Regulation of Carnitine Palmitoyltransferase (CPT) I during Fasting in Rainbow Trout (*Oncorhynchus mykiss*) Promotes Increased Mitochondrial Fatty Acid Oxidation

Andrea J. Morash*
Grant B. McClelland
Department of Biology, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada

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ABSTRACT

Periods of fasting, in most animals, are fueled principally by fatty acids, and changes in the regulation of fatty acid oxidation must exist to meet this change in metabolic substrate use. We examined the regulation of carnitine palmitoyltransferase (CPT) I, to help explain changes in mitochondrial fatty acid oxidation with fasting. After fasting rainbow trout (*Oncorhynchus mykiss*) for 5 wk, the mitochondria were isolated from red muscle and liver to determine (1) mitochondrial fatty acid oxidation rate, (2) CPT I activity and the concentration of malonyl-CoA needed to inhibit this activity by 50% (IC_{50}), (3) mitochondrial membrane fluidity, and (4) CPT I (all five known isoforms) and peroxisome proliferator-activated receptor (PPARα and PPARβ) mRNA levels. Fatty acid oxidation in isolated mitochondria increased during fasting by 2.5- and 1.75-fold in liver and red muscle, respectively. Fasting also decreased sensitivity of CPT I to malonyl-CoA (increased IC_{50}), by two and eight times in red muscle and liver, respectively, suggesting it facilitates the rate of fatty acid oxidation. In the liver, there was also a significant increase CPT I activity per milligram mitochondrial protein and in whole-tissue PPARα and PPARβ mRNA levels. However, there were no changes in mitochondrial membrane fluidity in either tissue, indicating that the decrease in CPT I sensitivity to malonyl-CoA is not due to bulk fluidity changes in the membrane. However, there were significant differences in CPT I mRNA levels during fasting. Overall, these data indicate some important changes in the regulation of CPT I that promote the increased mitochondrial fatty acid oxidation that occurs during fasting in trout.

* Corresponding author. Present address: Department of Physiology, Development and Neuroscience, Physiological Laboratory, Downing Site, University of Cambridge, Downing Street, Cambridge CB2 3ES, United Kingdom; e-mail: am2023@cam.ac.uk.

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Introduction

In a variety of animal species, bouts of fasting are experienced as a normal part of their natural life history. In most animals, these periods of fasting are fuelled principally by fatty acids. The use of lipids as a fuel is advantageous because they are highly reduced and can be stored in large quantities without hydration. The enzyme carnitine palmitoyltransferase (CPT) I helps control the entry of long-chain fatty acids into the mitochondria (McGarry et al. 1983), and its regulation is important in the modulation of fatty acid oxidation during periods of stress. Indeed, current evidence suggests that when food is unavailable or intake is suspended (e.g., during migration in fish) for extended periods of time, lipids are preferentially oxidized over other fuel sources in both mammals and fish. In contrast, carbohydrates are spared to provide glucose to the brain and central nervous system (Neumann-Haefelin et al. 2004), while proteins are usually oxidized as lipid stores decline (Frayn 1996). There are a host of metabolic modifications that must take place to increase lipid oxidation and decrease carbohydrate oxidation. As a first step to uncovering the regulatory changes that occur with fasting in fish we focus here on mitochondrial lipid oxidation and the regulation of CPT I.

Regulation of mitochondrial fat oxidation involves both genetic and nongenetic regulation of CPT I activity (McClelland 2004; Morash et al. 2008). CPT I isoforms are encoded by two different genes, CPT Iα and CPT Iβ, expressed in the liver and muscle, respectively (Britton et al. 1997). These isoforms are differentially sensitive to regulation by malonyl-CoA. The mammalian muscle CPT I has been shown to be approximately 100 times more sensitive to malonyl-CoA than the liver isoform (McGarry and Brown 1997). Previously, there was thought to be only one CPT I gene in fish (Gutieres et al. 2003). However, our recent research has demonstrated that CPT I in liver and muscle are differentially sensitive to malonyl-CoA, though unlike in CPT I in mammals, CPT I in trout liver is more sensitive to malonyl-CoA than CPT I in trout muscle is (Morash et al. 2008). Recently we have shown that these taxa-specific differences may be the result of genome duplications in bony fishes giving rise to five distinct CPT I isoforms in trout with tissue-specific expression patterns (Morash et al. 2010).

CPT I mRNA and protein expression in mammals is, in part, controlled by the transcription factor peroxisome proliferator-activated receptor (PPAR), specifically PPARα (Price et al.

