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Nutritional status and growth hormone regulate insulin-like growth factor binding protein (*igfbp*) transcripts in Mozambique tilapia

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Abstract

Growth in teleosts is controlled in large part by the activities of the growth hormone (Gh)/insulin-like growth factor (Igf) system. In this study, we initially identified *igf-binding protein (bp)1b, -2b, -4, -5a* and *-6b* transcripts in a tilapia EST library. In Mozambique tilapia (*Oreochromis mossambicus*), tissue expression profiling of *igfbps* revealed that *igfbp1b* and *-2b* had the highest levels of expression in liver while *igfbp4, -5a* and *-6b* were expressed at comparable levels in most other tissues. We compared changes in hepatic *igfbp1b, -2b* and *-5a* expression during catabolic conditions (28 days of fasting) along with key components of the Gh/Igf system, including plasma Gh and Igf1 and hepatic *gh receptor* (*ghr2*), *igf1* and *igf2* expression. In parallel with elevated plasma Gh and decreased Igf1 levels, we found that hepatic *igfbp1b* increased substantially in fasted animals. We then tested whether systemic Gh could direct the expression of *igfbps* in liver. A single intraperitoneal injection of ovine Gh into hypophysectomized tilapia specifically stimulated liver *igfbp2b* expression along with plasma Igf1 and hepatic *ghr2* levels. Our collective data suggest that hepatic endocrine signaling during fasting may involve post-translational regulation of plasma Igf1 via a shift towards the expression of *igfbp1b*. Thus, Igfbp1b may operate as a molecular switch to restrict Igf1 signaling in tilapia; furthermore, we provide new details regarding isoform-specific regulation of *igfbp* expression by Gh.

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Keywords

Growth hormone; Insulin-like growth factor; Binding proteins; Receptor; 56 Tilapia; Fasting

1. Introduction

Growth in vertebrates is largely controlled by the coordinated activities of the growth hormone (Gh)/insulin-like growth factor (Igf) system. In teleost fishes, the regulation of growth performance by the Gh/Igf system also seems to be highly conserved (Duan, 1997; Wood et al., 2005). Growth performance is often used as an indicator of the status of individuals and populations in culture and the wild, and therefore, major effort has been applied towards garnering a more comprehensive understanding of how multiple components of the Gh/Igf system interact to control growth and metabolism (Picha et al., 2008; Beckman, 2011).

Gh is a member of the Gh/prolactin/somatolactin family of pituitary hormones that regulate numerous physiological processes that include somatic growth, immune function, osmoregulation, lipid and protein metabolism and feeding behavior (Duan, 1997; Kawauchi and Sower, 2006). The physiological actions of Gh on target tissues are mediated via transmembrane receptors that activate the Jak/Stat signaling pathway Bole-Feysot et al., 1998). Genes encoding Gh receptors (*ghrs*) have been cloned from several teleost species and display a wide distribution of expression across tissues in accord with the pleiotropic actions of Gh (Reindl and Sheridan, 2012). We have identified two *ghr* sequences in Mozambique tilapia (*Oreochromis mossambicus*), denoted *ghr1* and *ghr2* (Kajimura et al., 2004; Pierce et al., 2007). Phylogenetic analyses, tissue expression patterns, and regulation by Gh of these two *ghrs* suggest that *ghr2* encodes the primary Gh receptor (Kajimura et al., 2004; Pierce et al., 2007; Pierce et al., 2012). Further evidence of divergent physiological roles for these two receptors comes from observations that *ghr1* and *ghr2* transcript expression is differentially responsive to fasting (Saera-Vila et al., 2005; Uchida et al., 2009; Fox et al., 2010), temperature (Gabillard et al., 2006), stressors (Saera-Vila et al., 2009), metabolic hormones (Reindl et al., 2009; Pierce et al., 2012), xenobiotics (Davis et al., 2009), and salinity (Pierce et al., 2007; Breves et al., 2011). Dynamic *ghr* expression within key metabolic tissues following alterations in nutritional, osmoregulatory and endocrine states occurs across teleosts (Reinecke, 2010; Reindl and Sheridan, 2012). Thus, the capacity to modulate *ghr* expression appears to serve as a fundamental mechanism to regulate the sensitivity of target tissues to Gh.

