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ORIGINAL PAPER

Temporal and spatial patterns of gene expression in skeletal muscles in response to swim training in adult zebrafish (*Danio rerio*)

Christophe M. R. LeMoine · Paul M. Craig · Kalindi Dhekney · Julie J. Kim · Grant B. McClelland

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Abstract In adult zebrafish, 4 weeks of exercise training is known to induce an increase in mitochondrial enzymes such as citrate synthase (CS) when determined in mixed (red and white) muscle. However, this remodeling is not accompanied by changes in PGC-1α mRNA, a potent inducer of mitochondrial biogenesis in mammals. To further understand this response, we examined absolute and relative changes in red muscle area by histochemistry after 4 weeks of swim training. We also examined fiber-type specific responses in the expression of metabolic genes and putative regulators in red and white muscle of adult zebrafish at 1 and 8 weeks of training and in recovery from a single bout of exercise. Total red muscle area was unaltered after 4 weeks of training. The mRNA expression of CS was unaffected in red muscle, while it was increased in white muscle after 1 week of training and remained elevated at 8 weeks of training, suggesting an increase in oxidative capacity of this fiber type. In contrast, PGC-1α mRNA was elevated in both muscles only after 1 week of training. In both muscles, an acute bout of exercise rapidly (within 0–2 h post-exercise) induced PGC-1α mRNA and a delayed (24 h) increase in CS mRNA post-exercise. These results suggest complex temporal and spatial adaptive molecular responses to exercise in the skeletal muscles of zebrafish.

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C. M. R. LeMoine (✉) · P. M. Craig · K. Dhekney · J. J. Kim · G. B. McClelland
Department of Biology, McMaster University, Hamilton, ON L8S 4K1, Canada
e-mail: clemoin@univmail.cis.mcmaster.ca

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Introduction

In vertebrates, skeletal muscle represents a significant portion of an animal's body mass and a major contributor to bioenergetic status. As muscle phenotype remains plastic throughout the life of an organism, it can be remodeled in response to developmental, energetic, and environmental cues. Although remodeling has been extensively studied in the context of the contractile apparatus, metabolic remodeling has also been found to play a pivotal role in the response of skeletal muscle to development, exercise, and nutritional status in mammals (reviewed in Biressi et al. 2007; Boveris and Navarro 2008; Hood et al. 2006; Matsakas and Patel 2009). Similarly, in fish skeletal muscle, metabolic adjustments have been documented during early growth (Johnston 2006; Ochi and Westerfield 2007), in response to temperature acclimation (Battersby and Moyes 1998; Guderley 2004; Hardewig et al. 1999; Johnston et al. 1990; Lucassen et al. 2006; McClelland et al. 2006; Van den Thillart and Modderkolk 1978), and exercise (Anttila et al. 2008; Farrell et al. 1991; McClelland et al. 2006). Unlike the highly heterogeneous muscle fibers found in mammals, fish skeletal muscles provide the advantage of exhibiting two main distinct and anatomically separated fiber types, red (type I/oxidative) and white (type II/glycolytic) muscles. Therefore, researchers can easily distinguish fiber type-specific remodeling. However, despite the multiple advantages of fish models, the molecular characteristics of skeletal muscle adaptation and phenotype plasticity in fish remain largely unexplored.

The effects of repeated exercise or training on skeletal muscle vary according to the training protocol used and the species studied. However, training programs at submaximal intensities, or endurance training, typically increase the oxidative capacities of muscles in both fish and mammals (see Davison 1997; Hoppeler et al. 2007; McClelland et al. 2006; Sänger 1993). This metabolic remodeling is often characterized by a greater reliance on fatty acids as fuels and increased flux capacity of the electron transport chain (Baldwin et al. 1972; Holloszy and Coyle 1984; Hoppeler et al. 2007; Russell et al. 2003). In addition, endurance training tends to promote the proliferation of slower twitch/more oxidative muscle fibers in most vertebrates (e.g., Andersen and Henriksson 1977; Davison 1997; Johnston and Moon 1980; Röckl et al. 2007). In mammals, these changes in phenotype are thought to be driven by a suite of transcriptional regulators central to mitochondrial gene expression, namely the nuclear respiratory factors (NRF), the peroxisome proliferator-activated receptors (PPAR) and their common coactivators the PPAR-γ coactivator-1α (PGC-1α) and PGC-1β (Barger and Kelly 2000; Scarpulla 2008; Handschin and Spiegelman 2006). However, although the DNA-binding proteins (i.e., NRF-1, PPARs) seem in some circumstances to assume an evolutionary conserved role in regulation of mitochondrial content and lipid homeostasis in vertebrates, current evidence suggests that the coactivators do not appear to coordinate the remodeling of muscle metabolic phenotype in response to temperature, exercise or diet in fish (McClelland et al. 2006; LeMoine et al. 2008).

