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Expression of genes associated with fatty acid metabolism during maturation in diploid and triploid female rainbow trout

Meghan L. Manor [a,*], Gregory M. Weber [b], Beth M. Cleveland [b], Jianbo Yao [a], P. Brett Kenney [a]

[a] Division of Animal and Nutritional Sciences, Davis College of Agriculture, Natural Resources, and Design, West Virginia University, PO Box 6108, Morgantown, WV 26506, United States
[b] National Center for Cool and Cold Water Aquaculture, ARS/USDA, 11861 Leetown Road, Kearneysville, WV 25430, United States

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ABSTRACT

To study the effects of sexual maturation on fatty acid metabolism in fish fed to satiation daily, expression of thirty-five genes involved in fatty acid metabolism was determined in sexually maturing diploid (2N; fertile) and triploid (3N; sterile) female rainbow trout. Gene expression was assessed in liver, white muscle, and visceral adipose tissues for fish that were 16 to 24 M of age. Previously, we reported minimal differences in most growth measurements between maturing 2N and 3N fish, but there were disparate changes in muscle proximate composition, visceral fat stores, and fatty acid contents of energy stores at 21 M with 3N females having greater lipid stores. Here, we report that gene expression profiles of liver and white muscle corresponded to the phenotypes with significant differences in expression at 20 M. Triploid females had increased expression of genes involved in fatty acid synthesis; including *gpat*, *srebp1*, *acyl*, *acc*, *fas*, and *scd1* in liver and *fas* in muscle. Conversely, 2N muscle had increased expression of β-oxidation genes *cpt1b*, *cpt2*, *ehhadh*, and *acat2* and TORC1 inhibitors *redd1*, *erk*, *mo25*, and *pras40*. Diploid muscle also had increased expression of *pparβ* along with increased expression of the fatty acid transporter gene *cd36*, and β-oxidation genes *cpt1a*, *cpt1c*, *aco*, and *acdhvl* at 20 M. Additionally, 2N visceral adipose tissue had increased *cpt1a* expression at 21 M. Overall, data suggest that 3N females are undergoing higher levels of fatty acid synthesis while 2N females have higher levels of β-oxidation during sexual maturation. Phenotypic data supports these findings with decreasing fatty acid stores in 2N females during this time period. Additionally, changes in gene expression are associated with altered expression within the mTOR and PPARβ signaling pathways.

1. Introduction

Although nutrient repartitioning is important to sexual maturation in female fish, little is understood about regulation of nutrient mobilization during this time period. Two pathways known to regulate nutrient metabolism in mammals are the mechanistic target of rapamycin (mTOR) and peroxisome proliferator activated receptors (PPAR) pathways (Laplante and Sabatini, 2011; Poulsen et al., 2012). Both pathways respond to nutrient availability and alter target gene expression of key enzymes involved in fatty acid metabolism. Albeit, both pathways are not specific to controlling lipid metabolism; they are active in a variety of other processes such as inflammation, immune function, apoptosis, protein metabolism, and stress resistance (Laplante and Sabatini, 2011; Poulsen et al., 2012). Sexual maturation in salmonids, such as rainbow trout, is not only a period of extensive restructuring of metabolism (Sumpter et al., 1991). Increased energy demand during gonadogenesis requires fat mobilization from muscle and visceral adipose tissue lipid stores (Gorgun and Akpinar, 2007; Jonsson et al., 1997; Kiessling et al., 2001; Memis and Gun, 2004; Nassour and Leger, 1989; Ribeiro et al., 2012; Salem et al., 2006; Shearer, 1994; Sumpter et al., 1991). This restructuring of metabolism to support a shift from somatic to gonadal growth and the importance of lipid metabolism during this time period make mTOR and PPAR signaling pathways primary candidates for regulating this process.