2000). It is assumed that fish CPT I genes also contain a PPAR response element, although regulation through this mechanism may be more complicated as multiple isoforms of PPARα and PPARβ are known to exist, at least in some fish species (Robinson-Rechavi et al. 2001; Leaver et al. 2007). Currently, only single isoforms of PPARα and PPARβ have been identified in trout. The effects of fasting on gene expression in fish muscle and liver are unclear. If these nuclear receptors act similarly in fish and in mammals, increases in cytosolic free fatty acids, which occur with fasting, could act as ligands for PPARs, thereby increasing transcription of many genes of the fatty acid oxidation pathway (Price et al. 2000). Also, differences in regulation of CPT I between fish and mammals suggest that there may be species-specific responses to fasting at the level of mitochondrial fatty acid oxidation.

In addition to genetic control, CPT I activity is also regulated allosterically by malonyl-CoA that is produced during the first committed step of fatty acid synthesis in the liver (McGarry and Brown 2000). Malonyl-CoA in the cytosol can inhibit CPT I activity and prevent the oxidation of any newly formed fatty acids. Regulation of fatty acid oxidation is dependent on the sensitivity of CPT I to this modulator. Most notably, changes in sensitivity can occur by changes in mitochondrial membrane fluidity (Kolodziej and Zammit 1990). This has been theorized to occur because the active site for malonyl-CoA is located on the cytosolic side of the transmembrane CPT I protein, and this area of the protein is adjacent to the mitochondrial membrane (Jackson et al. 2000). Increases in the fluidity of the mitochondrial membrane reduces the ability of malonyl-CoA to bind, as key amino acids at the N- and C-termini of the malonyl-CoA binding site of the CPT I protein become spatially separated (Faye et al. 2005). In mammals, mitochondrial membrane fluidity can be altered by a number of physiological states, including diabetes and starvation (Zammit et al. 1997) and by ingesting a diet high in polyunsaturated fatty acids (Power et al. 1994). In rat liver, starvation causes an increase in mitochondrial membrane fluidity (Zammit et al. 1998) as well as a decrease in CPT I sensitivity to malonyl-CoA (Drynan et al. 1996; Zammit et al. 1998). In fish, membrane composition and fluidity are highly malleable by temperature (Hazel 1984) and diet (Guderley et al. 2008; Morash et al. 2008), but the effect of fasting is currently unclear.

To date, most research on the effects of fasting on lipid oxidation has focused on a single tissue (liver) and taxon (mammals). However, many fish species experience bouts of fasting as part of their natural life history, which makes them a prime group for investigating the physiology of fasting. We previously showed that trout liver and red muscle CPT I show differential regulation and activity and that this regulation is quite different from that seen in mammals (Morash et al. 2008). For example, fish muscle CPT I is significantly less sensitive to M-CoA than liver CPT I is, while in mammals the liver is the less sensitive of the two tissues.

Our goal was to determine the responses in liver and red muscle mitochondria in rainbow trout to prolonged food deprivation. More specifically, we examined the genetic and non-genetic regulation of CPT I as a means to increase fatty acid oxidation capacity with fasting. After fasting trout for 5 wk, the trout mitochondria were isolated from red muscle and liver to determine (1) changes in mitochondrial fatty acid oxidation, (2) CPT I activity and sensitivity to malonyl-CoA, (3) membrane fluidity, and (4) CPT I isoforms and PPAR (α and β) mRNA levels.

Material and Methods

Experimental Fish and Conditions

Rainbow trout, *Oncorhynchus mykiss*, (~200 g) were obtained from a local hatchery (Humber Springs, Orangeville, ON) and maintained in 500-L tanks with circulating dechlorinated Hamilton tap water kept at 12°C and fed a commercial fish diet (Profishent Classic Floating Trout Grower, Martin Mills, Elmira, ON) until the start of the experiment. At the start of the experiment, fish were randomly separated into two 500-L tanks. For 5 wk, one group of fish was fed the same commercial diet daily to satiation while the other group was fasted. Five weeks of fasting was chosen to mimic natural lengths of fasting in migrating salmonids.

Mitochondrial Isolation

Mitochondria were isolated from red muscle and liver according to established methods (Suarez and Hochachka 1981; Moyes et al. 1988) previously described elsewhere (Morash et al. 2008). Briefly, each tissue was immediately excised (red muscle [~4 g] and whole liver) and placed, on ice, in mitochondrial isolation buffer (MIB) containing (in mM) 140 KCl, 10 EDTA, 5 $MgCl_2$, 20 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), and 0.5% bovine serum albumin (BSA; pH 7.0) for red muscle and 250 sucrose, 1 EDTA, 20 HEPES, and 0.5% BSA (pH 7.4) for liver. Tissues were homogenized three times, first using a chilled wide-clearance Potter-Elvehjem homogenizer, then three times with a narrow-clearance homogenizer to lyse cells. The resulting homogenates were centrifuged at 800 *g* for 10 min at 4°C. The supernatant was then spun at 9,000 *g* for 10 min at 4°C. Pellets were resuspended in the appropriate MIB lacking BSA and spun again at 9,000 *g* for 10 min at 4°C. The supernatant was discarded, and the mitochondrial pellet was resuspended in an appropriate volume of MIB lacking BSA and kept on ice for use in a CPT I assay and fatty acid oxidation assay.

