MOLECULAR CHARACTERIZATION AND EXPRESSION PATTERNS OF ATLANTIC BLUEFIN TUNA (*TUNNUS THYNNUS*) LEPTIN DURING THE REPRODUCTIVE SEASON

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Introduction:

The Atlantic bluefin tuna (BFT, *Thunnus thynnus*) is a highly prized fish comprising one of the world's most valuable commercial fisheries. Recently, with the intention of reducing fishing pressure on wild stocks, successful BFT breeding protocols were established, paving the way towards a self-sustained industry [1]. To further understand the endocrine mechanisms regulating reproduction in BFT, which is notable among fishes for its pseudo- endothermic metabolism and for its capacity for rapid lipid mobilization, the current study was focused on the molecular characterization of leptin. In mammals the latter hormone appears to transmit signals of the energy stores to the central nervous and endocrine systems allowing the onset of energy demanding situations such as puberty and reproduction [3, 4]. In order to look for parallels to these roles of leptin in BFT, our specific goals were to clone the cDNA encoding for BFT leptin (*bft*Lep), and follow the temporal expression profiles of the related gene during BFT natural spawning season within the Mediterranean Sea.

Methods:

Experimental fish and sampling procedures - Mature BFT captured at the Balearic Islands and held in floating cages (25-m diameter, 20-m deep) by the coast of Murcia, Spain (Tuna Graso S.A.), were sampled at three characteristic stages (April, n=15; May, n=12; July, n=12; 2008) during the natural spawning season within the Mediterranean Sea. Morphometric parameters were recorded and organ (i.e. gonad, liver, fat and viscera) indices (GSI, HIS, FSI and VSI, respectively) were calculated for individual fish. In addition, pituitary, brain, gonad and liver tissues were collected for gene expression analyses.

Cloning and gene expression analyses - The cDNA encoding for the BFT leptin (*bft*Lep) was cloned using the 5`-and 3`-RACE technique and degenerate primers that were designed according to the most conserved regions of leptin sequences available at the NCBI database. The identity of the isolated amplicon was confirmed as BFT leptin by BLAST (www.ncbi.nlm.nih.gov) and the tertiary structure of the hormone was validated using the I-TASSER server for protein 3D structure prediction (http://zhanglab.ccmb.med.umich.edu/I-TASSER/). The expression levels of *bft*Lep were profiled using real time-PCR quantification and were normalized to 18S as a housekeeping gene.

Results and Discussion:

The full length *bft*LepcDNA was 1126 bp long, encoding a precursor peptide of 160 amino acids including 19 residues of signal peptide. At the amino acid level, the *bft*Lep shows low homology with cognate tetrapod (~16%) and teleost (~26%) proteins, whereas its predicted tertiary structure reveals the four-alpha-helix bundle, characteristic of the class-I helical cytokine family.

The *bft*Lep mRNA was detectable in the liver and to a lesser extent in the hypothalamic, pituitary, fat and spleen tissues. Interestingly, similar temporal patterns were observed in both liver and hypothalamic tissues, with relatively low*bft*Lep transcript levels in fully mature fish that were sampled in May (GSI=1.85 ± 0.2 %), and elevated levels in fish sampled in July with regressed gonads (GSI= 0.62 ± 0.1 %). No sexual dimorphic patterns were observed. The organ indices HIS and FSI, declined concomitant with the progression of the reproductive season reaching minimal values during July, the end of the spawning season. Further regression analysis revealed a significant negative correlation between the HIS values and the hypothalamic*bft*Lep mRNA levels.

Conclusions:

The wide spectrum of tissues expressing the *bft*Lep gene may suggest the hormone's multiple physiological functions in BFT. The mechanisms by which leptin regulates reproductive functions are not yet fully

understood. However, the fact that leptin is being produced in the central nervous system (hypothalamus and pituitary) may suggest autocrine and/or paracrine regulation of the hypothalamic-pituitary-gonadal axis. Our striking findings that reveals a clear association between regressed gonads, depleted energy resources (as indicated by the low HIS and FSI values) and increased transcription of the *bft*Lep gene in the hypothalamic tissue, further attest to this notion. Nevertheless, since expression levels do not necessarily mirror the circulating hormone levels, further studies employing homologous immunoassays, are required to clarify the specific physiological roles of leptin in the control of gonadal function in BFT.

References:

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