and SERCA activities, and higher fast MHC concentration. Second, we tested whether locomotor performance and its underlying biochemical traits are modulated by the interaction between aerobic exercise and HDAC activity (experiment 2). We tested the hypotheses that aerobic exercise increases sustained locomotor performance, CS activity, SERCA activity, and slow MHC concentration. Furthermore, we predicted that the exercise response described above is enhanced by increasing histone acetylation resulting from inhibiting class I and II HDAC activity.

MATERIALS AND METHODS

Ethics statement. All animal handling and experimental procedures were conducted with the approval of the University of Sydney Animal Ethics Committee (approval no. 2014/587).

Animal collection and husbandry. Adult short fin zebrafish, *Danio rerio* [27.7 ± 0.26 (SE) mm standard length], were purchased from a commercial supplier (Livefish, Bundaberg, QLD, Australia) and kept in plastic tanks (645 × 423 × 276 mm) of dechlorinated water at a density of ~1 fish/liter. Each tank contained an air filter (Aquarium Bio-Filter, Fish 101) connected to an air pump (AC-9908; Resun, China), and a 30% water change was done once a week. Water was kept at 23°C (± 0.5°C) using subsensible heaters (200W; Aquaworld). Fish were fed fish food flakes (Wardley Total Tropical Flake; Hartz Mountain, Secaucus, NJ) until satiation once daily, and were exposed to a 12:12-h light/dark cycle. All fish were kept under these conditions for at least 1 wk before starting experimental treatments.

Locomotor performance. Sprint speed was measured following a previous protocol (37). Fish were placed in a tray (405 × 600 mm) with water kept at 23°C (± 0.5°C) and at 25 mm depth. Fish were lightly tapped on the tail with a rod to elicit an escape response, which was filmed from above using a camera (Exilim EX-ZR200; Casio) filming at 30 frames/s. Three escape responses were filmed for each fish and videos were analyzed in Tracker Video Analysis and Modeling Tool Software 4.01 (Open Source Physics, www.opensourcephysics.org). From the three responses the fastest speed was used as the maximum sprint speed.

Sustained swimming speed (22, 36) was measured immediately after sprint speed. Fish were swum in Blazka-type cylindrical flumes (26 mm diameter × 150 mm length), fitted over the intake end of a cylindrical subsensible pump (12V DC, iL500; Rule, Hertfordshire, UK). Water flow inside the flume was controlled by changing the water flow was ceased for 10 s and then gradually increased back to the maximal speed achieved. After the third attempt, the time swum at the final speed (\(T_f\)) and the final speed (\(U_f\)) were recorded and used to calculate \(U_{\text{cen}}(\text{m/s}) = U_i + (T_f/T_i) \times U_i\) (16).

Enzyme assays. Immediately after sprint and sustained speed were measured, fish were anesthetized in buffered ethyl 3-aminobenzoate methanesulfonate (MS222; 0.3 g/l; Sigma-Aldrich, Castle Hill, Australia) and euthanized by decapitation. Back and tail skeletal muscle was extracted, skinned, and halved along the axis of symmetry. Both lateral portions were immediately transferred to liquid nitrogen and stored at −80°C. One portion was used for measurements of metabolic enzyme activities and myosin heavy chain (MHC) protein concentrations, and the other for sarco/endoplasmic reticulum ATPase (SERCA) analysis. Muscle samples used for measurements of metabolic enzyme activities and MHC protein concentrations were homogenized (in a TissueLyser LT; Qiagen, Venlo, The Netherlands) in 9 vol RIPA buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% NP40, 1% sodium deoxycholate) and protease inhibitor cocktail (Complete, EDTA-free; Roche Life Sciences, Germany) solution. Homogenate was either further diluted by a factor 10 (to a final 1:100 dilution) for metabolic enzyme assays or aliquoted and stored at −20°C for MHC protein analysis. Citrate synthase (CS) and lactate dehydrogenase (LDH) are regulatory enzymes in aerobic and anaerobic metabolism, respectively, and these enzymes were chosen because of their relevance for differentiating between fiber types (35). Following published protocols (39), enzyme activities were determined using a UV/visible spectrophotometer (Ultrospec 2100 Pro; Biochrom, UK) with a temperature-controlled cuvette holder. Assays were performed in duplicate at 23°C.