The mTOR pathway is a central signaling cascade that plays a role in integrating energy-sensing pathways. Regulation of mTOR provides a mechanism for cells to transition between anabolic and catabolic states in response to nutrient and energy availability by responding to circulating insulin levels (Laplante and Sabatini, 2011). There are two main paths mTOR can act through; the assembly of mTOR Complex 1 (TORC1) and mTOR Complex 2 (TORC2). TORC1 elicits its effects on lipid metabolism by increasing expression of genes involved in fatty acid synthesis (Caron et al., 2010; Laplante and Sabatini, 2009). Whereas, TORC2 is believed to play a role in regulating the transcription of genes involved in fatty acid β-oxidation (Brown et al., 2007; Jones et al., 2009; Sipula et al., 2006). There has been some assessment of the fatty acid metabolism by investigating gene expression of *fas*, *srebp1*, and *cpt1* in salmonids (Lansard et al., 2009; Seiliez et al., 2011; Skiba-Cassy et al., 2009). Data suggest that there are metabolic differences in nutrient utilization

* Corresponding author at: Division of Animal and Nutritional Sciences, West Virginia University1042 Agricultural Sciences Building P.O. Box 6108, Morgantown, WV 26505-6108, United States. Tel.: +1 304 293 1896.
E-mail address: mmanor@mix.wvu.edu (M.L. Manor).

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between fish consuming altered protein (Seiliez et al., 2011) and fishmeal-replacement diets (Lansard et al., 2009). Divergently bred lines of rainbow trout (lean and fat) also have different nutrient utilization resulting in different phenotypes (Skiba-Cassy et al., 2009). The consensus among these studies is that the mTOR signaling pathway is involved in nutrient utilization in a variety of situations ranging from genetic selection to dietary alterations. These findings further support mTOR as a primary pathway of interest when investigating regulation of fatty acid metabolism during sexual maturation in fish.

Conversely, the PPAR signaling pathway is known to respond to lipids and elicit transcriptional changes on genes involved in lipid metabolism in mammals. PPARs are members of the nuclear receptor superfamily of ligand-activated transcription factors (Poulsen et al., 2012). Gender and stage of life cycle influence expression levels of all PPARs (α, β, and γ) in brown trout (Batista-Pinto et al., 2009) with estrogen appearing to play an important role in their differential expression. PPARγ affects transcription rates of a variety of lipogenic target genes such as *fabp*, *cd36*, *lpl*, *leptin*, *acc*, *fas*, *and scd1* (Lee and Hossner, 2002). Additionally, PPARα and PPARβ are responsible for regulating fatty acid β-oxidation (Varga et al., 2011). PPARs' involvement in fatty acid metabolism makes them prime candidates as regulators of fatty acid metabolism during sexual maturation in fish.

Previous work from this same research group has investigated the effects of ration level and sexual maturation on expression of thirty-five genes involved in fatty acid metabolism using Multiplex-PCR (Manor et al., 2015). Investigating only two time points during sexual maturation provided a brief glimpse into metabolic changes that occur in lipid stores when fish are moderately feed restricted. In general, ration levels did not meaningfully affect expression of genes included in the multiplex; however, sexual maturation did have distinct effects on gene expression between 20 and 22 M (Manor et al., 2015). It is apparent that mTOR and PPAR pathways are important signaling mechanisms during sexual maturation and that maturation-related signals, such as estrogen, may be regulators of these processes. The current study investigates changes in expression of thirty-five genes involved in fatty acid metabolism in fertile diploid (2N) and sterile triploid (3N) fish throughout sexual maturation from 16 to 24 M. Unlike 2N fish, 3N females produce only minimal gonadal tissue (Piferrer et al., 2009). This report is part of a larger, comprehensive investigation of growth parameters, fillet quality attributes, muscle collagen, muscle protein thermal stability, and fatty acid composition of liver, muscle, visceral adipose tissue, and ovaries of the same 2N and 3N female rainbow trout (Aussanasuwannakul et al., 2011, 2012; Manor et al., 2012; Salem et al., 2013). To summarize these results, 2N fish had reached the early secondary growth phase, vitellogenesis, by 16 M. Diploid HSI decreased from 21 to 24 M. The HSI of 3N fish was usually lower than HSI of 2N fish until 2N HSI began decreasing from 22 to 24 M. As reported in Aussanasuwannakul et al. (2011), we found no difference between 2N and 3N females in WBW or length and muscle protein content remained unchanged throughout sexual maturation. Muscle composition and texture were affected primarily by changes in fat content. In general, 3N females had greater fat stores than their 3N counterparts with 2N females mobilizing their lipid reserves to support gonadogenesis. Understanding how genes within pathways related to fatty acid metabolism are regulated during this important life stage will indicate mechanisms responsible for nutrient repartitioning during sexual maturation. Furthermore, identifying critical genes and pathways associated with phenotypic traits will enhance our knowledge of how management strategies can affect these mechanisms for more efficient food-fish production.

