General and Comparative Endocrinology 166 (2010) 573–580

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Tissue-specific regulation of the growth hormone/insulin-like growth factor axis during fasting and re-feeding: Importance of muscle expression of IGF-I and IGF-II mRNA in the tilapia

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ARTICLE INFO

Article history:
Received 25 August 2009
Revised 30 October 2009
Accepted 17 November 2009
Available online 20 November 2009

Keywords:
Growth hormone
Somatolactin
Insulin-like growth factor
Fasting
Re-feeding
Tilapia
Oreochromis mossambicus

ABSTRACT

The effects of prolonged nutrient restriction (fasting) and subsequent restoration (re-feeding) on the growth hormone (GH)/insulin-like growth factor (IGF) axis were investigated in the tilapia (*Oreochromis mossambicus*). Mean weight and specific growth rate declined within 1 week in fasted fish, and remained lower than controls throughout 4 weeks of fasting. Plasma levels of IGF-I were lower than fed controls during 4 weeks of fasting, suggesting a significant catabolic state. Following re-feeding, fasted fish gained weight continuously, but did not attain the weight of fed controls at 8 weeks after re-feeding. Specific growth rate increased above the continuously-fed controls during the first 6 weeks of re-feeding, clearly indicating a compensatory response. Plasma IGF-I levels increased after 1 week of re-feeding and levels were not otherwise different from fed controls. Plasma GH levels were unaffected by either fasting or re-feeding. No consistent effect of fasting or re-feeding was observed on liver expression of GH receptor (GH-R), somatolactin (SL) receptor (SL-R), IGF-I or IGF-II. In contrast, muscle expression of GH-R increased markedly during 4 weeks of fasting, and then declined below control levels upon re-feeding for weeks 1 and 2. Similarly, muscle expression of SL-R increased after 4 weeks of fasting, and reduced below control levels after 1 and 2 weeks of re-feeding. On the other hand, muscle expression of IGF-I was strongly reduced throughout the fasting period, and levels recovered 2 weeks after re-feeding. Muscle expression of IGF-II was not affected by fasting, but was reduced after 1 and 2 weeks of re-feeding. These results indicate that GH/IGF axis, particularly muscle expression of GH-R, SL-R and IGF-I and -II, is sensitive to nutritional status in the tilapia.

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1. Introduction

Growth in fish, as in higher vertebrates, is controlled in large part by the growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis in relation to available nutrients (Duan, 1997; Wood et al., 2005). Growth hormone is a member of the GH/prolactin (PRL)/somatolactin (SL) family of pituitary hormones which are involved in regulating numerous physiological processes besides somatic growth including behavior, immune function, lipid and protein metabolism, osmoregulation, and feeding behavior in fishes (Duan, 1997; Albalat et al., 2005; Kawauchi and Sower, 2006). Growth hormone acts directly on target tissue by stimulating mitosis and other aspects of energy metabolism, and indirectly by initiating the production and release of IGF-I in the liver and peripheral tissues of vertebrates, including fishes (Duan, 1997; Wood et al., 2005). These physiological actions of GH result from its binding to the single transmembrane-spanning Class I cytokine growth hormone receptor (GH-R) in target tissues (Pérez-Sánchez et al., 2002). Growth hormone receptors have recently been cloned from several teleost fish species, and display a wide distribution of expression in various tissues (Pérez-Sánchez et al., 2002; Wood et al., 2005; Saera-Vila et al., 2007). As sequences accumulated, the presence of two distinct phylogenetic clades of fish GH-R, GH-R1 and GH-R2, was recognized (Saera-Vila et al., 2005; Jiao et al., 2006; Fukamachi and Meyer, 2007). Fish GH-Rs have been shown to be differentially regulated by fasting (Saera-Vila et al., 2005), temperature (Gabillard et al., 2006b), feed composition (Benedito-Palos et al., 2007), pathogens (Sitjá-Bobadilla et al., 2008), stress (Saera-Vila et al., 2009), as well as by cortisol and testosterone (Jiao et al., 2006), suggesting different physiological roles for these receptors. In Mozambique tilapia (*Oreochromis mossambicus*), we have recently identified GH-R1 as SL-R and GH-R2 as GH-R, thus allowing for investigation into how these receptors are regulated in this important aquaculture species (Pierce et al., 2007).

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0016-6480/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.ygcen.2009.11.012

Fish are able to cope with long periods of nutrient restriction that may occur in nature in part by modulating the expression of a variety of genes associated with the GH/IGF-I system (MacKenzie et al., 1998; Wood et al., 2005; Gabillard et al., 2006a). When food supplies are restricted, energy is diverted away from growth and storage to support essential physiological processes. In many vertebrates, starvation increases plasma GH levels, shifting metabolism towards mobilization of energy substrates (lipolysis) to maintain basal metabolism. Simultaneously, decreased plasma IGF-I, hepatic expression of IGF-I and GH-R are induced in a catabolic state to inhibit the actions of GH and IGF-I (Thissen et al., 1994; Deng et al., 2004; Fukada et al., 2004). In several fish species, prolonged fasting produces a reduction in plasma IGF-I and liver expression of IGF-I mRNA, while plasma GH levels rise or remain unchanged (Pierce et al., 2005; Small and Peterson, 2005; Fox et al., 2006). This apparent paradox of GH resistance has also been observed in mammals (Thissen et al., 1994).

