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Role of the PGC-1 family in the metabolic adaptation of goldfish to diet and temperature

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SUMMARY

In mammals, the peroxisome proliferator-activated receptor (PPAR) γ coactivator-1 (PGC-1) family members and their binding partners orchestrate remodelling in response to diverse challenges such as diet, temperature and exercise. In this study, we exposed goldfish to three temperatures (4, 20 and 35°C) and to three dietary regimes (food deprivation, low fat and high fat) and examined the changes in mitochondrial enzyme activities and transcript levels for metabolic enzymes and their genetic regulators in red muscle, white muscle, heart and liver. When all tissues and conditions were pooled, there were significant correlations between the mRNA for the PGC-1 coactivators (both α and β) and mitochondrial transcripts (citrate synthase), metabolic gene regulators including PPARα, PPARβ and nuclear respiratory factor-1 (NRF-1). PGC-1β was the better predictor of the NRF-1 axis, whereas PGC-1α was the better predictor of the PPAR axis (PPARα, PPARβ, medium chain acyl CoA dehydrogenase). In contrast to these intertissue/developmental patterns, the response of individual tissues to physiological stressors displayed no correlations between mRNA for PGC-1 family members and either the NRF-1 or PPAR axes. For example, in skeletal muscles, low temperature decreased PGC-1α transcript levels but increased mitochondrial enzyme activities (citrate synthase and cytochrome oxidase) and transcripts for COX IV and NRF-1. These results suggest that in goldfish, as in mammals, there is a regulatory relationship between (i) NRF-1 and mitochondrial gene expression and (ii) PPARs and fatty acid oxidation gene expression. In contrast to mammals, there is a divergence in the roles of the coactivators, with PGC-1α linked to fatty acid oxidation through PPARα, and PGC-1β with a more prominent role in mediating NRF-1-dependent control of mitochondrial gene expression, as well as distinctions between their respective roles in development and physiological responsiveness.

Key words: PPAR, NRF-1, mitochondria, transcriptional regulation.

INTRODUCTION

Mitochondrial oxidative phosphorylation produces most of the ATP required by cells under aerobic conditions. Mitochondrial content differs among tissues as a result of development and can be adjusted in response to physiological stressors (Hood et al., 2006; Lyons et al., 2006; Moyes et al., 1997; Nelson, 1990; Nicholls et al., 1986). The coordination of the expression of suites of nuclear- and mitochondrial-encoded genes depends on both DNA-binding proteins and coactivators (for reviews, see Goffart and Wiesner, 2006; Scarpulla, 2006). Amongst the DNA-binding proteins, nuclear respiratory factors (NRFs) and peroxisome proliferators-activated receptors (PPARs) are paramount. NRF-1 regulates the expression of several subunits of the electron transport chain as well as the mitochondrial transcription factor mTFA (Choi et al., 2002; Virbasius and Scarpulla, 1994); PPARs form heterodimers that regulate genes involved in lipid metabolism (reviewed in Barger and Kelly, 2000; Berger and Moller, 2002; Van Bilsen et al., 2002). These transcription factors interact with coactivators from the PGC-1 (PPARγ coactivator 1) family (Handschin and Spiegelman, 2006; Monsalve et al., 2000; Puigserver et al., 1999; Scarpulla, 2006; Wallberg et al., 2003).

PGC-1α, the first characterized PGC-1 family member, was identified as a stimulator of thermogenin expression in brown adipose tissue of mice (Puigserver et al., 1998). The other family members, PGC-1β and PRC (PGC-1-related coactivator), were subsequently discovered through database searches for PGC-1α homologues (Andersson and Scarpulla, 2001; Lin et al., 2002). In general, PGC-1α and PGC-1β have similar coactivation responsibilities, correlating with both developmental differences in mitochondrial content (Lehman et al., 2000; Mortensen et al., 2006; Uldry et al., 2006) and physiological adaptation to stressors such as muscular activity (Baar et al., 2002; Goto et al., 2000) and nutritional status (Lin et al., 2002; Yoon et al., 2001). Although less is known about the function of PRC, this coactivator can stimulate mitochondrial biogenesis, as do its homologues, but it is constitutively and ubiquitously expressed (Gleyzer et al., 2005; Vercauteren et al., 2006).

These transcriptional networks have been studied primarily in mammalian systems. Given their highly conserved structures, the DNA-binding proteins probably have similar roles throughout vertebrates, as has been shown for PPARs (Kondo et al., 2007; Krey et al., 1993; Leaver et al., 2007). However, the role of the entire PGC-1 family in lower vertebrates has not been assessed. In this study, we explored the putative regulatory role of the coactivators and their binding partners in the metabolic adaptations of goldfish induced by two common stressors: temperature and diet. Goldfish and other fish species increase mitochondrial content in response to cold acclimation through a suite of qualitative and quantitative biochemical responses (Guderley, 1990; Johnston et al., 1990). This induction of mitochondrial biogenesis is much like the mammalian response to cold exposure and exercise, which is known to be mediated by these transcription factors (Hood et al., 2006; Liang and Ward, 2006; Puigserver and Spiegelman, 2003). Changes in

diet also have a pronounced effect on the tissue-specific metabolic strategy and mitochondrial phenotypes in most vertebrate species (Blasco et al., 1992; Chanseaume, 2007; Du et al., 2006; Ojano-Dirain et al., 2005). In fish, prolonged caloric restriction usually causes depletion of hepatic reserves and hypometabolism (Beamish, 1964; Black and Love, 1986; Blasco et al., 1992; Cui and Wang, 2007; Rios et al., 2002). In contrast, lipid-rich diets increase fatty acid metabolism and storage, and depress *de novo* lipogenic pathways (Kolditz et al., 2008; Lin et al., 1977; Poston and McCartney, 1974). In mammalian systems PPARs and both PGC-1α and PGC-1β have been associated with the regulation of fatty acid oxidation whereas only PGC-1β seems to regulate lipogenic pathways (Kamei et al., 2003; Lee et al., 2006; Mazzucotelli et al., 2007; Rodriguez-Cruz et al., 2006). Though these regulators have not been thoroughly studied in lower vertebrates, it is conceivable that they mediate similar metabolic adaptations. The present study will allow us to assess the patterns of expression of these important regulators in the context of developmental variation and physiological responsiveness in an early vertebrate model.