In liver and muscle, Gh can act directly by stimulating mitosis and differentiation among other cellular behaviors, and acts indirectly by initiating the production and release of Igfs (Wood et al., 2005; Duan et al., 2010). In mammals, Igf1 is regarded as the primary somatomedin during postnatal life. Igf2 exhibits minimal dependence upon endocrine Gh and its actions have been largely associated with fetal growth and development (Daughaday and Rotwein, 1989; Constancia et al., 2002). In a subset of teleosts, however, growing evidence supports the operation of Igf2, in addition to Igf1, as a somatomedin throughout the life cycle. For example, hepatic *igf2* expression and plasma Igf2 levels are stimulated by

Gh both *in vivo* and *in vitro* (reviewed by Reindl and Sheridan, 2012) and Igf2 administration stimulates growth in juvenile tilapia (Chen et al., 2000). These findings underscore the importance of considering both Igfs when characterizing the actions of Gh in teleosts.

Igf1 and Igf2 interact with a family of binding proteins, termed Igf binding proteins (Igfbps); the specific character of these interactions determines how the biological actions of Igfs are expressed because Igfbps affect hormone availability, transport and receptor binding (Duan et al., 2010). As in mammals, six Igfbps have been identified in fishes (Daza et al., 2011). Although the mechanisms of action are poorly understood, there is good evidence that Igfbps also exhibit ligand-independent activities (Firth and Baxter, 2002). Gh is an important regulator of *igfbp* expression and protein secretion in mammals (Yamada and Lee, 2009). This link in fishes, however, has only been investigated in a restricted number of salmonid species (Cheng et al., 2002; Pierce et al., 2006), with few studies aimed at characterizing the effects of Gh on *igfbp* expression *in vivo*.

We have previously characterized the responses of the Gh/Igf system to changes in metabolic status in Mozambique tilapia with particular attention to plasma Gh and Igf1 levels and the expression of hepatic *ghr2*, *igf1* and *igf2* transcripts (Uchida et al., 2003; Fox et al., 2006; Pierce et al., 2007; Peddu et al., 2009; Fox et al., 2010). In turn, the Mozambique tilapia is positioned as a key *in vivo* model from which to advance our understanding of how the Gh/Igf system, via the activities of Igfbps, responds to nutritional status. In this study, we identify *igfbp1b*, *-2b* and *-5a* as highly expressed hepatic transcripts and assess their regulation by nutrient restriction and Gh, and therefore contribute new details on the physiology of Igfbps in a widely cultured teleost.

2. Materials and methods

2.1. Animals

Male Mozambique tilapia (*O. mossambicus*) were maintained in re-circulating fresh water (FW) under artificial photoperiod (14 h light, 10 h dark) at the Department of Biological Sciences (University of Arkansas, Fayetteville, AK). Fish were fed Aquamax Starter Fingerling 300 (PMI Nutrition International, Brentwood, MO) and water temperatures were maintained between 20 and 22 °C. The Institutional Animal Care and Use Committee of the University of Arkansas approved all housing and experimental protocols.

2.2 igfbp sequences

Sequences for *igfbp1b* (Acc. No. XM_003438121), *igfbp2b* (Acc. No. XM_005450484), *igfbp4* (Acc. No. XM_003454633), *igfbp5a* (Acc. No. XM_003443250.2) and *igfbp6b* (Acc. No. XM_003441337) were identified in the Nile tilapia (*O. niloticus*) transcriptome (Lee et al., 2010) by searching with tBLASTn at the NCBI web resource using the relevant sequences known from rainbow trout (*Oncorhynchus mykiss)* (Kamangar et al., 2006). The sequence similarities of rainbow trout and Nile tilapia *igfbp1b*, *-2b*, *-4*, *-5a* and *-6b* were 79, 75, 76, 70 and 84%, respectively. Nucleotide similarities between Nile and Mozambique tilapia *igfbp* sequences were ≥98%.