Muscle samples used for SERCA assays were homogenized (in a TissueLyser LT; Qiagen) in nine parts homogenization buffer (250 mM sucrose, 5 mM EDTA, and 20 mM imidazole, pH 7.2). Under constant vortexing, 0.1% sodium deoxycholate dissolved in homogenization buffer was added to muscle tissue homogenate in equivalent amounts (wt/vol). Sodium deoxycholate-treated homogenates were preincubated for 10 min at 23°C in assay medium (25 mM imidazole, 0.2 mM CaCl₂, 80 mM KCl, 5 mM MgCl₂) with and without 10 μM of thapsigargin, a specific inhibitor of SERCA (34). Following a published protocol (42), the activity of SERCA was determined using a UV/visible spectrophotometer (Ultrospec 2100 Pro; Biochrom).

Assays were performed in duplicate. SERCA activity was measured by quantifying the liberation of inorganic phosphate. The assay was initiated by the addition of 3 mM ATP to homogenates, left to incubate for 5 min at 23°C, and the reaction was stopped by the addition of 0.4 M perchloric acid. The final solution was centrifuged (1,200 g, 15 min at 4°C) and the supernatant was added to 1 part color reagent (8 mM ammonium molybdate, 335 mM concentrated H₂SO₄, 145 mM FeSO₄) to quantify the amount of inorganic phosphate relative to a standard curve; the standard curve was determined with known concentrations of PO₄ (25–250 mM). After 10 min of color formation absorbance was read at 750 nm. SERCA activity (μmol of product-min⁻¹-g⁻¹ of wet tissue) was calculated as the difference in inorganic phosphate liberated in the presence and absence of thapsigargin.

*Myosin heavy chain protein concentrations.* The identification and quantification of slow and fast MHC isoforms, and acetylated histone 3 lysine 9 (acetyl-H3K9) were performed by capillary electrophoresis in a “Wes” Simple Western System (ProteinSimple) following the manufacturer’s instructions. Antibodies we used were EB165 (Developmental Studies Hybridoma Bank, University of Iowa) to determine fast MHC concentrations, BA-F8 (Developmental Studies Hybridoma Bank, University of Iowa) to determine slow MHC concentrations, C5B11 (Cell Signaling) to determine acetyl-H3K9, and 12G10 (α-tubulin; Developmental Studies Hybridoma Bank, University of Iowa) as internal control. Before protein assays, the concentrations of protein extracts were determined using a bicinchoninic acid assay kit (Sigma-Aldrich, Castle Hill, Australia) following manufacturer’s instructions.
Experiment 1: differences between high and low locomotor performers. Sprint and sustained speeds were determined in 48 fish. Based on differences in locomotor performance between individuals, we selected the 15 fastest (high performance) and 15 slowest (low performance) fish to make up different performance groups. Fish were grouped according to sprint and sustained swimming performance separately. We used all 15 fish within each performance group to determine differences in sprint speed, sustained speed, CS activity, and LDH activity, but we used 10 and 6 fish from each group for comparisons of SERCA activity and MHC concentrations between groups, respectively.

Pilot study: exercise training time course. Before conducting experiment 2, we conducted two pilot studies to determine, first, the time course over which an exercise training response was established and, second, whether the exercise training effect was diminished when fish were kept sedentary for 48 h, which was necessary for drug treatments (see below). To determine the time course over which an exercise training effect was established, 60 fish were randomly split into 2 treatments: an exercise-trained group and a control group (30 fish per treatment). Both treatments followed the exercise and control protocols described for experiment 2 below. The sustained speed of 10 exercise-trained fish was compared with that of 10 control fish at the end of each week for 3 wk of exercise training.

To determine whether the exercise training response diminished when fish were kept sedentary for 48 h, 30 fish were split into 2 treatments: an exercise-trained group (20 fish), and a control group (10 fish). For 3 wk, both treatments followed the exercise protocols described for experiment 2 below. After 3 wk, the exercise-trained group was split into 2 treatments: a group that continued exercise training, and a group in which fish were kept sedentary in 1-liter containers in pairs for 48 h, which was similar to the conditions during drug treatments (see below). The sustained speed was compared between fish from each of the 3 treatments.