Enzyme and Protein Assays

Fatty Acid Oxidation Assay. Fatty acid oxidation was assayed using a modified protocol for human skeletal muscle (Bezaire et al. 2006) and respiration buffer for fish (Leary et al. 2003). Palmitate oxidation was determined at room temperature by measuring trapped ^{14}C from labeled CO_2 production after a 30-min incubation of viable mitochondria in a sealed system. An 900-μL aliquot of respiration buffer (in mM, 140 KCl, 20 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES),

5 Na_2HPO_4, 0.5% fatty acid free–BSA supplemented with 5 ATP, 1 NAD^+, 0.5 carnitine, 0.1 coenzyme A, 0.5 malate) was added to a 20-mL glass scintillation vial at room temperature. The $^{14}CO_2$ produced during oxidation was trapped in150 μL of benzethonium hydroxide in a tube suspended within the reaction vial. Mitochondria (100 μL or ~1 mg mL^{-1}) were added to the system, which was then sealed with a rubber cap. The reaction was then started with the addition of 1 μCi of [1-^{14}C]-palmitate (specific activity 56 mCi/mmol; Amersham Biosciences, Baie d'Urfé, Quebec) in a final palmitate concentration of 100 μM via a syringe through the rubber cap. After 30 min at room temperature the reaction was terminated by the addition of 50 μL of 12N $HClO_4$. The remaining reaction mixture was acidified using 1 mL of 1M H_2SO_4. After 90 min at room temperature the suspended tube containing the benzethonium hydroxide and trapped $^{14}CO_2$ was then transferred to a new scintillation vial and radioactivity was counted on a Tricarb 2900 liquid scintillation analyzer (PerkinElmer).

CPT I Assay. Radioactive CPT I assay followed a published protocol (Morash et al. 2008) that was modified from previous methods for mammals (McGarry et al. 1983) to incorporate the appropriate changes for fish mitochondria (Rodnick and Sidell 1994). Briefly, 1 μCi of L-[methyl-^{3}H] carnitine hydrochloride (specific activity 82.0 Ci/mmol; Amersham Biosciences) was added to 70 μL of the assay mixture (in mM, 20 HEPES, 40 KCl, 1 EGTA, 220 sucrose, 0.1 DTT, 0.04 palmitoyl-CoA, 1 carnitine, and 1.3 mg/mL BSA, pH 7.0), which was placed in 1.5-mL Eppendorf tubes and incubated with 10 μL of 0.5–500 mM malonyl-CoA (for a final concentration of 0.05–50 μM) or H_2O in place of mitochondria for blanks or in place of malonyl-CoA and maximum activity. The reaction was initiated by the addition of 20 μL of mitochondria in MIB (~2 mg mL^{-1}) and incubated at room temperature for 8 min. The reaction was stopped by the addition of 60 μL of 1M HCl. The palmitoyl-[^{3}H]-carnitine was collected according to Starritt et al. (2000). Twenty microliters of the assay mixture with L-[methyl-^{3}H]-carnitine hydrochloride was also counted in duplicate for determination of individual specific activity. Background counts were determined from a blank sample containing aqueous counting scintillation fluid. The decays per minute (DPM) were read for 5 min per sample on a Tricarb 2900 TR liquid scintillation analyzer using QuantaSmart 1.31 (Packard Instrument) analysis software. The assay was performed at room temperature.

Citrate Synthase Assay. Citrate synthase was measured in isolated intact mitochondria, lysed isolated mitochondria and whole tissue according to Morash et al. (2008) in order to determine mitochondrial quality for the fatty acid oxidation and CPT I assays. Briefly, samples were placed in an assay buffer ([in mM] 20 TRIS [pH 8.0], 0.1 DTNB, and 0.3 acetyl-CoA), the reaction was started by the addition of 0.5mM oxaloacetate, and absorbance was monitored at 412 nm. Control rates lacking oxaloacetate were subtracted to account for variability in vivo substrate concentration during isolation.

Protein Content. Protein concentrations were determined by the Bradford method (Bradford 1976) using a BioRad kit (BioRad, Mississauga, Ontario).

