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

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10

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14

15 **Running Head:** Histone deacetylase modulates skeletal muscle plasticity

16

17 **Keywords:** myosin heavy chain, SERCA, metabolism, locomotion, sprint performance,
18 sustained performance.

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20 **Abstract**

21 Aerobic exercise has a positive impact on animals by enhancing skeletal muscle
22 function and locomotor performance. Responses of skeletal muscle to exercise involve
23 changes in energy metabolism, calcium handling, and the composition of contractile protein
24 isoforms, which together influence contractile properties. Histone deacetylases (HDAC)
25 can cause short-term changes in gene expression, and may thereby mediate plasticity in
26 contractile properties of skeletal muscle in response to exercise. The aim of this project was
27 to determine (in zebrafish, *Danio rerio*) the traits that mediate inter-individual differences
28 in sustained and sprint performance, and to determine whether inhibiting class I and II
29 HDACs mediates exercise-induced changes in these traits. High sustained performers had
30 greater aerobic metabolic capacity (citrate synthase [CS] activity), calcium handling
31 capacity (sarco/endoplasmic reticulum ATPase [SERCA] activity), and slow contractile
32 protein concentration (slow myosin heavy chain [MHC]) compared to low performers.
33 High sprint performers had lower CS activity and slow MHC concentrations compared to
34 low performers, but there were no significant differences in lactate dehydrogenase activity
35 or fast MHC concentrations. Four weeks of aerobic exercise training increased sustained
36 performance, CS activity, SERCA activity, and slow MHC concentration. Inhibiting class I
37 and II HDACs increased slow MHC concentration in untrained fish but not in trained fish.
38 However, inhibiting HDACs reduced SERCA activity, which was paralleled by a reduction
39 in sustained and sprint performance. The regulation of muscle phenotypes by HDACs could
40 be a mechanism underlying the adaptation of sustained locomotor performance to different
41 environmental conditions, and may therefore be of therapeutic and ecological significance.

42

43 **Introduction**

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

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

60 Modifications to muscle phenotypes result from repression or activation of
61 pathways that regulate transcription, particularly those mediated by AMP-activated protein
62 kinase (AMPK) (20, 26, 32). The increase in AMP:ATP ratio that is associated with
63 exercise activates AMPK in an intensity-dependent manner (8). At the same time, the
64 amplitude and duration of calcium flux between the SR and cytosol, which are proportional
65 to the force output, activate calmodulin-dependent protein kinase (CaMK) via
66 phosphorylation in an intensity-dependent manner (4). Once activated, both AMPK and

67 CaMK phosphorylate HDACs, thereby initiating their nuclear export (28) and relieving
68 their repressive effects on transcription (13).
69 HDACs are a family of enzymes that catalyse the removal of an acetyl group from
70 the lysine side chains of histones. Histone acetlytranferases (HATs) reverse this reaction. In
71 an acetylated state histones have looser contact with DNA and result in increased
72 transcription (31). The repressive activity of class IIa HDACs (HDAC 4, 5, 7, and 9)
73 requires a co-repressor complex with HDAC3 and SMRT/N-Cor (10). Class IIa HDAC co-
74 repressor complexes bind to the myocyte enhancer factor-2 (MEF2), a transcription factor
75 that regulates the expression of slow skeletal muscle proteins, and inhibit MEF2-dependent
76 transcription (29). Aerobic exercise causes nuclear export of HDAC4 and HDAC5 via the
77 AMPK and CaMK pathways, which increases MEF2 activity and histone 3 acetylation (28,
78 29). Histone 3 acetylation activates the enhancer and promoter regions such as the MEF2
79 binding sites in the promoter regions of slow muscle fibers genes (30) (7). Class IIa
80 HDACs may thereby regulate the aerobic exercise response. It was our aim to determine
81 whether inhibiting class I & II HDACs mediates the aerobic exercise-induced changes in
82 skeletal muscle proteins, enzyme activities, and locomotor performance.
83 We approached this aim in two steps. Firstly, we determined whether differences in
84 metabolic capacities (aerobic: citrate synthase activity, CS; anaerobic: lactate
85 dehydrogenase activity, LDH), calcium handling capacities (SERCA activity), and muscle
86 fiber compositions (slow and fast MHCs), could explain differences in locomotor
87 performance between high and low performing individuals (Experiment 1). We tested the
88 hypotheses that sustained performance is facilitated by higher CS and SERCA activities,
89 and higher slow MHC isoform concentration, while sprint performance is facilitated by
90 higher LDH and SERCA activities, and higher fast MHC concentration. Secondly, we

91 tested whether locomotor performance and its underlying biochemical traits are modulated
92 by the interaction between aerobic exercise and HDAC activity (Experiment 2). We tested
93 the hypotheses that aerobic exercise increases sustained locomotor performance, CS
94 activity, SERCA activity, and slow MHC concentration. Further, we predicted that the
95 exercise response described above is enhanced by increasing histone acetylation resulting
96 from inhibiting class I and II HDAC activity.
97

98 **Materials and Methods**

99 *Ethics statement*

100 All animal handling and experimental procedures were conducted with the approval
101 of the University of Sydney Animal Ethics Committee (approval number: 2014/587).
102

103 *Animal collection and husbandry*

104 Adult short fin zebrafish, *Danio rerio* (27.7 ± 0.26 [s.e.] mm standard length), were
105 purchased from a commercial supplier (Livefish, Bundaberg, QLD, Australia) and kept in
106 plastic tanks (645 x 423 x 276 mm) of dechlorinated water at a density of approximately 1
107 fish l^{-1}. Each tank contained an air filter (Aquarium Bio-Filter; Fish 101, Australia)
108 connected to an air pump (AC-9908; Resun, China), and a 30% water change was done
109 once a week. Water was kept at 23°C (± 0.5°C) using submersible heaters (200W;
110 AquaWorld, Australia). Fish were fed fish food flakes (Wardley Total Tropical Flake
111 Blend; Hertz, NJ, USA) until satiation once daily, and were exposed to a 12-hour light/12-
112 hour dark cycle. All fish were kept under these conditions for at least one week before
113 starting experimental treatments.

114

115 *Locomotor performance*

116 Sprint speed was measured following a previous protocol (37). Fish were placed in
117 a tray (405 x 600 mm) with water kept at 23°C (± 0.5°C) and at 25 mm depth. Fish were
118 lightly tapped on the tail with a rod to elicit an escape response, which was filmed from
119 above using a camera (Exilim EX-ZR200; Casio, USA) filming at 30 frames s^{-1}. Three
120 escape responses were filmed for each fish and videos were analysed in Tracker Video
121 Analysis and Modeling Tool Software 4.01 (Open Source Physics,
122 www.opensourcephysics.org). From the 3 responses the fastest speed was used as the
123 maximum sprint speed.

124 Sustained swimming speed (22, 36) was measured immediately after sprint speed.
125 Fish were swum in Blazka-type cylindrical flumes (26 mm diameter x 150 mm length),
126 fitted over the intake end of a cylindrical submersible pump (12V DC, iL500; Rule,
127 Hertfordshire, UK). Water flow inside the flume was controlled by changing the voltage
128 output to the pumps from DC-regulated power supplies (MP3090; Powertech, Sydney,
129 Australia), and changes in water flow were determined in real-time with flow meters
130 (DigiFlow 6710M; Savant Electronics, Taiwan) connected to each pump. Bundles of straws
131 at the intake end of the flume were used to maintain laminar flow, and a plastic grid at the
132 other end prevented fish from being sucked into the pump. Sustained speed was determined
133 as the critical sustained swimming speed, U_{crit} (36) at 23°C (± 0.5°C). Fish swam at an
134 initial water flow rate of 0.1 m s^{-1} for 600 s. Flow was increased incrementally by 0.06 m s^{-1}
135 (U_i) every 600 seconds (T_i) until fish could no longer maintain their position in the water
136 column and began resting against the back of the flume. Fish were given two additional
137 attempts, where water flow was ceased for 10 seconds and then gradually increased back to

138 the maximal speed achieved. After the third attempt, the time swum at the final speed (T_f)

139 and the final speed (U_f) were recorded and used to calculate U_{crit} (m s^{-1}) = $U_f + (T_f/T_i)*U_i$

140 (16).

