Genomic and Metabolic Preparation of Muscle in Sockeye Salmon *Oncorhynchus nerka* for Spawning Migration

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ABSTRACT

Prolonged endurance exercise and fasting are two major metabolic challenges facing Pacific salmon during spawning migrations that often occur over 1,000 km. Because both prolonged exercise and fasting stimulate the oxidation of lipids, particularly in heavily recruited tissues such as muscle, we sought to investigate the regulatory mechanisms that establish and maintain the capacity for substrate oxidation at four separate locations during the final 750 km of nonfeeding migration in sockeye salmon *Oncorhynchus nerka*. Transcript levels of multiple genes encoding for important regulators of lipid, carbohydrate, and protein oxidation as well as the activity of several important enzymes involved in lipid and carbohydrate oxidation were examined in red and white muscle. We found in both muscle types that the messenger RNA (mRNA) expression of carnitine palmitoyltransferase I isoforms, peroxisome proliferator–activated receptors α and β, and adenosine monophosphate–activated protein kinase β1 were all significantly higher at the onset compared to later stages of nonfeeding migration. However, the activities of β-hydroxyacyl-CoA dehydrogenase and citrate synthase were higher only early in migration and only in red muscle. Later in the migration and as muscle lipid stores were greatly depleted, the mRNA levels of hexokinase I and aspartate aminotransferase increased in white muscle. Overall, at the onset of migration, high transcript and metabolic enzyme activity levels in skeletal muscle of sockeye salmon may help support the high rates of lipid oxidation needed for endurance swimming. Furthermore, we suggest that the muscle capacity to use carbohydrates and proteins may be adjusted throughout migration on an as-needed basis to fuel burst exercise through very difficult hydraulic passages in the river and perhaps during mating activities.

Introduction

Pacific salmon (genus *Oncorhynchus*) swim distances of up to 1,500 km during the freshwater portion of their spawning migration (Groot and Margolis 1991). This monumental journey exposes them to numerous physiological challenges, including changes in salinity and temperature, sexual maturation, endurance exercise, and the cessation of feeding resulting in prolonged fasting (Hinch et al. 2006). Understanding the physiological adjustments required for transfer from seawater to freshwater has received much attention to date (Shrimpton et al. 2005; Cooperman et al. 2010; Flores et al. 2011), whereas the metabolic regulation necessary for endurance exercise and fasting are aspects of fish migration that have received comparatively less exploration. The genomic and metabolic alterations required by salmon to travel such great distances and to complete one of the most remarkable energetic feats in the animal world in a fasted state remain elusive. In addition to being an excellent model of extreme endurance exercise, migrating salmon are an important ecological and economic resource that is currently under stress from global climate change (Farrell et al. 2008; Eliason et al. 2011). Therefore, it is imperative to understand their physiology, particularly during their spawning migration.

Past research on sockeye salmon *Oncorhynchus nerka* used body composition analysis to show that spawning migrations are primarily fueled by lipid stores that are accumulated during the oceanic phase of the salmon life cycle (Idler et al. 1959; Gilhousen 1980; French et al. 1983). Proteins and carbohydrates supply relatively little energy during the majority of the migration but become crucial once body lipid stores have been almost fully depleted toward the end of migration (Gilhousen 1980; Mommsen et al. 1980) and whenever the fish employs burst swimming techniques. Thus, the regulation of substrate delivery to and oxidation by muscle is a vital factor to ensure that sufficient substrates are available at the appropriate times

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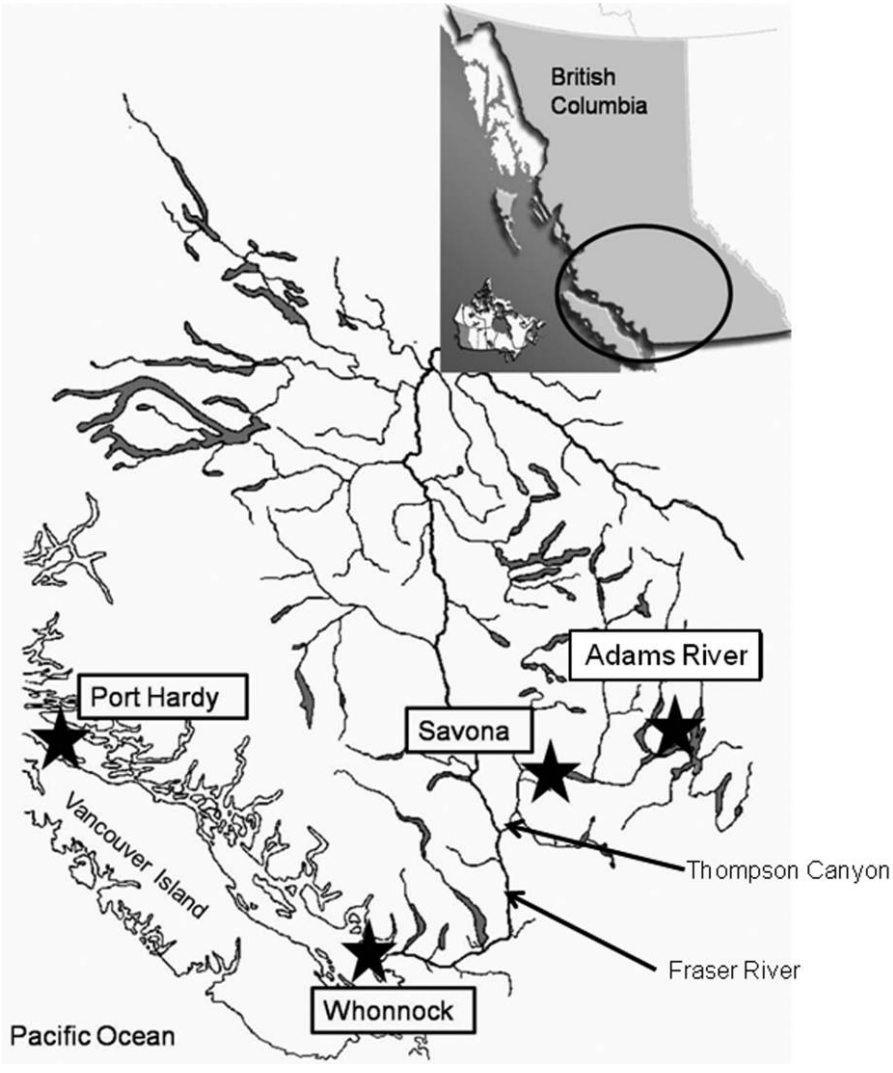