Experiment 2: exercise training and histone deacetylase activity. Seventy-two fish were randomly split into an aerobic exercise training treatment and a control treatment (36 fish per treatment). Fish from both treatments were dispersed across four treatment tanks in groups of nine fish, and kept at 23°C (± 0.5°C). Both control and exercise tanks (645 × 423 × 276 mm) were identical, except that there was no water flow in the control treatment (Fig. 1A). Within the tanks, fish were contained within an area (120 mm width) along the long end of the tank by a partition composed of a plastic island piece, and fish were prevented from leaving the area by fabric netting (Fig. 1A). Each tank contained submersible pumps (2 × 5 W, SP-900; Resun, China, and 1 × 24 W, JHQ-2000; Sunsun, China) at the opposite side of the tank to the area containing fish. The output from the pumps was directed to the area containing fish by a plastic sheet, and we mounted rounded plastic pieces in the corners to reduce turbulence (Fig. 1A). Before introducing fish, we measured flow rates within the areas containing fish using red dye and a camera (Exilim EX-ZR200; Casio) filming at 30 frames/s to ascertain that we could achieve the desired treatment flows.

![Fig. 1. A schematic representation of the exercise training tank and TSA dose responses.](http://ajpregu.physiology.org/)

In the exercise training tank (A), a submersible pump (S.P.) provided water flow (arrows), which was directed toward a linear swimming area by a plastic sheet (P.S.). The swimming area was bordered by a plastic island (I.P.) on one side and the edge of the tank on the other, and fish were prevented from leaving the area by fabric netting placed at either end. Rounded plastic corners (R.P.) reduced turbulence and helped maintain laminar flow. Histone deacetylase (HDAC) activity decreased significantly in fish that were exposed to 500 nM trichostatin A (TSA) dissolved in tank water. Values are means ± SE are shown. *Significant difference is the following: P < 0.05.
The exercise training protocol consisted of exposing fish three times daily to a water flow of 21 cm/s [~7 body lengths (BL)/s] for 6 days/wk. During the first week, each exercise training interval lasted 1 h and intervals were separated by 3-h rest periods. Exercise training intervals were incrementally increased by 0.5 h each week to a final of 2.5 h during the fourth week, whereas rest intervals were reduced by 0.5 h each week to a final 1.5 h. Outside training intervals, the water flow was 8 cm/s [2–3 BL/s], except during daily feeding (15 min). This regimen represented aerobic exercise with moderate intensity (25).

After the 4-wk training was completed, histone deacetylation was reduced pharmacologically by administering the HDAC class I and II (members: HDAC1-10) specific inhibitor Trichostatin A (TSA; Sigma-Aldrich) dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich). Both exercise-trained and control fish were randomly split into three treatments (12 fish each): TSA, DMSO only, and No drug. Fish in each treatment were kept in pairs in 1-liter containers at 23°C (±0.5°C) over 48 h. No-drug fish were not exposed to drugs. TSA-treated fish were kept in a 0.025% DMSO solution, and DMSO-treated fish were kept in a 500 nM solution of TSA dissolved in DMSO.

We conducted preliminary experiments with zebrafish not used elsewhere in the study to determine the efficacy of delivering TSA dissolved in tank water. We conducted three treatments (N = 9 fish each): 1) TSA dissolved in DMSO (0.025%) to give a final concentration of 100 nM TSA in tank water; 2) 500 nM concentration of TSA in tank water, and 3) DMSO dissolved in tank water at 0.025% final concentration (0.25 nM/L). After 2 days in the TSA treatments, fish were euthanized and muscle tissue was dissected to determine protein concentrations (25).

We determined HDAC activity with a commercial kit (cat. no. 566328, Merck Millipore) and following the manufacturer’s instructions. There was a significant decrease in HDAC activity (one-way ANOVA followed by Tukey post hoc tests) compared with controls (main effect of exercise training (Fig. 1B)). Hence, in the experiments below we exposed fish for 2 days to 500 nM TSA in tank water to test the effect of histone acetylation. A 48-h period for treatment with TSA was chosen, because this is sufficient to alter protein concentrations assuming a mean protein degradation rate of 2.2%/h (33). Immediately after the 48-h drug treatment, the sprint and sustained speeds were determined for each fish, and fish were euthanized for tissue collection.

