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Plasma non-esterified fatty acids of elasmobranchs: Comparisons of temperate and tropical species and effects of environmental salinity

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Abstract

We investigated the influence of environments with different average temperatures and different salinities on plasma NEFA in elasmobranchs by comparing species from tropical vs. cold temperate marine waters, and tropical freshwater vs. tropical marine waters. The influence of the environment on plasma NEFA is significant, especially with regard to essential fatty acids (EFA) and the *n*-3/*n*-6 ratio. *n*-3/*n*-6 ratios in tropical marine elasmobranchs were lower by two-fold or more compared with temperate marine elasmobranchs, because of higher levels of arachidonic acid (AA, 20:4*n*-6) and docosatetraenoic acid (22:4*n*-6), and less docosahexaenoic acid (DHA, 22:6*n*-3), in the tropical species. These results are similar to those in earlier studies on lipids in teleosts. *n*-3/*n*-6 ratios and levels of EFA were similar between tropical freshwater and tropical marine elasmobranchs. This suggests that the observation in temperate waters that marine fishes have higher levels of *n*-3 fatty acids and *n*-3/*n*-6 ratios than freshwater fishes may not hold true in tropical waters, at least in elasmobranchs. It also suggests that plasma NEFA are little affected by freshwater vs. seawater adaptation in elasmobranchs. Likewise, we found that plasma NEFA composition and levels were not markedly affected by salinity acclimation (2 weeks) in the euryhaline stingray *Himantura signifer*. However, in contrast to our comparisons of freshwater-adapted vs. marine species, the level of *n*-3 fatty acids and the *n*-3/*n*-6 ratio were observed to significantly decrease, indicating a potential role of *n*-3 fatty acids in salinity acclimation in *H. signifer*.

Keywords: Plasma; Non-esterified fatty acids; Lipids; Elasmobranch fish; Salinity acclimation; *n*-3/*n*-6 ratio; Tropical; Temperate

1. Introduction

Elasmobranchs are an ancient group of fishes that have an unusual metabolic organization characterized by heavy utilization of ketone bodies as oxidative fuel in the non-starved state and a low capacity for lipid oxidation in muscle (Speers-Roesch et al., 2006). Probably not coincidentally, elasmobranchs lack an albumin-like protein in the blood (Metcalf and Gemmell, 2005) and possess low levels of plasma non-esterified fatty acids (NEFA) (Ballantyne et al., 1993; Speers-Roesch et al., 2006), which represent a metabolically dynamic fraction of lipid in blood. Studies on teleosts and other ray-finned fishes (actinopterygians) have shown that the levels and composition of plasma NEFA reflect nutritional state and diet and indicate those fatty acids mobilized from extrahepatic or hepatic lipid stores for oxidation and certain anabolic processes in peripheral tissues (Henderson and Tocher, 1987). The physiological relevance of plasma NEFA in actinopterygians is supported by the few studies that have examined the effects on plasma NEFA of environmental factors such as temperature (e.g. Haman et al., 1997) and salinity (e.g. Jarvis and Ballantyne, 2003). Even less is known about how plasma NEFA composition is shaped by the environment in which a fish lives, especially in elasmobranchs. The aim of the present study, therefore, was to shed light on this question, via 1) comparisons of species living in separate ecosystems with different average environmental temperature (cold temperate vs. tropical oceans), 2) comparisons of species adapted to separate ecosystems with different environmental

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salinities (ocean vs. freshwater), and, 3) in relation to goal two, an evaluation of the effect of salinity acclimation on a euryhaline species.

The first goal of the present study was to investigate whether or not plasma NEFA composition varies with latitude in elasmobranchs, by comparison of species inhabiting cold temperate waters with those living in tropical waters. In particular, we were interested in the essential fatty acids (EFA) docosahexaenoic acid (DHA, 22:6*n*-3), eicosapentaenoic acid (EPA, 20:5*n*-3), and arachidonic acid (AA, 20:4*n*-6). These EFA and their elongation products play important physiological roles in fish, including membrane structure and function, reproduction, and eicosanoid metabolism (Tocher, 2003). One of the distinctive features of plasma NEFA (and tissue fatty acid composition) in temperate marine teleosts is a high ratio of *n*-3/*n*-6 fatty acids (4.7 to 14.4) due to elevated levels of DHA and EPA, which are common in cold-water food chains and have important roles in the function of cold-adapted membranes in fishes (Henderson and Tocher, 1987; Hazel and Williams, 1990; Ballantyne et al., 1993). The tissue lipids of tropical marine teleosts, on the other hand, contain less DHA and more AA and its elongation product docosatetraenoic acid (22:4*n*-6), resulting in lower *n*-3/*n*-6 ratios (Sinclair et al., 1983; Gibson et al., 1984; Hansel et al., 1993). In fact, Sinclair et al. (1984) demonstrated that the relative *n*-6 fatty acid content in fishes increased as sampling occurred closer to the equator. Whether or not these trends also exist in lipids from elasmobranchs is questionable, because Ballantyne et al. (1993) found relatively low *n*-3/*n*-6 ratios in plasma NEFA of some temperate marine elasmobranchs. To resolve this issue and provide insight into lipid metabolism of elasmobranchs from different thermal environments, we tested the hypothesis that temperate elasmobranchs possess a higher *n*-3/*n*-6 ratio in plasma NEFA compared with tropical species.