2.3. Fasting experiment

Tilapia (40–60 g) were distributed into 6 tanks representing two treatment groups (3 fed and 3 fasted). Fish were allowed to acclimate to the experimental tanks for 4 weeks prior to the beginning of the experiment. Following this initial acclimation period, food was withheld from 3 tanks while the animals contained in the other 3 tanks were fed at ~5% of their body weight twice daily.

Body weight and standard length were measured at each sampling point for calculation of condition factor: (body weight, g)/(standard length, cm)^3 × 100. At sampling, all fish were anesthetized in 2-phenoxyethanol (2-PE; 0.3 ml/l; Sigma, St. Louis, MO) and blood was collected from the caudal vasculature by a needle and syringe treated with ammonium heparin (200 U/ml, Sigma). Plasma was separated by centrifugation at 4 °C and stored at −80 °C until analyses for plasma glucose and Igf1. Liver tissue was collected, snap frozen in liquid nitrogen, and stored at −80 °C until RNA isolation.

2.4. Hypophysectomy and Gh injection

A Gh injection experiment was conducted at the Hawaii Institute of Marine Biology, University of Hawaii. Tilapia (70–150 g) were reared in outdoor tanks with a continuous flow of FW under natural photoperiod at 24–26 °C and fed a commercial diet *ad libitum* (Skretting, Tooele, UT). Hypophysectomy was performed by the transorbital technique (Nishioka, 1994). Briefly, tilapia were anesthetized by immersion in buffered tricaine methanesulfonate (100 mg/l, Argent Chemical Laboratories, Redmond, WA) and 2-PE (0.3 ml/l) in FW. Following removal of the right eye and underlying tissue, a hole was drilled through the neurocranium, and the pituitary was aspirated with a modified Pasteur pipette. The orbit was then packed with microfibrillar collagen hemostat (Ethicon, Somerville, NJ) and fish were allowed to recover in brackish water (12 ‰) composed of seawater (Kaneohe Bay, Hawaii) diluted with FW. Following recovery, fish were transferred to re-circulating experimental aquaria containing aerated brackish water and treated with kanamycin sulfate (National Fish Pharmaceuticals, Tucson, AZ). Upon transfer to experimental aquaria, food was withheld in all experiments to control for the possibility of confounding effects due to disparate feeding patterns between individuals. Water temperatures were maintained between 24 and 26 °C.

To test the effects of Gh on the Gh/Igf system, hypophysectomized fish maintained in brackish water for 3–4 days following surgery (n = 6–9) were administered ovine Gh (oGh; 5 μg/g body weight) or saline vehicle by a single intraperitoneal (IP) injection. oGh was obtained from the National Hormone and Peptide Program (NIDDK-oGH-15) and delivered in saline vehicle (0.9% NaCl; 1.0 μl/g body weight). Fish were lightly anaesthetized with 2-PE prior to injection. After injection, fish were returned to aquaria and left undisturbed for 12 h, after which time plasma and liver samples were collected as described above. At the time of sampling, completeness of hypophysectomy was confirmed by post-mortem inspection of the hypothalamic region. The Institutional Animal Care and Use Committee of the University of Hawaii approved all housing, surgical and experimental protocols.

2.5. Plasma measurements

Plasma glucose concentrations were assayed by the hexokinase method using a commercially available kit (Glucose Assay Kit, GAHK-20, Sigma) modified for a microplate reader (SpectraCount, Packard, Meriden, CT). Plasma Gh was measured by homologous radioimmunoassay as described by Yada et al. (1994). Total plasma Igf1 levels were measured in 25 μl of plasma extracted with 100 μl of acid-ethanol (87.5% ethanol and 12.5% 2 N HCl v/v) by heterologous radioimmunoassay using recombinant salmon Igf1 as the standard and anti-barramundi Igf1 (GroPep, Adelaide, Australia) (Shimizu et al., 1999; Kajimura et al., 2002).