Mitochondrial Membrane Fluidity

Aliquots of frozen mitochondria were thawed on ice and diluted in 3 mL of mitochondrial isolation buffer to a concentration of 50 μg mL^{-1} in a 3-mL quartz cuvette. Diluted mitochondria were then incubated in the dark with 1.5 μL 1,6-diphenyl 1,3,5-hexatriene (DPH) in 2 mM N′,N′-dimethyl formamide for 10 min at 10°C. Samples were stirred throughout the duration of the incubation and the assay with a micro–stir bar in a water-jacketed cell holder. Fluorescence polarization of DPH was measured at 10°C on a QuantaMaster model C-61 T-format scanning spectrofluorometer equipped with polarizing filters (Photon Technology, London, ON). Excitation of DPH was at 358 nm and fluorescence emissions were measured at 428 nm. Each sample was measured for 120 s at a rate of 1 reading s^{-1} using FeliX (ver. 1.41) software. Samples were measured in triplicate. Fluorescence polarization, an index of membrane fluidity, was calculated according to published methods (Litman and Barenholz 1982).

mRNA Quantification by Real-Time Polymerase Chain Reaction (PCR)

Total RNA was extracted from red muscle and liver using TRIzol Reagent (Invitrogen, Carlsbad, CA) based on the acid guanidinium thiocyanate-phenol-chloroform extraction method. RNA was quantified by UV spectroscopy at 260 nm and then diluted to 0.5μg μL^{-1}. We synthesized cDNA using 1 μg of DNase (Invitrogen) treated mRNA with SuperScript RNase H^- reverse transcriptase (Invitrogen). Real-time PCR was conducted using SYBR green with ROX as a reference dye in a Stratagene Mx3000P real-time PCR system. Each 25-μL reaction contained 12.5 μL SYBR green mix, 1 μL each of forward and reverse primer (5μM), 5.5 μL of DNase/RNase free water, and 5 μL of $\times 5$ diluted cDNA. Primers were designed using Primer3 software (Rozen and Skaletsky 2000) or previously published work (Morash et al. 2008, 2010), and the specific primer sequences appear in Table 1. The thermal program included 3 min at 95°C, 40 cycles of 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s for PPARα and PPARβ and 3 min at 95°C, 40 cycles of 95°C for 15 s, 58°C for 30 s, and 72°C for 30 s for the five CPT I isoforms. A no-template control and dissociation curve was performed to ensure only one PCR product was being amplified and stock solution was not contaminated. Standard curves were constructed for each gene using serial dilutions of stock cDNA to account for any differences in amplification efficiencies. All samples were normalized to the housekeeping gene EF1-α (used extensively in fish research, including other fasting studies; Gabillard 2006), and levels were not significantly different between tissues or between treatments ($P > 0.05$).

Table 1: Forward (F) and reverse (R) primers used for real-time polymerase chain reaction analysis of mRNA expression in rainbow trout liver and muscle during fasting

Gene	5′ to 3′ Forward Primer	5′ to 3′ Reverse Primer	T_m (°C)	Size (bp)
PPARα	ccaagttcagtttgccatga	attggggaagaggaaggtgt	60	204
PPARβ	ctggagctggatgacagtga	gtcagccatcttgttgagca	60	194
CPT Iβ1	gatgttccgtgagggtagga	ttgtcttgcatggctctgac	58	80
CPT Iβ2	gccgcaaactagagagagga	cccgtagtacagccacacct	58	199
CPT Iα1a	atgaggaatgccctcaagtg	gcttcctgccagagaacaac	58	120
CPT Iα1b	cgcttcaagaatggggtgat	caaccacctgctgtttctca	58	187
CPT Iα2	ccgttcctaacagaggtgct	acactccgtagccatcgtct	58	154
EF1α	cattgacaagagaaccattga	ccttcagcttgtccagcac	58	94

Note. PPAR = peroxisome proliferator-activated receptor, CPT I = carnitine palmitoyltransferase I, and EF1α = elongation factor 1α.

Statistical Analysis

All statistical analyses were performed using SigmaStat (Systat Software, San Jose, CA). A Student *t*-test was used to test for significance between control and fasted fish. Significance level was set at $\alpha = 0.05$.

Results

Fatty Acid Oxidation

Palmitate oxidation in isolated mitochondria from red muscle and liver was found to be significantly higher by 1.5- to 2.5-fold in fasted fish when compared with fed controls ($P < 0.05$; Fig. 1). Furthermore, red muscle mitochondria of both control and fasted fish oxidized palmitate at an approximately fourfold greater rate than those of liver ($P < 0.05$).

CPT I Activity

CPT I activity (U mg^{-1} mitochondrial protein) was approximately fivefold higher in red muscle compared with activity in liver ($P < 0.05$; Fig. 2). Fasting, however, did not increase CPT I activity in red muscle to a level significantly different from that in fed controls ($P = 1.51$; Fig. 2). In contrast, fasting resulted in a significant increase in liver CPT I activity fourfold ($P < 0.05$; Fig. 2).

CPT I Inhibition by Malonyl-CoA (IC_{50})

The IC_{50} for malonyl-CoA (one-half maximal inhibitory concentration) was determined for liver and red muscle tissue CPT I. The IC_{50} in both red muscle and liver was found to be significantly higher in fasted fish compared with levels in fed controls, indicating a decrease in sensitivity of the enzyme to its allosteric inhibitor ($P < 0.05$; Fig. 3).