141

142 *Enzyme assays*

143 Immediately after measuring sprint and sustained speed, fish were anesthetized in

144 buffered ethyl 3-aminobenzoate methanesulfonate (MS222; 0.3 g l^{-1}; Sigma-Aldrich, Castle

145 Hill, Australia) and euthanized by decapitation. Back and tail skeletal muscle was

146 extracted, skinned, and halved along the axis of symmetry. Both lateral portions were

147 immediately transferred to liquid nitrogen and stored at -80°C. One portion was used for

148 measurements of metabolic enzyme activities and myosin heavy chain (MHC) protein

149 concentrations, and the other for sarco/endoplasmic reticulum ATPase (SERCA) analysis.

150 Muscle samples used for measurements of metabolic enzyme activities and MHC

151 protein concentrations were homogenized (in a TissueLyser LT; Qiagen, Venlo,

152 Netherlands) in 9 volumes RIPA buffer (20mM TrisCl pH 7.5, 150 mM NaCl, 1 mM

153 EDTA, 1 mM EGTA, 1% NP40, 1% sodium deoxycholate) and protease inhibitor cocktail

154 (cƟmplete, EDTA-free; Roche Life Sciences, Germany) solution. Homogenate was either

155 further diluted by a factor 10 (to a final 1:100 dilution) for metabolic enzyme assays or

156 aliquoted and stored at -20°C for MHC protein analysis. Citrate synthase (CS) and lactate

157 dehydrogenase (LDH) are regulatory enzymes in aerobic and anaerobic metabolism,

158 respectively, and these enzymes were chosen because of their relevance for differentiating

159 between fiber types (35). Following published protocols (39), enzyme activities were

160 determined using a UV/visible spectrophotometer (Ultrospec 2100 Pro; Biochrom, UK)

161 with a temperature controlled cuvette holder. Assays were performed in duplicate at 23°C.

162 Muscle samples used for SERCA assays were homogenized (in a TissueLyser LT;
163 Qiagen, Venlo, Netherlands) in nine parts homogenization buffer (250 mM sucrose, 5 mM
164 EDTA and 20 mM imidazole, pH 7.2). Under constant vortexing, 0.1% sodium
165 deoxycholate dissolved in homogenization buffer was added to muscle tissue homogenate
166 in equivalent amounts (w/v). Sodium deoxycholate-treated homogenates were pre-
167 incubated for 10 minutes at 23°C in assay medium (25 mM imidazole, 0.2 mM, $CaCl_2$, 80
168 mM KCl, 5 mM $MgCl_2$) with and without 10 μM of thapsigargin, a specific inhibitor of
169 SERCA (34). Following a published protocol (42), the activity of SERCA was determined
170 using a UV/visible spectrophotometer (Ultrospec 2100 Pro; Biochrom, UK). Assays were
171 performed in duplicate. SERCA activity was measured by quantifying the liberation of
172 inorganic phosphate. The assay was initiated by the addition of 3 mM ATP to
173 homogenates, left to incubate for 5 minutes at 23°C, and the reaction was stopped by the
174 addition 0.4 M perchloric acid. The final solution was centrifuged (1200 *g,* 15 minutes at
175 4°C) and the supernatant was added to 1 part color reagent (8 mM ammonium molybdate,
176 335 mM concentrated H_2SO_4, 145 mM $FeSO_4$) to quantify the amount of inorganic
177 phosphate relative to a standard curve; the standard curve was determined with known
178 concentrations of PO_4 (25-250 nM). After 10 minutes of color formation absorbance was
179 read at 750 nm. SERCA activity (μmol of product $mins^{-1}$ g^{-1} of wet tissue) was calculated as
180 the difference in inorganic phosphate liberated in the presence and absence of thapsigargin.
181
182 *Myosin heavy chain protein concentrations*
183 The identification and quantification of slow and fast MHC isoforms, and acetylated
184 histone 3 lysine 9 (acetyl-H3K9) were performed by capillary electrophoresis in a “Wes”
185 Simple Western System (ProteinSimple, CA, USA) following the manufacturer's

186 instructions. Antibodies (from Developmental Studies Hybridoma Bank, University of
187 Iowa, USA) we used were EB165 (Developmental Studies Hybridoma Bank, University of
188 Iowa, USA) to determine fast MHC concentrations, BA-F8 (Developmental Studies
189 Hybridoma Bank, University of Iowa, USA) to determine slow MHC concentrations,
190 C5B11 (Cell Signaling, USA) to determine acetyl-H3K9, and 12G10 (α-tubulin;
191 Developmental Studies Hybridoma Bank, University of Iowa, USA) as internal control.
192 Before protein assays, the concentrations of protein extracts were determined using a
193 bicinchoninic acid assay kit (Sigma-Aldrich, Castle Hill, Australia) following
194 manufacturer's instructions.
195
196 *Experiment 1: differences between high and low locomotor performers*
197 Sprint and sustained speeds were determined in 48 fish. Based on differences in
198 locomotor performance between individuals, we selected the 15 fastest (high performance)
199 and 15 slowest (low performance) fish to make up different performance groups. Fish were
200 grouped according to sprint and sustained swimming performance separately. We used all
201 15 fish within each performance group to determine differences in sprint speed, sustained
202 speed, CS activity, and LDH activity, but we used 10 and 6 fish from each group for
203 comparisons of SERCA activity and MHC concentrations between groups, respectively.
204
205 *Pilot study: exercise training time course*
206 Before conducting Experiment 2, we conducted two pilot studies to determine,
207 firstly, the time course over which an exercise training response was established and,
208 secondly, whether the exercise training effect was diminished when fish were kept
209 sedentary for 48 hours, which was necessary for drug treatments (see below). To determine

210 the time course over which an exercise training effect was established, 60 fish were
211 randomly split into 2 treatments: an exercise-trained group and a control group (30 fish per
212 treatment). Both treatments followed the exercise and control protocols described for
213 Experiment 2 below. The sustained speed of 10 exercise-trained fish was compared to that
214 of 10 control fish at the end of each week for 3 weeks of exercise training.

215 To determine whether the exercise training response diminished when fish were
216 kept sedentary for 48 hours, 30 fish were split into 2 treatments: an exercise-trained group
217 (20 fish), and a control group (10 fish). For 3 weeks, both treatments followed the exercise
218 protocols described for Experiment 2 below. After 3 weeks, the exercise-trained group was
219 split into 2 treatments: a group that continued exercise training, and a group in which fish
220 were kept sedentary in 1 litre containers in pairs for 48 hours, which was similar to the
221 conditions during drug treatments (see below). The sustained speed was compared between
222 fish from each of the 3 treatments.

223

224 *Experiment 2: Exercise training and histone deacetylase activity*

225 72 fish were randomly split into an aerobic exercise training treatment and a control
226 treatment (36 fish per treatment). Fish from both treatments were dispersed across four
227 treatment tanks in groups of nine fish, and kept at 23°C (± 0.5°C). Both control and
228 exercise tanks (645 x 423 x 276 mm) were identical, except that there was no water flow in
229 the control treatment (Fig. 1A). Within the tanks, fish were contained within an area (120
230 mm width) along the long end of the tank by a partition comprised of a plastic island piece,
231 and fish were prevented from leaving this rectangular area by fabric netting (Fig. 1A). Each
232 tank contained submersible pumps (2 x 5W, SP-900; Resun, China, and 1 x 24W, JHQ-
233 2000; Sunsun, China) at the opposite side of the tank to the area containing fish. The output

234 from the pumps was directed to the area containing fish by a plastic sheet, and we mounted
235 rounded plastic pieces in the corners to reduce turbulence (Fig. 1A). Before introducing
236 fish, we measured flow rates within the areas containing fish using red dye and a camera
237 (Exilim EX-ZR200; Casio, USA) filming at 30 frames s^{-1} to ascertain that we could achieve
238 the desired treatment flows.

239 The exercise training protocol consisted of exposing fish three times daily to a water
240 flow of 21 cm s^{-1} (~7 BL s^{-1}) for 6 days a week. During the first week, each exercise
241 training interval lasted 1 hour and intervals were separated by 3 hour rest periods. Exercise
242 training intervals were incrementally increased by 0.5 hours each week to a final of 2.5
243 hours during the fourth week, while rest intervals were reduced by 0.5 hours each week to a
244 final 1.5 hours. Outside training intervals, the water flow was 8 cm s^{-1} (2-3 BL s^{-1}), except
245 during daily feeding (15 minutes). This regimen represented aerobic exercise with moderate
246 intensity (25).