**Statistical analysis.** For experiment 1, two-tailed, independent sample t-tests were used to compare sprint speed, sustained speed, CS activity, LDH activity, SERCA activity, and MHC concentrations and ratios between high and low performers. We analyzed data from the first pilot study with a two-way ANOVA to test for differences in sustained speed between exercise-trained and control fish (factor 1) after each week for 3 wk of exercise training (factor 2); note that independent fish were used to measure swimming performance at each time point. Data from the second pilot study were analyzed with a one-way ANOVA to test for differences in sustained speed between control, exercise-trained, and exercise-trained fish that remained sedentary for 48 h. Here and below, we used Tukey’s HSD post hoc test to determine differences between means.

We analyzed data from experiment 2 with separate two-way ANOVAs to test for differences in sprint speed, sustained speed, CS activity, LDH activity, SERCA activity, MHC concentrations, and H3K9 acetylation between exercise-trained and control fish (factor 1) within drug treatments (control, DMSO, and TSA; factor 2). However, because DMSO did not affect locomotion significantly it was excluded from the analysis of protein concentrations.

Data were analyzed in IBM SPSS 20 and all data were tested for homogeneity of variance using Levene’s test, and no significant results were found. The truncated product method (44) was used to combine all the P values in this study to determine whether there is a bias from multiple hypothesis testing. The truncated product method P value was <0.001, showing that the results are not biased.

**RESULTS**

**Differences between high and low locomotor performers (experiment 1).** As expected, both sustained and sprint speed were higher in high performers than in low performers (t = 17.35, P < 0.001, Fig. 2A; t = 15.33, P < 0.001, Fig. 2B, respectively). CS activity was higher in high sustained performers than in low sustained performers (t = 2.09, P = 0.049, Fig. 2C). In contrast, CS activity was lower in high sprint performers compared with low sprint performers (t = 2.11, P = 0.048, Fig. 2D). LDH activity did not differ significantly between high and low performers with respect to either sustained or sprint speed (t = 1.13, P = 0.267, Fig. 2E; t = 1.20 P = 0.241, Fig. 2F, respectively). SERCA activity was higher in high performers than in low performers with respect to both sustained and sprint speed (t = 4.12, P = 0.003, Fig. 2G; t = 3.11, P = 0.024, Fig. 2H, respectively).

The concentration of slow MHC isoforms relative to α-tubulin did not differ significantly between high and low sustained performance groups (t = 0.27, P = 0.792, Fig. 3A). In contrast, low sprint performers had higher slow MHC concentrations compared with high sprint performers (t = 3.07, P = 0.012, Fig. 3B). Fast MHC concentrations did not differ significantly between low and higher performers with respect to either sustained or sprint speed (t = 1.25, P = 0.241, Fig. 3C) or sprint (t = 0.03, P = 0.982; Fig. 3D) speed. The ratio between slow- to fast isoform concentrations was significantly greater in high compared with low sustained performers (t = 4.83, P < 0.001, Fig. 3E). Conversely, the slow-to-fast MHC ratio was significantly higher in low sprint performers compared with high sprint performers (t = 2.25, P = 0.048, Fig. 3F).

**Exercise training time course (pilot data).** Exercise training for longer than 1 wk increased sustained swimming speed compared with controls (main effect of exercise training F = 18.78, P < 0.001, Fig. 4A), and increasing the length of training did not have any further significant effect (main effect of time F = 0.72, P = 0.492, Fig. 4A). Exercise training followed by a 48-h sedentary period did not decrease swimming performance significantly compared with fish that were exercised without the sedentary period, and swimming performance of both groups of trained fish (exercise only and exercise followed by 48 h being sedentary) was significantly greater than that of control fish (F = 9.97, P < 0.001, Fig. 4B).