Figure 1. Map of the Fraser River system with sampling locations used in this study indicated by stars. Adams River = spawning grounds; Port Hardy = ocean site; Savona = midriver site; Whonnock = river entry site.

during migration. Indeed, sockeye salmon invest all of their energy resources into a single reproduction event at the expense of their own lives (semelparity). A recent large-scale functional genomics study of the white muscle in migrating sockeye salmon examined the putative environmental cues affecting transcriptional regulation of metabolic machinery. Fasting and changes in circulating hormones were identified as major triggers responsible for initial shifts in transcriptional patterns in coastal return migrations, at least in white muscle (Miller et al. 2009). Yet it is unclear how these triggers may affect the lipid oxidation machinery in red and white muscles that are necessary to capitalize on the large lipid reserves used to complete the extensive aerobic swimming involved in this journey. For example, is muscle phenotypic plasticity induced only in response to early environmental and energetic cues as part of preparatory changes triggered well in advance of metabolic need, do some phenotypic changes occur on an as-needed basis later in migration, or is a combination of both mechanisms involved?

Lipid oxidation is regulated, in part, by carnitine palmitoyltransferase (CPT) I, an enzyme that determines the entry of long-chain fatty acids into the mitochondria (McGarry et al. 1983). Recently, we have shown that another salmonid species, rainbow trout *Oncorhynchus mykiss*, expresses five distinct isoforms of CPT I encoded by separate genes (Morash et al. 2010). To date, no CPT I genes have been identified or kinetically characterized in sockeye salmon. However, given the phylogenetic relatedness to rainbow trout at the genus level, we predict a similar isoform complement with a similar cross-tissue expression pattern in this species. The expression of CPT I is transcriptionally regulated in mammals by the peroxisome proliferator–activated receptors family of transcription factors (PPARs; Price et al. 2000). The PPARα isoform is highly expressed in tissues exhibiting high rates of lipid oxidation, such

Table 1: Primers used to amplify and sequence peroxisome proliferator–activated receptor α (PPARα)

Primer	5′ to 3′ forward primer	5′ to 3′ reverse primer	T_m (°C)	Size (bp)
PPARα S1	catctacgaggcctacctcaa	agacttggcgaactcggtta	58	259
PPARα S2	cgaggcctacctcaagaact	tggagtgacgtgtcctctgt	60	741
PPARα S3	tcttctaacctggagtgtcg	agggtggagtgcttgtcttg	60	424

Note. S1, S2, and S3 represent individual sections of the gene, which were sequenced individually. bp = base pairs; T_m = melting temperature.

as liver, heart, and skeletal muscle; is stimulated by high cytosolic fatty acid content; and induces the expression of several key metabolic genes involved in lipid metabolism in mammals (Desvergne and Wahli 1999) and likely in fishes (Leaver et al. 2005, 2007). In contrast, PPARβ is expressed ubiquitously in all tissues and appears to help maintain a basal level of lipid metabolism (Desvergne and Wahli 1999). Although the function of PPARs in nonmammalian vertebrates such as fish is poorly understood, given their central role in lipid metabolism, PPARs may be involved in regulating metabolic changes in muscle during migration (Batista-Pinto et al. 2005; McClelland et al. 2006; Morash et al. 2008; LeMoine et al. 2010). Both PPARα and CPT I activity are subject to regulation by adenosine monophosphate–activated protein kinase (AMPK), a major sensor and regulator of cellular energy production (Lee et al. 2006). AMPK works concurrently to stimulate fatty acid oxidation and glucose uptake in liver and muscle while inhibiting lipogenesis (Winder and Hardie 1999). In mammals, AMPK also upregulates muscle hexokinase (HK) activity, which can increase glycolytic flux capacity (Holmes et al. 1999). Overall, AMPK acts to stimulate adenosine triphosphate (ATP)–generating processes, particularly during large changes in cellular energy status, such as with exercise and fasting (Winder et al. 2000; Ojuka 2004).