2. Materials and methods

2.1. Experimental design

Experimental design was reported in detail by Manor et al. (2012) and Aussanasuwannakul et al. (2011). Briefly, a two by two by six ($2 \times 2 \times 6$) factorial, randomized-complete block design was used. In this design, family became the blocking variable. Independent variables included two sex conditions (fertile, 2N females and sterile, 3N females) and six sampling periods or harvest endpoints (16, 18, 20, 21, 22, and 24 M of age). Five fish for each treatment combination were randomly selected at each sampling, equaling 20 fish sampled at each endpoint and a total of 120 fish in the study.

2.2. Animals

Fish care and experimentation followed guidelines outlined by the US Department of Agriculture (USDA) and the National Center for Cool and Cold Water Aquaculture (NCCCWA; USDA—Agricultural Research Service; Leetown, WV, USA) Animal Care and Use Committee, which are in line with the National Research Council publication *Guide for Care and Use of Laboratory Animals*. Two families, each containing 2N and 3N rainbow trout, were generated and maintained at the NCCCWA. Fish were confirmed as 2N or 3N by flow cytometry (Allen, 1983; Hershberger and Hostuttler, 2007). Animals were fed a commercial feed, Zeigler GOLD Floating 5.0 mm pelleted feed (42% protein, 16% fat, and 2% fiber; 316520-36-44; Zeigler Brothers, Inc.; Gardners, Pennsylvania, U.S.A.), throughout the course of the experiment. Fish were fed on a tank-by-tank basis. Part of the daily ration was delivered by a belt feeder. At the end of the day fish were fed by hand to apparent satiation. The amount of feed delivered by the belt feeder was altered depending on appetite. From 16 to 19 M fish were fed at 1% of body weight; between 19 and 21 M, fish were fed at 0.8%; and between 21 and 24 M, fish were fed at 0.3%. Fish were initially maintained as part of stocks in five, 1.22 m diameter tanks. In July, each of the five tanks was stocked with thirty-five fish, totaling 175 fish for this study. The thirty-five fish assigned to each tank consisted of 2N and 3N fish from each of the two families. At each sampling period, fish were shifted to a different tank to reduce biases associated with tank. Similar tank densities were maintained during the study. To avoid temperature effects, water temperatures were maintained between 12.0 and 13.5 °C. A simulated ambient photoperiod was maintained with artificial lighting. Passive integrated transponders (Avid Identification Systems Inc., Norco, CA) were implanted in the musculature below the dorsal fin as tags for individual fish identification.

2.3. Sampling

All fish were weighed, and length (L) was measured (fork length) once a month between July (16 M post hatching) and March (24 M). Fish were held off feed 24 h prior to sampling and were anesthetized using 150 mg/L tricaine methanesulfonate (tricane-S; Western Chemical, Inc., Ferndale, WA, USA). Liver, white muscle, and visceral adipose tissue samples were frozen in liquid nitrogen and stored at −80 °C until further processing. Fish were manually filleted the following day at West Virginia University's Muscle Foods Laboratory. Gravimetric and morphometric measurements and chemical analyses are reported in Salem et al. (2013), Manor et al. (2012), and Aussanasuwannakul et al. (2011, 2012).

2.4. Gene expression analysis

2.4.1. Multiplex analysis

The GenomeLab GeXP genetic analysis system (Beckman Coulter Inc.; Pasadena, CA, USA) was used to simultaneously analyze expression of thirty-nine genes in liver, white muscle, or visceral adipose tissue. Within the multiplex, thirty-five genes were associated with fatty acid metabolic pathways and four served as potential reference genes. Primers were designed using eXpress Designer software (Beckman Counter Inc.; Pasadena, CA, USA) and primer sequences were compared against other rainbow trout gene sequences using the BLAST function within the NCBI database to reduce unintended sequence amplification.