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Review

# Fatty acid metabolism in fish species as a biomarker for environmental monitoring☆

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ABSTRACT

Pollution by Organic Contaminants (OC) in aquatic environments is a relevant issue at the global scale. Lipids comprised of Fatty Acids (FA) play many important roles in the physiology and life history of fishes. Toxic effects of OC are partly dependent on its bioaccumulation in the lipids of aquatic organisms due its physicochemical properties. Therefore, there is an increasing interest to investigate the gene expression as well as the presence and activity of proteins involved in FA metabolism. The attention on Peroxisome Proliferation Activate Receptors (PPARs) also prevails in fish species exposed to OC and in the transport, biosynthesis and β-oxidation of FA. Several studies have been conducted under controlled conditions to evaluate these biological aspects of fish species exposed to OC, as fibrates, endocrine disrupting compounds, perfluoroalkyl acids, flame retardants, metals and mixtures of organic compounds associated with a polluted area. However, only fibrates, which are agonists of PPARs, induce biological responses suitable to be considered as biomarkers of exposure to these pollutants. According to the documented findings on this topic, it is unlikely that these physiological aspects are suitable to be employed as biomarkers with some noticeable exceptions, which depend on experimental design. This emphasises the need to investigate the responses in fish treated with mixtures of OC and in wild fish species from polluted areas to validate or refute the suitability of these biomarkers for environmental or fish health monitoring.



## 1. Introduction

Lipids comprised of Fatty Acids (FA) are a class of biomolecules that possess a wide diversity of functions in structure and biological process through interactions with proteins (Dowhan et al., 2008; Sul and Smith, 2008). In fish species, lipids and proteins are the main organic constituents, and play many important roles in the fish's life history and physiology, which includes growth, movement, reproduction and migration (Tocher, 2003). The main role of lipids in these organisms is the storage and provision of energy in the form of Adenosine Triphosphate (ATP) through the β-oxidation of fatty acids (Froyland et al., 2000). A lot of studies had documented the toxic responses of fish species from polluted aquatic environments at a global scale. In this regard, Organic Contaminants (OC), as in the cases of polyaromatic hydrocarbons (PAH), polychlorinated hydrocarbons, pesticides, perfluoroalkyl acids and pharmaceutical products, among others, are the most investigated. These pollutants are hydrophobic, and due to their physicochemical properties are able to accumulate in the lipids of aquatic organism in a dose-dependent manner (Kainz and Fisk, 2009). There is an increase in the number of studies exploring the responses of proteins involved in Fatty Acid Metabolism (FAM).

## 2. Proteins involved in FA metabolism

The digestion, transport, synthesis and oxidation of FA are relevant processes in the metabolism of these biomolecules in fish species. Although there are large numbers of proteins whose presence and activity are responsible for FAM, only a few of them have been employed to evaluate their responses in fish exposed to OC. To provide good background knowledge of this topic, we describe the most studied proteins in this regard.

Triacylglycerol is the main lipid component in the diet of marine and freshwater fish (Tocher, 2003). Lipoprotein lipase (LPL; EC

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3.1.1.34) is an enzyme responsible for the hydrolysis of triacylglycerol and it plays a central role in the overall lipid metabolism and transport (Mead et al., 2002). The product of this hydrolysis provides non-esterified FA and 2-monoacylglycerol for their use in the tissues, which could be useful for the re-esterification involved in the storage of energy as triacylglycerol (Cryer, 1981).

Generally, the intracellular transport of FA is performed by Fatty Acid Binding Proteins (FABP) which are highly conserved cytoplasmic proteins possessing low molecular weight between 14 and 15 kDa (Tocher, 2003; Schulz, 2008). FABP are involved in some physiological processes, such as sequestering FA and other lipophilic compounds, transporting FA to the mitochondria, storage, modulating the cell proliferation and the FAM by Nuclear Receptors (NRs) (Meunier-Durmort et al., 1996; Bernlohr et al., 1997; Poirier et al., 2001; Leaver et al., 2005; Schulz, 2008).

FA oxidation is the major source of energy for many fish species. This physiological process occurs in cellular organelles as mitochondria and peroxisomes by a different set of enzymes (Tocher, 2003). FA oxidation is also known as β-oxidation, since this degradation begins in the third carbon atom (β-carbon) on the FA chain; the resulting β-keto acid is cleaved between the α-carbon and β-carbon to yield FA, shortened by two carbon atoms that generate acetyl-CoA, which supports gluconeogenesis, ketogenesis and the production of ATP through the Krebs cycle (Kompare and Rizzo, 2008; Schulz, 2008). The β-oxidation is a biochemical process catalysed in the mitochondria and peroxisomes (Schulz, 2008) with some peculiar differences.