Fasting and long-term endurance exercise are known to induce a greater rate of fatty acid oxidation in mammals. Salmonid fish respond to chronic exercise training and fasting by increasing the capacity of muscle for fatty acid oxidation (Johnston and Moon 1980; Farrell et al. 1990). This muscle remodeling may reflect a response to high levels of circulating lipids during long-distance exercise (Magnoni et al. 2006), but the potential interaction between fasting and exercise on muscle phenotype in fish remains unknown. The degree to which muscle remodeling occurs in migrating salmon is unclear, and what potential genomic changes might be responsible for this plasticity is also uncertain. Coordination between transcriptional regulation for metabolic genes and the appropriate muscle metabolic phenotype for endurance locomotion would be a requisite for successful migration. To address these transcriptional and metabolic changes, we examined major transcriptional (PPARs) and catalytic regulators of lipid metabolism (CPT Is, β-hydroxyacyl-CoA dehydrogenase [HOAD], citrate synthase [CS], and AMPK β1) and, to a lesser extent, indexes of carbohydrate (HK1) and protein (aspartate aminotransferase [AST]) metabolism in sockeye salmon at four different points along the migration route from the ocean to the spawning area.

Material and Methods

Experimental Species and Sampling Locations

These studies were approved by the University of British Columbia Animal Care Committee in accordance with the Canadian Council of Animal Care. Sockeye salmon were sampled along their migration route in southern British Columbia, Canada, between August and October of 2006. All fish used in the study were collected from standard stock assessment sampling platforms and were caught at four geographically distant locations: (1) Port Hardy in the Johnson Strait (PH; ocean; nonfeeding; 270 km from Fraser River estuary), (2) Whonnock (WH; immediately after river entry; full freshwater), (3) Savona (SA; midriver; 391 km upriver), and (4) Adams River (AR; arrival at spawning area but not paired for spawning; 480 km upriver; fig. 1). Fish were measured for fork length (cm), body mass (g), and gonad mass (g). Red and white muscle samples were excised and immediately frozen on dry ice and stored at −80°C before analysis. A subsample of dorsal muscle tissue (200–300 g) for estimation of body constituents was cut from the left side of the body and frozen at −20°C, similar to the method described in Clark et al. (2010). Stomach contents were removed and weighed at PH and WH to confirm that feeding had essentially ceased, because only trace amounts of stomach contents were present at both locations (mean, <2 g at PH and <1 g at WH). Only female fish from the Late Shuswap stock were selected for additional analysis. Stock identification was confirmed by DNA stock assignment analysis (Beacham et al. 2005). Proximate constituent analysis on the dorsal section was completed for water content, ash, and lipid using methods described elsewhere (Crossin et al. 2004), and protein was calculated as the difference between the above constituents and 100% (e.g., Hendry et al. 1999). Only results for protein and lipid are presented herein. Condition factor (CF) was calculated using the equation $CF = 100\, w/l^3$, where w is the mass of the fish in grams and l is the length of the fish in centimeters.

RNA Extraction and Complementary DNA (cDNA) Synthesis

Frozen tissues were powdered in a liquid N_2–chilled mortar and pestle. Total RNA was extracted from each tissue using

Table 2: Primer sequences used for real-time polymerase chain reaction (PCR) analysis of red and white muscle

Gene	5′ to 3′ forward primer	5′ to 3′ reverse primer	T_m (°C)	Size (bp)	Accession no.
PPARα	ccaagttcagtttgccatga	attggggaagaggaaggtgt	60	173	JN971011
PPARβ	ctggagctggatgacagtga	gtcagccatcttgttgagca	60	195	NM_001197207
CPT Iβ1	gatgttccgtgagggtagga	ttgtcttgcatggctctgac	58	80	AJ606076
CPT Iβ2	gccgcaaactagagagagga	cccgtagtacagccacacct	58	199	NM_001124735
CPT Iα1a	atgaggaatgccctcaagtg	gcttcctgccagagaacaac	58	120	NM_001246330
CPT Iα1b	cgcttcaagaatggggtgat	caaccacctgctgtttctca	58	187	AJ619768
CPT Iα2	ccgttcctaacagaggtgct	acactccgtagccatcgtct	58	154	AJ620356
HK	ctgggacgctgaagaccaga	cggtgctgcatacctccttg	58	159	AY864082
AST	gacctgtggctttgacttcc	gcaatctccttccactgctc	58	135	EF675996
AMPK	actgtgttccgttggacagg	tcaatcatgagggcatcaaa	58	272	NM_001165146
EF1α	cattgacaagagaaccattga	ccttcagcttgtccagcac	58	94	AF498320

Note. T_m = melting temperature; bp = base pairs.

TRIzol Reagent (Invitrogen, Carlsbad, CA) based on the acid guanidinium thiocyanate-phenol-chloroform extraction method. The RNA was quantified by UV spectroscopy at 260 nm and then diluted to 0.5 μg/μL. The cDNA was synthesized using 1 μg of DNase (Invitrogen) treated messenger RNA (mRNA) with SuperScript RNase H^- reverse transcriptase (Invitrogen), as described elsewhere (Morash et al. 2008).