Previous research in zebrafish showed that 4 weeks of training increased mitochondrial enzymes (COX and CS) activities and gene expression (CS mRNA) in mixed (red and white) muscle (McClelland et al. 2006). These changes were paralleled, to some extent, by the levels of NRF-1, a potential modulator of mitochondrial biogenesis, but not by its putative regulator PGC-1α. However, it was unclear if the changes seen in mixed muscle oxidative capacities were either a reflection of changes in metabolic properties and/or proportion of either red or white muscle, or if the absence of changes in PGC-1α expression were due to a distinct temporal expression pattern for this coregulator. Therefore, this study had two main objectives: (1) to determine if changes seen at 4 weeks of training in mixed muscle were due to changes in relative proportion of muscle fiber types, and (2) to determine changes in gene expression spatially, temporally and with increasing duration and intensity of training. Consequently, adult zebrafish were used to investigate the changes in metabolic gene expression and their putative regulators in both red and white muscles in response to 1 and 8 weeks of endurance training. To further define temporal patterns, we also examined fiber-type specific gene expression changes during recovery after a single bout of exercise.

Materials and methods

Exercise training

Adult zebrafish were commercially obtained (PetsMart, Ancaster, ON) and acclimated for approximately 1 week in dechlorinated tap water tanks maintained at 28°C and fed tropical fish chow daily (Top Fin Tropical Flakes, Phoenix, AZ). A control group of fish remained in these conditions for the duration of the experiment. An exercise trained group was transferred into a 200 L Brett-style swim flume maintained at 28°C and after a 1-week acclimation period training was started as described previously (McClelland et al. 2006). Briefly, 5 days a week fish were exercised twice daily for 3 h separated by a 2-h rest period, feeding occurred immediately after each training session. Training speed began at 6 cm s^{-1} (equivalent to two body lengths (BL) s^{-1}) and was increased weekly by 3 cm s^{-1} (1BL s^{-1}) up to 15.0 cm s^{-1} (5BL s^{-1}) at week 4. At week 5, the training speed was increased to 21 cm s^{-1} (7BL s^{-1}) with a weekly increase of 3 cm s^{-1}, equivalent to a maximum speed of 30 cm s^{-1} (10BL s^{-1}) by the end of week 8. This maximal training speed represented only a moderate exercise regime reaching approximately 50% critical swimming speed (Ucrit) of zebrafish (McClelland et al. 2006). At all times other than during feeding and training, the water was run at a constant speed of 3 cm s^{-1} (1BL s^{-1}). Fish were sampled at the end of week 1 and week 8 of the training program for mRNA measurements and at the end of week 4 in a separate group of fish for histochemistry (sample sizes appear in figure legends). Water quality was monitored weekly and several liters of water were changed in the swim flume daily as described previously (McClelland et al. 2006).

Exercise recovery

Adult zebrafish (DAP International, Etobicoke, ON, Canada) were acclimated in the swim flume maintained at 28°C at a constant speed of 3 cm s^{-1} except during feeding (twice a day). Control fish were sampled after a week of acclimation, while the remainder of the animals were subjected to a single 3 h bout of exercise at 9 cm s^{-1} (3BL s^{-1}). Immediately post-exercise a group of fish ($n = 5$) was removed from the swim flume and sampled, while the remaining fish were allowed to recover and sampled at 2, 6, and 24 h post-exercise ($n = 5$ each).

Muscle sampling

At the time of sampling fish were killed by a single blow to the head. The body mass and length were recorded, and used to evaluate the condition of the fish using the Fulton index or condition factor ($k = 100 \times$ mass $(\text{length}^{-1})^3$). For samples

destined for histochemistry, the fish were rapidly decapitated and sectioned to obtain the trunk section between the tail and anal fin. The section was immediately frozen in liquid N_2-cooled isopentane as described previously (Dubowitz 1985). For gene expression, the fish were euthanized as described above, and the red and white skeletal muscles were rapidly excised and frozen in liquid N_2. All tissues were subsequently stored at −80°C until further analysis.

