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Vitamins C and E concentrations in muscle of elasmobranch and teleost fishes

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ABSTRACT

In fish, vitamins are part of the first line of the antioxidant defense, they are directly related to stress and disease, and they are involved in the maintenance of various physiological processes and metabolic reactions. In general, fish are unable to synthesize vitamin C due to a deficiency in gulonolactone oxidase (GLO), the enzyme responsible for its *de novo* synthesis. Vitamin E is involved in the immune response and perhaps one of its main physiological functions is to protect membranes from oxidative damage (lipid peroxidation) associated with free radical production. In fish muscle, vitamin E has an important role as an antioxidant *in vivo* and its content is highly related to the stability of lipids and fats. The aim of this study was to determine the content of vitamins C and E in muscle from different species of elasmobranch and teleost fishes. The concentrations of vitamins C and E were determined by high performance liquid chromatography (HPLC). The concentration of vitamin C found for the group of elasmobranchs was lower ($p = 0.001$) than that for teleosts. For *Mustelus henlei* vitamin C was found in only one individual; in *Tetrapturus audax* and *Totoaba macdonaldi* vitamin C concentration was below the detection limit. The concentration of vitamin E was lower in the group of elasmobranchs ($p = 0.03$) compared with that of teleosts. The main differences in the antioxidant system between teleosts and elasmobranchs appear to be the specific type and levels of antioxidant compounds, as well as the synergistic interactions among the antioxidants present in their tissues.

1. Introduction

The antioxidant system, composed of nutrients, enzymes and small molecular weight molecules, differs between tissues, is related to the lifestyle and depends on the evolutionary history of a species; for instance, differences in the antioxidant system between elasmobranchs and teleosts have been reported (Wilhelm-Filho and Boveris, 1993; Wilhelm-Filho et al., 1993; López-Cruz et al., 2010; Faramarzi, 2012). In fish, vitamins are part of the first line of the antioxidant defense, are directly related to stress and disease, and are involved in the maintenance of various physiological processes and metabolic reactions (Martínez-Álvarez et al., 2005; Kumari and Sahoo, 2005; Lopera-Barrero and Poveda-Parra, 2009). Vitamins are organic compounds obtained mainly through the diet because of either a lack of key enzymes involved in their synthesis, or failure to produce them in sufficient amounts (Weber, 1995; Drouin et al., 2011). Although the specific requirements are not equal for all species, vitamins A, D, E, K and C are essential nutrients in the fish diet, and insufficient intake of vitamins C and E can cause nutritional and growth deficiencies (Weber, 1995; Corredor and Landines, 2009; Lopera-Barrero and Poveda-Parra, 2009).

Vitamin C (ascorbic acid or, under physiological conditions, ascorbate) is a water-soluble nutrient required for the synthesis of red blood cells and collagen, which is a component of connective tissues, blood vessels, bone matrix, cartilage and tissue repair (Fracalossi et al., 1998). Vitamin C is also involved in iron metabolism, in the formation of neurotransmitters such as serotonin, in the transformation of dopamine to noradrenaline and other hydroxylation reactions, as well as in the biosynthesis of catecholamines, which are part of the primary response to stressful situations (Verlhac and Gabaudan, 1997; Torres et al., 2002; Kumari and Sahoo, 2005; Corredor and Landines, 2009). In general, fish are unable to synthesize vitamin C due to a deficiency in gulonolactone oxidase (GLO; EC 1.1.3.8), the enzyme responsible for its *de novo* synthesis (Verlhac and Gabaudan, 1997; Fracalossi et al., 2001; Faramarzi, 2012). The majority of teleosts (bony fish) have no functional GLO genes; however, GLO activity was found in the kidney of several primitive non-teleost fishes, such as sharks, sturgeon and lamprey, suggesting that these species can synthesize vitamin C (Cho et al., 2007). Species that cannot synthesize this vitamin absorb it by a sodium (Na)-dependent active transport mechanism, which apparently acts at low micronutrient concentrations, whereas at higher concentrations absorption can occur via passive diffusion (Verlhac and

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Gabaudan, 1997). Physical and physiological effects of vitamin C deficiency are varied; including internal bleeding, immunosupression, increased susceptibility to bacterial infections, reduced growth, skeletal muscle injuries, structural deformities, abnormal pigmentation and poor reproductive performance, among others (Alava et al., 1993; Verlhac and Gabaudan, 1997; Fracalossi et al., 1998; Mæland and Waagbø, 1998; Fracalossi et al., 2001; Kumari and Sahoo, 2005; Cho et al., 2007).