Mitochondrial Membrane Fluidity

Mitochondrial membrane fluidity was measured using DPH anisotropy where fluidity is inversely proportional to anisotropy. There were no statistically significant changes in mitochondrial membrane fluidity in either red muscle or liver tissue of fasted fish when compared with fed controls (Fig. 4). However, red muscle mitochondrial membranes were found to be significantly more fluid than those of the liver ($P < 0.05$).

mRNA Expression

Changes in levels of PPARα mRNA were not induced in either red muscle or liver with fasting (Fig. 5). However, fasting did have a tissue-specific effect on changes in expression for PPARβ mRNA which significantly decreased in red muscle (Fig. 5), whereas in the liver, PPARβ significantly increased during fasting ($P < 0.05$; Fig. 5). Most of the CPT isoforms mRNA expression was uneffected by fasting but there was a significant induction of CPT I α1a expression in liver during fasting ($P < 0.05$; Fig. 6). Moreover, there was a significant reduction of CPT I α1b in red muscle during fasting ($P < 0.05$; Fig. 6).

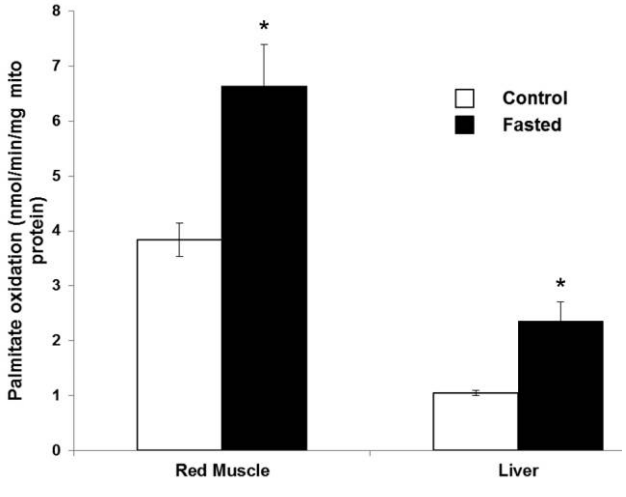

Figure 1. Palmitate oxidation (in nmol/min/mg mitochondrial protein) in isolated mitochondria from red muscle and liver of control and fasted rainbow trout. Values are means ± SE, $n = 5$. Asterisk denotes significant differences between control (white bars) and fasted (black bars) fish ($P < 0.05$).

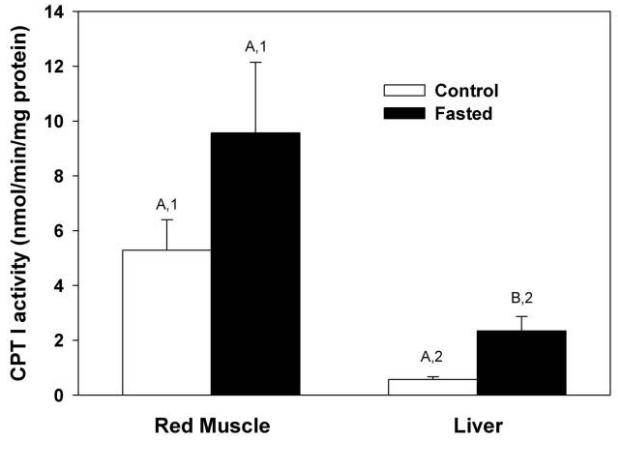

Figure 2. Apparent V_{max} of carnitine palmitoyltransferase (CPT) I in red muscle and liver of control (white bars) and fasted (black bars) rainbow trout. Values are means ± SE, $n = 4$. Letters denotes significance ($P < 0.05$) between control and fasted fish within each tissue, while numbers denote significance between red muscle and liver in both control and fasted fish ($P < 0.05$).

Discussion

In this study we demonstrate that after 5 wk of fasting in rainbow trout, there was an increase in mitochondrial fatty acid oxidation in both liver and red muscle (Fig. 1). Concurrently, there was a decrease in the sensitivity of CPT I to the allosteric inhibitor malonyl-CoA (IC_{50}) in both tissues and an increase in the apparent V_{max} for CPT I in liver but not in red muscle (Figs. 3 and 2, respectively). Previously we observed a correlation between indices of mitochondrial membrane fluidity and IC_{50} of CPT I for malonyl-CoA (Morash et al. 2008). Thus, we anticipated that increased mitochondrial membrane fluidity would help explain the increase in IC_{50}. However, we observed no change in mitochondrial membrane fluidity in either tissue with fasting (Fig. 4). In contrast, fasting did effect mRNA expression in two of the five trout CPT I isoforms and in PPARβ. These data suggest that upregulation of mitochondrial fatty acid oxidation during fasting in fish occurs through both genomic and nongenomic mechanisms acting on CPT I to increase mitochondrial fatty acid uptake and oxidation.

