Marine Environmental Research 121 (2016) 40–48

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

PCB and OCP accumulation and evidence of hepatic alteration in the Atlantic bluefin tuna, *T. thynnus*, from the Mediterranean Sea

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ARTICLE INFO

Article history:
Received 2 October 2015
Received in revised form
7 March 2016
Accepted 13 March 2016
Available online 17 March 2016

Keywords:
Fish
Pelagic
Thunnus thynnus
POPs
Biomagnification
Liver
Lipid metabolism

ABSTRACT

Persistent organic pollutants (POPs) are known to act as "obesogens", being fat-soluble and affecting lipid metabolism. The Atlantic bluefin tuna, *Thunnus thynnus*, are top pelagic predators prone to bioaccumulate and biomagnify environmental contaminants. This study aimed at evaluating POPs-induced ectopic lipid accumulation in liver of adult tuna from the Mediterranean Sea. PCBs and organochlorine pesticides were measured in tuna liver, and marked morphological changes observed, namely poorly compacted tissues, intense vacuolization, erythrocyte infiltration and presence of melanomacrophages. The expression of perilipin, a lipid-droplet marker, positively correlated with the gene expression of PPARγ, a master regulator of adipogenesis, and its heterodimeric partner, RXRα. Changes in metabolites involved in fatty acid biosynthesis and ketogenesis were also observed. Although male bluefin tuna appeared to be more sensitive than females to the adverse effects of environmental obesogens, the alterations observed in tuna liver of both sexes suggest a potential onset of hepatic steatosis.

1. Introduction

Environmental contamination by persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), has been worldwide of great concern because of their persistence and toxicity to humans and wildlife (Corsolini et al., 2005). PCBs, a group of halogenated aromatic hydrocarbons consisting of 209 congeners (Hutzinger et al., 1974), were widely used from the 1930s as dielectric and hydraulic fluids in transformers and capacitors, and as plasticizers in paint and rubber sealants (Abrha and Raghavan, 2000), while OCPs were massively used in agricultural production and for sanitation purposes. With the aim to protect human health and the environment from the negative effects of these chemicals, the Stockholm Convention (United Nations Environment Programme, UNEP, 2009) was adopted as an international action deemed necessary to reduce or eliminate the production, use and releases of POPs. Although PCBs and OCPs were banned in the 1970s, considerable amounts of these persistent compounds continue to cycle the ecosphere (Minh et al., 2000). In combination with their persistence, the lipophilicity of PCBs and OCPs contributes to their high bioaccumulation potential and trophic transfer along the food chain. As a matter of fact, organisms occupying a top trophic position in a food web accumulate the highest concentrations of these lipophilic contaminants, and can become more vulnerable to their toxic effects (Storelli et al., 2008; Ueno et al., 2002). Recent studies have reported that PCBs and OCPs have a possible obesogenic effect, affecting both carbohydrate and lipid metabolism, which in turn leads to metabolic disease and non alcoholic fatty liver disease (Boucher et al., 2015; Dirinck et al., 2011; Gadupudi et al., 2015; Shi et al., 2012; Wahlang et al., 2013).

The Atlantic bluefin tuna, *Thunnus thynnus* (Linnaeus, 1758), is an important ecological and commercial species within the Atlantic and Mediterranean ecosystems (Whitehead et al., 1984). This

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http://dx.doi.org/10.1016/j.marenvres.2016.03.003
0141-1136/

species is worldwide considered one of the most highly valuable fishery resources, particularly for the Japanese market, and its catch and trade support an important production chain. The elevated fishing pressure on this resource has determined a decrease of eastern Atlantic and Mediterranean bluefin tuna stock during the last century (Sumaila and Huang, 2012). Therefore, in order to manage and provide recommendations on the stock status of the bluefin tuna, the International Commission for the Conservation of Atlantic Tunas (ICCAT) was established in 1996, and sustainable management actions for the conservation of this species have been launched (EC Regulation 302/2009; EU Regulation 500/2012). Moreover, bluefin tuna has been listed in the International Union for Conservation of Nature (IUCN) Red List (A2bd ver 3.1, 2011) as "endangered species".

The conservation of bluefin tuna stock has important ecological consequences because this species assume a significant role in the pelagic trophic web. Indeed, as several top predators, it plays a role in the control of the ecosystem balance and prey biomass by a constant predation, assuring a positive control on biodiversity (Battaglia et al., 2013a, 2013b; Karakulak et al., 2009; Romeo et al., 2011; Sarà and Sarà, 2007). Furthermore, due to its continuous migratory behaviour and long lifespan (Rooker et al., 2007; Santamaria et al., 2009), bluefin tuna is especially prone to bioaccumulate and biomagnify environmental toxic compounds, in particular the lipophilic POPs (Corsolini et al., 2007; Di Bella et al., 2006; Sprague et al., 2012; Storelli et al., 2008).