2.6. RNA extraction, cDNA synthesis and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from tissue by the TRI Reagent procedure (MRC, Cincinnati, OH) according to the manufacturer's protocols. RNA concentration and purity were assessed by spectrophotometric absorbance (Nanodrop 1000, Thermo Scientific, Wilmington, DE). First strand cDNA was synthesized with a High Capacity cDNA Reverse Transcription Kit (Life Technologies, Carlsbad, CA). Relative mRNA expression was determined by qRT-PCR using the StepOnePlus real-time PCR system (Life Technologies, Carlsbad, CA). Primer pairs for *ghr1, ghr2, igf1, igf2* and *ef1*αare previously described (Pierce et al., 2007; Breves et al., 2011; Pierce et al., 2011; Pierce et al., 2012). Additional primer pairs used were (5'-3'): *igfbp1b* (forward, CCTTCCCTTTGATCACCAAG; reverse, GTGTGACATGGACCCTGTTG; amplicon size = 102 nt), *igfbp2b* (forward, CCGACTTCCCTTTACAGCAG; reverse, TCAGTCCCATGCACCTCATA; amplicon size = 112 nt), *igfbp4* (forward, ATCCCCATACCCAACTGTGA; reverse, TGATCCACACACCAGCATTT; amplicon size = 98 nt), *igfbp5a* (forward, AACTGGACGGGATCATTCAG; reverse, GCACTGTTTGCGTTTGAAGA; amplicon size = 107 nt), and *igfbp6b* (forward, TCCTACCTGCAGAGGAAAGC; reverse, CGCAGCTCAGAGTGTAGACG; amplicon size = 104 nt).

The qRT-PCR reactions were setup as previously described (Pierce et al., 2007). Briefly, 200 nM of each primer, 3 μl cDNA and 12 μl of SYBR Green PCR Master Mix (Life Technologies) were added to a 15 μl final reaction volume. The following cycling parameters were employed: 2 min at 50 °C, 10 min at 95 °C followed by 40 cycles at 95 °C for 15 sec and 60 °C for 1 min. After verification that expression did not vary across treatments, *ef1*αexpression levels were used to normalize target genes. Reference and target genes were calculated by the relative quantification method with PCR efficiency correction (Pfaffl, 2001). Standard curves were prepared from serial dilutions of untreated liver cDNA and included on each plate to calculate the PCR efficiencies for target and normalization genes. Relative gene expression ratios between groups are reported as a fold-change from controls.

2.7. Statistics

Tissue expression profiles were analyzed by one-way ANOVA followed by Tukey's HSD. The fasting experiment was analyzed by two-way ANOVA. When the interaction between main effects (treatment and time) was significant, Dunn's test was employed at each time point. Otherwise, significant main effects are indicated in the figure. Group comparisons in

the Gh injection experiment were analyzed by Student's t-test. When necessary, data were transformed to satisfy homogeneity of variance requirements. If transformation was not sufficient, data were analyzed with a nonparametric Kruskal-Wallis analysis. Significance for all tests was set at $P < 0.05$ and asterisks in the figures indicate significance levels: *$P <$ 0.05, **$P < 0.01$ and ***$P < 0.001$. All tests were performed using GraphPad Prism 5.0 (San Diego, CA).

3. Results

3.1. Tissue distribution

The distribution of *igfbp1b, -2b, -4, -5a* and *-6b* expression was analyzed in tissues of fed, FW-acclimated, tilapia. *igfbp1b* and *-2b* were expressed at greater than 4- and 3-orders of magnitude higher, respectively, in liver than other examined tissues (Fig. 1A,B). *igfbp4* expression was highest in kidney, brain and liver, with comparable expression in other tissues (Fig. 1C). *igfbp5a* exhibited the highest expression levels in kidney, muscle and liver with low levels found elsewhere (Fig. 1D). *igfbp6b* expression was highest in intestine, with low levels in other examined tissues (Fig. 1E).