MATERIALS AND METHODS

Animals and treatments

Goldfish (*Carassius auratus* L.) were maintained in the lab at 20°C in dechlorinated water in a flow-through tank and fed goldfish chow *ad libitum* (30% protein, 3% fat; Hagen, Montreal, QC, Canada). Fish were divided in five treatment groups. One group of control fish was held at 20°C and fed goldfish chow *ad libitum*. Two groups were subjected to thermal acclimation regimes (and fed goldfish chow *ad libitum*). For these two groups, water temperature was changed by about 1°C every 2 days until the temperature reached the desired endpoint; one group was held at 4°C for 6 weeks and the other group was held at 35°C for 3 weeks. The last two groups were subjected to a dietary regime (and held at 20°C). For 3 weeks, one group was held without feeding while the other was fed a high fat diet (50% protein, 20% fat; Corey Aquafeed, Fredericton, NB, Canada).

Fish were lethally anaesthetized with tricaine methane sulfonate (0.8 g l^{-1} sodium bicarbonate, 0.4 g l^{-1} MS-222), weighed and measured, and then tissue samples were rapidly collected and flash frozen. Samples were powdered in liquid nitrogen and stored at –80°C. The computed condition factor (K) post-treatment did not indicate any significant difference between groups (data not shown).

Enzyme analysis

Approximately 50 mg of powdered tissue was homogenized in 20 volumes of extraction buffer (20 mmol l^{-1} Hepes, 1 mmol l^{-1} EDTA, 0.1% Triton X-100, pH 7.4) with a ground glass tissue homogenizer on ice. Specific enzyme activities were spectrophotometrically assayed in 96 well plates. Immediately following homogenization, cytochrome *c* oxidase (COX) activity was assayed at 550 nm in a solution composed of 20 mmol l^{-1} Tris (pH 8.0), 0.5% Tween 20 and 50 μmol l^{-1} cytochrome *c*. Cytochrome *c* was reduced using ascorbic acid, then dialysed exhaustively in 10 mmol l^{-1} Tris (pH 8.0) and frozen in aliquots. Citrate synthase (CS) activity was measured at 412 nm in 20 mmol l^{-1} Tris (pH 8.0), 0.1 mmol l^{-1} 5,5-dithio-bis(2-nitrobenzoic acid), 0.3 mmol l^{-1} acetyl CoA and 0.5 mmol l^{-1} oxaloacetate (omitted for control). Liver samples were assayed for CS immediately after COX assay, whereas muscle homogenates were subjected to a single freeze–thaw cycle (–80°C) prior to CS assay.

RNA analysis

Total RNA was extracted from 30–50 mg of powdered tissue using the Qiagen RNeasy kit (Mississauga, ON, Canada) according to the manufacturer's instructions. RNA was reverse transcribed using (per μg RNA): 0.125 μg oligo-dT primers, 0.375 μg random hexamers and 15 U of Promega AMV RT (Madison, WI, USA). Real-time analysis was performed on an ABI 7500 RT-PCR system (Foster City, CA, USA) using the following conditions: an initial denaturation for 10 min at 95°C followed by 40 cycles of 15 s denaturation at 95°C, 30 s annealing at optimal primer temperature (Table 1) and 36 s extension at 72°C. Each sample was assayed in duplicate in a 25 μl reaction volume containing 2.5 μl cDNA, 12.5 μl SYBR green master mix (Qiagen) and 0.58 μmol l^{-1} of each primer. Negative controls (no template or selected untranscribed RNA) were run as well to ensure the absence of contamination. Analysis was performed according to the ΔCt method using elongation factor-1α (EF-1α) as the housekeeping gene. Specific primers for each gene were designed to amplify a single product (using goldfish published sequences or consensus primers, see Table 1), as checked by regular PCR and dissociation curve analysis post-real-time PCR.

Statistical analysis

For the treatment comparisons, all data are presented as mean values ± s.e.m., and are expressed relative to the control treatment (fish held at 20°C and fed goldfish chow). Significance between treatments was tested using a Kruskall–Wallis test followed by a Mann–Whitney *U*-test. Least-squares linear regressions were established using the log-transformed gene expression data from all the treatments and all the tissues.