**Effects of exercise training and histone deacetylation activity (experiment 2).** Sustained speed (Fig. 5A), but not sprint speed (Fig. 5B), was higher in exercise-trained fish than control fish. There was an interaction between exercise training and drug treatment in determining sustained (interaction F = 3.20, P = 0.047, Fig. 5A) and sprint (interaction F = 3.18, P = 0.048, Fig. 5B) speed; TSA reduced both in control fish but it did not have a significant effect on exercise-trained fish. DMSO had no significant effect on sustained or sprint speed, and DMSO-treated fish did not differ significantly from no-drug controls for any response variable. For clarity, we therefore did not show responses of DMSO-treated fish here or below.

Exercise training increased CS activity (main effect F = 23.09, P < 0.001, Fig. 5C) but not LDH activity (main effect F = 1.19, P = 0.279, Fig. 5D). TSA had no significant effect on CS or LDH activities (main effects F = 0.08, P =...
Fig. 2. Differences between high- and low-performing individuals. Sustained (A) and sprint (B) speed were significantly higher in high-performing (black bars) than in low-performing (gray bars) fish \((N = 15\) for each group; BL = body lengths). Citrate synthase (CS) activity \((N = 15\) per group) was greater in high sustained performers compared with low sustained performers (C), but the reverse was the case for sprint performance (D). There was no significant difference in lactate dehydrogenase (LDH; \(N = 15\) per group) activity between high- and low-performing fish with respect to either sustained or sprint performance (E and F). However, high sustained and sprint performers had greater sarco/endoplasmic reticulum ATPase (SERCA; \(N = 10\) per group) activities compared with low performers \((G\) and \(H\)). Values are means ± SE are shown. Significant differences are the following: *\(P < 0.05\); **\(P < 0.01\); ***\(P < 0.001\).

Fig. 3. Differences in myosin heavy chain (MHC) isoform concentrations between high (black bars) and low (gray bars) locomotor performers. Concentration of slow MHC (normalized to \(\alpha\)-tubulin) did not differ significantly between high and low sustained performers (A), but high sprint performers had significantly lower amounts of slow MHC compared with low sprint performers (B). Fast MHC isoform concentrations did not differ significantly between high and low sustained (C) and sprint (D) performers. However, the ratio of slow-to-fast MHC isoform concentrations was significantly lower in low sustained performers compared with high sustained performers (E), but the reverse was the case for sprint performers (F). Representative protein bands of slow MHC, fast MHC, and \(\alpha\)-tubulin detected by capillary electrophoresis are shown for high and low sustained \((G\) and \(H\)) and sprint \((G\) and \(H\)) performers. Values are means ± SE are shown \((all \(N = 6\) per group). Significant differences are the following: *\(P < 0.05\); ***\(P < 0.001\).
Fig. 4. Exercise training time course. One week of exercise training (Ex) increased sustained swimming performance compared with nonexercised control fish, but swimming speed did not increase further with an additional 2 wk of training (A). Sustained swimming performance (BL = body length) of fish that were exercise-trained for 2 wk and then kept sedentary for 48 h (ExS) did not differ significantly from that of fish that were exercise trained for 2 wk but not kept sedentary (Ex). Swimming performance of control fish (C) that were not exercise-trained was significantly lower than that of fish from either exercise training treatment (B). Significant differences are the following: **P < 0.01.

0.923, and $F_{2,66} = 0.05$, $P = 0.951$, respectively). SERCA activity was higher in exercise-trained fish (main effect $F_{1,66} = 9.93$, $P = 0.002$, Fig. 5E), and TSA reduced SERCA activity significantly (main effect $F_{1,66} = 3.46$, $P = 0.037$).

There was an interaction between exercise training and drug treatment in determining the concentration of slow MHC (interaction $F_{1,20} = 4.57$, $P = 0.045$, Fig. 6A) as well as the slow-to-fast MHC ratio (interaction $F_{1,20} = 9.31$, $P = 0.006$, Fig. 6C), and TSA increased both in control fish but it had no significant effect on exercise-trained fish. Neither exercise training (main effect $F_{1,20} = 3.84$, $P = 0.064$) nor drug treatment (main effect $F_{1,20} = 0.71$, $P = 0.409$) affected concentration of fast MHC (Fig. 6B). TSA, but not exercise training, significantly increased H3K9 acetylation (main effects $F_{1,20} = 19.87$, $P < 0.001$, and $F_{1,20} = 1.69$, $P = 0.209$, respectively; Fig. 6E).

