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Dynamics of fatty acid metabolism in a cell line from southern bluefin tuna (*Thunnus maccoyii*)

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ABSTRACT

Bluefin tunas are large predatory marine fish of great commercial value but little is known of their specific nutritional requirements. The three species are farmed in sea cages in Australia, the Mediterranean, Mexico and Japan where they are fed small oily fish sourced from wild-catch fisheries. This may not be sustainable and, therefore, it is important to investigate the possible consequences of the replacement of wild-catch fisheries products (fish oil and fish meal) with alternative oil and meal sources in feeds for these fish. To this end we have studied fatty acid metabolism in a recently developed southern bluefin tuna (SBT, *Thunnus maccoyii*) cell line designated SBT-E1. The predominant fatty acids in the total lipid of the SBT-E1 cells were 16:0, 18:0 and 18:1n−9. There were also substantial amounts of 20:4n−6, 22:5n−3 and 22:6n−3 but only very limited amounts of 18:2n−6, 18:3n−3 or 20:5n−3. The fatty acid composition of the cells reflected that of the culture medium except that 20:4n−6, 22:5n−3 and 22:6n−3 were substantially more abundant in the cells than in the medium. Fatty acid esterification occurred predominantly into phosphatidylcholine (PC) and phosphatidylethanolamine (PE), the two most abundant classes of lipids. The SBT-E1 cells showed very limited Δ6 fatty acyl desaturase (Fads) activity towards either 18:3n−3 or 18:2n−6 but substantial elongation of very long chain fatty acids (Elovl) activity towards 20:5n−3. The latter activity is usually attributable to an Elovl5 enzyme. Surprisingly though, there were much higher levels of Δ6 Fads compared with Elovl5 gene expression in the SBT-E1 cells, suggesting that a different Elovl enzyme may catalyse this reaction in SBT. The cells also showed substantial β-oxidation of 18:3n−3 and 20:5n−3 but much less activity towards 18:0, 18:1n−9 or 18:2n−6. These results may explain the high 22:6n−3 to 20:5n−3 ratios found in the SBT tissue lipids, especially in the phospholipids. The results are discussed in terms of the presumed nutritional requirements of bluefin tunas given their high trophic level in marine food webs.

1. Introduction

Bluefin tunas are large predatory marine fish of great commercial value but little is known of their specific nutritional requirements other than from studies of their natural prey (Itoh et al., 2011; Logan et al., 2011; Masuma et al., 2008; Metian et al., 2014; Miyake et al., 2010; Mylonas et al., 2010; Shimose et al., 2013; Skirtun et al., 2013; Woodhams et al., 2013). The three bluefin tuna species are farmed in sea cages in the Spencer Gulf of South Australia (southern bluefin tuna, *Thunnus maccoyii*), the Mediterranean Sea (Atlantic bluefin tuna, *Thunnus thynnus*) and along the Pacific coast of Mexico and the southern coast of Japan (Pacific bluefin tuna, *Thunnus orientalis*). For the most part, this involves the on-growing and/or fattening of wild-caught juveniles due to the limited success of captive breeding (Bubner et al., 2012; De Metrio et al., 2010; Masuma et al., 2008; Okada et al., 2014; Sawada et al., 2005; Tsuda et al., 2012; Yúfera et al., 2014). The capture of bluefin tunas from the wild is limited by strict catch quotas due to concerns regarding their declining stocks (CCSBT; ICCAT). Therefore, the purpose of bluefin tuna farming is to maximise commercial returns from the limited number of fish available. This is done by on-growing the fish to a larger size, increasing the fat content of their muscle (desired by Japanese sashimi consumers) and holding fish back from the market at times when over supply or currency fluctuations bring sub-optimal prices (Miyake et al., 2010; Mylonas et al., 2010; Skirtun et al., 2013; Woodhams et al., 2013). The main market for bluefin tunas is Japan and the main use is for the production of the Japanese raw fish delicacies sushi and sashimi. On-growing and fattening of bluefin tunas is achieved by feeding them small pelagic fish (*e.g.* sardines, mackerels, herrings) sourced from wild-catch fisheries (Miyake et al., 2010; Musgrove et al., 2011; Mylonas et al., 2010). This mimics their natural diets and so presumably satisfies their nutritional requirements but is not a sustainable practice (Mourente and Tocher, 2009).