Gene expression

Whole red and white skeletal muscles were homogenized in Trizol (Invitrogen, Burlington, ON, Canada), and total RNA was extracted following the acid–phenol chloroform extraction protocol. Total RNA was treated with DNAseI (Invitrogen, Burlington, ON, Canada) and reverse transcribed using Superscript II reverse transcriptase (Invitrogen) following manufacturer's instructions. Real-time PCR analysis was performed on a Stratagene MX3000P real-time PCR thermocycler (La Jolla, CA, USA) using the following conditions: an initial denaturation for 3 min at 95°C, followed by 40 cycles of 15 s denaturation at 95°C, 30 s annealing at 60°C, and 30 s extension at 72°C. Relative gene expression of each sample was assayed in duplicate in a 25 µl reaction containing 5 µl cDNA, 12.5 µl SYBR green SuperMix Low ROX (QANTA Biosciences, Gaithersburg, MD, USA), and 0.2 µM of each primer. Negative controls with no template were run with each assay to ensure absence of contamination. Gene expression analysis was performed according to the ΔΔCt method using the elongation factor 1α (EF1α) as the reference gene. Specific primers for each gene were designed to amplify a single product in zebrafish (Table 1), as checked by Real-time PCR and dissociation curve analysis post-real-time PCR.

Table 1 Primers used in the real-time PCR analysis of mRNA expression (see footnote for GENBANK accession numbers)

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
CS[a]	agcgctgctatgaatggtct	ctgaggaagacagaccctcg
EF1α[b]	gtgctgtgctgattgttgct	tgtatgcgctgacttccttg
MCAD[c]	caatggtcagaaaatgtggat	ggcccatgtttaattccttt
PGC-1α[d]	tgaggaaaatgaggccaact	agcttcttcagcagggaagg
PGC-1β[e]	tggggaagaggaggtctgc	ccgtccaggctgtctgtg
PPARβ$_1$[f]	gcgtaagctagtcgcaggtc	tgcaccagagagtccatgtc
PPARβ$_2$[g]	tggctttgtggatctcttcc	ctcactgaatggcttgcgta

[a] BC045362
[b] AY422992
[c] NM213010
[d] AY998087
[e] XM_678566
[f] AF342937
[g] BC162174

Histochemistry

The frozen trunks were serially sectioned (11 µm) at −20°C (CRYO-CUT II microtome, American Optical, US). Sections were taken at different points along the length of the fish as red and white muscle area is not constant from trunk to tail in other fish (e.g., Devincenti et al. 1988). These cryosections were then transferred to silane-coated slides for myosin ATPase histochemistry. The muscle fiber types were characterized according to their ATPase activity following acidic pH incubation as described previously (Stewart et al. 2004) with minor modifications. Slides were first incubated at 25°C in acidic solutions (50 mM $C_2H_3O_2K$, 18 mM $CaCl_2$) at pH 4.30 for 5 min, pH 4.54 for 7.5 min, and pH 4.60 for 6.5 min. The slides were then rinsed with distilled water (ddH_2O) and incubated for 45 min at 37°C in an alkaline ATP solution (3 mM ATP, 75 mM glycine, 75 mM NaCl, 41 mM $CaCl_2$, 675 mM NaOH; Dubowitz 1985). Following ATP incubation, the slides were rinsed twice and transferred into a calcium chloride solution (68 mM $CaCl_2$) for 3 min at 25°C. Slides were washed and placed in a cobalt chloride solution (84 mM $CoCl_2$) for 3 min at 25°C, thoroughly rinsed in ddH_2O and incubated in a 1% w/v ammonium sulphide ($(NH_4)_2S$) solution for 10–15 s at 25°C and rinsed again in ddH_2O. Sections were slowly dehydrated in successive ethanol solutions (50, 75, 95 and 100%) for 3 min each. Slides were cleared in xylene for 2 min and mounted.

Digital microscopic images were acquired with a standalone digital camera (Canon Powershot A620 for whole cross sections) or a Q-imaging digital camera (Meyer Instruments, Houston, TX) on an Olympus BX60 microscope (Olympus, New York, NY) using Northern Eclipse Elite software (EMPIX imaging Inc, Mississauga, ON, Canada). The areas of stained red muscle fibers and whole cross section were quantified using ImageJ software (Girish and Vijayalakshmi 2004). The quantification procedure was done blindly and in random order.

Statistical analyses

Data are presented as means ± SEM statistical significance was established ($P < 0.05$) following analysis by Students t tests (histochemistry), One way ANOVA with Holm-Sidak post hoc tests, and Mann–Whitney rank sum tests (gene expression, physical characteristics).