Polymerase Chain Reaction (PCR) and Sequencing

For all of the CPT I isoforms, elongation factor 1 α (EF1α), PPARβ1, AMPK β1, HK I, and AST, primers from rainbow trout *Oncorhynchus mykiss* were used because of their genetic similarity to sockeye salmon. A small section of each gene was amplified by PCR (0.2 mM dNTP, 1.5 mM $MgCl_2$, 0.5 μM of each forward and reverse primer [tables 1, 2], 1 unit of Taq polymerase [Fermentas, Burlington, Ontario], and 1 × Taq amplification buffer). The PCR products were purified using QiaQuick gel extraction kit (Qiagen, Mississauga, Ontario) and directly sequenced at the Mobix laboratory (McMaster University) to ensure that the correct gene was amplified in salmon. For PPARα1, sequences were aligned from mammals and other fish species, and specific PCR primers were designed from conserved regions using Primer3 software (Rozen and Skaletsky 2000). For PPARα1, the full-length sequence was obtained by three overlapping sections (table 1). The PCR products were purified as mentioned above, then cloned using the pGEM-T easy vector system (Promega, Nepean, Ontario) and sequenced at the Mobix laboratory.

mRNA Quantification by Real-Time PCR

The expression of each mRNA was quantified using real-time PCR with SYBR green and ROX as a reference dye using a Stratagene M × 3000P (Stratagene, Cedar Creek, TX) real-time PCR system. Each 25-μL reaction contained 12.5 μL SYBR green mix, 1 μL each of forward and reverse primer (5 μM), 5.5 μL of DNase/RNase free water, and 5 μL of 5 × diluted cDNA. Primers were designed using Primer3 software (Rozen and Skaletsky 2000; table 2). The thermal program included 3 min at 95°C, 40 cycles of 95°C for 15 s, 58°C for 30 s, and 72°C for 30 s. A no-template control and dissociation curve were performed to ensure that only one PCR product was being amplified and stock solutions were 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, EF1α, whose expression did not change significantly between sites.

Enzyme Analysis

All assays were performed in triplicate at room temperature in a 96-well format using a Spectramax Plus 384 spectrophotometer (Molecular Devices, Sunnyvale, CA), and the data were collected using Softmax Pro 4.7.1 software (Molecular Devices). Frozen tissues were powdered using a liquid N_2–chilled mortar and pestle and homogenized in 20 volumes of homogenization buffer (100 mM potassium phosphate, 5 mM EDTA, and 0.1% Triton at pH 7.2) using a glass-on-glass homogenizer.

CS. The CS was measured according to previously published protocols (McClelland et al. 2005). Briefly, the CS assay buffer contained 20 mM TRIS (pH 8.0), 0.1 mM DTNB, and 0.3 mM acetyl-CoA. The reaction was initiated by the addition of 0.5 mM oxaloacetate, and absorbance was measured for 5 min at 412 nm. Control samples were assayed without oxaloacetate to control for background hydrolase activity.

HOAD. HOAD was measured according to previously published methods (McClelland et al. 2005) and consisted of 50 mM imidazole (pH 7.4), 0.1 mM acetoacetyl-CoA, 0.15 mM NADH, and 0.1% Triton X-100 at 340 nm.

HK. The HK assay was modified from Houle-Leroy et al. (2000) for use in fish. The assay buffer contained 4 mM ATP, 10 mM

Table 3: Physical characteristics of salmon at each sampling point along the migration route

Site	Fork length (cm)	Fish mass (kg)	Condition factor	HSI (%)	Lipid percentage	Protein percentage	Ovary mass (g)
Port Hardy	57.7 ± .5	2.34 ± .037	1.3 ± .03	1.33 ± .08^{AD}	15.6 ± .6^{A}	18.4 ± .6	83.1 ± 3.2^{A}
Whonnock	58.0 ± .7	2.35 ± .081	1.2 ± .02	1.63 ± .07^{AC}	10.3 ± 1.4^{B}	19.1 ± .5	156.9 ± 9.3^{B}
Savona	58.0 ± 1.0	2.20 ± .137	1.1 ± .10	1.90 ± .05^{BC}	6.4 ± 1.0^{C}	19.6 ± 1.5	281.3 ± 16.4^{C}
Adams River	58.9 ± .8	2.34 ± .063	1.2 ± .03	1.25 ± .12^{D}	2.5 ± .2^{D}	17.9 ± .4	425.2 ± 17.2^{D}

Note. Lipid and protein percentage values are for dorsal muscle. Values are means ± standard error of the mean ($n = 5$). Letters indicate a statistically significant difference between sampling sites. HSI = hepatosomatic index.

$MgCl_2$, 0.5 mM NADP, and 1 U glucose-6-phosphate dehydrogenase in 50 mM HEPES (pH 7.0). The reaction was initiated by the addition of 5 mM d-glucose (omitted in control reactions).

Statistical Analysis

All statistical analyses were performed using SigmaStat, version 3.5 (Systat Software, San Jose, CA). One-way ANOVA and Holm-Sidak posttests were used to test for significance between tissues and sampling locations. Significance level was set at $P < 0.05$.

Results

Condition of Sockeye Salmon during Migration

The fish mass, length, hepatosomatic index (HSI), lipid percentage and protein percentage of dorsal muscle, and ovary mass were measured in salmon throughout migration (table 3). There were no significant changes in fork length, mass, or condition factor of the sockeye salmon among sampling sites. Although the lipid percentage of the dorsal muscle significantly decreased as the fish progressed in their migration ($P < 0.05$), protein percentage did not change significantly. The HSI increased throughout migration ($P < 0.05$) but returned to oceanic levels at the spawning ground ($P > 0.05$). Ovary mass also increased significantly at each sampling site up to the spawning grounds ($P < 0.05$).