Fatty Acid Oxidation

The rate of palmitate oxidation in isolated mitochondria increased after 5 wk of fasting by 2.5- and 1.5-fold in red muscle and liver, respectively (Fig. 1). This enhanced oxidation of abundant fatty acids during fasting serves to (1) provide cellular energy in both liver and muscle, (2) preserve scarce carbohydrate stores and valuable protein reserves, (3) provide glycerol as a backbone for gluconeogenesis, and (4) condense the acyl-CoA formed during fat oxidation to form ketone bodies in liver, an essential fuel for the brain when glucose becomes depleted (Soengas et al. 1998). Previously we demonstrated that red muscle had a greater capacity for fatty acid oxidation (Morash et al. 2008). Here, using direct measurement of mitochondrial palmitate oxidation we show that red muscle mitochondria oxidize fatty acids at a greater rate than liver mitochondria.

CPT I Activity and Sensitivity to Malonyl-CoA

The kinetic activity of CPT I and the cellular concentration of its allosteric modulator, malonyl-CoA, are two of the major factors determining entry of fatty acids into the mitochondria (McGarry et al. 1983; Murthy and Pande 1987). During fasting, we found that the activity of CPT I was increased by 2.5-fold in liver but did not significantly increase in red muscle (Fig. 2). The change in CPT I activity in the liver during fasting may be due, in part, to increased expression of CPT I α1a mRNA (Fig. 6) or to changes in sensitivity to malonyl-CoA. Indeed, there were substantial decreases in the sensitivity of CPT I to malonyl-CoA in both tissues, by approximately two and eight times, in red muscle and liver, respectively (Fig. 3). A similar response has been noted for mammalian liver-type CPT I iso-

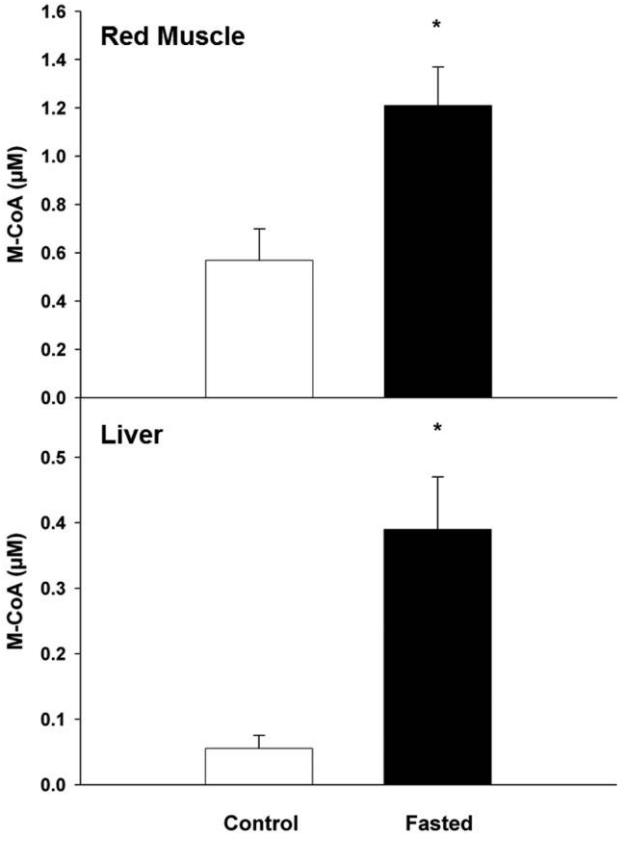

Figure 3. The concentration of malonyl-CoA that reduces carnitine palmitoyltransferase (CPT) I activity by 50% (IC_{50}) in red muscle and liver of control and fasted rainbow trout. Values are means ± SE, $n = 4$. Asterisk denotes significance ($P < 0.05$) between control and fasted fish.

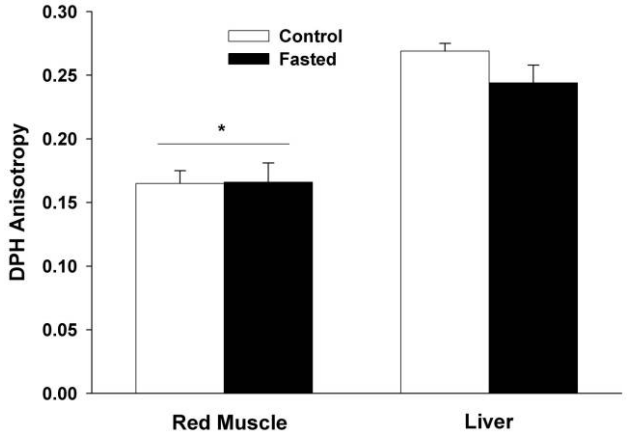

Figure 4. The 1,6-diphenyl 1,3,5-hexatriene (DPH) anisotropy values from red muscle and liver of control and fasted rainbow trout mitochondria. Mitochondrial membrane fluidity is inversely proportional to anisotropy. Values are means ± SE, $n = 4$. Asterisk denotes significance between red muscle and liver ($P < 0.05$).

form (CPT Iα) in response to 24 h of fasting (Drynan et al. 1996; Zammit et al. 1998).