Results and discussion

In most vertebrates, endurance training promotes a variety of morphological and metabolic changes in skeletal muscles. Although these adaptations vary as a function of the

species or the training protocol used, low intensity exercise training generally elicits changes toward a more oxidative phenotype (reviewed in Davison 1997; Hawley 2002; Holloszy and Coyle 1984; Sänger 1993). Previous work demonstrated the considerable phenotypic plasticity of adult zebrafish mixed muscle in response to exercise, as 4 weeks of training produced a more aerobic phenotype (McClelland et al. 2006). Although some gene expression changes appeared to be conserved between fish and mammals (e.g., NRF-1), training also induced zebrafish-specific responses. Most notably, expression of PGC-1α and the PPAR (α, β_1), homologues of key regulators responsible for muscle remodeling in mammals, were not induced by 4 weeks of training in mixed muscle of zebrafish (McClelland et al. 2006). Thus, in this study we further examined changes in gene expression patterns associated with exercise-induced phenotypic plasticity in the axial muscles of adult zebrafish. To this aim, we evaluated both the temporal and spatial response to chronic and acute endurance exercise. This allowed further differentiation of the contribution of each muscle fiber type, and any distinct temporal patterns of gene expression in the skeletal muscles of zebrafish. In addition, to determine the potential contribution of differential fiber-type hypertrophy to overall remodeling we examined morphometric changes in red relative to white muscle in exercise trained zebrafish.

Physical characteristics and muscle fiber types

All the fish used in this study were in good physical condition (Table 2). Although most treatment groups had relatively comparable condition factors (k), 8 weeks of training significantly increased k of the fish as compared to 1 week of training and control fish (Table 1). Even though this increase in the condition of the fish is relatively modest (approximately 10%), it is in line with previous reports that exercise training in most fish species tends to improve the overall condition of the animals (see Davison 1997; Sänger 1993).

Previously, we showed that in zebrafish, 4 weeks of training leads to increased oxidative capacity of mixed muscle (McClelland et al. 2006). Therefore, we decided to evaluate if changes in oxidative capacity were due to a proliferation of more oxidative fibers (i.e., red muscle), as has been documented in some fish species (e.g., Hinterleitner et al. 1992; Young and Cech 1993). Training had a limited impact on the absolute red muscle area in all sections examined by histochemical analyses (Fig. 1). Both control and exercised fish showed some variability in absolute red muscle area across the different body sections from pectoral fin to tail (data not shown), though when combined the cross sections did not show any significant differences between the two treatments (Fig. 1a). In addition, the proportion of red muscle relative to the entire fish cross section appeared to be relatively unaffected by the training protocol (Fig. 1b). Overall, the data show that in trained fish there was a non-significant trend for a lower absolute red muscle area and a slightly higher (approximately 10%) proportion of red muscle. Although these results may appear contradictory, they suggest that trained fish had slightly smaller axial muscle areas (both red and white) but that the proportion of both fiber type was only slightly affected. Moreover, gene expression for fast and slow myosin heavy chains (fMyHC

Table 2 Physical characteristics of adult zebrafish used in the study

Treatment group	Weight (g)	Length (cm)	Condition factor (k)
Exercise training (gene expression)			
Controls 1 week	0.38 ± 0.06	2.90 ± 0.13	1.49 ± 0.07
Controls 8 weeks	0.45 ± 0.04	3.14 ± 0.07[a]	1.42 ± 0.07
Trained 1 week	0.35 ± 0.03	3.00 ± 0.11	1.30 ± 0.08
Trained 8 weeks	0.45 ± 0.04	3.06 ± 0.04	1.57 ± 0.06[a,b]
Exercise training (histology)			
Controls 4 weeks	0.43 ± 0.07	2.85 ± 0.10	1.80 ± 0.17
Trained 4 weeks[#]	0.54 ± 0.10	3.03 ± 0.15	1.85 ± 0.11
Acute exercise (gene expression)			
Controls	0.39 ± 0.04	2.85 ± 0.09	1.71 ± 0.14
Post-exercise 0 h	0.35 ± 0.03	2.79 ± 0.08	1.62 ± 0.13
Post-exercise 2 h	0.37 ± 0.03	2.84 ± 0.06	1.62 ± 0.18
Post-exercise 6 h	0.36 ± 0.03	2.75 ± 0.06	1.72 ± 0.10
Post-exercise 24 h	0.39 ± 0.03	2.80 ± 0.07	1.76 ± 0.08

Values are expressed as mean ± SEM, $n = 5$ for all groups except [#]$n = 4$

[a] Indicates significantly different from other time point in same treatment

[b] Indicates significantly different from same time point in the other groups

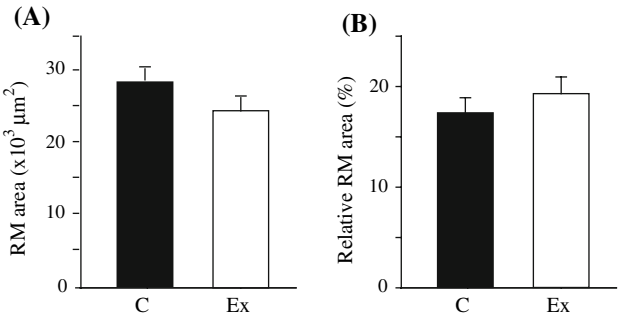

Fig. 1 Skeletal muscle phenoptype in trained zebrafish. Absolute ($n = 4$) and relative ($n = 3$) red muscle cross-sectional areas were assessed in skeletal muscle of control (C) and 4 weeks trained (Ex) adult zebrafish. The absolute (**a**) and relative (**b**) red muscle area was estimated by histochemistry in different sections of the axial musculature along the length of fish. No significant differences ($P > 0.05$) were detected between treatments