Gene Expression

White and red muscle underwent significant changes in gene expression from ocean sampling site to the spawning grounds. The mRNA expression of CPT I isoforms was highest in both red and white muscle when the fish were in the open ocean (PH; i.e., at the onset of the nonfeeding portion of migration). Immediately after the salmon entered the river system, mRNA in red muscle for CPT I isoforms β1a, β1b, α1a, and α2 significantly decreased compared with levels in fish from the open ocean (PH vs. WH; fig. 2*A*; $P < 0.05$). White muscle CPT I isoforms showed a similar response at this stage of the migration, with the exception that the β1a isoform mRNA expression was unchanged (fig. 2). Most of the CPT I isoforms showed a significant reduction in mRNA levels at the midriver section (SA) and on entering the spawning grounds (AR) in red and white muscle with the exception of the α2 isoform, which was unchanged in red muscle, and the α1b isoform, which remained unchanged throughout the migration (fig. 2). AMPKβ1 mRNA expression in red and white muscle was maintained throughout migration before decreasing significantly at the spawning area in both tissues (fig. 3; $P < 0.05$). Changes in the mRNA expression of both the transcription factors, PPARα and β, and for the glycolytic enzyme HK I showed similar trends in both muscle types, decreasing significantly just after the river entry (WH), peaking at the midriver site (SA) near the difficult migratory passages, and decreasing again at the spawning site (AR; fig. 3; $P < 0.05$). The expression of AST was significantly induced at both the river entry (WH) and spawning sites (AR) when compared with the ocean (PH) and midriver (SA) sites in both tissues (fig. 3; $P < 0.05$).

Enzyme Activity

In red muscle, the activity of the β-oxidation enzyme HOAD was highest at the first sampling site (PH). HOAD significantly decreased by ~65% at the river entry site (WH; fig. 4*A*; $P < 0.05$) and again by 35% at the SA site to an activity level that was maintained until the end of the migration (AR; fig. 4*A*; $P < 0.05$). The mass specific activity of the Kreb's cycle enzyme CS in red muscle, an indicator of mitochondrial content, was significantly reduced at WH and AR (fig. 4*B*; $P < 0.05$). In contrast, for white muscle, HOAD and CS activity was unchanged during migration (fig. 4*D*, 4*E*; $P > 0.05$). HK activity in red muscle, one indicator of glycolytic flux capacity, was significantly higher at WH and SA sites than at the first sampling site, but it was only significantly higher at SA in white muscle (fig. 4*C*, 4*F*; $P < 0.05$). In both tissues, HK activity significantly decreased at the spawning ground (AR), returning to a similar level of activity seen at PH (fig. 4*C*, 4*F*; $P < 0.05$).

Discussion

The ability of Pacific salmon to successfully complete their spawning migration relies on the capacity for substrate oxidation to meet the energetic demands of the muscle tissue during chronic exercise, in the absence of energy intake. Here, we show evidence that the muscles of migrating sockeye salmon are transcriptionally and metabolically tailored for lipid oxidation at the onset of nonfeeding migration but remodel throughout migra-