247 After completing the four week training, histone deacetylation was reduced
248 pharmacologically by administering the HDAC class I & II (members: HDAC1-10)
249 specific inhibitor Trichostatin A (TSA; Sigma-Aldrich, Castle Hill, Australia) dissolved in
250 dimethyl sulfoxide (DMSO; Sigma-Aldrich, Castle Hill, Australia). Both exercise-trained
251 and control fish were randomly split into 3 treatments (12 fish each): TSA, DMSO only,
252 and No drug. Fish in each treatment were kept in pairs in 1 liter containers at 23°C (±
253 0.5°C) over 48 hours. No drug fish were not exposed to drugs, DMSO treated fish were
254 kept in a 0.025% DMSO solution, and TSA treated fish were kept in a 500 nM solution of
255 TSA dissolved in DMSO at a final concentration of 0.025%.

256 We conducted preliminary experiments with zebrafish not used elsewhere in the
257 study to determine the efficacy of delivering TSA dissolved in tank water. We conducted

258 three treatments (N = 9 fish each): a) TSA dissolved in DMSO (0.025%) to give a final
259 concentration of 100 nM TSA in tank water; b) 500 nM concentration of TSA in tank
260 water, and c) DMSO dissolved in tank water at 0.025% final concentration (0.25 ml l^{-1}).
261 After two days in the TSA treatments, fish were euthanized and muscle tissue was dissected
262 to determine activity of class I and II histone deacetylases (HDAC). We determined HDAC
263 activity with a commercial kit (catalogue #566328, Merck Millipore, USA) and following
264 the manufacturer's instructions. There was a significant decrease in HDAC activity (one-
265 way ANOVA followed by Tukey post hoc tests $F_{2,24} = 4.03$, $p = 0.029$) following the 500
266 nM TSA treatment (Fig. 1B). Hence, in the experiments below we exposed fish for two
267 days to 500 nM TSA in tank water to test the effect of histone acetylation. A 48 hour period
268 for treatment with TSA was chosen, because this is sufficient to alter protein concentrations
269 assuming a mean protein degradation rate of 2.2% per hour (33). Immediately after the 48 h
270 drug treatment, the sprint and sustained speeds were determined for each fish, and fish were
271 euthanized for tissue collection.

272

273 *Statistical analysis*

274 For Experiment 1, two tailed, independent sample t-tests were used to compare
275 sprint speed, sustained speed, CS activity, LDH activity, SERCA activity, and MHC
276 concentrations and ratios between high and low performers.

277 We analysed data from the first pilot study with a two-way ANOVA to test for
278 differences in sustained speed between exercise-trained and control fish (factor 1) after
279 each week for 3 weeks of exercise training (factor 2); note that independent fish were used
280 to measure swimming performance at each time point. Data from the second pilot study

was analysed 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 hours. Here and below, we used Tukey's HSD post hoc test to determine differences between means.

We analysed 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 analysed 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 to 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

305 sprint speed ($t = 1.13$, $p = 0.267$, Fig. 2E; $t = 1.20$ $p = 0.241$, Fig. 2F, respectively). SERCA
306 activity was higher in high performers than in low performers with respect to both
307 sustained and sprint speed ($t = 4.12$, $p = 0.003$, Fig. 2G; $t = 3.11$, $p = 0.024$, Fig. 2H,
308 respectively).

309 The concentration of slow MHC isoforms relative to α-tubulin did not differ
310 significantly between high and low sustained performance groups ($t = 0.27$, $p = 0.792$, Fig.
311 3A). In contrast, low sprint performers had higher slow MHC concentrations compared to
312 high sprint performers ($t = 3.07$, $p = 0.012$, Fig. 3B). Fast MHC concentrations did not
313 differ significantly between low and higher performers with respect to either sustained ($t =$
314 1.25, $p = 0.241$, Fig. 3C) or sprint ($t = 0.03$, $p = 0.982$; Fig. 6D) speed. The ratio between
315 slow:fast isoform concentrations was significantly greater in high compared to low
316 sustained performers ($t = 4.83$, $p < 0.001$, Fig. 3E). Conversely, the slow:fast MHC ratio
317 was significantly higher in low sprint performers compared to high sprint performers ($t =$
318 2.25, $p = 0.048$, Fig. 3F).

319

320 *Exercise training time course (Pilot data)*

321 Exercise training for longer than one week increased sustained swimming speed
322 compared to controls (main effect of exercise training $F_{1, 54} = 18.78$, $p < 0.001$, Fig. 4A),
323 and increasing the length of training did not have any further significant effect (main effect
324 of time $F_{2, 54} = 0.72$, $p = 0.492$, Fig. 4A). Exercise training followed by a 48 h sedentary
325 period did not decrease swimming performance significantly compared to fish that were
326 exercised without the sedentary period, and swimming performance of both groups of
327 trained fish (exercise only and exercise followed by 48 h being sedentary) was significantly
328 greater than that of control fish ($F_{2, 27} = 9.97$, $p < 0.001$, Fig. 4B).

329

330 *Effects of exercise training and histone deacetylase activity (Experiment 2)*

331 Sustained speed (Fig. 5A), but not sprint speed (Fig. 5B), was higher in exercise-
332 trained fish than control fish. There was an interaction between exercise training and drug
333 treatment in determining sustained (interaction $F_{2, 66} = 3.20$, $p = 0.047$, Fig. 5A) and sprint
334 (interaction $F_{2, 66} = 3.18$, $p = 0.048$, Fig. 5B) speed; TSA reduced both in control fish but it
335 did not have a significant effect on exercise-trained fish. DMSO had no significant effect
336 on sustained or sprint speed, and DMSO treated fish did not differ significantly from no-
337 drug controls for any response variable. For clarity, we therefore did not show responses of
338 DMSO treated fish here or below.

339 Exercise training increased CS activity (main effect $F_{1, 66} = 23.09$, $p < 0.001$, Fig.
340 5C) but not LDH activity (main effect $F_{1, 66} = 1.19$, $p = 0.279$, Fig. 5D). TSA had no
341 significant effect on CS or LDH activities (main effects $F_{2, 66} = 0.08$, $p = 0.923$, and $F_{2, 66} =$
342 0.05, $p = 0.951$, respectively). SERCA activity was higher in exercise-trained fish (main
343 effect $F_{1, 66} = 9.93$, $p = 0.002$, Fig. 5E), and TSA reduced SERCA activity significantly
344 (main effect $F_{1, 66} = 3.46$, $p = 0.037$).

345 There was an interaction between exercise training and drug treatment in
346 determining the concentration of slow MHC (interaction $F_{1, 20} = 4.57$, $p = 0.045$, Fig. 6A)
347 as well as the slow:fast MHC ratio (interaction $F_{1, 20} = 9.31$, $p = 0.006$, Fig. 6C), and TSA
348 increased both in control fish but it had no significant effect on exercise-trained fish.
349 Neither exercise training (main effect $F_{1, 20} = 3.84$, $p = 0.064$) nor drug treatment (main
350 effect $F_{1, 20} = 0.71$, $p = 0.409$) affected the concentration of fast MHC (Fig. 6B). TSA, but
351 not exercise training, significantly increased H3K9 acetylation (main effects $F_{1, 20} = 19.87$,
352 $p < 0.001$, and $F_{1, 20} = 1.69$, $p = 0.209$, respectively; Fig 6E).

353

354 **Discussion**

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

365 As predicted, CS activity, an indicator of mitochondrial capacity (23), was a good
366 predictor of sustained performance. Additionally, we found that sustained performance was
367 facilitated by an aerobically poised myosin heavy chain composition. Slow MHCs have
368 lower ATPase activity and lower force production, which makes them optimal for low
369 intensity contractions (14). These results indicate that fatigue resistance is facilitated both
370 by a decrease in ATP demand and an increase in ATP production via aerobic metabolism.
371 We found that anaerobic metabolic capacity, measured by lactate dehydrogenase activity
372 (LDH), did not enhance sprint performance significantly, which corroborates earlier results
373 (38). Low sprint performance was associated with higher aerobic metabolic capacities and
374 slow MHC concentrations, which indicates that higher sprint performance is mediated by a
375 reduction in the capacity for sustained performance rather than an increase in the capacity
376 for sprint performance, thereby shifting the ratio of slow:fast muscle fibers. However,

377 sprint performance was not reduced by exercise training-induced increases in CS activity
378 and slow MHC expression, perhaps due to concomitant increases in SERCA activity and an
379 indication that fast MHC expression was increased (insignificant: $p = 0.064$). Contrary to a
380 previous study on zebrafish (27), we found no increase in LDH activity following exercise
381 training. An increase in LDH activity is likely due to the more anaerobically poised
382 exercise training speed which the previous study employed (12 BL s^{-1} compared to our 7
383 BL s^{-1})(43).