and sMyHC) was found to be unaltered in mixed muscle after 4 weeks of training (McClelland et al. 2006). These results suggest that overall the axial muscle contractile phenotype remains relatively unaltered following 4 weeks of moderate intensity exercise training in zebrafish, and that these minor changes are unlikely to account for the 40% increase in oxidative capacity of mixed muscle previously described (McClelland et al. 2006). In contrast, endurance training in mammals has been typically associated to fiber-type switching from fast to slow twitch fibers (Andersen and Henriksson 1977; Demirel et al. 1999; Fitts 2003; Röckl et al. 2007; Russell et al. 2003; Short et al. 2005). Similarly, in some other fish species studied, low intensity exercise training triggered red muscle proliferation (Davison 1997; Hinterleitner et al. 1992; Johnston and Moon 1980; Sänger 1992; Sänger 1993; Sänger and Pötscher 2000; Young and Cech 1993). However, zebrafish red muscle does not respond to 4 weeks of exercise training with an increase in either absolute or relative area (Fig. 1). Similarly, juvenile zebrafish endurance-trained for 10 weeks do not exhibit dramatic changes in absolute and relative abundance of red and white fiber types (Van der Meulen et al. 2006). Furthermore, both red and white muscle fibers respond differently to repeated contractions in zebrafish with a modulation of gene expression (see below).

Exercise training gene expression

The swim training treatment induced metabolic gene expression in red and white muscle but with distinct effects depending on the fiber type (Fig. 2a, b). CS mRNA approximately doubled in the white muscle of exercise trained fish compared to controls at both time-points examined (Fig. 2a), while remaining unchanged in red muscle (Fig. 2b). In contrast, the β-oxidation gene encoding for the medium chain acyl-CoA dehydrogenase (MCAD) enzyme did not change regardless of the muscle type or the training period (Fig. 2a, b). This was consistent with our previous results determined in mixed muscle showing no induction of another lipid oxidation enzyme, β-hydroxyacyl-CoA dehydrogenase (HOAD, McClelland et al. 2006). Thus, the changes in oxidative capacity previously observed in zebrafish mixed muscle (McClelland et al. 2006) are likely a reflection of the metabolic remodeling of the white muscle through changes in the transcription of respiratory genes. As the majority of the fish axial musculature is composed of glycolytic white muscle (Sänger and Stoiber 2001), it seems logical that most of the phenotypic plasticity in oxidative capacity seen in mixed muscle (McClelland et al. 2006), originates from changes in mitochondrial capacity of white muscle. Similar increases of mitochondrial indicators in response to training have been documented in a variety of vertebrate species (Baldwin et al. 1972; Houmard et al. 1993; Leek et al. 2001; Pilegaard et al. 2003; Serrano et al. 2000; Short et al. 2003; Taylor et al. 2005; Yeo et al. 2008). However, in several of these studies, the capacity for fatty acid oxidation increased in parallel to mitochondrial content, suggesting in these experimental models that there is a tight coupling between increases in the oxidative capacity of muscle and ability to oxidize lipids (e.g., Farrell et al. 1991; Johnston and Moon 1980; Pilegaard et al. 2003). Although the capacity of skeletal muscle to oxidize lipids is regulated at multiple levels (e.g., cellular and mitochondrial import of lipids), both the mRNA expression profiles (MCAD, HOAD) and enzyme levels (HOAD) strongly suggest a minimal effect of training on this pathway in zebrafish (Fig. 2a, b; McClelland et al. 2006).

The levels of the nuclear receptor PPAR β isoforms 1 and 2 were relatively unaffected by 1 week of training in either fiber types (Fig. 2c, d), similar to humans where PPARβ is largely unaffected by training (Russell et al. 2003). Interestingly, while in zebrafish red muscle the ratio of the PPARβ_1/β_2 isoforms remained unchanged after 1 week of training, the relative abundance of PPARβ_1 versus PPARβ_2 increased approximately twofold in white muscle ($P < 0.05$). These results could indicate that the fish-specific duplicated isoforms of PPARβ exhibit different responsiveness to a common stressor. However, the roles of these duplicated nuclear hormone receptors in fish remain largely unknown, and as one of their putative targets, by homology with mammals (MCAD), did not change with our treatment, we cannot elaborate further on their physiological significance. Nonetheless, the differential expression of these duplicated genes may suggest that they assume different roles under these conditions, and warrants further investigation in this relatively unexplored area of research.