3.2. Effects of fasting on the Gh/Igf system

We described responses of the Gh/Igf system to catabolic conditions by fasting tilapia for 7, 14 and 28 days and assaying systemic hormone levels and hepatic gene expression. There was a significant main effect of fasting on standard length (Fig. 2A), body weight (Fig. 2B), condition factor (Fig. 2C), and plasma glucose (Fig. 2D). There was a significant interaction between time and fasting on plasma Gh levels. Plasma Gh was significantly elevated from fed controls after 14 and 28 days of fasting (Fig. 2E). Similarly, there was a significant effect of fasting on plasma Igf1 levels (Fig. 2F). With respect to hepatic gene expression, there were no clear effects of fasting on *ghr2, igf1* or *igf2* expression (Fig. 3A–C). There was a significant interaction between time and fasting on *igfbp1b* levels, with elevated expression observed after 14 and 28 days of fasting (Fig. 3D); alternatively, *igfbp2b* and *-5a* were not responsive the fasting (Fig. 3E–F).

3.3. Effects of Gh injection

To examine whether systemic Gh could affect the expression of *igfbp* transcripts, we injected hypophysectomized tilapia with purified oGh and quantified hepatic gene expression at 12 h post injection. oGh injection did not impact plasma glucose (Fig. 4A). Plasma Igf1 roughly doubled following oGh injection (Fig. 4B). oGh injection stimulated hepatic *ghr2* by 2.6-fold from the levels of vehicle-injected fish (Fig. 4C). There was no effect of oGh on *ghr1* expression (data not shown). While *igf1* expression was not significantly increased by oGh (Fig. 4D), oGh stimulated *igf2* expression by 2.5-fold (Fig. 4E). There were no effects of oGh on the expression of *igfbp1b* or *-5a* (Figs. 4F,H). Alternatively, oGh stimulated *igfbp2b* levels by 6.2-fold from the levels of vehicle-injected fish (Fig. 4G).

4. Discussion

This study aimed to provide new details on how nutritional conditions and endocrine Gh drive activities within the Gh/Igf system of Mozambique tilapia, with special attention to *igfbps* expressed in liver. Following the identification of five *igfbp* transcripts in a tilapia EST library (Lee et al., 2010), we investigated expression patterns across multiple tissues and found that *igfbp1b*, *-2b* and *-5a* were robustly, and nearly exclusively, expressed in liver (Fig. 1A,B,D). Currently, it is not clear how these transcripts correspond to Igfbps of different sizes (20–40 kDa) previously described by Park et al. (2000) and Kajimura et al. (2003). However, the high levels of expression for *igfbp1b and -2b* in liver is similar to patterns reported in other teleosts, including chinook salmon (*Oncorhynchus tshawytscha*), gilthead sea bream (*Sparus aurata*), yellowtail (*Seriola quinqueradiata*), fine flounder (*Paralichthys adspersus*) and zebrafish (*Danio rerio*) (Funkenstein et al., 2002; Kamei et al., 2008; Zhou et al., 2008; Pedroso et al., 2009; Shimizu et al., 2011a,b; Safian et al., 2012). With *igfbp1b*, *-2b* and *-5a* identified as transcripts with robust expression in liver, we first turned to characterizing their expression dynamics under a nutrient restriction paradigm.