384 During muscle contraction, nerve signals stimulate voltage gated calcium channels,
385 dihydropyridine receptors, to release calcium into the cell. Dihydropyridine receptors also
386 stimulate ryanodine receptors (RyR) to release additional calcium stores from the
387 sarcoplasmic reticulum (SR) (1). The resultant calcium signal acts on troponin and causes
388 myosin and actin to interact, thereby leading to muscle contraction. Muscle relaxation is
389 facilitated by the activity of SERCA that pumps calcium back into the SR (1). The
390 capacities of different calcium cycling steps are often linked to enhancing either sustained
391 or sprint performance. For example, reducing the activity of RyR caused a decrease in
392 sprint performance but an increase in the fatigue resistance of isolated carp muscle (37).
393 The reduced sprint performance could be explained by a reduction in free calcium leading
394 to fewer actin-myosin cross-bridges and thereby reduced muscle force production, while
395 enhanced fatigue resistance may result from reduced depletion of calcium stores in the SR.
396 We found that increased SERCA activity facilitated both sprint and sustained performance.
397 Similarly, zebrafish with high sprint and sustained performance expressed more SERCA
398 mRNA than low performers (38). Additionally, the inhibition of SERCA in rat muscle
399 decreased fatigue resistance (21). Perhaps SERCA is important to both locomotor modes
400 because it enables the high relaxation rates that are associated with sprint performance, and

401 replenishes calcium stores for subsequent contractions to promote fatigue resistance. As
402 further support for this suggestion, the overexpression of parvalbumin, a calcium binding
403 protein that increases the efficiency of SERCA, increased both sprint and sustained
404 performance (38).

405 The transcriptional regulators that mediate aerobic exercise-induced changes have
406 not been fully elucidated (9, 14). However, class IIa HDACs (HDAC 4, 5, 7, and 9) are of
407 particular interest, because of their repressive effects on the transcriptional activity of the
408 myocyte enhancer factor-2 (MEF2) (29). MEF2 is a transcription factor that regulates
409 muscle fiber differentiation and development by inducing expression of mitochondrial
410 proteins and slow MHC in cardiac and skeletal muscle (6). We have shown that inhibiting
411 class I & II HDACs with trichostatin A (TSA) caused an increase in the expression of slow
412 MHCs in control fish, but had no effect on the expression of slow MHCs in exercise-
413 trained fish. Increased expression of slow MHCs may be due to TSA relieving the
414 suppressive effect that HDACs have on MEF2. Post-exercise, there was no reduction in the
415 concentration of HDAC 4 or 5 directly, but there was an increase in histone 3 acetylation at
416 lysine 36 in humans (28). This result may be explained by AMPK and CaMK-mediated
417 phosphorylation of class IIa HDACs, which allowed chaperone proteins to bind and export
418 HDAC 4 and 5 from the nucleus (6, 28). Hence, aerobic exercise may cause the nuclear
419 export of HDACs via the activation of AMPK and CaMK pathways. Inhibiting class I and
420 II HDACs in exercise-trained fish did not further increase slow MHC expression, possibly
421 because exercise training had already caused nuclear export of HDAC 4 and 5, thereby
422 permitting MEF2 dependent transcription. TSA treatment, but not exercise, increased
423 histone 3 acetylation at lysine 9 (H3K9). Similarly, treatment with a specific class IIa
424 HDAC inhibitor increased H3K9 acetylation (12), while exercise did not alter acetylation at

425 lysine 9 (28). TSA-mediated increase in the expression of slow MHCs in control fish was
426 not accompanied by increases in CS activity, which may be because CS has a longer half-
427 life and slower expression rate than the 48 hour period over which we inhibited HDACs.
428 For example, the half-life of cytochrome c, another mitochondrial protein, is 6-8 days (2).
429 Additionally, the TSA-mediated increase in slow MHCs of control fish was not paralleled
430 by increased sustained performance. In fact, inhibition of HDACs decreased both sprint and
431 sustained performance in control fish, but had no effect on the performance of exercise-
432 trained fish. The reduction in sprint and sustained performance may be due to the reduction
433 in SERCA activity that resulted from inhibiting HDACs. SERCA activity is important to
434 both locomotor modes, and a reduction in SERCA activity could be the result of reduced
435 SERCA expression, or an increase in the expression and activity of inhibitory proteins,
436 such as phospholamban (15).
437
438 *Perspectives and significance*
439 We have shown that inhibiting class I and II HDACs mediated increases in slow
440 MHC expression, which resembled the effects of aerobic exercise. However, inhibiting
441 class I and II HDACs also reduced SERCA activity and locomotor performance, and the
442 parallel changes in SERCA activity and locomotion suggest a casual relationship. Aerobic
443 exercise must therefore activate a pathway that leads to increases in SERCA activity not
444 mediated by class I and II HDACs. For example, HDACs may inhibit the expression of
445 proteases that can degrade SERCA, while aerobic exercise training increases the expression
446 of protease inhibitors such as calpastatin (43) and these relationships should be tested
447 experimentally. It is likely, however, that the exercise training response is mediated by
448 specific class IIa HDAC isoforms that only regulate slow MHC expression and not SERCA

449 activity. TSA inhibits all HDAC class I and II isoforms, and further studies should employ
450 isoform-specific inhibitors to determine which HDAC isoforms are responsible for
451 regulating slow MHC expression and SERCA activity. Understanding the complexities of
452 the so-called "histone code" will be one of the greatest challenges in biology, and is likely
453 to have future medical and ecological significance. We have identified class I & II HDACs
454 as transcriptional regulators of slow MHC expression and SERCA activity, and the
455 regulation of biochemical traits by class I & II HDACs could be a mechanism underlying
456 the adaptation of locomotor performance to different environmental conditions.
457 Additionally, there is therapeutic potential in manipulating specific HDAC isoform activity
458 to induce the expression slow fiber proteins to counteract muscular diseases such as
459 dystrophy, or lifestyle-related diseases such as type 2 diabetes.
460

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463

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465 No conflicts of interest, financial or otherwise, are declared by the author(s).

466 **References**
467 1. **Berchtold MW**, **Brinkmeier H**, **Müntener M**. Calcium ion in skeletal muscle: its
468 crucial role for muscle function, plasticity, and disease. *Physiol Rev* 80: 1215–1265,
469 2000.
470 2. **Booth FW**, **Holloszy JO**. Cytochrome-c turnover in rat skeletal-muscles. *J Biol*
471 *Chem* 252: 416–419, 1977.

472 3. **Booth FW**, **Roberts CD**, **Laye MJ**. Lack of exercise is a major cause of chronic
473 diseases. *Compr Physiol* 2: 1143–1211, 2012.
474 4. **Chin ER**. Intracellular Ca^{2+} signalling in skeletal muscle: decoding a complex
475 message. *Ex Sport Sci Rev* 38: 76–85, 2010.
476 5. **Clobert J**, **Oppliger A**, **Sorci G**, **Ernande B**. Trade-offs in phenotypic traits:
477 endurance at birth, growth, survival, predation and susceptibility to parasitism in a
478 lizard, *Lacerta vivipara*. *Funct Ecol* 14: 675–684, 2000.
479 6. **Cohen TJ**, **Choi M-C**, **Kapur M**, **Lira VA**, **Yan Z**, **Yao T-P**. HDAC4 regulates
480 muscle fiber type-specific gene expression programs. *Mol Cells* 38: 343–348, 2015.
481 7. **ENCODE Project Consortium**. An integrated encyclopedia of DNA elements in
482 the human genome. *Nature* 488: 57–74, 2012.
483 8. **Egan B**, **Carson BP**, **Garcia-Roves PM**, **Chibalin AV**, **Sarsfield FM**, **Barron N**,
484 **McCaffrey N**, **Moyna NM**, **Zierath JR**, **O'Gorman DJ**. Exercise intensity-
485 dependent regulation of peroxisome proliferator-activated receptor coactivator-1
486 mRNA abundance is associated with differential activation of upstream signalling
487 kinases in human skeletal muscle. *J Physiol (Lond)* 588: 1779–1790, 2010.
488 9. **Egan B**, **Zierath JR**. Exercise metabolism and the molecular regulation of skeletal
489 muscle adaptation. *Cell Metab* 17: 162–184, 2013.
490 10. **Fischle W**, **Dequiedt F**, **Hendzel MJ**, **Guenther MG**, **Lazar MA**, **Voelter W**,
491 **Verdin E**. Enzymatic activity associated with class II HDACs is dependent on a
492 multiprotein complex containing HDAC3 and SMRT/N-CoR. *Mol Cell* 9: 45–57,
493 2002.
494 11. **Fry CS**, **Noehren B**, **Mula J**, **Ubele MF**, **Westgate PM**, **Kern PA**, **Peterson CA**.