Although changes in IC_{50} may be due to changes in isoform expression, another intriguing hypothesis is that increased mitochondrial membrane fluidity leads to decreased CPT I sensitivity to malonyl-CoA. However, we did not observe any change in bulk total mitochondrial membrane fluidity in either tissue with fasting (Fig. 4). However, CPT I is located in the outer mitochondrial membrane and may only interact with specific membrane microdomains; therefore, the possibility exists that distinct changes in fluidity in the areas surrounding CPT I may have been masked by the bulk fluidity of the rest of the membrane. Beyond changes in fluidity, other mechanisms may be responsible for the fasting-induced changes in CPT I kinetics, such as changes in cytosolic pH (Bezaire et al. 2004), covalent modulation (Harano et al. 1985), or the differential expression of isoforms (Morash et al. 2010) or splice variants (Kim et al. 2002) with altered malonyl-CoA sensitivities. Furthermore, cellular concentrations of M-CoA may also play a role in modulating changes in fatty acid oxidation. During fasting, lipogenesis, the main reaction producing M-CoA, is limited. Thus, we expect that M-CoA concentrations would decrease, and, in combination with a decrease in sensitivity (Fig. 3), would intensify the increase in lipid oxidation.

Gene Expression

Trout express five different CPT I isoforms in both red muscle and liver, and sequence analysis suggests differential expression can affect overall tissue fatty acid oxidation (Morash et al. 2010). The induced expression of CPT I in mammals is regulated, in part, by the transcription factors and nuclear receptors, PPARα and PPARβ (Price et al. 2000). During fasting, plasma and cytosolic free fatty acids are elevated (Pottinger et al. 2003). These fatty acids may act as ligands to activate PPARα and induce expression of CPT I in both liver and muscle. However, we saw no change in either liver or red muscle PPARα mRNA levels after 5 wk of fasting (Fig. 5). Even in the absence of its putative transcriptional regulator, there was an increase in CPT I α1a mRNA in the liver while the level of CPT I α1b mRNA in red muscle decreased (Fig. 6). Based on previous genomic analysis, these isoforms have amino acid substitutions that may affect their sensitivity to malonyl-CoA (Morash et al. 2010). In this way differential expression of the various isoforms may be contributing to changes in CPT I IC_{50} (Morash et al. 2010). Unlike mammals, multiple CPT I isoforms may provide fish with greater spatial and temporal control of protein expression and regulation during physiological perturbations where an increase in fatty acid oxidation is required.

In contrast to PPARα, PPARβ mRNA expression was significantly upregulated in the liver, while in the red muscle it was significantly downregulated (Fig. 5). The exact role of PPARβ has yet to be elucidated, but the ubiquitous expression across tissues suggests that it functions at a basal level of metabolism in both rats (Braissant and Wahli 1998) and fish (Batista-Pinto et al. 2005). Moreover, there is evidence sug-

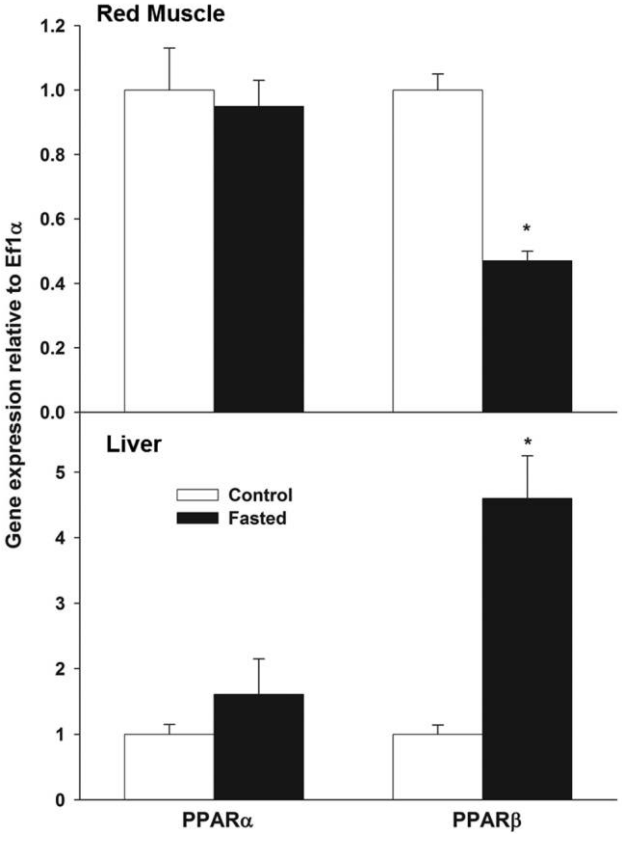

Figure 5. Real-time polymerase chain reaction gene expression of peroxisome proliferator activated receptor α (PPARα) and PPARβ in red muscle and liver. Expression is relative to Ef1α. Values are means ± SE, $n = 4$. Asterisk denotes significance ($P < 0.05$) between control and fasted fish for each gene.