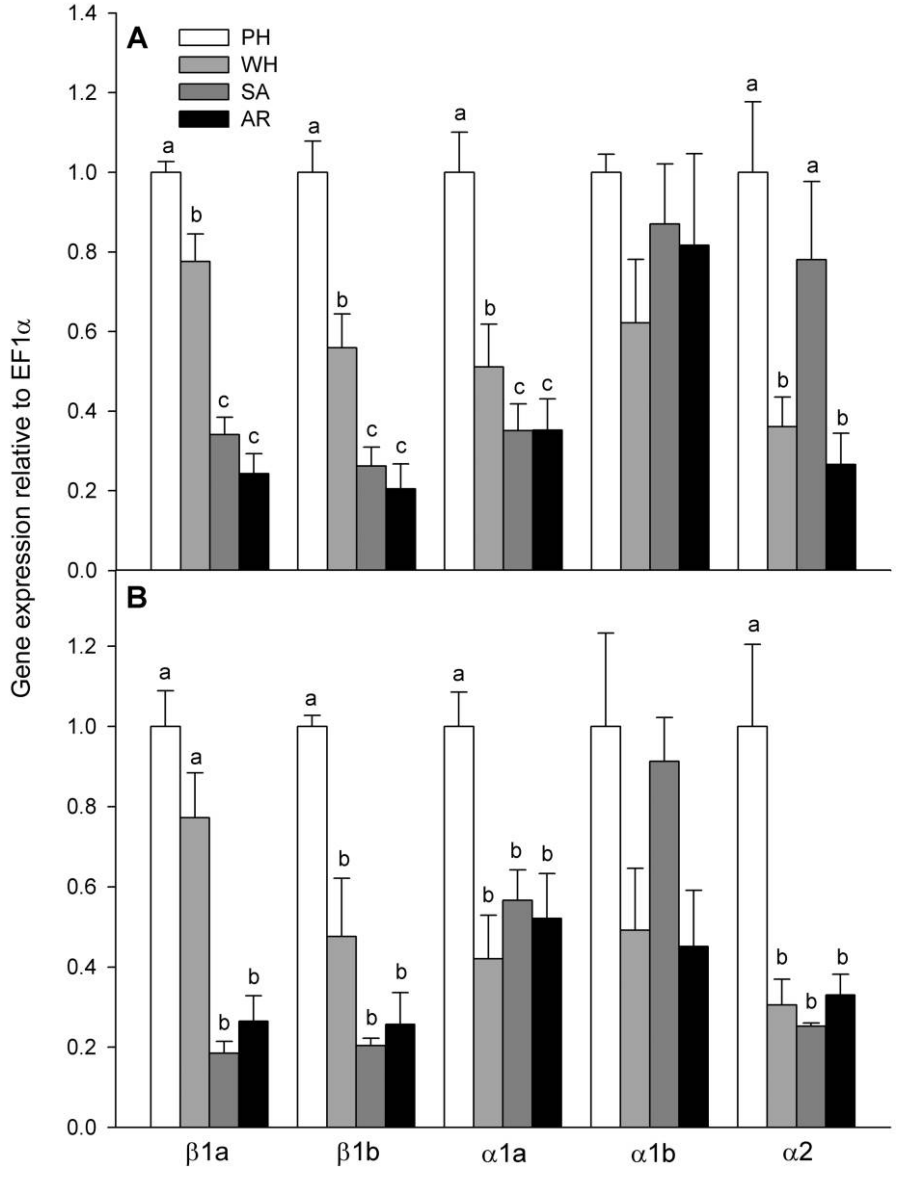

Figure 2. Messenger RNA (mRNA) expression of five carnitine palmitoyltransferase I isoforms in salmon red muscle (*A*) and white muscle (*B*) across four sampling sites during migration. Values are means ± SEM, with $n = 5$ and normalized to PH expression. Letters indicate significance between sites for each individual isoform. AR = Adams River; PH = Port Hardy; SA = Savona; WH = Whonnock.

tion, likely to match energetic demand with substrate supply. Indeed, there was a massive genomic response as transcript levels of genes encoding lipid oxidizing proteins were found to be as much as tenfold higher at the beginning of migration than at later stages. Earlier research has characterized metabolic changes during migration using a biochemical approach to assess changes in fuel stores. We show here the molecular and enzymatic alterations that may be required to exploit the limited substrate availability in the muscles throughout migration.

In both red and white muscle, transcript levels for genes encoding lipid metabolizing proteins and transcription factors important for lipid oxidation were highest in fish sampled at the seawater location (PH). These individuals were not feeding but still faced a nearly 300-km coastal migration, at speeds of 20–30 km/d (English et al. 2005), before starting the strenuous upriver segment of migration. Thus, transcripts for these particular genes are elevated at least 10 d ahead of the start of the more energetically demanding phase of spawning migration through the Fraser and Thompson River canyons (Rand and Hinch 1998; Young et al. 2006), which is accomplished typically at the same ground speed (English et al. 2005). The significant and continual decline of intramuscular lipids from the dorsal muscle region is consistent with high levels of fat oxidation early on and at middle stages of migration and is in line with other field (Gilhousen 1980; Magnoni et al. 2006) and laboratory measurements (Patterson et al. 2004; table 3). Thus, initially high levels of HOAD