495 Fibre type-specific satellite cell response to aerobic training in sedentary adults. *J*
496 *Physiol (Lond)* 592: 2625–2635, 2014.
497 12. **Gaur V**, **Connor T**, **Sanigorski A**, **Martin SD**, **Bruce CR**, **Henstridge DC**, **Bond**
498 **ST**, **McEwen KA**, **Kerr-Bayles L**, **Ashton TD**, **Fleming C**, **Wu M**, **Pike Winer**
499 **LS**, **Chen D**, **Hudson GM**, **Schwabe JWR**, **Baar K**, **Febbraio MA**, **Gregorevic P**,
500 **Pfeffer FM**, **Walder KR**, **Hargreaves M**, **McGee SL**. Disruption of the class IIa
501 HDAC corepressor complex increases energy expenditure and lipid oxidation. *Cell*
502 *Reports* 16: 2802–2810, 2016.
503 13. **Gräff J**, **Tsai L-H**. Histone acetylation: molecular mnemonics on the chromatin. *Nat*
504 *Rev Neurosci* 14: 97–111, 2013.
505 14. **Gundersen K**. Excitation-transcription coupling in skeletal muscle: the molecular
506 pathways of exercise. *Biol Rev* 86: 564–600, 2011.
507 15. **Gustavsson M**, **Traaseth NJ**, **Karim CB**, **Lockamy EL**, **Thomas DD**, **Veglia G**.
508 Lipid-mediated folding/unfolding of phospholamban as a regulatory mechanism for
509 the sarcoplasmic reticulum Ca^{2+}-ATPase. *J Mol Biol* 408: 755–765, 2011.
510 16. **Hammill E**, **Wilson RS**, **Johnston IA**. Sustained swimming performance and
511 muscle structure are altered by thermal acclimation in male mosquitofish. *J Therm*
512 *Biol* 29: 251–257, 2004.
513 17. **Hood DA**. Contractile activity-induced mitochondrial biogenesis in skeletal muscle.
514 *J Appl Physiol* 90: 1137–1157, 2001.
515 18. **Husak JF**, **Fox SF**, **Lovern MB**, **Van Den Bussche RA**. Faster lizards sire more
516 offspring: sexual selection on whole-animal performance. *Evolution* 60: 2122–2130,
517 2006.
518 19. **Husak JF**. Does survival depend on how fast you can run or how fast you do run?

519 *Funct Ecol* 20: 1080–1086, 2006.

520 20. **Irrcher I**, **Ljubicic V**, **Kirwan AF**, **Hood DA**. AMP-activated protein kinase-
521 regulated activation of the PGC-1α promoter in skeletal muscle cells. *PLoS ONE* 3:
522 e3614, 2008.

523 21. **James RS**, **Walter I**, **Seebacher F**. Variation in expression of calcium-handling
524 proteins is associated with inter-individual differences in mechanical performance of
525 rat (*Rattus norvegicus*) skeletal muscle. *J Exp Biol* 214: 3542–3548, 2011.

526 22. **Kolok A**. Interindividual variation in the prolonged locomotor performance of
527 ectothermic vertebrates: a comparison of fish and herpetofaunal methodologies and a
528 brief review of the recent fish literature. *Can J Fish Aquat Sci* 56: 700–710, 1999.

529 23. **Larsen S**, **Nielsen J**, **Hansen CN**, **Nielsen LB**, **Wibrand F**, **Stride N**, **Schroder**
530 **HD**, **Boushel R**, **Helge JW**, **Dela F**, **Hey-Mogensen M**. Biomarkers of
531 mitochondrial content in skeletal muscle of healthy young human subjects. *J Physiol*
532 *(Lond)* 590: 3349–3360, 2012.

533 24. **Le Galliard J-F**, **Clobert J**, **Ferrière R**. Physical performance and Darwinian
534 fitness in lizards. *Nature* 432: 502–505, 2004.

535 25. **LeMoine CMR**, **Craig PM**, **Dhekney K**, **Kim JJ**, **McClelland GB**. Temporal and
536 spatial patterns of gene expression in skeletal muscles in response to swim training
537 in adult zebrafish (*Danio rerio*). *J Comp Physiol B* 180: 151–160, 2010.

538 26. **Ljubicic V**, **Miura P**, **Burt M**, **Boudreault L**, **Khogali S**, **Lunde JA**, **Renaud JM**,
539 **Jasmin BJ**. Chronic AMPK activation evokes the slow, oxidative myogenic
540 program and triggers beneficial adaptations in mdx mouse skeletal muscle. *Human*
541 *Mol Gen* 20: 3478–3493, 2011.

542 27. **McClelland GB**, **Craig PM**, **Dhekney K**, **Dipardo S**. Temperature- and exercise-

543 induced gene expression and metabolic enzyme changes in skeletal muscle of adult
544 zebrafish (*Danio rerio*). *J Physiol (Lond)* 577: 739–751, 2006.
545 28. **McGee SL**, **Fairlie E**, **Garnham AP**, **Hargreaves M**. Exercise-induced histone
546 modifications in human skeletal muscle. *J Physiol (Lond)* 587: 5951–5958, 2009.
547 29. **McGee SL**, **Hargreaves M**. Exercise and myocyte enhancer factor 2 regulation in
548 human skeletal muscle. *Diabetes* 53: 1208–1214, 2004.
549 30. **McGee SL**, **Hargreaves M**. Histone modifications and exercise adaptations. *J Appl*
550 *Physiol* 110: 258–263, 2011.
551 31. **Mihaylova MM**, **Shaw RJ**. Metabolic reprogramming by class I and II histone
552 deacetylases. *Trends Endocrinol Metab* 24: 48–57, 2013.
553 32. **O'Neill HM**, **Holloway GP**, **Steinberg GR**. AMPK regulation of fatty acid
554 metabolism and mitochondrial biogenesis: Implications for obesity. *Mol Cell*
555 *Endocrinol* 366: 135–151, 2013.
556 33. **Pratt JM**, **Petty J**, **Riba-Garcia I**, **Robertson DHL**, **Gaskell SJ**, **Oliver SG**,
557 **Beynon RJ**. Dynamics of protein turnover, a missing dimension in proteomics. *Mol*
558 *Cell Proteomics* 1: 579–591, 2002.
559 34. **Ramnanan CJ**, **McMullen DC**, **Bielecki A**, **Storey KB**. Regulation of
560 sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA) in turtle muscle and liver during
561 acute exposure to anoxia. *J Exp Biol* 213: 17–25, 2009.
562 35. **Schiaffino S**, **Reggiani C**. Fiber types in mammalian skeletal muscles. *Physiol Rev*
563 91: 1447–1531, 2011.
564 36. **Seebacher F**, **Ducret V**, **Little AG**, **Adriaenssens B**. Generalist–specialist trade-off
565 during thermal acclimation. *R Soc Open Sci* 2: 140251, 2015.
566 37. **Seebacher F**, **Pollard SR**, **James RS**. How well do muscle biomechanics predict

567 whole-animal locomotor performance? The role of Ca^{2+} handling. *J Exp Biol* 215:
568 1847–1853, 2012.
569 38. **Seebacher F**, **Walter I**. Differences in locomotor performance between individuals:
570 importance of parvalbumin, calcium handling and metabolism. *J Exp Biol* 215: 663–
571 670, 2012.
572 39. **Seebacher F, Guderley H, Elsey, RM, Trosclair PL III.** Seasonal acclimatisation
573 of muscle metabolic enzymes in a reptile (*Alligator mississippiensis*). *J Exp Biol*
574 206: 1193–1200, 2003.
575 40. **van der Meulen T**, **Schipper H**, **van den Boogaart JGM**, **Huising MO**,
576 **Kranenbarg S**, **van Leeuwen JL**. Endurance exercise differentially stimulates heart
577 and axial muscle development in zebrafish (*Danio rerio*). *Am J Physiol Regul Integr*
578 *Comp Physiol* 291: R1040–8, 2006.
579 41. **Vina J**, **Rodriguez-Mañas L**, **Salvador-Pascual A**, **Tarazona-Santabalbina FJ**,
580 **Gomez-Cabrera MC**. Exercise: the lifelong supplement for healthy ageing and
581 slowing down the onset of frailty. *J Physiol (Lond)* 594: 1989–1999, 2016.
582 42. **Walter I**, **Seebacher F**. Endothermy in birds: underlying molecular mechanisms. *J*
583 *Exp Biol* 212: 2328–2336, 2009.
584 43. **Wang L**, **Duan L**, **Li X**, **Li G**. Acute-exercise-induced alterations in calpain and
585 calpastatin expression in rat muscle. *J Sport Rehab* 18: 213–228, 2009.
586 44. **Zaykin D**, **Zhivotovsky L**, **Westfall PH**, **Weir BS**. Truncated product method for
587 combining P-values. *Gen Epidemiol* 22: 170–185, 2002.
588
589

Figure captions

Figure 1 A schematic representation of the exercise training tank and TSA dose responses. In the exercise training tank (A)**,** a submersible pump (S.P.) provided water flow (blue arrows), which was directed towards 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 contained within the swimming area by fabric netting placed at either end. Rounded plastic corners (R.P.) reduced turbulence and helped maintain laminar flow. Histone deacetylase (HDAC) activity (B) decreased significantly in fish that were exposed to 500 nM trichostatin A (TSA) dissolved in tank water. Means ± s.e.m. are shown, and asterisks indicate significant differences (* $p < 0.05$; ** $p< 0.01$; *** $p < 0.001$).