The term vitamin E includes two families of compounds, tocopherols and tocotrienols, which have similar structure and differ only in their saturation; α-tocopherol is the most active and abundant isomer in organelles and cell membranes of vertebrates (Torres et al., 2002; Wilhelm-Filho, 2007; Faramarzi, 2012). Vitamin E is a fat-soluble vitamin that is absorbed in the small intestine by passive diffusion; it is stored primarily in the liver, from which it can be mobilized quickly, but can also deposited in adipose tissue and muscle where it shows a slow turnover (Torres et al., 2002). Vitamin E is involved in the immune response and perhaps one of its main physiological functions is to protect membranes from oxidative damage (lipid peroxidation) associated with free radical production and/or with an imbalance between antioxidant and prooxidant molecules (Weber, 1995; Wilhelm-Filho, 2007). In fish muscle, vitamin E has an important role as an antioxidant *in vivo* and its content is highly related to the stability of lipids and fats (Pazos et al., 2005). Both vitamin C and vitamin E can act as antioxidants in a synergistic manner. Vitamin C acts as a terminal element in the protection against tissue damage caused by free radicals, but when both vitamins are present, the major function of vitamin C is restoration of vitamin E (Verlhac and Gabaudan, 1997; Torres et al., 2002).

The requirements of antioxidant vitamins intake are directly related to the levels of highly unsaturated fatty acids (HUFA), given their susceptibility to oxidation (Faramarzi, 2012). Tissues and organs with an elevated unsaturated lipid content, as is the case of fish tissues, are expected to have elevated antioxidant vitamin content. Given the lifestyle and evolutionary history, elasmobranch fishes are expected to have higher antioxidant vitamin content than teleost fishes (Wilhelm-Filho and Boveris, 1993; Wilhelm-Filho et al., 1993; López-Cruz et al., 2010; Faramarzi, 2012). Despite the importance of fish as part of the aquatic food chains, studies of the antioxidants, including vitamin E and vitamin C, in fish are scarce. The aim of this study was to determine and compare the content of vitamins C and E in muscle from elasmobranch and teleost fishes. Three species of each group (elasmobranch and teleost) were selected based on their ecological relevance.

2. Materials and methods

2.1. Sample collection

White muscle samples (approximately 5 g) were collected from the caudal area of six different elasmobranch and teleost fish species. The sampled elasmobranch species include *Mustelus henlei* (Carcharhiniformes, Triakidae) a species that lives mainly in the intertidal zone and continental shelf where it feeds mostly on crustaceans; *Prionace glauca* (Carcharhiniformes, Carcharhinidae), an oceanic and pelagic shark, which feeds on squid and other smaller pelagic fishes, and *Isurus oxyrinchus* (Lamniformes, Lamnidae), a coastal and oceanic shark, feeding primarily on other fishes and squid. Sampled teleosts included *Totoaba macdonaldi* (Perciformes, Sciaenidae), which primarily inhabits coastal waters and rocky bottoms and feeds mainly on crustaceans; *Coryphaena hippurus* (Perciformes, Coryphaenidae) is an oceanic pelagic species, whose feeding habits are based on crustaceans, squid and other fish, and *Kajikia audax* (Perciformes, Istiophoridae), an oceanic and epipelagic species that feeds on fish, crustaceans and cephalopods (Fischer et al., 1995; Compagno, 2002). Data of the sampled species are summarized in Table 1. Samples were collected at fishing camps (San Lazaro, Punta Lobos), in sportfishing docks (Cabo San Lucas) and during scientific sampling trips (Ensenada de Muertos, El Sargento, Upper Gulf of California) from April to December 2011. All samples were stored in the dark and frozen at −80°C, for a period not exceeding six months from the time samples were collected, until analyzed.