**DISCUSSION**

We have shown that class I and II HDACs modulate responses to exercise, but not necessarily as predicted. We accept our hypotheses that citrate synthase (CS) activity, slow myosin heavy chain (MHC) composition, and sarco/endoplasmic reticulum (SERCA) activity are biochemical traits that are associ-
ated with differences in sustained and sprint performance between individuals. We also accept the hypotheses that aerobic exercise training increased sustained performance, CS activity, slow MHC expression, and SERCA activity. However, inhibition of class I and II HDAC caused an increase in slow MHC expression in control fish only, but had no effect on exercise-trained fish. In contrast to our hypothesis, inhibiting class I and II HDACs caused a decrease in SERCA activity and locomotor performance.

As predicted, CS activity, an indicator of mitochondrial capacity (23), was a good predictor of sustained performance. Additionally, we found that sustained performance was facilitated by an aerobically poised myosin heavy chain composition. Slow MHCs have lower ATPase activity and lower force production, which makes them optimal for low intensity contractions (14). These results indicate that fatigue resistance is facilitated both by a decrease in ATP demand and an increase in ATP production via aerobic metabolism. We found that anaerobic metabolic capacity, measured by lactate dehydrogenase activity (LDH), did not enhance sprint performance significantly, which corroborates earlier results (38). Low sprint performance was associated with higher aerobic metabolic capacities and slow MHC concentrations, which indicates that higher sprint performance is mediated by a reduction in the capacity for sustained performance rather than an increase in the capacity for sprint performance, thereby shifting the ratio of slow-to-fast muscle fibers. However, sprint performance was not reduced by exercise training-induced increases in CS activity and slow MHC expression, perhaps due to concomitant increases in SERCA activity and an indication that fast MHC expression was increased (insignificant: $P = 0.064$). Contrary to a previous study on zebrafish (27), we found no increase in LDH activity following exercise training. An increase in LDH activity is likely due to the more anaerobically poised exercise training speed which the previous study employed (12 BL/s compared with our 7 BL/s) (43).

During muscle contraction, nerve signals stimulate voltage-gated calcium channels, dihydropyridine receptors, to release calcium into the cell. Dihydropyridine receptors also stimulate ryanodine receptors (RyR) to release additional calcium stores from the sarcoplasmic reticulum (SR) (1). The resultant calcium signal acts on troponin and causes myosin and actin to interact, thereby leading to muscle contraction. Muscle relaxation is facilitated by the activity of SERCA that pumps calcium back into the SR (1). The capacities of different calcium cycling steps are often linked to enhancing either sustained or sprint performance. For example, reducing the activity of RyR caused a decrease in sprint performance but an

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Values are means ± SE are shown (all $N = 6$ per group). Significant difference is the following: 

![Graph F](image6.png)
increase in the fatigue resistance of isolated carp muscle (37). The reduced sprint performance could be explained by a reduction in free calcium leading to fewer actin-myosin cross bridges and thereby reduced muscle force production, whereas enhanced fatigue resistance may result from reduced depletion of calcium stores in the SR. We found that increased SERCA activity facilitated both sprint and sustained performance. Similarly, zebrafish with high sprint and sustained performance expressed more SERCA mRNA than low performers (38). Additionally, the inhibition of SERCA in rat muscle decreased fatigue resistance (21). Perhaps SERCA is important to both locomotor modes because it enables the high relaxation rates that are associated with sprint performance, and replenishes calcium stores for subsequent contractions to promote fatigue resistance. As further support for this suggestion, the overexpression of parvalbumin, a calcium-binding protein that increases the efficiency of SERCA, increased both sprint and sustained performance (38).