The transcript levels of coactivators of metabolic gene expression (PGC-1α and PGC-1β) showed similar temporal patterns (Fig. 2c, d). PGC-1α mRNA levels were increased three- and fourfold in red and white muscles, respectively, after 1 week of training, but returned to pre-training levels by week 8 (Fig. 2c, d). This is consistent with previous data using mixed muscle samples where PGC1α was at control levels after 4 weeks of training (McClelland et al. 2006). PGC-1β levels followed a similar trend with an approximate twofold increase in white muscle (although not statistically significant), and a threefold increase in red muscle after a week of training, also returning to baseline levels by 8 weeks (Fig. 2c, d). These results suggest a transient induction of expression of these coactivators, with a rapid return to pre-training levels, a trend distinct from that observed in mammals. Indeed, most mammalian studies show sustained elevated levels of PGC-1α mRNA and a dowregulation of PGC-1β with 1–16 weeks of training (see

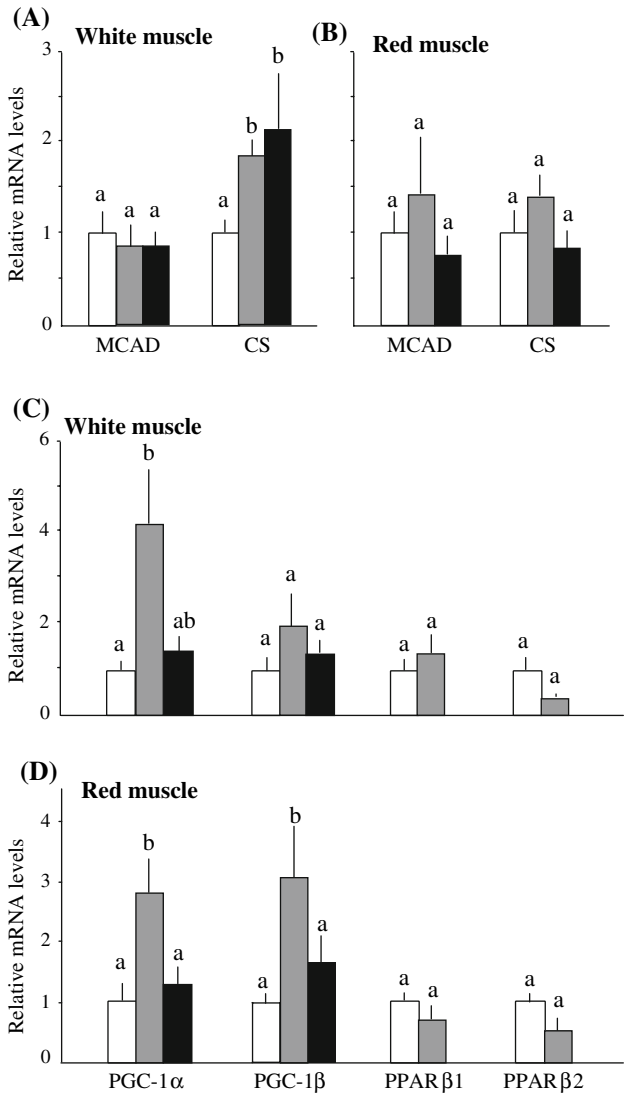

Fig. 2 Training-induced gene expression changes in zebrafish axial muscles. Relative mRNA levels were evaluated by real-time PCR in white (**a**, **c**) and red (**b**, **d**) muscles of controls (*white bars*), 1 week (*gray bar*), and 8 weeks (*black bar*) trained zebrafish normalized to EF1α. Shared letters indicate no significant differences ($P > 0.05$) between treatments ($n = 4$ or 5)

Goto et al. 2000; Mortensen et al. 2007; Pilegaard et al. 2003; Russell et al. 2003; Short et al. 2003; Taylor et al. 2005). Zebrafish show patterns of expression of both coactivators in red and white muscle that do not directly correspond to the temporal pattern of CS mRNA. This may suggest that these coactivators are not directly involved in the regulation of mitochondrial content in response to exercise training in this species. Similarily, in temperature-challenged goldfish, there does not seem to be a direct correlation between PGC-1α or PGC-1β mRNA levels and respiratory gene transcripts in muscles (LeMoine et al. 2008). However, as most coactivators, the activity of PGC-1α and PGC-1β is post-transcriptionally regulated, at least in mammals (e.g., Jäger et al. 2007; Puigserver et al. 2001). Thus, their respective transcript levels may not be a direct reflection of their coactivating abilities. Nevertheless, considering the divergent patterns of CS and PGC-1α/β gene expression observed in this study and previously (LeMoine et al. 2008; McClelland et al. 2006), the coactivators do not seem to be directly implicated in the long-term changes in oxidative capacity of skeletal muscle. However, this does not preclude them from having additional regulatory roles in the initial adaptation of skeletal muscle to exercise training.