In the past 15–20 years, the growth of wild-catch fisheries production has failed to keep pace with the growth of aquaculture and as a

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result the use of wild-caught fish to feed farmed fish has been called into question (Metian et al., 2014; Naylor et al., 2009; Tacon and Metian, 2013). In other farmed fish species, such as Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), significant progress has been made in the replacement of wild-catch fishery products (fish oil and fish meal) with alternatives such as vegetable oils and plant meals but this has not been the case for tunas (Metian et al., 2014; Tacon and Metian, 2013). There are several reasons for this. The first is that Japanese sushi and sashimi consumers prefer wild-caught tunas or, at the very least, farmed tunas that have been fed their natural diet (Ottolenghi, 2008). The second is that classical feeding trials with appropriate numbers of biological replicates have not been possible due to the high commercial value of wild-caught bluefin tunas, the high costs of maintaining them in sea cages or purpose built facilities on land and the limited success of captive breeding programmes to produce small fish for research (Mourente and Tocher, 2009; Mylonas et al., 2010). The third reason is that replacement of fish oils with vegetable oils in feeds for farmed fish reduces their flesh concentrations of the omega-3 (n−3) long-chain polyunsaturated fatty acids (LC-PUFAs), eicosapentaenoic acid (20:5n−3) and docosahexaenoic acid (22:6n−3) (Miller et al., 2008; Turchini et al., 2009). These fatty acids have a range of important human health benefits including reduced risk of cardiovascular disease and amelioration of the symptoms of inflammatory disorders such as rheumatoid arthritis (Monteiro et al., 2014; Yates et al., 2014).

Fish oils contain high concentrations of 20:5n−3 and 22:6n−3 whereas vegetable oils completely lack these fatty acids (Turchini et al., 2009). Some vegetable oils, such as linseed or canola oil, contain high concentrations of α-linolenic acid (18:3n−3), the precursor of 20:5n−3 and 22:6n−3. However many fish species, especially marine piscivores, have only limited capacity to convert 18:3n−3 to 20:5n−3 or 22:6n−3 (Ghioni et al., 1999; Tocher, 2003; Tocher and Ghioni, 1999). It is assumed, though it has not been tested, that tunas would also have limited capacity for endogenous production of LC-PUFAs from C_{18} PUFAs (Mourente and Tocher, 2009).

In vertebrates, including fish, *de novo* synthesis of 20:5n−3 and 22:6n−3 from 18:3n−3 proceeds via a series of reactions catalysed by fatty acyl desaturase (Fads) and elongation of very long chain fatty acids (Elovl) enzymes (Fig. 1). The most commonly observed pathway is as follows. In the first step, 18:3n−3 is desaturated to 18:4n−3 by a Δ6 Fads. In the second step, 18:4n−3 is elongated to 20:4n−3 by an Elovl5. This is followed by desaturation of 20:4n−3 to 20:5n−3 catalysed by a Δ5 Fads and elongation of 20:5n−3 to 22:5n−3 catalysed by the same Elovl5 as mentioned above. Subsequently, 22:5n−3 is elongated to 24:5n−3 by an Elovl2 and 24:5n−3 is desaturated to 24:6n−3 by the same Δ6 Fads as mentioned above. Finally, partial β-oxidation of 24:6n−3 to yield 22:6n−3 occurs in the peroxisomes. It is important to note that there is competition between different intermediates within this pathway for the same enzymes. There is also competition from the corresponding omega-6 (n−6) fatty acids that are abundant in certain vegetable oils such as linoleic acid (18:2n−6) in soybean oil.