The transcriptional regulators that mediate aerobic exercise-induced changes have not been fully elucidated (9, 14). However, class IIa HDACs (HDAC 4, 5, 7, and 9) are of particular interest, because of their repressive effects on the transcriptional activity of the myocyte enhancer factor-2 (MEF2) (29). MEF2 is a transcription factor that regulates muscle fiber differentiation and development by inducing expression of mitochondrial proteins and slow MHC in cardiac and skeletal muscle (6). We have shown that inhibiting class I and II HDACs with trichostatin A (TSA) caused an increase in the expression of slow MHCs in control fish, but had no effect on the expression of slow MHCs in exercise-trained fish. Increased expression of slow MHCs may be due to TSA relieving the suppressive effect that HDACs have on MEF2. Postexercise, there was no reduction in the concentration of HDAC 4 or 5 directly, but there was an increase in histone 3 acetylation at lysine 36 in humans (28). This result may be explained by AMPK and CaMK-mediated phosphorylation of class IIa HDACs, which allowed chaperone proteins to bind and export HDAC 4 and 5 from the nucleus (6, 28). Hence, aerobic exercise may cause the nuclear export of HDACs via the activation of AMPK and CaMK pathways. Inhibiting class I and II HDACs in exercise-trained fish did not further increase slow MHC expression, possibly because exercise training had already caused nuclear export of HDAC 4 and 5, thereby permitting MEF2-dependent transcription. TSA treatment, but not exercise, increased histone 3 acetylation at lysine 9 (H3K9). Similarly, treatment with a specific class IIa HDAC inhibitor increased H3K9 acetylation (12), whereas exercise did not alter acetylation at lysine 9 (28). TSA-mediated increase in the expression of slow MHCs in control fish was not accompanied by increases in CS activity, which may be because CS has a longer half-life and slower expression rate than the 48-h period over which we inhibited HDACs. For example, the half-life of cytochrome c, another mitochondrial protein, is 6–8 days (2). Additionally, the TSA-mediated increase in slow MHCs of control fish was not paralleled by increased sustained performance. In fact, inhibition of HDACs decreased both sprint and sustained performance in control fish, but had no effect on the performance of exercise-trained fish. The reduction in sprint and sustained performance may be due to the reduction in SERCA activity that resulted from inhibiting HDACs. SERCA activity is important to both locomotor modes, and a reduction in SERCA activity could be the result of reduced SERCA expression, or an increase in the expression and activity of inhibitory proteins, such as phospholamban (15).

**Perspectives and Significance**

We have shown that inhibiting class I and II HDACs mediated increases in slow MHC expression, which resembled the effects of aerobic exercise. However, inhibiting class I and II HDACs also reduced SERCA activity and locomotor performance, and the parallel changes in SERCA activity and locomotion suggest a casual relationship. Aerobic exercise must therefore activate a pathway that leads to increases in SERCA activity not mediated by class I and II HDACs. For example, HDACs may inhibit the expression of proteases that can degrade SERCA, whereas aerobic exercise training increases the expression of protease inhibitors such as calpastatin (43) and these relationships should be tested experimentally. It is likely, however, that the exercise training response is mediated by specific class IIa HDAC isoforms that only regulate slow MHC expression and not SERCA activity. TSA inhibits all HDAC class I and II isoforms, and further studies should employ isoform-specific inhibitors to determine which HDAC isoforms are responsible for regulating slow MHC expression and SERCA activity. Understanding the complexities of the so-called “histone code” will be one of the greatest challenges in biology, and is likely to have future medical and ecological significance. We have identified class I and II HDACs as transcriptional regulators of slow MHC expression and SERCA activity, and the regulation of biochemical traits by class I and II HDACs could be a mechanism underlying the adaptation of locomotor performance to different environmental conditions. Additionally, there is therapeutic potential in manipulating specific HDAC isoform activity to induce the expression of slow fiber proteins to counteract muscular diseases such as dystrophy, or lifestyle-related diseases such as type 2 diabetes.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

A.I.M.S. performed experiments; A.I.M.S. analyzed data; A.I.M.S. and F.S. interpreted results of experiments; A.I.M.S. and F.S. prepared figures; A.I.M.S. and F.S. drafted manuscript; A.I.M.S. and F.S. edited and revised manuscript; A.I.M.S. and F.S. prepared figures; A.I.M.S. and F.S. approved final version of manuscript; F.S. conceived and designed research.

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