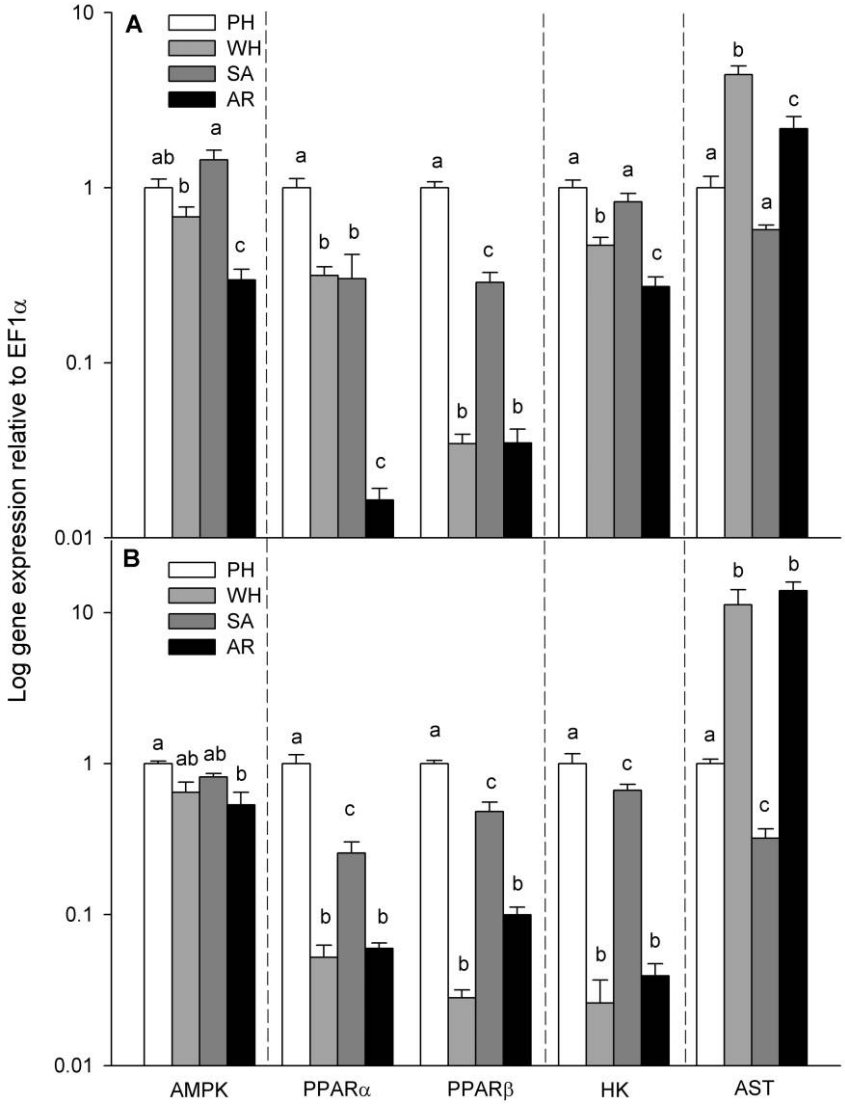

Figure 3. Adenosine monophosphate–activated protein kinase (AMPK), peroxisome proliferator–activated receptor (PPAR) α, PPARβ, hexokinase (HK), and aspartate amino transferase (AST) messenger RNA expression in salmon red muscle (*A*) and white muscle (*B*) across four sampling sites during migration. Values are means ± SE, with $n = 5$ and normalized to PH expression. Letters indicate significance between sites for each individual isoform. AR = Adams River; PH = Port Hardy; SA = Savona; WH = Whonnock.

activity and expression of genes encoding for lipid oxidizing machinery help ensure that muscles can capitalize on the large lipid stores when they are needed for endurance locomotion. Indeed, we found mRNA expression of all but one (CPT Iα1b) of the CPT I isoforms were highest in both red and white muscle at the onset of the nonfeeding phase of migration (fig. 2). High mRNA expression likely helps maintain the high enzyme activities necessary for mitochondrial oxidation. Free fatty acids are known ligands for PPAR activation, and their high circulating concentrations seen during salmon migration (Magnoni et al. 2006) potentially activate PPARs and induce the expression of PPAR target genes, such as CPT I. Expression of these genes, however, decreases in concert with decreasing lipid stores, implying that substrate supply and capacity of catabolic machinery may be linked in migrating salmon. However, multiple PPARα and β isoforms are expressed by some salmonid species (Leaver et al. 2007), and possibly each isoform has a unique response to stress to affect genes for metabolic pathways. It is also unclear how changes in the CPT I isoform complement of a tissue affect the kinetics of mitochondrial fat oxidation. Indeed, the role of multiple additional isoforms of metabolic genes or metabolic regulators in fishes compared with mammals is an area ripe for future research.

Because it can be activated by changes in cellular energy status, AMPK has been referred to as an energy-sensing molecule (Winder and Hardie 1999). In mammals, this protein is known to activate both PPARα and peroxisome proliferator–activated receptor γ co-activator 1 α to increase the expression

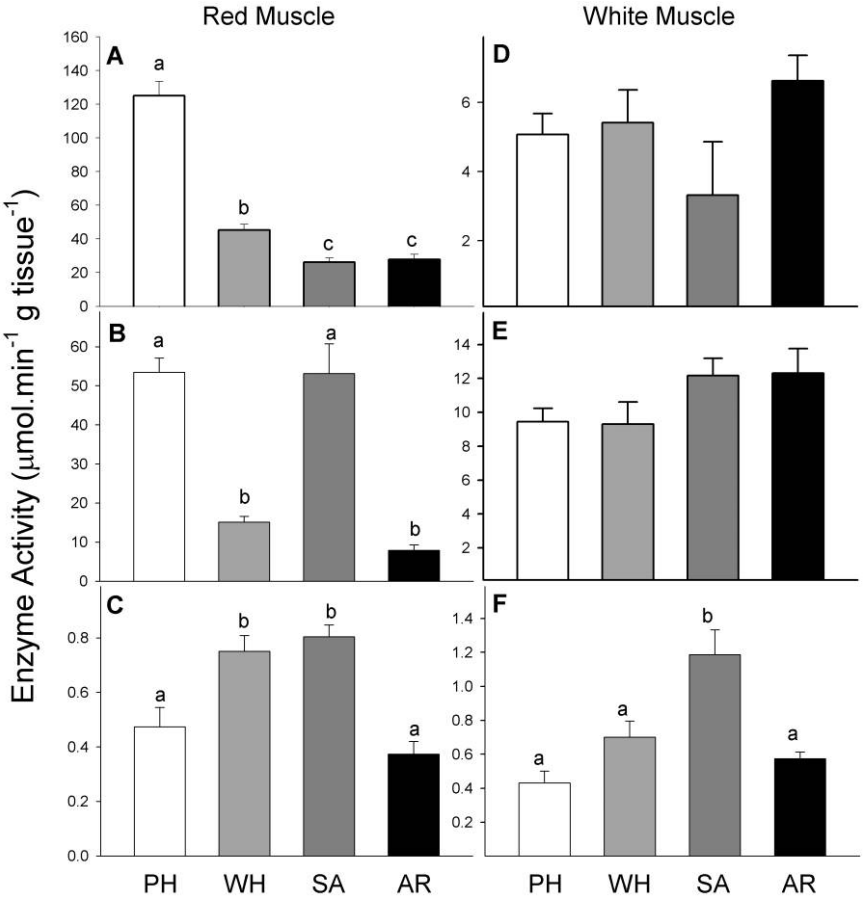

Figure 4. Apparent maximum reaction velocity of β-hydroxyacyl-CoA dehydrogenase (HOAD; *A*, *D*), citrate synthase (CS; *B*, *E*), and hexokinase (HK; *C*, *F*) expressed per gram of tissue in salmon red and white muscle. Values are means ± SE, with $n = 5$ and normalized to expression at the PH sampling site. Letters indicate significance between sites. AR = Adams River; PH = Port Hardy; SA = Savona; WH = Whonnock.