Figure 2 Differences between high- and low-performing individuals. Sustained (A) and sprint (B) speed were significantly higher in high (black bars) than in low (grey bars) performing fish (N = 15 for each group; BL = body lengths). Citrate synthase (CS) activity (N = 15 per group) was greater in high sustained performers compared to 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, F). However, high sustained and sprint performers had greater sarco-endoplasmic reticulum ATPase (SERCA; N= 10 per group) activities compared to low performers (G, H). Means ± s.e.m. are shown, and asterisks indicate significant differences (* $p < 0.05$; ** $p< 0.01$; *** $p < 0.001$).

614 **Figure 3** Differences in myosin heavy chain (MHC) isoform concentrations between high
615 (black bars) and low (grey bars) locomotor performers. Concentration of slow MHC
616 (normalised to α-tubulin) did not differ significantly between high and low sustained
617 performers (A), but high sprint performers had significantly lower amounts of slow MHC
618 compared to low sprint performers (B). Fast MHC isoform concentrations did not differ
619 significantly between high and low sustained (C) and sprint (D) performers. However, the
620 ratio of slow:fast MHC isoform concentrations was significantly lower in low sustained
621 performers compared to high sustained performers (E), but the reverse was the case for
622 sprint performers (F). Representative protein bands of slow MHC, fast MHC, and α-
623 tubulin detected by capillary electrophoresis are shown for high and low sustained (G) and
624 sprint (H) performers. Means ± s.e.m. are shown (all $N = 6$ per group), and asterisks
625 indicate significant differences (* $p < 0.05$; ** $p< 0.01$; *** $p < 0.001$).
626
627 **Figure 4** Exercise training time course. One week of exercise training (Ex) increased
628 sustained swimming performance compared to non-exercised control fish (C), but
629 swimming speed did not increase further with an additional two weeks of training (A).
630 Sustained swimming performance (BL = body length) of fish that were exercise-trained for
631 two weeks and then kept sedentary for 48 h (ExS) did not differ significantly from that of
632 fish that were exercise trained for two week but not kept sedentary (Ex). Swimming
633 performance of control fish (C) that were not exercise-trained was significantly lower than
634 that of fish from either exercise training treatment (B). Means ± s.e.m. are shown (all N =
635 10 per group), and asterisks indicate significant differences (* $p < 0.05$; ** $p< 0.01$; *** p <
636 0.001).

637

638 **Figure 5** Effect of exercise and inhibition of histone deacetylases with trichostatin A (TSA)
639 on swimming performance and enzyme activities. Sustained (A) and sprint performance
640 (B) decreased with TSA treatment (grey bars) compared to no-drug controls (No drug,
641 clear bars), but the effect of TSA was more pronounced in non-exercised control fish (C)
642 compared to exercise-trained fish (Ex; Exercise*drug interaction). Exercise-trained fish had
643 greater citrate synthase (CS) activity compared to controls, but there was no effect of the
644 drug treatment (C). There was no significant effect of either exercise training or drug
645 treatment on lactate dehydrogenase activity (LDH; D). Sarco-endoplasmic reticulum
646 (SERCA) activity increased with exercise training (indicated by horizontal bars with
647 different letters), and it decreased with TSA treatment (E). Means ± s.e.m. are shown (all N
648 = 12 per group), and asterisks indicate significant differences (* $p < 0.05$; ** $p < 0.01$; *** p
649 < 0.001).

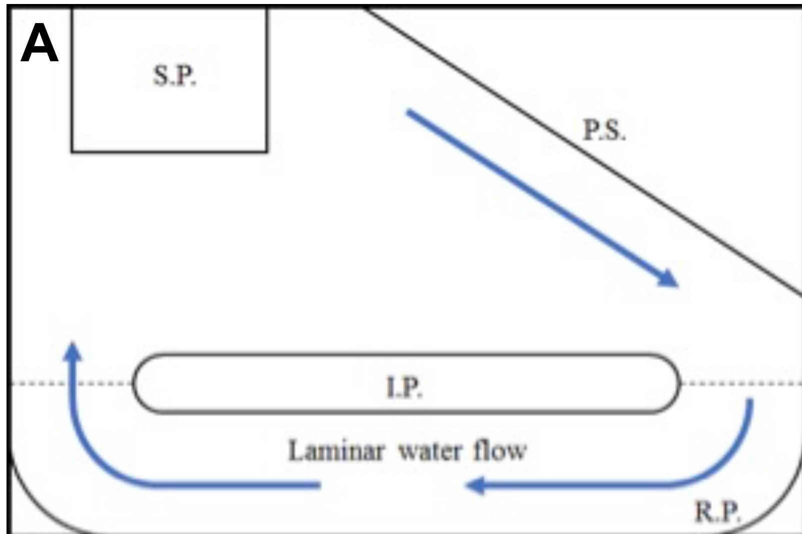
650

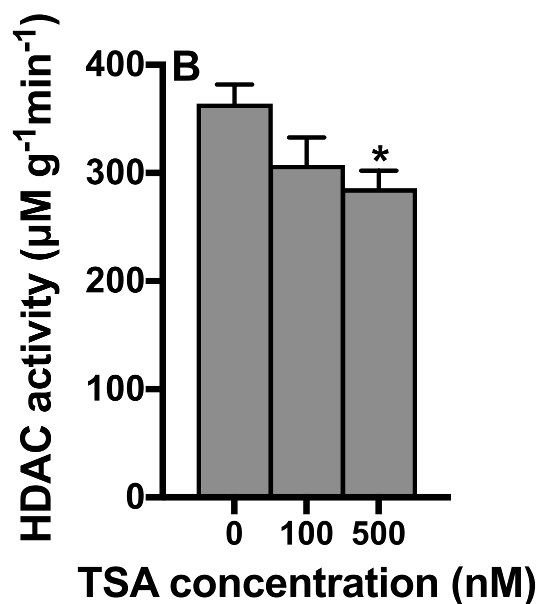
651 **Figure 6** Effect of exercise and inhibition of histone deacetylases with trichostatin A (TSA)
652 on myosin heavy chain (MHC) isoforms and histone 3 lysine 9 acetylation (acetyl-H3K9).
653 Compared to no-drug control treatments (clear bars), TSA (grey bars) increased slow MHC
654 in non-exercised control fish (C) but not in exercise-trained (Ex) fish (A; exercise*drug
655 interaction). There was no effect of either exercise training or drug treatment on fast MHC
656 (B). However, similar to slow MHC, the ratio of slow:fast MHC isoforms increased with
657 TSA treatment in non-exercised control fish (C; exercise*drug interaction). Representative
658 protein bands detected by capillary electrophoresis are shown for slow MHC, fast MHC,
659 and α-tubulin (D). TSA treatment increased acetyl-H3K9 in exercised-trained and control

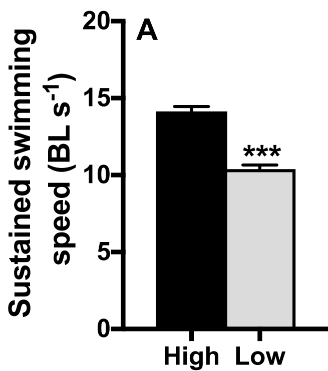
660 fish (E), and representative bands detected by capillary electrophoresis are shown (F).