RESULTS

Mitochondrial enzyme activities

Fish acclimated at colder temperatures exhibited increased oxidative capacity in skeletal muscles (Fig. 1). In red muscle

Table 1. Real-time PCR primers

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
PGC-1α[1]	atggcgtgggacaggtgta	tgattggtcactgtaccacttgag
PGC-1β[2]	tggggaagaggaggtctgc	ccgtccaggctgtctgtg
PRC[3]	cagttatgagcaggtggaca	tctcgcctctatctgaatgg
PPARα[4]	gagcccaagtttcagtttgc	ggaagaggaactcgttgtcg
PPARβ[5]	tggctttgtggatctcttcc	gatctcgctgaaaggtttgc
NRF-1[6]	aggccctgaggactatcgtt	gctccagtgccaacctgtat
CS[7]	atggacttgatcgccaagc	ccaccctcatggtcactgt
COX IV[8]	agggatcctggctgcact	tcaaaggtatgggggacatc
COX I[9]	gtgcctgagccggaatagt	tcagtttccgaatcctccaat
MCAD[10]	caatggtcagaaaatgtggat	ggcccatgtttaattccttt
FAS[11]	ccaacacctcagtgcagttc	ggaacgtcacaccttgctc
EF-1α[12]	atgcggtggaatcgacaa	cagagagcaatgtcaatggtg

Gene-specific primers were designed on goldfish or consensus sequences. CS, citrate synthase; COX, cytochrome oxidase; EF-1α, elongation factor-1α; FAS, fatty acid synthase; MCAD, medium chain acyl CoA dehydrogenase; NRF-1, nuclear respiratory factor-1; PPAR, peroxisome proliferator activator receptor; PGC-1, PPARγ coactivator-1; PRC, PGC-1-related coactivator. GenBank accession numbers: [1]EU426842, XM678107; [2]EU426839, XM678566; [3]EU426841, XM001338200, CAAB01000022.1, CA371089; [4]AY198322, AM230808, AY590299; [5]AY894894, AF342937, AJ416953, AY590301, AY356399; [6]AF087671; [7]BC045362, DQ059757, AY382596; [8]EU426840; [9]AB111951, DQ656543, NC001606, AY996924; [10]NM213010, NM016986, NM000016; [11]XM682295, NM007988, NM004104, NM205155; and [12]AB056104, NM1312.

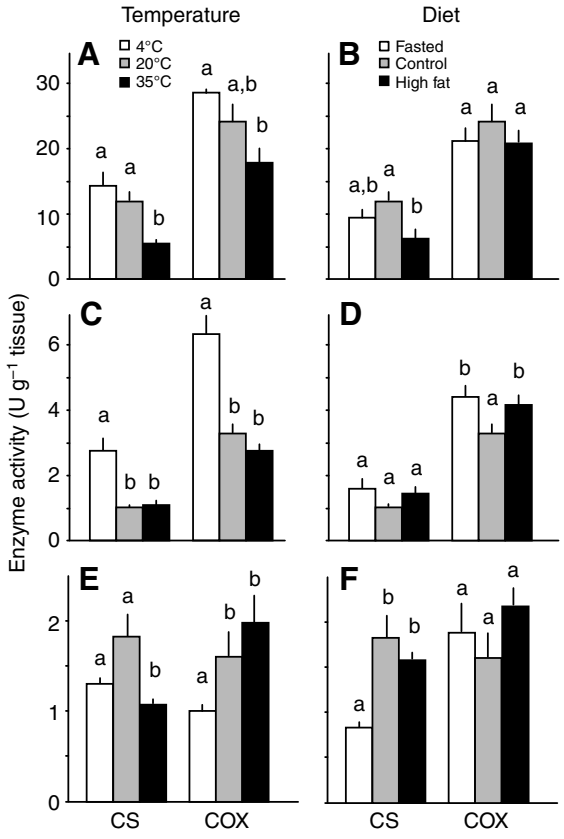

Fig. 1. Mitochondrial enzyme activities in red muscle (A,B), white muscle (C,D) and liver (E,F) of goldfish exposed to three acclimation temperatures (4, 20, 35°C) and three diets (fasted, control and high fat diet). Values are expressed as means + s.e.m., and are labelled with different letters to indicate statistical significance according to a Kruskall–Wallis test followed by a Mann–Whitney *U*-test, with *N*=5–7 for each treatment. CS, citrate synthase; COX, cytochrome oxidase.

