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ScienceDirect
Domestic Animal Endocrinology 37 (2009) 74–83
DOMESTIC ANIMAL ENDOCRINOLOGY
www.domesticanimalendo.com

Effects of fasting on IGF-I, IGF-II, and IGF-binding protein mRNA concentrations in channel catfish (*Ictalurus punctatus*)

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Received 27 January 2009; received in revised form 25 March 2009; accepted 26 March 2009

Abstract

The effects of fasting on insulin-like growth factor (IGF)-I, IGF-II, and IGF-binding protein (IGFBPs) mRNA in channel catfish were examined. Fed control fish (Fed) were compared to fish that had been fasted for 30 d followed by 15 d of additional feeding (Restricted). Sequence alignment and similarity to orthologous proteins in other vertebrates provided structural evidence that the 3 catfish sequences identified in the present research were IGFBP-1, -2, and -3. Prolonged fasting (30 d) reduced body weight approximately 60% ($P < 0.001$) and decreased IGF-I mRNA in the liver and muscle ($P < 0.01$). Fifteen days of re-feeding restored concentrations of hepatic and muscle IGF-I mRNA. Liver IGF-II mRNA was not affected by fasting but was increased 2.2-fold after 15 d of re-feeding ($P < 0.05$). Abundance of muscle IGF-II mRNA was similar between the fed control group and the restricted group throughout the experimental period. Fasting also increased liver IGFBP-1 mRNA ($P < 0.05$) and decreased IGFBP-3 mRNA ($P < 0.01$), whereas abundance of IGFBP-2 mRNA was not significantly affected. Interestingly, re-feeding for 15 d did not restore concentrations of IGFBP-1 and IGFBP-3 mRNA relative to fed control concentrations. The IGF results suggest that IGF-I and IGF-II are differently regulated by nutritional status and probably have a differential effect in promoting muscle growth during recovery from fasting. Similar to mammals, IGFBP-1 mRNA in catfish is increased during catabolism, whereas IGFBP-3 mRNA is decreased during inhibited somatic growth. The IGFBP results provide additional evidence of the conserved nature of the IGF-IGFBP-growth axis in catfish.
Published by Elsevier Inc.

Keywords: Fasting; IGF-I; IGF-II; IGF-binding proteins; Catfish

1. Introduction

The growth hormone-insulin-like growth factor (GH-IGF) axis has been implicated in the regulation of somatic growth and nutrient metabolism in both mammals and teleost fishes [1,2]. In fish and mammals, GH is a potent regulator of hepatic IGF-I expression [3,4], and several authors have reported that the IGF-II gene is also regulated by GH, as administration of exogenous GH increases concentrations of IGF-II mRNA [5–8]. The activity of IGFs is regulated not only by GH and other endocrine modulators that enhance or suppress local and systemic IGF concentrations, but also by the presence of IGF binding proteins (IGFBPs). These binding proteins are crucial for modulating the half-lives of IGFs and coordinating and transporting IGFs in circulation [9–14]. In mammals, the IGFBPs include a family of 6 proteins (IGFBP-1 to -6) that bind to IGFs with high affinity and specificity [15]. Insulin-like growth factor binding proteins have been identified in several teleost species [16–22], including channel catfish

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0739-7240/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.domaniend.2009.03.004

[23–25]. Three teleost IGFBPs, ranging in size from 24 to 50 kDa, are commonly reported. The IGFBP family has recently expanded to include the IGFBP-related proteins (IGFBP-rPs), which may also play a role in regulating IGF activity [26]. The IGFs evoke their biological responses through their respective receptors on target tissues, resulting in growth promotion.

It is widely accepted that fasting or reduced feeding decreases concentrations of IGF-I in channel catfish (*Ictalurus punctatus*) and other teleosts [27–33], whereas the effects of nutritional status on IGF-II are less defined. Handling stress, confinement stress, and starvation result in decreased plasma concentrations of IGF-II in Atlantic salmon (*Salmo Salar*) and rainbow trout (*Oncorhynchus mykiss*) [34–35]. In channel catfish, IGF-II mRNA is up-regulated after GH administration and is greater in fast-growing compared to slow-growing families [8,36,37]. However, the effect of reduced feeding or starvation on IGF-II mRNA in channel catfish is not known.