Overall, these results suggest that endurance training triggered an increase in oxidative capacity (largely in white muscle) as assessed by the mitochondrial marker CS mRNA, similar to other vertebrate models. In contrast to the current model of muscle remodeling in mammals where PGC-1α seems to play a preponderant role (e.g., Russell et al. 2003; Short et al. 2003; Taylor et al. 2005), the regulatory pathways of muscle plasticity in zebrafish do not seem to implicate PGC-1α directly in the response to exercise training.

Exercise recovery time course

To assess the responsiveness of zebrafish muscle to an acute exercise bout we examined changes in gene expression with recovery from a single 3 h training session at moderate intensity. Both fiber types showed induction of gene expression during the recovery period (Fig. 3a, b). In red and white muscles, the transcript levels of CS increased approximately twofold 24 h into recovery. This temporal response of CS expression is delayed compared to mammalian models, where CS and other mitochondrial gene transcript levels tend to increase relatively rapidly after an exercise bout (Neufer and Dohm 1993; Schmutz et al. 2006; Wright et al. 2007).

The acute bout of exercise also brought about some distinct changes in the expression of PGC-1α and PGC-1β (Fig. 3). In white muscle, PGC-1α was only significantly elevated 2 h post-exercise compared to 6 h post-exercise (Fig. 3a). Although not significantly different from controls, there is a trend for a gradual increase of PGC-1α mRNA in white muscle immediately after and 2 h post-exercise. In red muscle, PGC-1α transcript levels increased immediately post-exercise but did not reach statistical significance (Fig. 3b). These changes are relatively similar to that in mammalian muscle, which typically translates into a rapid upregulation of PGC-1α mRNA post-exercise (Baar et al. 2002; Leick et al. 2008; Pilegaard et al. 2003; Russel et al. 2005; Terada et al. 2002) but with a much greater magnitude (e.g., up to tenfold in Pilegaard et al. 2003). This difference in magnitude may reflect differences in the exercise intensities used in the training programs, or reveal

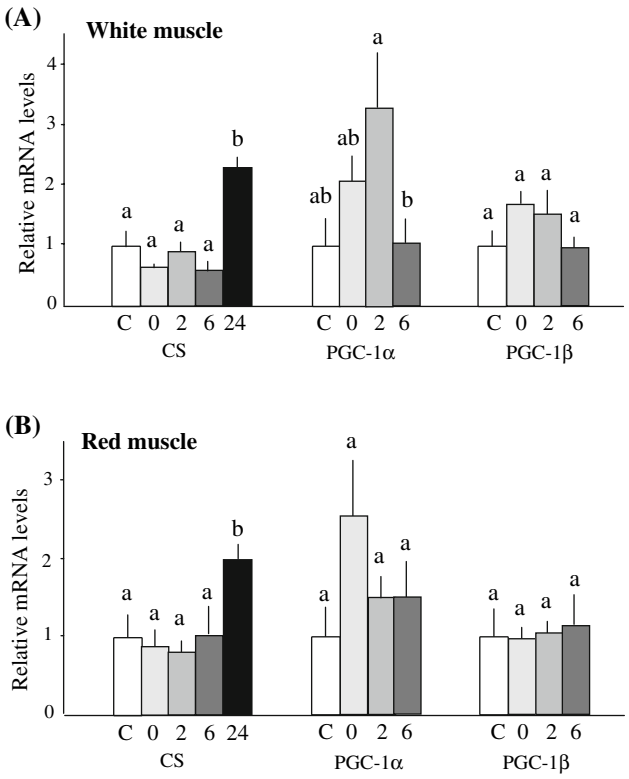

Fig. 3 Muscular metabolic gene expression during recovery to a single-bout of exercise. Relative mRNA levels were measured in white (**a**) and red (**b**) muscles of adult zebrafish before (**c**, *white bars*) and 0, 2, 6, and 24 h (*gray* and *black bars*) after an acute exercise bout normalized to EF1α. Different letters indicate significant differences ($P < 0.05$) between time points ($n = 5$)

intrinsic differences in the degree of phenotypic plasticity potential of the species under investigation.