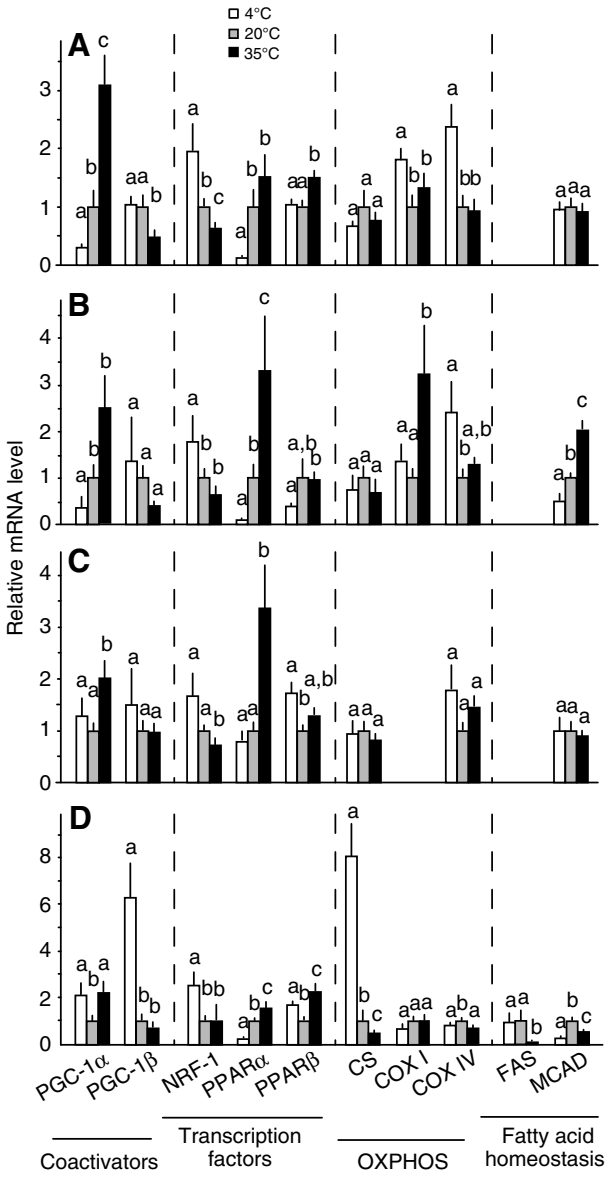

Fig. 2. Gene expression in red muscle (A), white muscle (B), heart (C) and liver (D) of goldfish exposed to three acclimation temperatures (4, 20, 35°C). Values are expressed as means + s.e.m. relative to controls (20°C and control diet fish). Different letters indicate statistically significant differences between treatments according to a Kruskall–Wallis test followed by a Mann–Whitney *U*-test (*N*=7–17). MCAD, medium chain acyl CoA dehydrogenase; NRF-1, nuclear respiratory factor-1; PPAR, peroxisome proliferator-activated receptor; PGC-1, PPARγ coactivator-1; OXPHOS, oxidative phosphorylation; other abbreviations as in Fig. 1.

(Fig. 1A), the activity of the Krebs cycle enzyme CS and of the electron transport chain enzyme COX was approximately 2- and 1.6-fold higher in goldfish acclimated at 4°C *vs* 35°C. These changes were even more pronounced in white muscle of cold-acclimated fish (Fig. 1C), as CS and COX enzyme activity was, respectively, 2.3- and 3-fold higher than in the 35°C group. The temperature acclimation had less impact on enzyme activity in liver (Fig. 1F). There was no consistent pattern in CS activity (statistically highest at 20°C) whereas COX activity increased at elevated temperature. Thus, skeletal muscles, but not liver, exhibited positive thermal compensation with both mitochondrial enzymes.

Diet had negligible effects on mitochondrial enzymes in most tissues, with results showing either non-significant patterns or minor but significant changes (Fig. 1). Compared with the normal diet counterparts, there was an approximately 50% decrease in CS activity in red muscle in response to the high fat diet (Fig. 1B) and in liver in response to starvation (Fig. 1F). COX activity was virtually invariant with dietary treatments (Fig. 1B,D,F).

We measured both COX and CS for independent assessment of mitochondrial content. The stoichiometry between COX and CS was approximately 2 in both skeletal muscles and was largely unaffected by stressors. In liver, there was relatively less CS, such that the activity ratio of COX:CS was close to 1 under all conditions, but with food deprivation the decline in CS altered the stoichiometry (COX:CS about 2).

Temperature acclimation and mRNA

Skeletal muscles showed increases in mitochondrial enzyme activity in cold-acclimated fish, but the mRNA pattern was less uniform. For example, in red and white muscle, CS mRNA was unaffected by temperature (Fig. 2A,B). Low temperature significantly increased COX IV mRNA (a nuclear-encoded subunit) in both skeletal muscles, whereas COX I (a mitochondrial-encoded subunit) was significantly elevated only in red muscle; in white muscle, COX I

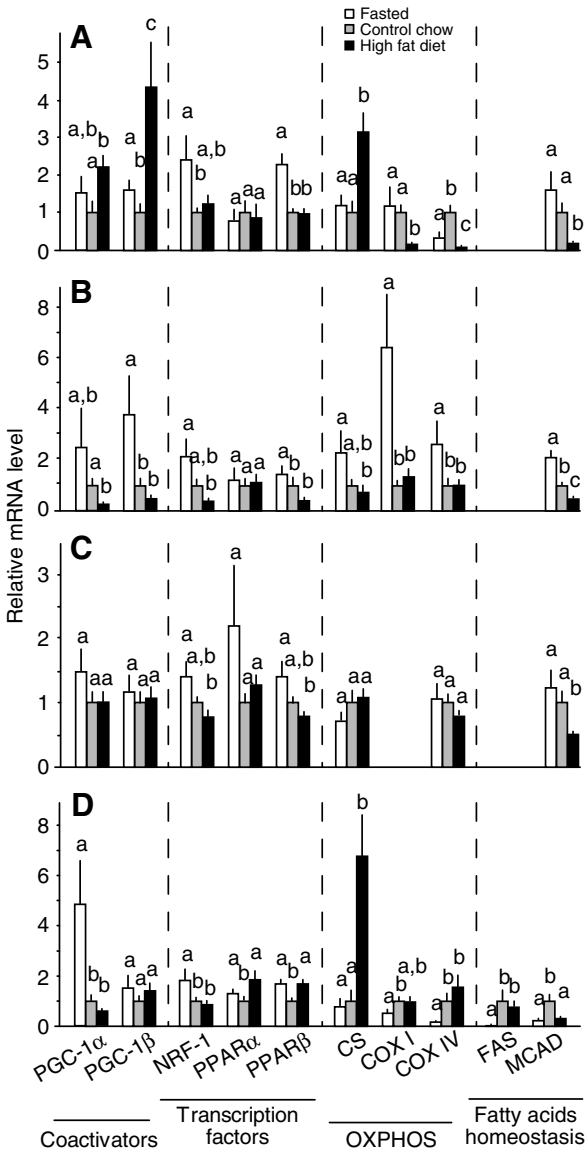

Fig. 3. Gene expression in red muscle (A), white muscle (B), heart (C) and liver (D) of goldfish exposed to three diets (fasted, control and high fat diet). Values are expressed as means + s.e.m. relative to controls (20°C and control diet fish). Different letters indicate statistically significant differences between treatments according to a Kruskall–Wallis test followed by a Mann–Whitney *U*-test (N=7–17). Abbreviations as in Fig. 2.

was highest in the 35°C group (Fig. 2A,B). In heart, temperature had no effect on either CS or COX IV mRNA level. In liver, CS transcript level was inversely related to temperature (Fig. 2D), but in comparison COX I and COX IV were relatively unaffected. Thus, temperature-induced changes in the mRNA of COX IV (but not COX I) were consistent with patterns of COX enzymatic activity, and there was little relationship between CS transcript level and enzymatic activity.