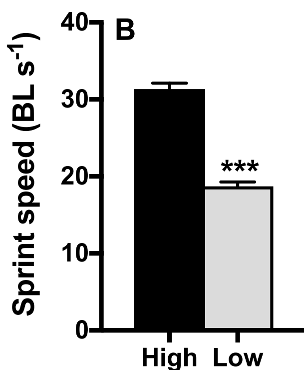
661 Means ± s.e.m. are shown (all N = 6 per group), and asterisks indicate significant

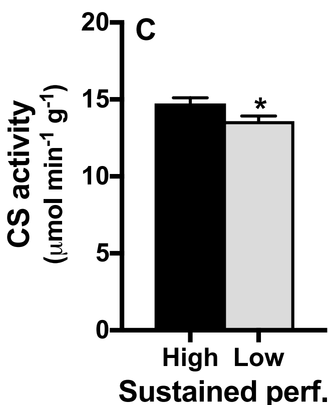
662 differences (* $p < 0.05$; ** $p< 0.01$; *** $p < 0.001$).

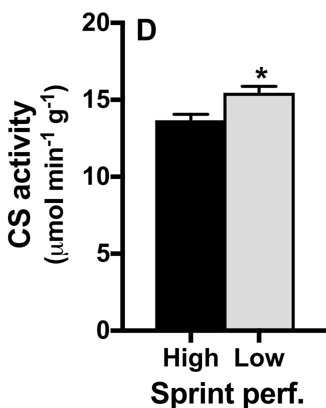

A
S.P.
P.S.
I.P.
Laminar water flow
R.P.
S.P. Submersible pumps
P.S. Plastic sheet
I.P. Plastic island piece
R.P. Rounded plastic
Water flow
Fabric netting

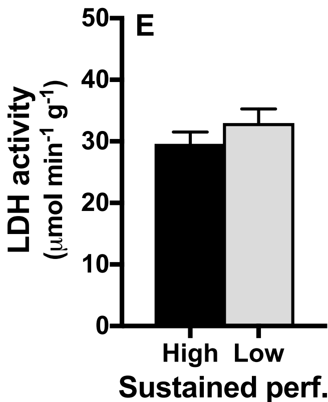

B
HDAC activity (μM g-1min-1)
400
300
200
100
0
*
0
100
500
TSA concentration (nM)

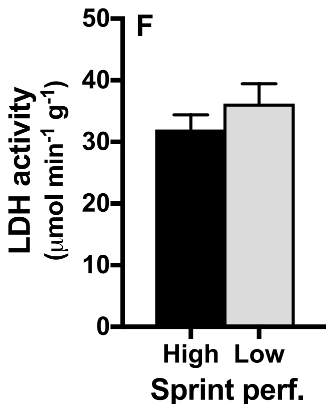

A
Sustained swimming speed (BL s-1)
20
15
10
5
0
***
High
Low

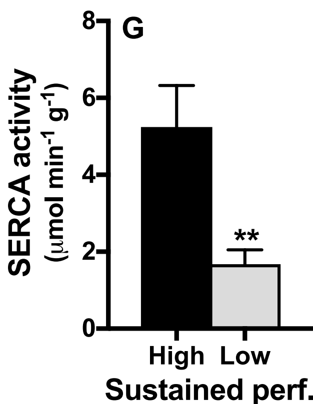

B
Sprint speed (BL s-1)
40
30
20
10
0
***
High
Low

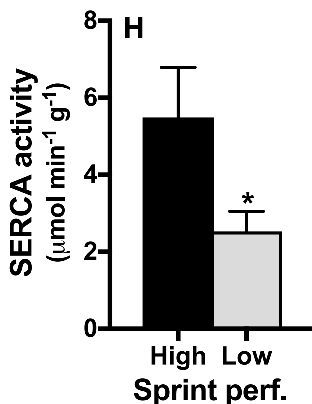

C
CS activity
(μmol min-1 g-1)
20
15
10
5
0
*
High
Low
Sustained perf.

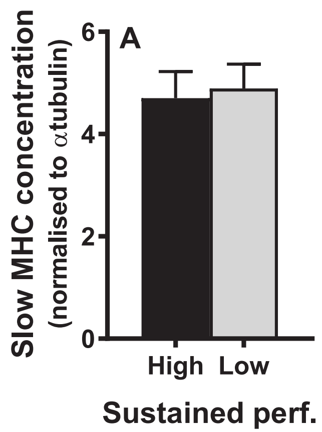

D
CS activity
(μmol min-1 g-1)
20
15
10
5
0
*
High
Low
Sprint perf.

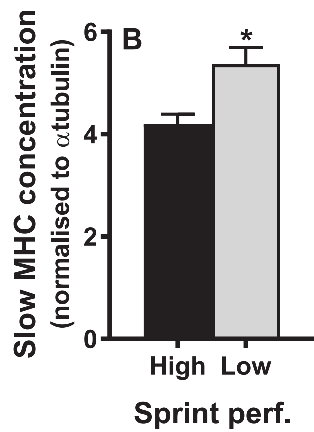

E
LDH activity
(μmol min-1 g-1)
50
40
30
20
10
0
High
Low
Sustained perf.

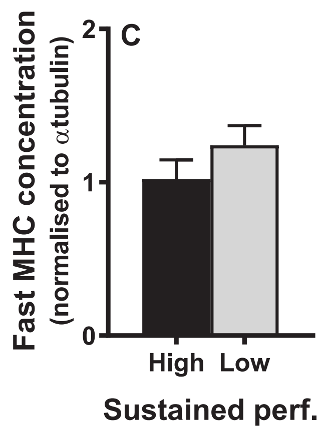

F
LDH activity
(μmol min-1 g-1)
50
40
30
20
10
0
High
Low
Sprint perf.

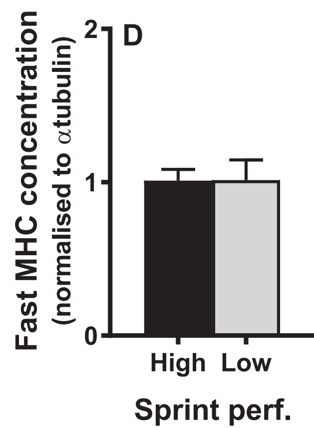

G
SERCA activity
(μmol min-1 g-1)
8
6
4
2
0
**
High
Low
Sustained perf.

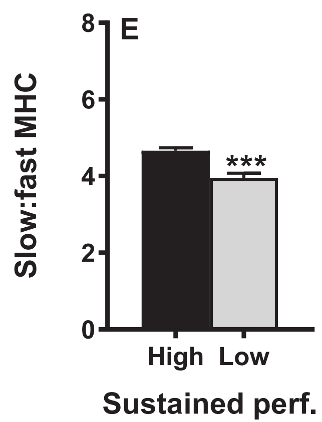

H
SERCA activity
(μmol min-1 g-1)
8
6
4
2
0
*
High
Low
Sprint perf.

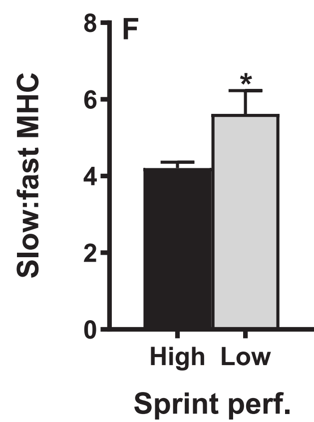

A
Slow MHC concentration
(normalised to αtubulin)
0
2
4
6
High
Low
Sustained perf.

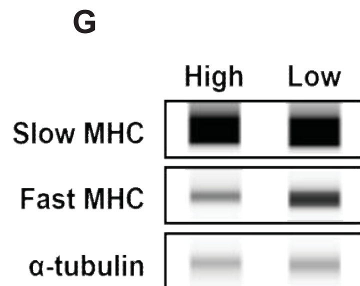

B
Slow MHC concentration
(normalised to αtubulin)
0
2
4
6
*
High
Low
Sprint perf.

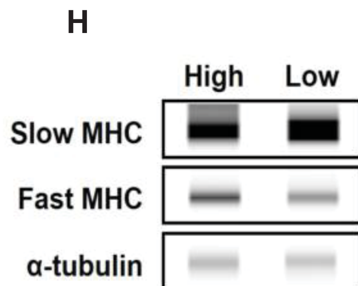

C
Fast MHC concentration
(normalised to αtubulin)
0
1
2
High
Low
Sustained perf.