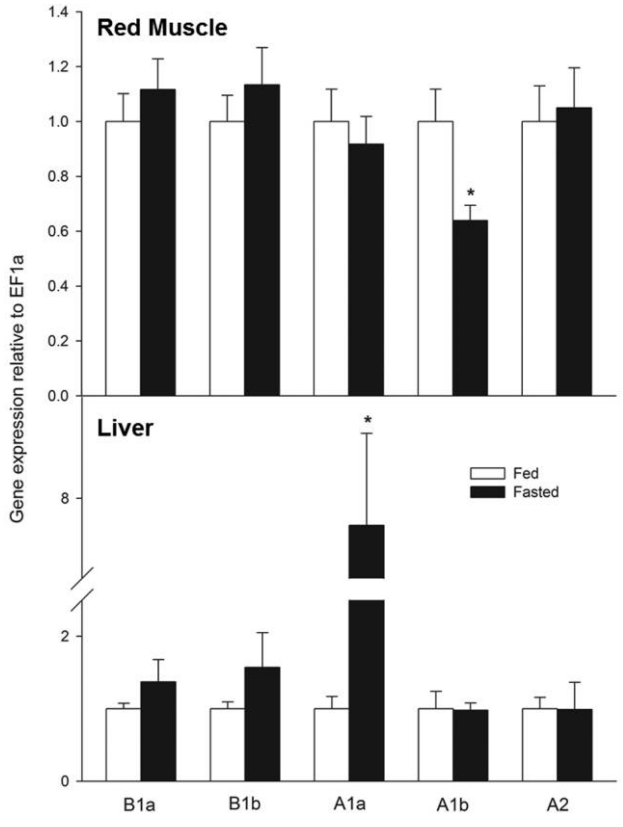

Figure 6. Real-time quantitative polymerase chain reaction gene expression of carnitine palmitoyltransferase (CPT) I isoforms in red muscle and liver. Expression is relative to Ef1α. Values are means ± SE, $n = 4$. Asterisk denotes significance ($P < 0.05$) between control and fasted fish for each gene.

gesting that PPARβ may be involved in lipid storage (Peters et al. 2000) and inhibition of PPARα (Leaver et al. 2007); however, there is also evidence that it may be involved in lipid oxidation (Dressel et al. 2003; Wang et al. 2003). In addition, fish appear to have multiple isoforms of both PPARα and PPARβ, as shown in a variety of species including zebrafish (Robinson-Rechavi et al. 2001) and Atlantic salmon (Leaver et al. 2007). These isoforms have not yet been identified in rainbow trout, but it is possible that liver and red muscle of trout express different isoforms of PPARα and β at different times during fasting and that these isoforms have very specific roles in the regulation of lipid metabolism. For a more comprehensive picture of fasting-induced gene expression in this species, identification of specific isoforms, their tissue distribution and inducibility, as well as their ligand sensitivity need to be fully established.

Fasting in Mammals versus Fish

It appears that reliance on fatty acids during fasting is conserved across vertebrate taxa, but with distinct regulatory mechanisms. We show here that increases in mitochondrial fatty acid oxidation most likely results from a variety of different mechanisms that are distinct between liver and muscle and between fish and mammals. Decreasing the sensitivity of CPT I to malonyl-CoA is a conserved mechanism to increase mitochondrial fatty acid oxidation in both liver and muscle of fish and mammals in response to fasting (Drynan et al. 1996; Zammit et al. 1998). Distinct from mammals, the genomic control of fatty acid oxidation is quite different in fish, particularly between the tissues studied here. This has broad ecological relevance as the ability of fish to regulate lipid oxidation through a variety of mechanisms may offer an advantage in adapting to their dynamic environment as many fish species experience periods of food limitation, or undergo voluntary fasting during migration.

Conclusions

Fish are good models to examine the regulation of lipid oxidation as they fast naturally as part of their life history. We have shown that there are many tissue-specific responses to fasting. Although red muscle and liver respond differently, ultimately they achieve the same goal; an increased ability to oxidize fats as a fuel source in the mitochondria. Fasting induced increased fatty acid oxidation rates in mitochondria from both tissues examined, and this was achieved partially through modifications in CPT I allosteric modulation and changes in gene expression. Interestingly, modifications in mitochondrial membrane fluidity do not seem to play a role in fasting. This suggest that genomic regulation through the induced expression of other less malonyl-CoA-sensitive CPT I isoforms may be the predominate level of control to enhance fat oxidation with fasting.

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