Temperature had no effect on the transcript level of medium chain acyl CoA dehydrogenase (MCAD) in either red muscle (Fig. 2A) or heart (Fig. 2C), but white muscle MCAD mRNA level was directly related to temperature (Fig. 2B). In liver, MCAD expression was greatest at an intermediate temperature, 70% lower in the cold and 45% lower in the warm. The mRNA level of the lipogenic enzyme fatty acid synthase (FAS) was only reliably detected in liver, where the transcript level was depressed by 90% in the warm temperature group (Fig. 2D).

Overall, NRF-1 mRNA level was inversely related to temperature whereas PPARα mRNA level was directly related to temperature (Fig. 2). PPARβ mRNA expression was more complex, showing a tissue-specific pattern. PGC-1α expression was directly related to temperature in the three striated muscles (Fig. 2A,B,C), but was unaffected in liver (Fig. 2D). In contrast, PGC-1β mRNA was inversely related to temperature in red muscle and liver, but heart was unaffected. The same inverse trend occurred in white muscle but there was no significant temperature effect. With respect to the PGC-1 family members, changes in PGC-1α and PGC-1β mRNA were typically in opposite directions.

Considering the mRNA profiles in relation to temperature, with some exceptions there was a general relationship between (i) PGC-1β, NRF-1 and mitochondrial genes, and (ii) PGC-1α and PPARs.

Dietary treatments and mRNA

As with the temperature treatments, the dietary regimes revealed a lack of correlation between CS transcript and enzyme activity. Unlike with the temperature treatments, COX mRNA expression pattern also changed in tissues that displayed no change in enzymatic activity. CS mRNA levels increased 3-fold in response to the high fat diet in red muscle (Fig. 3A), whereas CS activity declined by 50% (Fig. 1B). Likewise, CS mRNA in liver increased 6-fold (Fig. 3D) whereas enzymatic activity was unaffected (Fig. 1F). Food deprivation decreased the levels of mRNA for COX subunits in liver (COX I and IV; Fig. 3D) and red muscle (COX IV; Fig. 3A), but increased COX I and COX IV transcript levels in white muscle (Fig. 3B), where a small but significant increase in activity was observed (Fig. 1D). The mRNA levels for CS and COX IV were unaffected by any dietary regime in heart (Fig. 3C).

The high fat diet reduced MCAD expression 40–80% in each tissue (Fig. 3). Food deprivation increased the MCAD mRNA level 2-fold in white muscle (Fig. 3B) and decreased it by 50% in liver (Fig. 3D). While relatively unchanged in the livers of high fat-fed fish, FAS mRNA levels were reduced by over 95% in the fasted animals (Fig. 3D).

A high fat diet had no significant effect on NRF-1 mRNA level in any tissue. Likewise, the high fat diet had no effect on PPAR mRNA in most tissues, though liver demonstrated a modest increase in both PPARα and PPARβ (Fig. 3D). Caloric restriction had a negligible effect on the expression of NRF-1 and PPARα in heart (Fig. 3C) and white muscle (Fig. 3B), but increased PPARβ in skeletal muscles (Fig. 3A,B), NRF-1 in red muscle (Fig. 3A) and expression of all three in liver (Fig. 3D).

The pattern of response of PGC-1α and PGC-1β mRNA to dietary manipulations differed among tissues. PGC-1α and PGC-1β showed parallel responses in the muscles. In heart, neither dietary regime affected the mRNA for either coactivator (Fig. 3C). In red muscle, the high fat diet and food deprivation elevated both PGC-1β and PGC-1α mRNA, though in fasted fish the 50% increase in PGC-1α mRNA was not significant (Fig. 3A). In white muscle, the mRNA level for both coactivators tended to decrease with a high fat diet and to increase with food deprivation (Fig. 3B). The dietary treatments did not affect the hepatic levels of PGC-1β, but the starved fish exhibited an approximately 5-fold increase in PGC-1α transcript level (Fig. 3D).