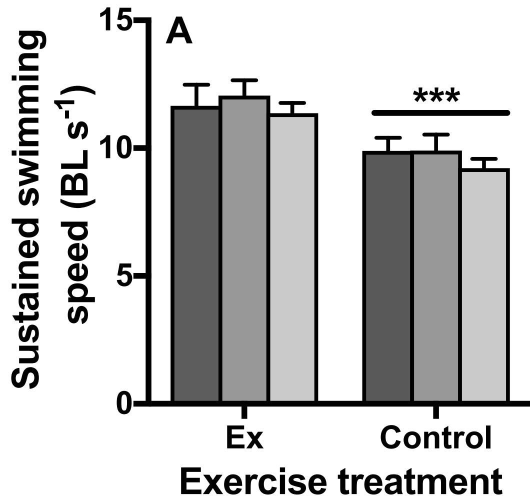

D
Fast MHC concentration
(normalised to αtubulin)
0
1
2
High
Low
Sprint perf.


E
Slow:fast MHC
0
2
4
6
8
***
High
Low
Sustained perf.

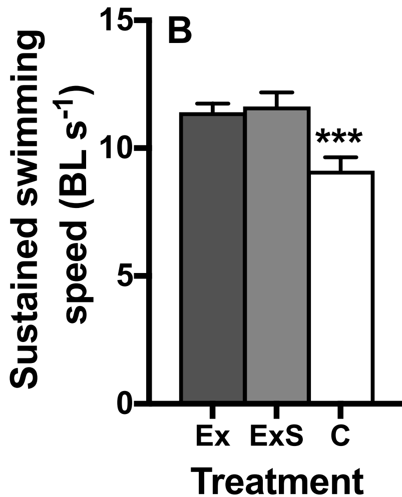

F
Slow:fast MHC
0
2
4
6
8
*
High
Low
Sprint perf.


G
High
Low
Slow MHC
Fast MHC
α-tubulin
Sustained perf.

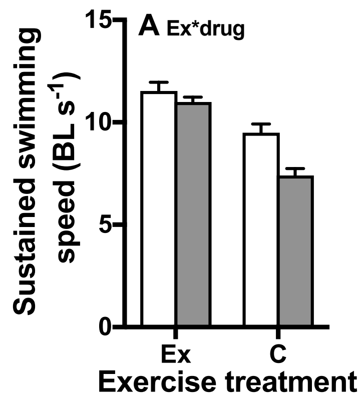

H
High
Low
Slow MHC
Fast MHC
α-tubulin
Sprint perf.

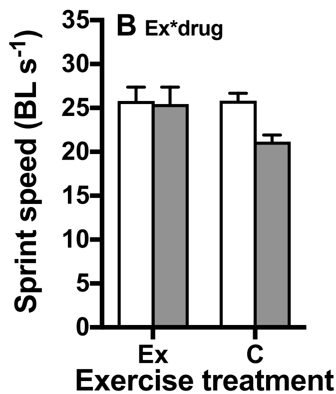


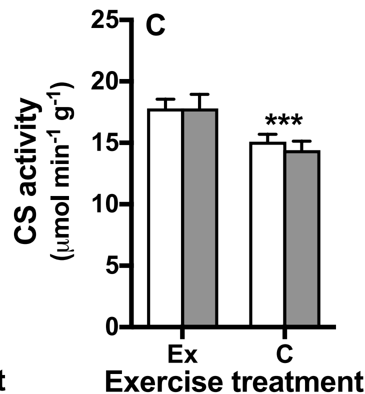
A
15
10
5
0
Sustained swimming speed (BL s-1)
***
Ex
Control
Exercise treatment
week 1
week 2
week 3

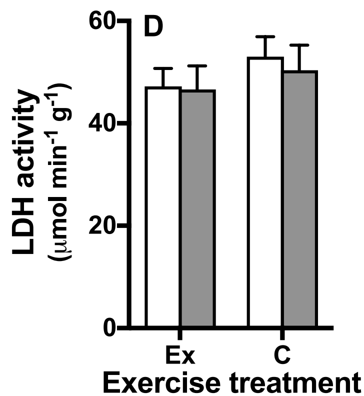


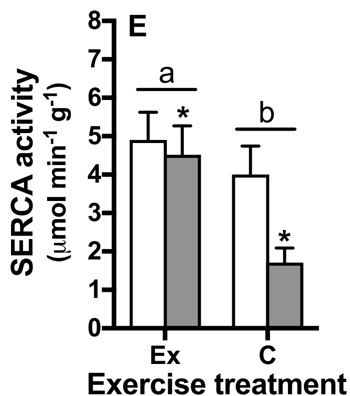
B
15
10
5
0
Sustained swimming speed (BL s-1)
***
Ex
ExS
C
Treatment



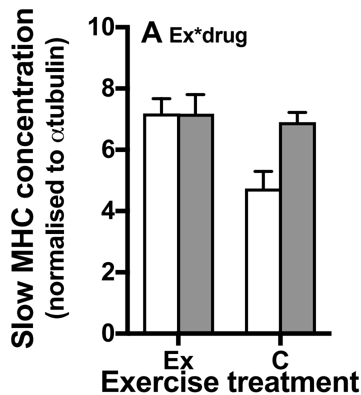


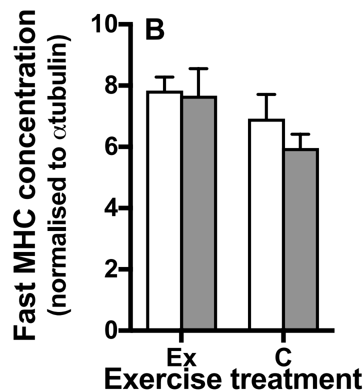


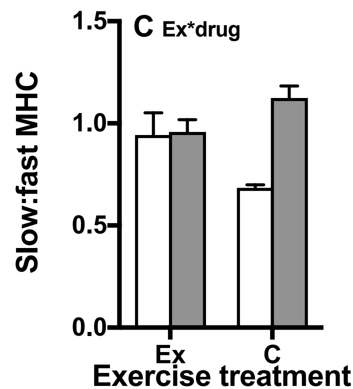


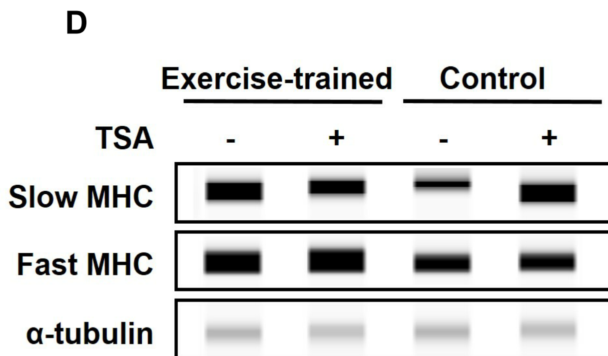


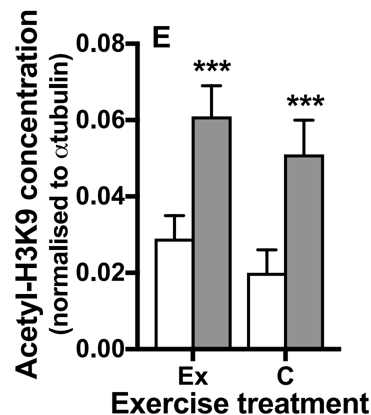
No drug
TSA

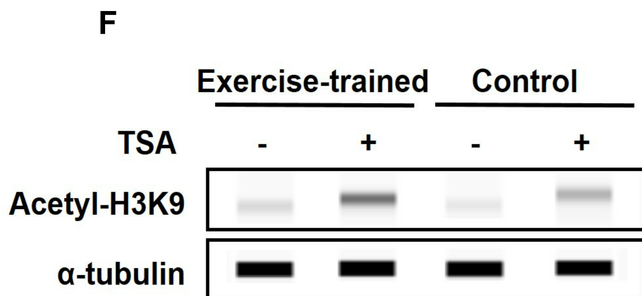

A Ex*drug
Slow MHC concentration (normalised to αtubulin)
10
8
6
4
2
0
Ex
C
Exercise treatment


B
Fast MHC concentration (normalised to αtubulin)
10
8
6
4
2
0
Ex
C
Exercise treatment


C Ex*drug
Slow:fast MHC
1.5
1.0
0.5
0.0
Ex
C
Exercise treatment


D
Exercise-trained
Control
TSA
-
+
-
+
Slow MHC
Fast MHC
α-tubulin


E
Acetyl-H3K9 concentration (normalised to αtubulin)
0.08
0.06
0.04
0.02
0.00
***
***
Ex
C
Exercise treatment
No drug
TSA


F
Exercise-trained
Control
TSA
-
+
-
+
Acetyl-H3K9
α-tubulin