of PPARα target genes and stimulate mitochondrial biogenesis, respectively (Winder et al. 2000; Lee et al. 2006). We found that transcript levels of AMPK in red and white muscle remained high throughout most of the migration. On entry into the spawning ground, AMPK mRNA decreased significantly compared with the first sampling site (fig. 3). AMPK transcription may be elevated at the onset of migration to induce specific metabolic changes associated with ATP production in nonfeeding migrating salmon, although this remains to be clarified through investigations of the phosphorylation and activation state of AMPK. It is important to note that AMPK is subject to a series of posttranslational modifications; therefore, changes in mRNA expression alone do not necessarily equate to changes in activity. The large-scale changes in AMPK mRNA expression warrant additional research to determine its role in muscle remodeling during migration. Both red muscle HOAD and CS activity (fig. 4*A*) displayed a similar temporal pattern to that of AMPK expression (fig. 3*B*), which suggests that there was a stimulation of mitochondrial biogenesis in red muscle early in migration. In contrast, CS and HOAD activities remained unchanged in white muscle during migration. This is perhaps not surprising given that red muscle plays a more important role in the sustained aerobic exercise that characterizes locomotion for most of the migration.

As a regulator of metabolic homeostasis, AMPK can also stimulate the activity of enzymes of the glycolytic pathway (Winder and Hardie 1999). However, carbohydrate metabolism is thought to be reserved for burst activity involved in spawning at the very end of migration, and limited glycogen reserves are spared for brain metabolism (Standen et al. 2002). We found that HK activity was increased in both red and white muscle (fig. 4*C*, 4*F*) in the middle stages of migration. However, this stage increasingly involves high-intensity burst swimming and the use of anaerobic metabolism to negotiate formidable river rapids (Rand and Hinch 1998). Unexpectedly, there was a mismatch between HK1 mRNA expression and HK activity at different sampling points of migration (figs. 3, 4). This temporal mismatch between observed transcript expression and protein activity may be the result of (1) long distances and time between sampling, during which the mRNA peaks occurred, or (2) a down regulation of mRNA expression due to a longer half-life of the protein (Miller et al. 2009). Alternatively, the total activity of HK is a combination of

different isoforms, each with distinct kinetics, whereas our expression data is for the one currently known isoform, HK I.

The mRNA expression of AST, a marker of protein catabolism, was low at the beginning of migration, as expected, but then increased by 4.5- and 11-fold in red and white muscle, respectively, on river entry. These results suggest either that protein oxidation increases much earlier in migration than anticipated or that this represents a preparatory change in capacity for protein oxidation in advance of lipid depletion. Although earlier research has indicated that proteins play a relatively small role in graded aerobic exercise in trout (~20%; Lauff and Wood 1996; Richards et al. 2002), an elevation early in migration may serve to provide intermediates for the TCA cycle during the large increases in lipid metabolism (Owen and Hochachka 1974). Furthermore, amino acids are thought to be used to replenish carbohydrate stores near the end of migration to support spawning behaviors (Mommsen 1980, 2004; French et al. 1983). Indeed, the change in HSI in these fish is likely a result of glycogenesis and subsequent release of glycogen stores from the liver to the muscles at the spawning grounds. Alternatively, there may be large-scale protein shifts from the muscle to the gonads for vitellogenin deposition in the eggs as female salmon complete sexual maturation during the migration. In fact, ovary mass increased fivefold in this study from the ocean location to the spawning grounds (table 3), which is similar to findings in other work on mature sockeye salmon (Kiessling 2004; Mommsen 2004). At the end of the migration, the mRNA expression of AST was increased again, this time by 2- and 14-fold in red and white muscle, respectively. On the spawning grounds, the elevated expression of AST is most likely related to a preparation for increased protein oxidation at a time when other fuels are greatly depleted. The massive increase in AST in white muscle is consistent with severe wasting of this tissue by protein catabolism seen in earlier studies of salmon sampled after spawning (Mommsen 1980), but an unchanged protein percentage (table 3) observed in this study suggests the elevation in AST on arrival at the spawning area signifies the initiation of this process. All of the enzymes that we measured were lowest at the spawning site, possibly as a result of decreasing muscle metabolic capacity as the tissue protein starts to be depleted (Mommsen 1980) and tissue damage increases (Miller et al. 2009). At this point in the migration, fat stores are largely depleted, and protein oxidation has become the primary fuel for muscles (Mommsen 1980).

Conclusion and Perspectives

This study shows changes in transcript and enzyme activities that help explain how migrating salmon can capitalize on large lipid stores to power locomotion during the early stages of migration. Elevated mRNA expression and the activity of key enzymes involved in fatty acid oxidation occur at the onset of coastal migration to support high capacities for lipid oxidation. The activity of HK and expression of AST appear to be induced on an as-needed basis, perhaps triggered by sustained exercise and/or sexual maturity. However, the ultimate triggers for these genomic regulators of metabolic modification may be fasting, changes in water temperature, and/or hormonal signals associated with spawning migration (Miller et al. 2009). Taken together, our data on the metabolic remodeling of skeletal muscle provide a window into the molecular and mechanistic explanation that is consistent with previously presented physiology of these animals (Idler and Bitners 1958; Gilhousen 1980; Mommsen 1980; French et al. 1983; Magnoni et al. 2006; Miller et al. 2009).

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