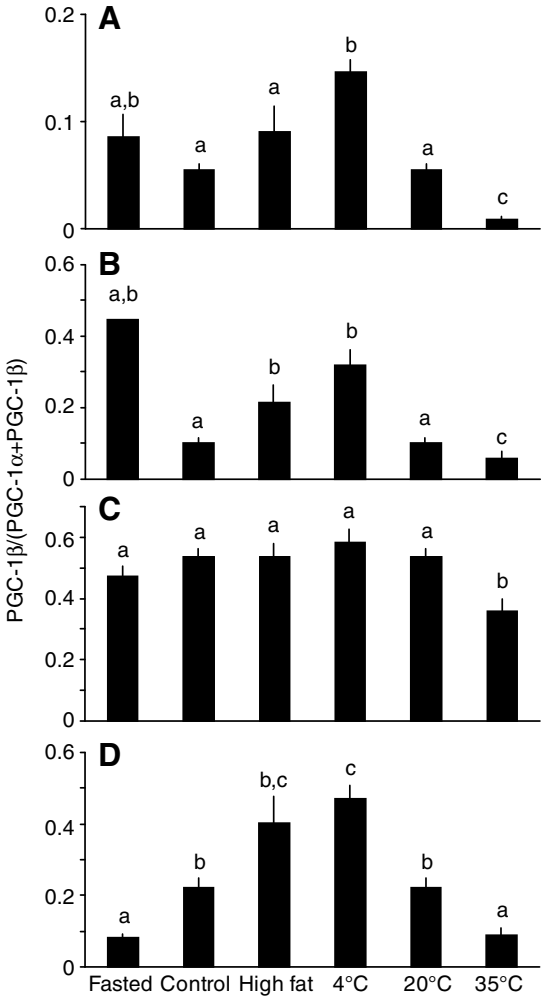

Fig. 4. PGC-1β mRNA expressed as a proportion of PGC-1α + β transcript levels in red muscle (A), white muscle (B), heart (C) and liver (D) of goldfish exposed to three acclimation temperatures (4, 20, 35°C) and three dietary treatments (fasted, control and high fat diet). Bars represent means + s.e.m. Different letters indicate statistically significant differences between treatments according to a Kruskall–Wallis test followed by a Mann–Whitney *U*-test (N=7–17). Abbreviations as in Fig. 2.

Relative abundance of PGC-1 family members

We were interested in the role of the transcriptional regulators and among them the PGC-1 family, so we looked at the relative importance of the family members in different tissues. We analysed PRC in each tissue of control fish and found that it was ubiquitously expressed but at low levels (<5% of PGC-1α level) in each tissue we examined (data not shown). Thus, the transcripts of PGC-1α and PGC-1β were the predominant isoforms in each tissue, with their relative abundance presented in Fig. 4. In control fish, PGC-1β mRNA represented only about 10% of the total for the two coactivators in red and white muscle, about 25% in liver and 50% in heart. In heart, the relative proportion of the two coactivators was unaffected by any treatment. In the other tissues, PGC-1β transcript level was proportionally higher with decreasing temperature. Fasting decreased the relative abundance of PGC-1β in liver by about 50% but increased it about 5-fold in white muscle (Fig. 4D).

Global patterns in transcriptional regulators

When all the tissues and treatments were combined, there were significant correlations between mRNA levels of the PGC-1 coactivators and their transcription factor binding partners (NRF-1, PPARα, PPARβ) and regulatory targets (CS, COX IV). PGC-1α was the better predictor of mRNA levels for PPARα (Fig. 5A *vs* 5B) and PPARβ (Fig. 5C *vs* 5D). The coactivators had a weaker correlation with MCAD mRNA level, and there was little difference in the regression coefficient with PGC-1α *vs* PGC-1β. MCAD mRNA was better correlated with PPARα (R^2=0.48) and PPARβ (R^2=0.46).

Of the two coactivators, PGC-1β mRNA was the better predictor of mRNA levels for NRF-1 (Fig. 5H *vs* 5G), CS (Fig. 5J *vs* 5I) and COX IV (Fig. 5L *vs* 5K). There was also a strong relationship between CS and NRF-1 mRNA levels (R^2=0.54, data not shown). As well, transcript levels for COX IV and COX I exhibited a strong correlation (R^2=0.71) in skeletal muscle (data not shown).

DISCUSSION

Animal tissues differ in their mitochondrial phenotype in terms of metabolic rate and the relative importance of mitochondrial metabolism in energy production and fuel selection. In addition to constitutive differences in mitochondrial content among tissues, each tissue can respond to metabolic stress to fine tune the bioenergetics of the cell. Studies in mammals have identified several factors as central regulators of these adaptations (e.g. PGC-1s, NRFs, PPARs), but less is known about the role of these regulators in lower vertebrates. In the present study we subjected goldfish to diet and temperature challenges to investigate the metabolic response elicited, and the putative role of transcriptional regulators in this response.

Mitochondrial enzyme activities

In our comparisons between tissues and treatments, we assessed both COX and CS to distinguish between enzyme-specific changes and changes in mitochondrial content. The COX:CS stoichiometries (COX:CS about 2) were relatively unaffected by treatment and similar among skeletal fiber types (Fig. 1). Liver showed a lower COX:CS stoichiometry (close to 1), except with food deprivation, when CS decreased. The relatively higher CS activity in liver, and the changes in response to food deprivation probably underscore its role in fatty acid synthesis rather than oxidative metabolism *per se*.

The dietary and thermal stressors caused different responses in individual tissues. Diet exerted its main effects on liver (primarily on CS), whereas temperature affected mainly the muscles. The increase in mitochondrial enzymes at low temperature is qualitatively similar to that seen in numerous other studies in fish (Battersby and Moyes, 1998; McClelland, 2006; van den Thillart and Modderkolk, 1978). Likewise, the absence of thermal compensation in liver is similar to findings in previous studies (Hardewig et al., 1999; Lucassen et al., 2006; van den Thillart and Modderkolk, 1978). Our goal was to use both intertissue differences and adaptive remodelling of tissues to generate a collection of conditions in which the relationships between transcriptional regulators and their putative targets could be assessed.

Regulation of expression of genes encoding mitochondrial enzymes

Though cold temperature increased CS and COX enzyme activities to a similar extent in skeletal muscles, the transcript level of each enzyme indicated differential modes of regulation.