Research with channel catfish has shown that nutritional status is a key regulator of IGFBP-1 and IGF-I in channel catfish. There is no information on how nutritional status governs IGFBP-2, IGFBP-3, or IGF-II mRNA concentrations in channel catfish, and the primary amino acid sequences of catfish IGFBPs are not known. The objectives of this study were to examine the effects of fasting on IGF-I, IGF-II, IGFBP-1, IGFBP-2, and IGFBP-3 mRNA in channel catfish, the primary cultured species in the southeastern United States.

2. Materials and methods

2.1. Research animals

Fish used in this study were a channel catfish strain (NWAC103) maintained by the National Warmwater Aquaculture Center (NWAC) and housed at the USDA-ARS Catfish Genetics Research Unit aquaculture facility in Stoneville, MS. Prior to experimentation, approximately 50 fish from each of 3 different families were placed into a 120-L holding tank. The following day, 100 fish (mean initial size 72.4 ± 2.1 g) were randomly assigned to ten 76-L tanks (10 fish/tank) and allowed to acclimate for 12 days. The fish were fed once per day to apparent satiation using a commercial 36% crude protein floating catfish diet (Farmland Industries, Inc., Kansas City, MO) and reared in 26.7 ± 0.2 °C flow-through well water under a 14:10 L:D photoperiod. Water quality (pH approximately 8.5; dissolved oxygen concentrations > 5.0 mg/L) and flow rates (1.0 L/min) were similar between tanks.

The fish were randomly separated into 2 treatment groups with 5 replicates each. One group served as fed controls (Fed) (commercial catfish diet, fed daily to apparent satiation), whereas the other group of fish was subjected to a 30-d fast followed by 15 additional days of refeeding (Restricted). On day 0 of the experiment, all fish were anesthetized with 0.1 g/L of tricainemethane sulfonate (MS-222; Western Chemical Inc., Ferndale, WA) and weighed. Five fish were sampled from the 120-L holding tanks and served as time 0 controls. On days 30 and 45, all fish were weighed and 15 fish per treatment (3 fish/tank) were euthanized with an overdose of MS-222 (0.3 g/L) for sample collection. Approximately 100 mg of liver and muscle (transverse slice of fast muscle located beneath the dorsal fin) were removed for RNA extraction. No mortalities were observed throughout the study. Studies were conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee, United States Department of Agriculture/Agriculture Research Service Catfish Genetics Research Unit.

2.2. Sample preparation and RNA isolation

After collection, samples were immediately placed in 1 mL TRI-Reagent (Molecular Research Center, Inc., Cincinnati, OH), flash-frozen in liquid nitrogen, and stored at −80 °C until RNA isolation. Total RNA was isolated, quantified by measuring the absorbance at 260 nm using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE), and the integrity of the RNA was verified by visualization of the 18S and 28S ribosomal bands stained with ethidium bromide after electrophoresis on 2.0% agarose gels.

Extracted RNA was treated with DNase I to remove co-extracted DNA using a TURBO DNA-freeTM kit (Ambion, Austin, TX). An aliquot of the extracted RNA (15 μL) was treated at 37 °C for 30 min with 0.1 volume of buffer (1.5 μL) and 2 U (1 μL) of DNase I. The enzyme was inactivated with 2 μL of inactivation reagent at room temperature for 2 min. Samples were centrifuged at $10\,000 \times g$ for 1 min, and the supernatant (16 μL) was transferred to a clean RNase-free microcentrifuge tube and stored at -80 °C before the reverse transcriptase step.

2.3. Identification and quantitation of catfish IGFBP mRNAs

Partial sequences for channel catfish IGFBP-1, -2, and -3 mRNAs were identified by sequence similarity with zebra fish (*Danio rerio*) IGFBPs using BlastX searches of catfish expressed sequence tags (ESTs). The full