Systemic signaling towards the control of growth and metabolism is vital; however, autocrine/paracrine production of IGF-I in non-hepatic tissues contributes significantly as well (Reinecke et al., 2005). On the other hand, the metabolic roles of IGF-II in fish are virtually unknown. In mammals, the IGF-II gene is expressed chiefly during embryonic development, whereas IGF-II is widely expressed in both juvenile and adult fish (Reinecke et al., 2005). Insulin-like growth factor-II shares a high structural homology to IGF-I, and gene expression of both mitogens appear to be regulated by GH in several tissue types in fish (Vong et al., 2003). Administration of both IGF-I and IGF-II stimulate growth in tilapia *in vivo* (Chen et al., 2000), and both IGFs stimulate mitogenesis in rainbow trout muscle cell culture (Codina et al., 2008). Both IGFs also act through the same receptor, suggesting that they share overlapping physiological roles in fish (Reinecke et al., 2005).

While the relationship between GH and IGF-I during altered nutritional condition has been well-characterized in fish, very little is known about the function of SL. Fukada et al. (2005) observed the highest levels of SL-R mRNA in the liver and fat tissue of salmon, which supports an earlier study suggesting SL's roles in lipid metabolism in sea bream and sea bass (Vega-Rubin de Celis et al., 2003). Nonetheless, inconsistent changes in plasma SL levels during fasting have been observed in salmonids (Company et al., 1999; Pottinger et al., 2003). We have recently cloned SL in the tilapia and observed significant reductions in pituitary SL mRNA levels after fasting in both fresh water and seawater, suggesting a possible role for this hormone in energy metabolism (Uchida et al., 2009).

Upon re-feeding, animals have been shown to gain weight at an accelerated rate; this phenomenon, termed compensatory growth, has been observed in numerous vertebrate species including fish (Ali et al., 2003). While previous experiments have examined compensatory growth in the tilapia (Wang et al., 2005), endocrine function during re-feeding has not yet been addressed. In the current study, we examined effects of fasting and subsequent re-feeding on the coordinate regulation of the GH/IGF-I axis in the tilapia. In an effort to better understand the roles of these parameters during growth suppression and recovery, we followed the time course of changes in plasma levels of GH and IGF-I concomitantly with variations in gene expression of IGF-I, IGF-II, GH-R, and SL-R in muscle and liver.

2. Materials and methods

2.1. Animals

Male tilapia (*O. mossambicus*), were reared outdoors in freshwater flow-through tanks under natural photoperiod at the Hawaii Institute of Marine Biology. Animals were fed approximately 5% of their body weight per day by belt feeder (12-h belt feeder, Aquatic Ecosystems Inc., Apopka, FL) with Silver Cup Trout Chow (Nelson and Sons, Murray, UT). All experiments were conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee, University of Hawaii.

2.2. Fasting and re-feeding

One hundred and twenty sexually-mature fish, weighing approximately 38 g, were tagged individually with passive integrated transponder tags (PIT tags; Biomark, Boise, ID) and divided at random into four tanks representing two treatment groups (two fed and two fasted/re-fed). Four separate flow-through tanks were used. The animals were then maintained in outdoor oval 700 l fiberglass aquaria (30 fish in each tank). Water temperature was maintained between 27 and 28 °C using submersible aquarium heaters (Ebo-Jager Inc., El Segundo, CA). The fish were allowed to acclimate to the tanks for 2 weeks prior to the beginning of the experiment. Following the acclimation period, food was withheld from two tanks for a period of 4 weeks, while the remaining two tanks were fed continuously throughout the experiment by automatic belt feeder. After the 4 weeks, fasted animals were fed continuously for the remaining 8 weeks. The tanks were siphoned daily, and regularly cleaned to remove algae. Twelve fish, six fed and six fasted/re-fed (three fish from each of the four tanks), were terminally sampled in buckets containing 2-phenoxyethanol (0.2 ml/l) at 8 time points; weeks -4, -3, -2, 0, 1, 2, 4, and 8. Week -4 represents the beginning of the fasting period, while week 0 represents the beginning of the re-feeding period, and week 8 represents the termination of the experiment. To minimize the effect of crowding on growth rate, stocking densities were maintained at approximately 5 g/l by adjusting the height of the vertical standpipe as fish were removed for sampling.

2.3. Sampling

Body weight and standard length were measured at each sampling time point from the beginning of the experiment. Condition factor, (body weight, g)/(standard length, cm)^3 × 100, and specific growth rate (ln W_f–ln W_i)/t × 100, where W_f is the final weight (g), W_i is the initial weight (g) at each time interval and t is growth time (days), were calculated at each time point. At the time of sampling, all fish were netted, anesthetized, and blood was collected from the caudal vasculature by a needle and syringe treated with ammonium heparin (200 U/ml, Sigma–Aldrich, St. Louis, MO). Plasma was separated by centrifugation at 1000*g* for 10 min at 4 °C, and stored at −20 °C until analyses for GH and IGF-I. Fish were rapidly decapitated and approximately 100 mg of liver and muscle tissues were collected, snap frozen and stored at −80 °C until analyses for mRNA expression of IGF-I, IGF-II, GH-R, and SL-R.

2.4. Plasma measurements

Plasma GH levels were measured by homologous radioimmunoassay according to Yada et al. (1994). Plasma IGF-I levels were measured from 25 μl of plasma that was extracted with 100 μl of acid–ethanol as described by Shimizu et al. (1999). Total IGF-I levels were measured by heterologous RIA using recombinant salmon IGF-I as the standard and anti-barramundi IGF-I (GroPep, Adelaide, Australia) following Shimizu et al. (1999).

2.5. RNA extraction, cDNA Synthesis, and qPCR

Liver, and muscle mRNA levels were determined by quantitative real-time RT-PCR (qPCR). Total RNA was extracted from individual