The second goal of the present study was to evaluate how plasma NEFA composition, in particular EFA, differs between tropical elasmobranchs that are adapted to seawater and those that are adapted to life in freshwater. In temperate freshwater teleosts, lower *n*-3/*n*-6 ratios (0.5 to 3.8) are found due to greater input of terrestrial plant-derived fatty acids, which have relatively higher levels of *n*-6 vs. *n*-3 fatty acids (Henderson and Tocher, 1987; Ballantyne et al., 1993). The *n*-3/*n*-6 ratio has therefore been suggested as a distinguishing feature between marine and freshwater teleosts (Henderson and Tocher, 1987). However, there is reason to hypothesize that this general rule does not hold true for comparisons of plasma NEFA or lipid composition between freshwater and marine fishes from tropical waters because, as previously mentioned, tissue lipids of warm-water marine teleosts contain less DHA and more AA and 22:4*n*-6, resulting in low *n*-3/*n*-6 ratios (Sinclair et al., 1983; Gibson et al., 1984; Hansel et al., 1993). We tested this hypothesis and evaluated the influence of adaptation to differing environmental salinity on plasma NEFA by comparing plasma NEFA composition between two tropical marine elasmobranchs and two tropical freshwater elasmobranchs.

To further investigate the effects of salinity on plasma NEFA in elasmobranchs, we measured the effects of a 2 week acclimation to half-strength seawater (15‰) on the levels and composition of plasma NEFA in the tropical freshwater euryhaline stingray *Himantura signifer*. The effects of salinity acclimation on plasma NEFA levels in an elasmobranch have not been previously measured, and are poorly known in other fishes. This experiment allowed us to evaluate whether acute salinity acclimation causes changes in the *n*-3/*n*-6 ratio in plasma NEFA, and whether these changes are similar at all to the patterns seen in lipids of freshwater-adapted vs. marine elasmobranchs. Changes in *n*-3 fatty acids, in particular, have been observed to occur in phospholipids and neutral lipids of some fishes during salinity acclimation although the exact functional role is unclear (e.g. Leray et al., 1984; Cordier et al., 2002; Martinez-Álvarez et al., 2005). Plasma NEFA also have been suggested in sturgeon to be an important fuel source for costly osmoregulatory processes during salinity acclimation (Jarvis and Ballantyne, 2003). Osmoregulatory tissues (e.g. rectal gland, kidney) in elasmobranchs have a high capacity for lipid oxidation (Speers-Roesch et al., 2006), so we hypothesized that levels of total NEFA and individual NEFA important in fatty acid oxidation would increase during salinity acclimation of *H. signifer*.

2. Materials and methods

2.1. Animals, salinity acclimation of H. signifer, and sampling

Spiny dogfish (*Squalus acanthias*) (temperate marine) (1.3 ± 0.1 kg, mean ± SEM) and little skate (*Leucoraja erinacea*) (temperate marine) (657 ± 73 g) were obtained by otter trawl (70–100 m depth) from *R/V W.B. Scott* in Passamaquoddy Bay, New Brunswick, in August, 2004. Fishes were maintained in flow-through seawater aquaria at Huntsman Marine Science Centre (St. Andrews, NB) and sampled within 2 days of capture. The dogfishes were all male; the little skates were of mixed sex. Examination of stomach contents showed all fishes had been feeding. Water temperature could not be measured at trawl depth (70–100 m) but available oceanographic data suggest that the temperature was approximately 8 °C.

Longnose skate (*Raja rhina*) (temperate marine) (7.6 ± 1.5 kg) and *Bathyraja* sp. (temperate marine) (1.2 and 3.4 kg) were captured by otter trawl off northern Vancouver Island, British Columbia, on a Department of Fisheries and Oceans (Canada) groundfish survey aboard *CCGS W.E. Ricker* in May, 2004. Depth of capture was 120–200 m and bottom temperature was 6–7 °C. Animals were of mixed sexes. Responsive animals were sampled upon retrieval of the trawl contents. Examination of stomach contents showed that the animals had been feeding.

Amazonian ocellate river stingray (*Potamotrygon motoro*) (tropical freshwater) (69 ± 4 g) and white-edge whip ray (*H. signifer*) (tropical euryhaline freshwater) (139 ± 36 g) were obtained in July 2004 from a fish farm in Singapore, where they were fed bloodworms (freshwater chironomid larvae); blue-spotted ribbontail stingray (*Taeinura lymma*) (tropical marine) (480 ± 33 g) and brownbanded bamboo shark (*Chiloscyllium punctatum*) (tropical marine) (1988 ± 221 g) were purchased at a live fish market in Singapore about 6 h after being caught in the ocean in July 2004. All species were of mixed sexes. Holding