2.2. Vitamin C concentration

Concentrations of vitamin C were quantified by high performance liquid chromatography (HPLC) (Waters Model 2695; Milford, MA, USA) following the methods of Carvajal et al. (1997), Ledezma-Gairard (2004) and Agilent Technologies Company (2001). The extraction of water-soluble compounds was carried out using a solution of metaphosphoric acid (3%), acetic acid (8%), and EDTA (0.01 M). Following homogenization (Polytron 3100, Switzerland), samples were centrifuged at 23895 *g* at 4°C for 10 min. The supernatant was filtered using a cellulose membrane (0.45 μm). The extract was separated in a Hypersil BDS C8 column (5 μm, 250 mm in length, 4.6 mm internal diameter; Bellefonte, PA, USA) by a gradient separation system with two mobile phases, deionized water (pH 2.4) and acetonitrile (100%). Vitamin C was detected at 245 nm wavelength with a retention time of 6.7 min (PAD UV detector; Waters). Vitamin C content in samples was derived from a calibration curve of L-ascorbic acid (1, 5, 10, 25, 50, 100, 150 and 200 ng μL^{-1}). When the concentrations were below the minimum detection limit (MDL, 0.01 ng μL^{-1}) a manual integration based on the retention time and absorption spectrum of vitamin C was performed. Results are expressed as $\mu g\ g^{-1}$ of wet tissue.

2.3. Vitamin E concentration

Concentrations of vitamin E in fish muscle samples were quantified by HPLC (Waters) according to the methods of Alava et al. (1993) and Supelco (2003/2004). Samples were homogenized with anhydrous sodium sulfate (0.5 g), hexane, ethanol and distilled water (7:3:2 v/v). Subsequently, butylated hydroxytoluene (BHT, 504 mM) was added, and the sample was mixed and centrifuged at 944 *g* at 10 °C for 10 min. The supernatant was recovered and evaporated between 35 and 45°C. The precipitate was again subjected to extraction by adding hexane (98.5%), centrifuged and the recovered supernatant was mixed with the first extraction product. The extracted mixture was completely evaporated with nitrogen gas. Each concentrate was reconstituted in ethanol, pyrogallol (1%) and potassium hydroxide (50%). Tubes were placed at 65°C for 25 min. Tubes were cooled in a mixture of BHT (504 mM), hexane and distilled water, shaken, and the hexane phase was recovered; this step was repeated twice. The mixture was filtered through cellulose acetate filters (0.45 μm) and completely evaporated with nitrogen gas. Finally, the lipidic extract was diluted in acetonitrile (100%) and separated in a Supelcosil LC18 column (3 μm, 150 mm in length, 4.6 mm internal diameter) by a gradient separation system with mobile phase consisting of acetonitrile:water (9:1 v/v). Vitamin E was detected at 285 nm wavelength with a retention time of 13 min (Waters PAD detector UV). Vitamin E concentration in samples was derived from a calibration curve of DL-α-tocopherol acetate (1, 6.25, 12.5, 25, 50 and 100 ng μL^{-1}). When the concentrations were below the MDL (0.001 ng μL^{-1}), a manual integration based on the retention time and absorption spectrum of vitamin E was performed. Results are expressed as $\mu g\ g^{-1}$ of wet tissue.

2.4. Statistical analyses

Because the data did not meet the criteria for normality and homoscedasticity, non-parametric statistical analyses were performed. In two species, vitamin C concentration was found to be below the detection limit precluding statistical analyses within teleosts. In samples where vitamin concentrations were below the MDL the methodology of simple value replacement reported by Helsel (1990) was applied in order to run the statistical analyses. Kruskal–Wallis and Mann–Whitney tests were applied to probe for significant differences in the concentrations