Marine fish have an abundance of 20:5n−3 and 22:6n−3 in their natural diets and this probably explains their limited capacity to synthesise these fatty acids *de novo* (Tocher, 2003). Whatever the case may be, and in light of the great commercial value of bluefin tuna aquaculture, it is important to gain a better understanding of their fatty acid metabolism to support the development of more sustainable artificial feeds. To this end, we have taken advantage of a recently established tuna cell line derived from southern bluefin tuna (*T. maccoyii*) (Bain et al., 2013). Using this cell line, we have investigated the esterification into various lipid classes and the metabolism via desaturation, elongation and/or β-oxidation of a selection of fatty acids known to be quantitatively important in tuna tissues.

2. Materials and methods

2.1. Cell culture

For routine maintenance, the SBT-E1 cell line established from an anterior body cross-section of a captive-bred southern bluefin tuna fingerling was cultured as previously described (Bain et al., 2013; Scholefield and Schuller, 2014). The cell line was originally a primary culture and is now presumed to be immortalized. The experiments described in this paper were conducted between passages 43 and 54. The standard culture medium consisted of Leibovitz's L-15 medium supplemented with 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.4) and 10% (v/v) foetal bovine serum (FBS)

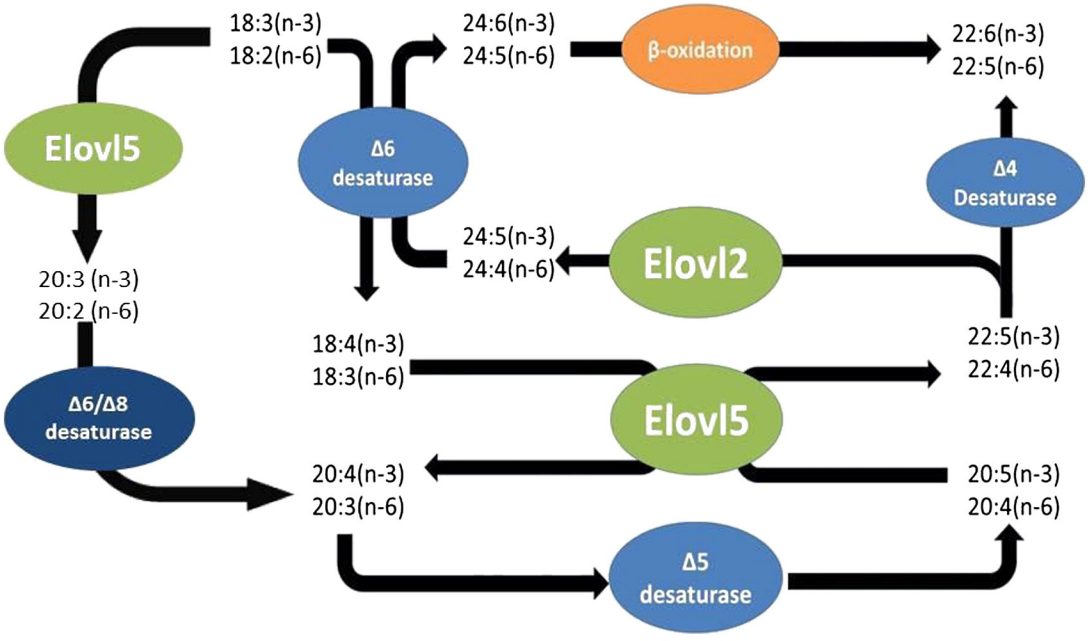

Fig. 1. Alternative pathways for the *de novo* synthesis of long-chain polyunsaturated fatty acids (LC-PUFAs) from their C_{18} precursors in fish (Li et al., 2010; Monroig et al., 2011; Scholefield and Schuller, 2014).

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