Mitochondria are cellular organelles that provide useful energy by utilizing part of the free energy released during the consumption of hydrogen in the form of ATP, NADPH, ΔpH, and ΔΨ derived from different food, such as carbohydrates, FA and proteins (Panov et al., 2014). Mitochondrial β-oxidation is a specific process for long, medium and short chain of FA, which are taken from the liver and the muscle where they are activated to their coenzyme A (CoA) esters through the metabolism of long-chain acyl-CoA synthetase (ACSL; EC 6.2.1.3) (Hashimoto, 1999; Kompare and Rizzo, 2008). Long FA esters, which typically contain between 16 and 18 atoms of carbon, are transported to mitochondria in a system dependent on carnitine/acyl-carnitine balance named as “carnitine shuttle” (Kerner and Hoppel, 2000; Kompare and Rizzo, 2008). In this regard, the carnitine palmitoyltransferase I (CPT I; EC 2.3.1.21) is the enzyme responsible for the conversion of acyl-CoA compounds to their acylcarnitine metabolites at the outer membrane of the mitochondria and it plays a pivotal role in the regulation of β-oxidation by the rate-limiting step of carnitine transport (Kerner and Hoppel, 2000; Kompare and Rizzo, 2008). In contrast, medium chains FA are independent of carnitine (Hashimoto, 1999). Each cycle of mitochondrial β-oxidation requires the sequential metabolism of the following enzymes: acyl-CoA dehydrogenase, 2-enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase and 3-ketoacyl-CoA thiolase. The last three comprised the mitochondrial trifunctional protein (Kompare and Rizzo, 2008). The shortened acyl-CoA is subject to another β-oxidation process to generate shorter FA, whereas their final products are involved in the gluconeogenesis, ketogenesis and ATP generation (Kompare and Rizzo, 2008).

The peroxisomes are single membrane-delimited organelles found in almost all eukaryotic cells that undergo diverse metabolic processes, and they differ between organisms according to their developmental stages or as a response to environmental conditions (Wanders and Waterham, 2006; Baker et al., 2015). Peroxisomes contain almost 50 enzymes that are involved in the metabolism of $H_2O_2$, FA, amino acids and uric acid (Cooper, 2000). Specific endogenous substrates for peroxisomal β-oxidation are very long chain FA, methyl-branched carboxylic acids, prostaglandins, dicarboxylic acids, leukotrienes, isoprenoid-derived fat soluble vitamins, pristanic acid and xenobiotic compounds (Hashimoto, 1999; Poirier et al., 2006). The substrates for β-oxidation enter to the peroxisomes via ATP-binding cassette transporters of subfamily D and are activated by specific acyl CoA synthetases (ACS; EC 6.2.1.1) for their metabolism (Baker et al., 2015). In addition, the proteins of peroxisome membrane of 70 and 69 KDa are also involved (Schulz, 2008). Although the transport of FA in the matrix of peroxisomes is independent of carnitine, the presence of carnitine octanoyl transferase (CROT; EC 2.3.1.137) has been documented (Miyazawa et al., 1983). CROT is responsible for the catalysis of the shortened FA to acyl carnitine favouring in this way its output of the peroxisome (Le Borgne et al., 2011). Peroxisomal β-oxidation is comprised of CoA oxidase, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase bifunctional protein (these two enzymes conform to the L-bifunctional protein), and 3-ketoacyl-CoA thiolase (Hashimoto, 1999; Poirier et al., 2006). However, the metabolism of palmitoyl-CoA oxidase, which is a specific enzyme of the type acyl-CoA oxidase (AOX; EC 1.3.3.6), oxidizes the CoA esters of straight chain FA (Van Veldhoven et al., 1992; Reddy and Mannaerts, 1994). This enzyme was more studied in fish species exposed to OC while being employed as a biomarker.

The main pathway of lipogenesis is catalysed in the cytosol of the cell by the Fatty Acid Synthase (FAS; EC 2.3.1.85), a multienzyme complex characterized in fish species (Tocher, 2003). Mainly, the products of de novo FAS are saturated FA of 16 and 18 atoms of carbon (palmitic and stearic acids, respectively), including the nicotinamide adenine dinucleotide phosphate in almost all organisms, including fish species (Stoops and Wakil, 1981; Tocher, 2003; Sul and Smith, 2008).

The malonyl-CoA is a molecule that plays important roles in FAM, and it is able to inhibit the activity of CPT I; therefore, it favours the decrease on the transference of FA residues from CoA to carnitine and their translocation to mitochondria. In this way, it causes the depression of β-oxidation (Schulz, 2008). However, the malonyl-CoA is also a co-substrate for the condensation reaction of the cytosolic FAS complex and is a co-substrate for the FA elongation system, which occurs in the membrane of the endoplasmic reticulum (Saggerson, 2008). The malonyl-CoA is synthesized by the carboxylation of acetyl-CoA through the metabolism of acetyl-CoA carboxylases (ACC; EC 6.4.1.2), and both enzymes are dependent on the biotin (Kim, 1997; Harwood, 2005). In mammals and fish species, two isoforms of ACC exist: *i*) ACC1, located in the cytosol produces malonyl-CoA utilized for FA biosynthesis, and *ii*) ACC2 is found in the mitochondria generating malonyl-CoA involved in the inhibition of β-oxidation (Cheng et al., 2011; Zu et al., 2013).

## 3. Peroxisome proliferator-activated receptors (PPARs)

The PPARs are a cluster of NRs, which participate in some biological processes as in the cases of development, growth, reproduction, metabolism, immune response, apoptosis and cancer, among others (Berger and Moller, 2002; Poulsen et al., 2012). The name “PPARs” was derived from the ability of one of these receptors to induce the Peroxisome Proliferation (PP) in the hepatocytes of rodents (Christodoulides and Vidal-Puig, 2010). The PPARs are sensitive to dietary lipids ingest or to essential FA metabolites and participates on the FA signals as key regulators of lipid metabolism (Varga et al., 2011). In vertebrates, three subtypes of PPAR have

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