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Temperature and metal exposure affect membrane fatty acid composition and transcription of desaturases and elongases in fathead minnow muscle and brain

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ABSTRACT

In this study, we tested the hypothesis that metal exposure affected the normal thermal response of cell membrane FA composition and of elongase and desaturase gene transcription levels. To this end, muscle and brain membrane FA composition and FA desaturase (*fads2*, *degs2* and *scd2*) and elongase (*elovl2*, *elovl5* and *elovl6*) gene transcription levels were analyzed in fathead minnows (*Pimephales promelas*) acclimated for eight weeks to 15, 25 or 30 °C exposed or not to cadmium (Cd, 6 µg/l) or nickel (Ni, 450 6 µg/l). The response of membrane FA composition to temperature variations or metal exposure differed between muscle and brain. In muscle, an increase of temperature induced a decrease of polyunsaturated FA (PUFA) and an increase of saturated FA (SFA) in agreement with the current paradigm. Although a similar response was observed in brain between 15 and 25 °C, at 30 °C, brain membrane unsaturation was higher than predicted. In both tissues, metal exposure affected the normal thermal response of membrane FA composition. The transcription of desaturases and elongases was higher in the brain and varied with acclimation temperature and metal exposure but these variations did not generally reflect changes in membrane FA composition. The mismatch between gene transcription and membrane composition highlights that several levels of control other than gene transcription are involved in adjusting membrane FA composition, including post-transcriptional regulation of elongases and desaturases and *de novo* phospholipid biosynthesis. Our study also reveals that metal exposure affects the mechanisms involved in adjusting cell membrane FA composition in ectotherms.

1. Introduction

Cell membranes are highly sensitive to temperature, affecting their physical properties and consequently the functioning of embedded proteins (Hochachka and Somero, 2002). Under cold temperatures, the overall packing order of membrane phospholipids increases, causing a decrease of membrane fluidity. At the opposite, an increase of temperature induces phospholipid disorder and enhances fluidity. To counteract temperature effects, poikilotherms remodel membrane phospholipid fatty acid (PLFA) composition, a process known as homeoviscous adaptation (Hazel and Williams, 1990; Henderson et al., 1995; Sinensky, 1974; Wodtke and Cossins, 1991).

Long chain polyunsaturated fatty acids (LC-PUFA) such as arachidonic acid (ARA, 20:4n-6), eicosapentanoic acid (EPA, 20:5n-3) and docosahexanoic acid (DHA, 22:6n-3) are essential for cell functioning. As major constituents of membrane phospholipids, they control membrane fluidity and influence protein activity and membrane function (Hashimoto et al., 2006; Horrocks and Farooqui, 2004; Stillwell and Wassall, 2003). In addition, DHA and ARA have an important role in neural function and development (Innis et al., 1999; Salem et al., 2001). Two groups of enzymes, desaturases and elongases, are responsible for the regulation of membrane PLFA composition following changes in temperature (Hazel and Livermore, 1990; Trueman et al., 2000). The biosynthesis of LC-PUFA from 18:2n-6 and 18:3n-3 involves desaturases and elongases. Desaturases incorporate double bonds at a specific position of the acyl chain (Guillou et al., 2010) and can be divided into two families: steroyl-CoA desaturases (SCD) and fatty acid desaturases (FADS) (Marquardt et al., 2000). Elongases catalyze the elongation

Abbreviations: ARA, arachidonic acid; Cd, cadmium; CI, condition index; DHA, docosahexanoic acid; EPA, eicosapentanoic acid; FA, fatty acid; FADS, fatty acid desaturases; HIS, hepatosomatic index; HVA, homeoviscous adaptation; LC-PUFA, long chain polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; Ni, nickel; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid

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process by inserting 2 carbons at a time (Jakobsson et al., 2006). Recently, a variety of desaturases and elongases involved in the PUFA biosynthetic pathway have been cloned and numerous desaturase families have been identified in marine and freshwater species (Tocher et al., 2006; Zheng et al., 2004). The Δ6 FADS2 desaturase appears to be more common compared to the Δ5 FADS2 desaturase. Desaturases vary among species. In Atlantic salmon, a unifunctional Δ5 FADS2 desaturase has been reported, while in zebrafish (*Danio rerio*), rabbitfish (*Siganidae Siganus*) and pike silverside (*Chirostoma estor*) three bifunctional Δ6/Δ5 FADS2 desaturases have been identified (Fonseca-Madrigal et al., 2014; Hastings et al., 2004). Regarding elongases, the first that has been reported, ELOVL5, was characterized in zebrafish (*Danio rerio*) (Agaba et al., 2004) and subsequently in several other species (Agaba et al., 2005; Monroig et al., 2013). As for ELOVL2, to date it has been reported in many species, such as Atlantic salmon, rainbow trout and zebrafish (Gregory and James, 2014; Monroig et al., 2009, 2013). The extent to which fish can convert 18:2n6 and 18:3n3 to LC-PUFA varies among species and depends on their assemblages of desaturase and elongase enzymes. Palmitic acid (16:0) and stearic acid (18:0) are converted to 16:1n7 and 18:1n9 by SCD, that performs a desaturation at the Δ9 position of these fatty acids (Guillou et al., 2010). Since they do not possess Δ12 or Δ15 desaturases to desaturate 18:1n9 to 18:2n6 (LOA) and then to 18:3n3 (ALA), fish need to acquire these essential fatty acids through food. Then, LOA and ALA are converted to LC-PUFA through a series of enzymatic reactions (Fig. 1). DHA can be synthesised by two pathways. In the first one, often referred to as the "Sprecher shunt pathway", EPA undergoes two elongations to obtain 24:5n-3 followed by a Δ6 desaturation and a chain shortening (Sprecher, 2000). The second one is more direct and it involves Δ4 desaturation of 22:5n-3 (Li et al., 2010). It was long considered that vertebrates produced DHA from EPA only via the Sprecher shunt pathway and did not possess a Δ4 desaturation step, but the existence of an alternative pathway for DHA production from EPA via direct Δ4-desaturation has been recently demonstrated (Li et al., 2010). Once produced, PUFA are incorporated into membrane phospholipids by specific acyltransferases. It appears that freshwater fish have the enzymatic capacity to perform LC-PUFA biosynthesis (Agaba et al., 2005; Hastings et al., 2004; Morais et al., 2009) while marine fish exhibit low activity of desaturases and elongases such as Δ5 FADS2 desaturase and ELOVL2 elongase (Morais et al., 2012; Tocher et al., 2006). This difference may be explained by the higher abundance of LC-PUFA in marine compared to freshwater food webs. In marine ecosystems, the higher availability of LC-PUFA may have induced the loss of biosynthetic capacities for LC-PUFA in fish, while in contrast their lower availability in freshwater food webs may be the responsible for the persistence of desaturases and elongases (Leaver et al., 2008).

The effects of temperature on metal uptake in aquatic organisms have been abundantly studied and consistently reported to increase

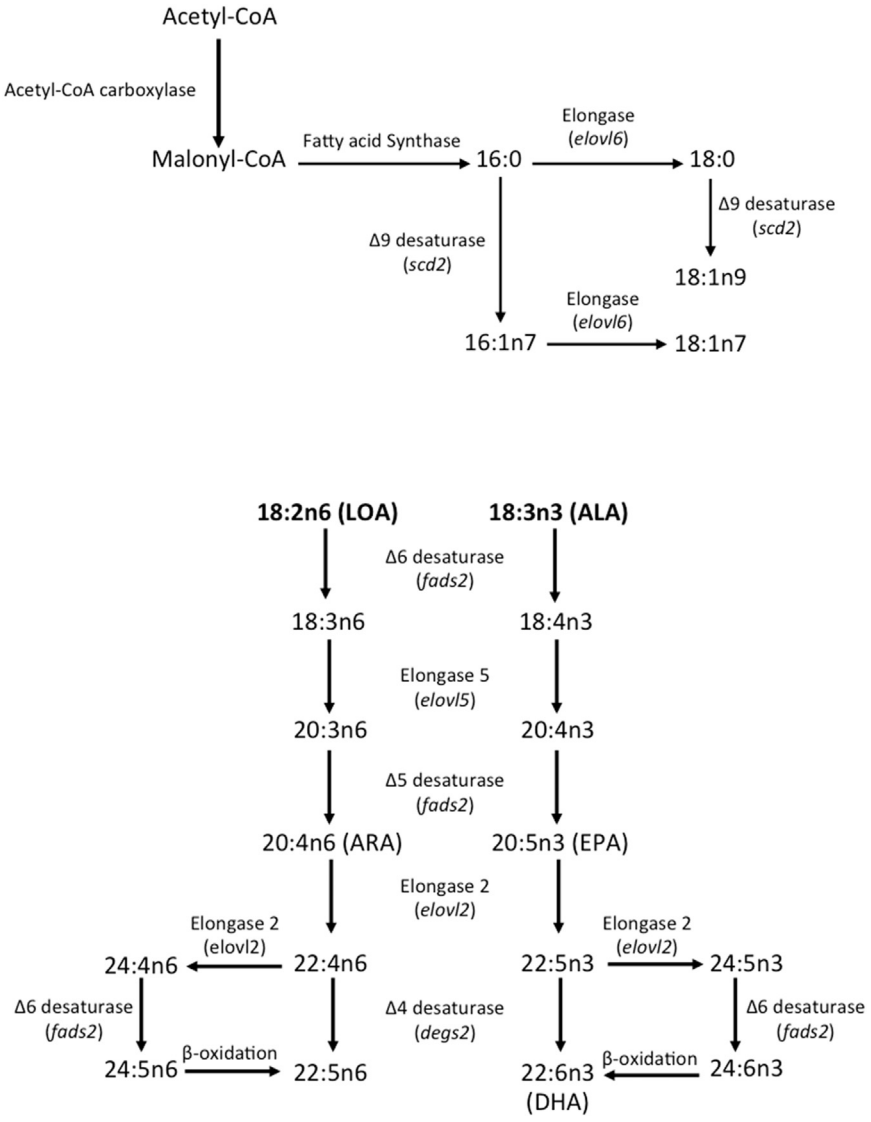

Fig. 1. The biosynthesis pathway of monounsaturated and long-chain polyunsaturated fatty acids in fish.