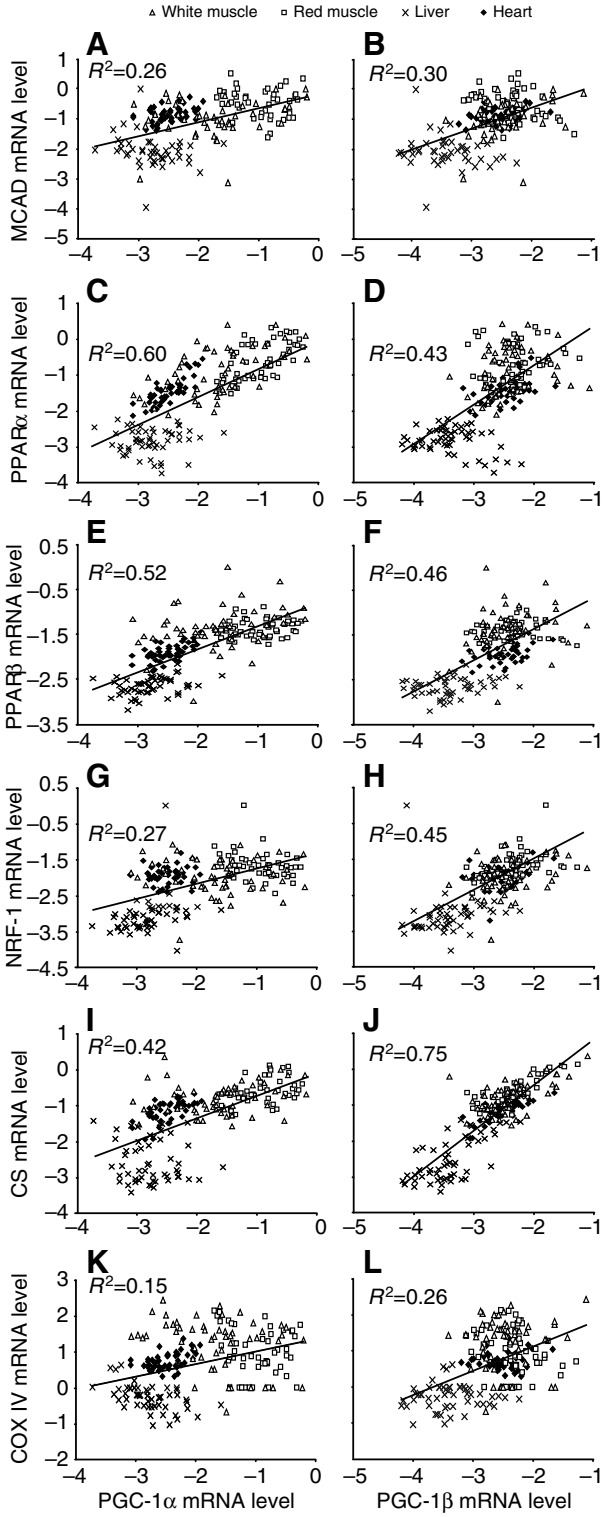

Fig. 5. Putative regulators of metabolic gene expression in goldfish tissue. Paired relative mRNA values for all tissues and all treatments were used; R^2 values were determined by linear regression; all correlations were found to be significant (P<0.05; N=46–53 per tissue). Abbreviations as in Fig. 2.

CS is a homodimer with no isoforms, making it easier to develop links between gene regulation and enzyme abundance. Though CS enzyme activity differed among tissues, and changed in individual tissues with stressors, there was very little relationship between CS enzyme activity and CS mRNA level. This situation is usually interpreted as evidence of post-transcriptional determination of protein levels. The lack of relationship is particularly clear in cold acclimation, where CS mRNA levels did not vary (Fig. 2) despite positive thermal compensation of the enzyme (Fig. 1), as well as diet, where CS mRNA increased without corresponding changes in enzyme activity. Post-transcriptional regulation of CS has been implicated in various contexts, including intertissue comparisons in trout (Leary et al., 1998), evolutionary and developmental variation in scombrids (Dalziel et al., 2006), and thermal compensation in fish (Lucassen et al., 2006; McClelland et al., 2006). While transcriptional regulation did not account for enzymatic changes, it is still worthwhile to assess the nature of the regulation of CS gene expression. CS mRNA changed dramatically in two tissues; in both cases there was a corresponding change in PGC-1β mRNA: an increase in liver with low temperature (Fig. 2D), and an increase in red muscle with a high fat diet (Fig. 3A). Furthermore, where intertissue differences were considered in combination with treatments (Fig. 5), there was a very strong correlation between CS and PGC-1β mRNA (R^2=0.75). Conversely, there was little relationship between mRNA levels of CS and PGC-1α with treatment. For example, increases in PGC-1α mRNA occurred in muscles at high temperature without changes in CS mRNA (Fig. 2A–C). Although, when all tissues and treatments were pooled, there was a correlation between CS mRNA and PGC-1α mRNA (Fig. 5I), this relationship was driven by the liver data. If only muscles are considered, there was a weak correlation between the transcript levels of CS and PGC-1α (R^2=0.29; data not shown). Despite its common use as a marker of mitochondrial content, relatively little is known about the regulation of the CS gene. Promoter analysis in mammals suggests a role for Sp1 (Kraft et al., 2006). NRF-1 appears to potentiate Sp1 effects on respiratory gene expression (Seelan et al., 1996; Virbasius and Scarpulla, 1994). The level of CS mRNA also correlated with NRF-1 mRNA (R^2=0.54), suggesting that PGC-1β and NRF-1 may each influence the regulation of CS expression.