Fish consumption is a source of human exposure to environmental contaminants including POPs (Corsolini et al., 2005; Sidhu, 2003). Hence, the present study aimed to evaluate the potential toxicity of PCBs and OCPs in liver of adult individuals of *T. thynnus* caught from the central Mediterranean Sea. To this end, the levels of individual PCB congeners and OCPs were determined in the Atlantic bluefin tuna livers. Histomorphological observations of liver tissue were performed in order to highlight the presence of lipid droplets, whose fat composition was ascertained by immunohistochemical localization of perilipin, a lipid-droplet marker protein involved in packaging of fat droplets and control of lipolysis (Tansey et al., 2004). The induction of adipogenesis was evaluated by investigating on the gene expression of the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ), a master regulator of adipogenesis and fatty acid metabolism (Lehrke and Lazar, 2005), and retinoid-X-receptor-α (RXRα), which is a heterodimeric partner for PPARγ (Nielsen et al., 2008). Furthermore, an environmental metabolomics approach, which has been demonstrated to be an effective tool for studying organism–environment interactions and for evaluating the health status of organisms at the molecular level (Brandão et al., 2015; Cappello et al., 2013, 2015, 2016; Fasulo et al., 2012; Viant et al., 2003), was applied in order to elucidate changes on tuna liver in metabolites involved in energy metabolism. Overall, this study aimed at obtaining information useful for the assessment of fish well-being as well as of possible risks for human health.

2. Materials and methods

2.1. Sample collection

Adult Atlantic bluefin tuna, *T. thynnus*, were caught in the Strait of Messina, central Mediterranean Sea (Fig. 1), during October–November 2011, by small crafts using hand lines and hooks (Di Natale et al., 2005). At landings, the gilled and gutted weight (GGWT range: 30–55 kg) of twenty randomly selected individuals was recorded, and sex was determined by macroscopic observation of gonads. Liver samples of individual fish were removed and frozen at −18 °C until processed for chemical analysis, or flash-frozen in liquid nitrogen and stored at −80 °C until processed for molecular and metabolomics analyses. Furthermore, small pieces of each dissected tissue were taken for histological and immunohistochemical analyses.

2.2. Determination of PCBs and OCPs

2.2.1. Fat extraction and clean-up

Tuna liver tissue was homogenate before the extraction according to Kalachova et al. (2011). Subsequently, a mixture of distilled water and ethyl acetate with QuEChERS (4:1 magnesium sulfate and sodium chloride) was added to sample in a polypropylene tube. It was shaken vigorously and centrifuged at 11,000 rpm for 5 min. Then, the upper organic phase and the solvent were evaporated using a vacuum rotary evaporator, and fat concentration was recovered and clean-up with a silica glass column. The glass column, packed with glass wool and silica gel, was conditioned with *n*-hexane:dichloromethane (3:1). The elution was performed with 30 mL of a mixture of *n*-hexane:dichloromethane (3:1) and finally, the solvent collected was evaporated using a vacuum rotary evaporator and re-dissolved in 1 mL of *n*-hexane containing 50 ng/mL of bromophos-methyl. Each sample was injected into a Shimadzu gas chromatograph mass spectrometer (TQ8030 HRGC-MS/MS Shimadzu) and analysed with Selected Reaction Monitoring (SRM) method.

2.2.2. Gas chromatography–mass spectrometry (HRGC-MS/MS)

Confirmation of residues was performed by HRGC-MS/MS using a Shimadzu TQ8030 equipped with a ZB-5MS (5% biphenyl, 95% methyl polysiloxane) (30 m × 0.25 mm; 0.25 μm film thickness) capillary column. The pressure at the head of the column was 29.2 KpA. Helium was used as a carrier gas at a rate of 30 cm/s and a flow of 0.68 mL/min. The injector temperature was maintained at 250 °C, in Splitless mode for 1 min. The temperature of interface was 300 °C. The column oven was temperature programmed from an initial value of 60 °C for 1 min to 150 °C at a rate of 15 °C/min, to 270 °C at a rate of 10 °C/min, and then to 300 °C (2 min hold) at a rate of 2 °C/min. The electronic impact (EI) source was 70 eV, the acquisition of spectra was performed in Multiple Reaction Monitoring analysis (MRM) using argon as collision gas at the pressure of 200 kPa. The accuracy and repeatability of the method were assessed by performing a spike-and-recovery test on certified standards (Supelco). The recoveries (%) and repeatabilities of the measurement were calculated from three replicate analyses of 'blank' tuna in which a mixture of target analytes were added at the concentration of 50 ng/g. Recovery values for OCPs and PCBs were around 90%. Also the limits of detection (LOD) and quantification (LOQ) were calculated using a signal-to-noise ratio equal to 3.3 and 10, respectively (EURACHEM guide 2000).

2.3. Histological analysis

For histological assessment, liver tissues were preserved in 4% paraformaldehyde in 0.1 M phosphate buffered solution (PBS, pH 7.4) at 4 °C. After dehydration in a graded series of ethanol baths, tissues were embedded in paraffin (Bio-Optica, Italy). Histological sections (4 μm thick) were cut with a rotary automatic microtome (Leica Microsystems, Wetzlar, Germany), and stained with haematoxylin/eosin (Bio-Optica, Italy) to evaluate morphological features.

2.4. Immunohistochemical analysis

For the immunohistochemical assessment, liver histological sections were treated using an indirect immunofluorescence