In the current study, we employed a 28-day nutrient restriction paradigm that we expected to induce catabolic conditions in tilapia (Uchida et al., 2003; Fox et al., 2008; Fox et al., 2010). Indeed, accompanying strong reductions in body weight, condition factor and plasma glucose, we observed increased levels of circulating Gh in parallel with marked reductions in plasma Igf1 levels (Fig. 2B–F). The disparate responses of plasma Gh and Igf1 are in strong accord with patterns previously described in fasted tilapia (Uchida et al., 2003; Fox et al., 2006; Fox et al., 2010) and therefore substantiate the efficacy of the experimental paradigm employed in this study. Interestingly, this apparent uncoupling of plasma Gh from hepatic Igf1 synthesis and secretion (termed Gh-resistance") has been documented during catabolic conditions in a number of teleosts including coho salmon (*Oncorhynchus kisutch*), chinook salmon and channel catfish (*Ictalurus punctatus*) (Duan and Plisetskaya, 1993; Pierce et al., 2005; Small and Peterson, 2005). Because it is commonly held that liver is the primary source of circulating Igfs in both mammals and fishes (Reinecke et al., 1997; Ohlsson et al., 2009; Reinecke, 2010; Reindl and Sheridan, 2012), we next turned to describing hepatic transcriptional responses to nutrient restriction. Despite marked changes in plasma Gh and Igf1 levels, we found that *ghr2*, *igf1* and *igf2* expression levels were not impacted by fasting (Fig. 3A–C). Nonetheless, of the investigated *igfbp* transcripts, *igfbp1b* was markedly changed after 14 and 28 days of fasting (Fig. 3D) suggesting that transcriptional regulation of this particular *igfbp* isoform plays a key role in the response to nutrient restriction.

While some Igfbps promote Igf1 activity, others are inhibitory, and some exhibit ligand-independent activities; the regulatory systems controlling Igfbp production are complex and remain largely unclear (Duan et al., 2010; Reindl and Sheridan, 2012). A strong association between elevated plasma Igfbp1 and fasting conditions appears to be conserved from teleosts to mammals (Lee et al., 1997; Siharath et al., 1996). In mammals, Igfbp1 inhibits somatic growth and glucose metabolism and is regulated by metabolic hormones, including insulin, glucocorticoids and thyroid hormones (Lee et al., 1997). Recently, Kamei et al. (2008) established that *igfbp1b* underlies growth and developmental retardation of

embryonic zebrafish. In accord with highly conserved activities linked to diminished Igf1 signaling, Igfbp1 responds to nutritional status in a variety of species. For example, a 22 kDa Igfbp in chinook salmon plasma understood to be a homolog of human Igfbp1 was induced by a reduction in feeding ration (Shimizu et al., 2005; Shimizu et al., 2009). Similarly, *igfbp1* expression was increased following 30 and 45 days of fasting in channel catfish (Peterson and Weldbieser, 2009) and chronic hypoxia in Atlantic croaker (*Micropogonias undulates*) (Rahman and Thomas, 2011) Albeit based on a restricted number of species, it appears that Igfbp1 plays a conserved role in the response to fasting in teleosts (Kelley et al., 2001; Shimizu et al., 2005; Shimizu et al., 2006; Wood et al. 2005). With this response now identified in tilapia, we propose that an important hepatic response to fasting in tilapia may involve regulation of plasma Igf1 via a shift towards the expression of *igfbp1b* to restrict Igf signaling. Future work (i.e., development of a radioimmunoassay for Igfbp1b) should resolve the relationship between the induction of *igfbp1b* gene expression and circulating levels of this Igfbp isoform.