In contrast to the situation with CS, the complex structure of COX, with 13 subunits (10 nuclear genes and 3 mitochondrial genes), makes it more challenging to develop models of transcriptional regulation. Nonetheless, there was a strong relationship between COX enzyme activity and mRNA for COX IV (nuclear encoded) and, to a lesser extent, COX I (mitochondrial encoded). The relationship was most clear in thermal remodelling of skeletal muscle, where COX IV transcript paralleled increased COX enzyme activity in both red and white muscles. Other studies have found that changes in COX activity arise in response to transcriptional regulation (Hardewig et al., 1999). In addition, we found a strong correlation between COX I mRNA and COX IV mRNA in skeletal muscles with the various stresses (R^2=0.71). In general, the various nuclear genes encoding COX subunits are regulated in a coordinated fashion (Evans and Scarpulla, 1990; Ongwijitwat et al., 2006; Wiesner et al., 1992), and most studies in mammals suggest that several COX genes are regulated in a NRF-1/PGC-1α-dependent manner (see Handschin and Spiegelman, 2006; Scarpulla, 2006). In our study, NRF-1 mRNA was in general a good indicator of COX IV fluctuations in muscles; the relationship was clearest in thermal compensation of white muscle (Fig. 2B). Surprisingly, there was no relationship between PGC-1α mRNA and the transcript level of either NRF-1 or COX subunits; the lack of relationship is best illustrated in the context of thermal acclimation, where mitochondrial enzymes, mitochondrial transcripts and NRF-1 mRNA all increased at low temperature while PGC-1α mRNA markedly decreased (Figs 1 and 2). Though there was a modest correlation between NRF-1 and PGC-1α mRNA when the data were pooled (Fig. 5G), this relationship is driven primarily by the liver data.

There is no correlation between these parameters when only muscles are considered (R^2=0.03, data not shown). There was a stronger relationship between mRNA of NRF-1 and PGC-1β in relation to the stressors in individual tissues (Figs 2 and 3), as well as the global relationship across tissues (Fig. 5H).

These patterns of expression may not necessarily reflect direct interactions between the different factors and may involve additional regulators. However, collectively the data support a role for PGC-1β, but not PGC-1α, in the control of mitochondrial gene expression in fish, and in the determination of intertissue differences and adaptive responses to physiological stressors.

Lipid homeostasis gene expression

In addition to changes in mitochondrial content, many physiological stressors lead to changes in fuel selection through effects on the mitochondrial enzymes involved in fatty acid oxidation. MCAD, an enzyme in the β-oxidation of fatty acids, is an established target of PPAR-dependent regulation (Gulick et al., 1994). MCAD levels were only slightly affected by temperature, although a strong induction of MCAD expression was seen in white muscle in the warm-acclimated group (Fig. 2B). MCAD mRNA levels decreased in all tissues with a high fat diet (Fig. 3). This would seem counterintuitive, given that an abundance of fat in the diet should lead to increased reliance on lipid. However, it is conceivable that the reduction in MCAD reflects a response to fatty acid chain length profile rather than lipid content *per se*. Food deprivation caused a marked reduction in FAS, consistent with a shift away from lipogenesis, as seen in other models (Field et al., 2003; Kim et al., 2003; Li et al., 2006; Paulauskis and Sul, 1988).

In most tissues, the changes in MCAD mRNA were paralleled by changes in mRNA of one or both PPARs. This is consistent with the situation in mammals (Cheng et al., 2004; Tanaka et al., 2003; Vega et al., 2000). When reconciling changes in MCAD with the coactivators during physiological remodelling, there was a relatively weak relationship between MCAD and both PGC-1α and PGC-1β. In situations where MCAD mRNA level changed (e.g. liver with diet), the mRNA for PGC-1α and PGC-1β either failed to change, or changed in the opposite direction. Furthermore, when all tissues were pooled, there was a weak correlation between MCAD and both PGC-1α and PGC-1β. There was a stronger relationship between the coactivators and both PPARα and PPARβ expression (Fig. 5), though in both cases the correlation was stronger with PGC-1α. Although these correlations could indicate shared sensitivity to common regulatory pathways, overall the results are similar to the scenario seen in mammals, where the role for PPARs is clear (Vega et al., 2000; Wang et al., 2003).

The PGC-1 family

In mammals, PGC-1α and PGC-1β have overlapping roles, with similar capacities to induce mitochondrial biogenesis when overexpressed (Lehman et al., 2000; St-Pierre et al., 2003; Wu et al., 1999). As well, both PGC-1α and PGC-1β correlate with the oxidative capacity of tissues (Esterbauer et al., 1999; Kressler et al., 2002; Larrouy et al., 1999; Puigserver et al., 1998). Though PGC-1α garners most attention as a master controller of mitochondrial content, and it typically occurs at higher levels than PGC-1β (Andersson and Scarpulla, 2001; Kressler et al., 2002), the relative importance of PGC-1β has not been established. Null mutants for both PGC-1α and PGC-1β present distinct mitochondrial dysfunctions as well as tissue metabolic derangements that are not compensated by the respective functional family members (Lin et al., 2004; Lelliott et al., 2006; Sonoda et al., 2007; Uldry et al., 2006). Though there may be redundancy in their capacities to regulate mitochondrial biogenesis as suggested in mammals (Uldry et al., 2006), there appear to be specific capabilities unique to PGC-1β. In our study, we found that in each tissue PGC-1α mRNA was greater than PGC-1β mRNA (Fig. 4). However, the differential expression of the PGC-1α and PGC-1β isoforms in the various stressors is further support for distinct roles for the two coactivators. While the DNA-binding proteins behaved much as expected from mammalian studies, there was an unexpected lack of coordination between mitochondrial gene expression and the putative 'master controllers' of mitochondrial biogenesis.

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