The differences in magnitude and temporal response seen in PGC-1α mRNA and CS mRNA suggest that the coactivator does not play a direct role in the late increase in one of its putative target gene (CS) expression. However, the latency between the increases in gene expression of PGC-1α and CS could just be an indication of additional regulatory steps. Nevertheless, these gene expression results in response to acute and chronic exercise stimulation, taken together with previous results (LeMoine et al. 2008; McClelland et al. 2006) suggest that PGC-1α may not be directly involved in the regulation of CS expression in fish muscle. Although relatively little is known about the control of CS expression in mammals, transcription factors such as sp1 and NRF-1 appear to have a role in its transcriptional regulation (Kraft et al. 2006; Seelan et al. 1996). Interestingly, in both zebrafish and goldfish NRF-1 mRNA seems to parallel CS expression patterns, suggesting a possible regulatory relationship that warrants further investigation (LeMoine et al. 2008; McClelland et al. 2006). Besides its role in regulating mitochondrial content, PGC-1α has recently been associated with other homeostatic responses in muscles, including orchestrating reestablishment of glycogen stores, and regulating fatty acid oxidation (Lee et al. 2006; Wende et al. 2007); both crucial for skeletal muscle recovery from exercise (Keins and Richter 1998; Richards et al. 2002; Wende et al. 2007). Therefore, it is conceivable that PGC-1α could have an evolutionary conserved role in the reestablishment of fuel homeostasis post-exercise.

In contrast to its paralogue, PGC-1β did not change in either fiber types during the post exercise recovery period (Fig. 3). This is similar to mammals where in general PGC-1β expression is unaffected by changes in muscle activity (Meirhaeghe et al. 2003; Mortensen et al. 2007). Thus, PGC-1β does not seem play a role in the increase in CS mRNA content in zebrafish muscle in response to a single bout of exercise. In mammals, both PGC-1α and PGC-1β are important regulators of mitochondrial content and have shared and unique respective roles in metabolic adaptations (e.g., Lehman et al. 2000; St-Pierre et al. 2003; Wu et al. 1999). Here our results suggest that similar to their mammalian counterparts, PGC-1α and PGC-1β in zebrafish show distinct pattern of expression in response to an acute exercise bout (Fig. 3). However, their exact roles in the muscular response to exercise or other stressors in non-mammalian species are unclear and warrant further investigation.

Chronic versus acute exercise response

Overall, our results indicate relatively distinct responses of zebrafish skeletal muscles to a single bout of exercise compared to prolonged exercise training (Figs. 2, 3). Indeed, CS mRNA was stimulated in both muscles after one bout of exercise (Fig. 3), but only in white muscle after exercise training (Fig. 2a). Considering this delayed response in CS expression in recovery to one exercise bout (Fig. 3a, b), it is possible that the elevated CS mRNA levels in white muscle of trained animals (Fig. 2a) reflect the response to a previous exercise bout (approximately 24 h before sampling) rather than sustained constitutive levels. Nevertheless, our results indicate a differential response of red and white muscle to exercise training, suggesting a reduced responsiveness of CS expression in red muscle compared to white muscle between acute exercise and exercise training. PGC-1α and PGC-1β also exhibited strikingly different response to the acute and chronic exercise stimulation in both muscles (Figs. 2c, d, 3). In both muscles, 1 week of exercise training-induced a more pronounced increase in PGC-1α than a single exercise bout (Figs. 2c, d, 3). However, by week 4 and 8 of training, this response was absent in mixed muscle and red and white muscle, respectively (McClelland et al. 2006; Figs. 2c, d), suggesting a reduced responsiveness of PGC-1α to exercise training past week 1. In contrast, PGC-1β was only responsive to exercise after

1 week of training in red muscle (Figs. 2c, d, 3), indicating both a temporal and fiber-type-specific response of that coactivator to exercise training.

Conclusions

Zebrafish muscle responds to endurance training with an increase in the aerobic capacity of white muscle, but without any changes in their capacity to oxidize fatty acids. Contrarily to most teleost species (see Davison 1997; Sänger 1993), endurance training did not increase red muscle in zebrafish. The reason for this is unclear, although it is interesting to note that unlike larger teleosts, zebrafish seem to exhibit determinate growth (Biga and Goetz 2006). Perhaps a different response to exercise training would be observed in another species, the giant danio (*Danio aequipinnatus*), a close relative of the zebrafish that exhibits indeterminate growth (Biga and Goetz 2006).

These results show that like mammals, zebrafish remodel muscle in response to exercise producing a more aerobic phenotype. At the molecular level, our results strongly argue that the PGC-1 family of coactivators does not play a direct regulatory role in these adaptations in fish warranting further physiological elucidation of these regulatory pathways (LeMoine et al. 2008; McClelland et al. 2006).

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