In teleosts, the regulatory systems controlling *igfbp* gene expression remain largely unknown (Duan and Xu, 2005; Duan et al., 2010; Reindl and Sheridan, 2012). Since Gh is a known regulator of *igfbp* expression and protein secretion in mammals Yamada and Lee, 2009), we conducted a Gh injection experiment to address whether Gh is a potential regulator of *igfbp* expression in tilapia. First, we observed a clear induction of hepatic *ghr2* mRNA in hypophysectomized animals following Gh treatment Fig. 4C), a pattern consistent with the proposition that *ghr2* encodes the primary Gh receptor in tilapia (Kajimura et al., 2004; Pierce et al., 2007; Pierce et al., 2012). As reported previously (Chen et al., 2007), plasma Igf1 increased following oGh injection Fig. 4B). Following similar patterns reported in rainbow trout, coho salmon, common carp (*Cyprinus carpio)*, channel catfish, gilthead sea bream and Japanese eel (*Anguilla japonica*) (Shamblott et al., 1995; Tse et al., 2002; Vong et al., 2003; Carnevali et al., 2005; Petersen et al., 2005; Moriyama et al., 2008; Pierce et al., 2010), animals in this study showed markedly elevated hepatic *igf2* expression following Gh treatment (Fig. 4E), similar to responses in intact animals (Eppler et al., 2010; Pierce et al., 2011; Shved et al., 2011). While multiple lines of evidence support the operation of Igf1, as opposed to Igf2, as the primary somatomedin in post-embryonic mammals (Le Roith et al., 2001), in light of the demonstrated bioactivity of native recombinant Igf2 to stimulate growth in tilapia (Chen et al., 2000) our data linking Gh with *igf2* in hypophysectomized animals suggest that Igf2 should be further investigated as a possible somatomedin in tilapia. In any event, the positive relationship among Gh injection and plasma Igf1 and hepatic *igf2* suggests that animals were in an anabolic state at the time of sampling.

Igfbp3, which in mammals may account for up to 90% of Igf1 binding (Baxter, 1994), was reported in tilapia to be highly expressed in liver and inducible by Gh (Cheng et al., 2002). Despite this, however, the prevailing 40–50 kDa Igfbp in circulation in salmonids, and possibly other teleosts, is not Igfbp3 as it is in mammals, but an Igfbp2 paralog (Shimizu et al., 2011a). Here, we found that hepatic *igfbp2b* expression was strongly stimulated by oGh (Fig. 4G), a pattern consistent with previous reports showing that the 40–50 kDa Igfbps in teleost plasma are responsive to Gh (Shimizu et al., 1999; Shimizu et al., 2003). When

considering that *igfbp2b* expression is subject to Gh regulation in tilapia along with the known regulation of Igfbp3 by Gh in rat (Schmid et al., 1994), the conserved actions of Gh on Igfbps from mammals to fish appear to be more closely related to their prevalence in circulation rather than to their molecular structure *per se*. Since Igfs are known to directly modulate *igfbp* expression in mammals (Conover, 1990), Gh-stimulated plasma Igf1 (Fig. 4B) may indirectly link Gh with *igfbp2b* expression. The potential for direct actions of Gh and Igf1 on hepatic *igfbp2b* expression should be addressed in future studies employing primary hepatocyte culture (Pierce et al., 2006; Pierce et al., 2011).

In summary, our principal findings include the identification of *igfbp1b* as a transcript that is highly responsive to nutritional conditions in tilapia. In addition to plasma Igf1 and hepatic *igf2*, we identified hepatic *igfbp2b* as a Gh-responsive transcript in hypophysectomized animals. Since the regulation of *igfbp* expression in teleosts undoubtedly extends beyond Gh to include peripheral signals under pituitary control such as cortisol and thyroid hormones (Kajimura et al., 2003; Pierce et al., 2006), the hypophysectomized tilapia model is well suited to further resolve how the pituitary mediates *igfbp* transcriptional responses to a variety of physiological challenges (nutrient restriction, salinity challenge, handling stress). Inasmuch as tilapiine species are among the most important aquaculture resources worldwide, they continue to represent key models from which to gain an improved understanding of how the Gh/Igf system coordinates growth and metabolism in teleosts.

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Highlights

▶ *igfbp1b*, -2b, -4 and -5a are highly expressed in liver ▶ Fasting stimulated plasma Gh and decreased plasma Igf1 ▶ Hepatic *igfbp1b* was enhanced 60 after 14 and 28 days of fasting ▶ Gh injection stimulated hepatic *igfbp2b* in hypophysectomized tilapia

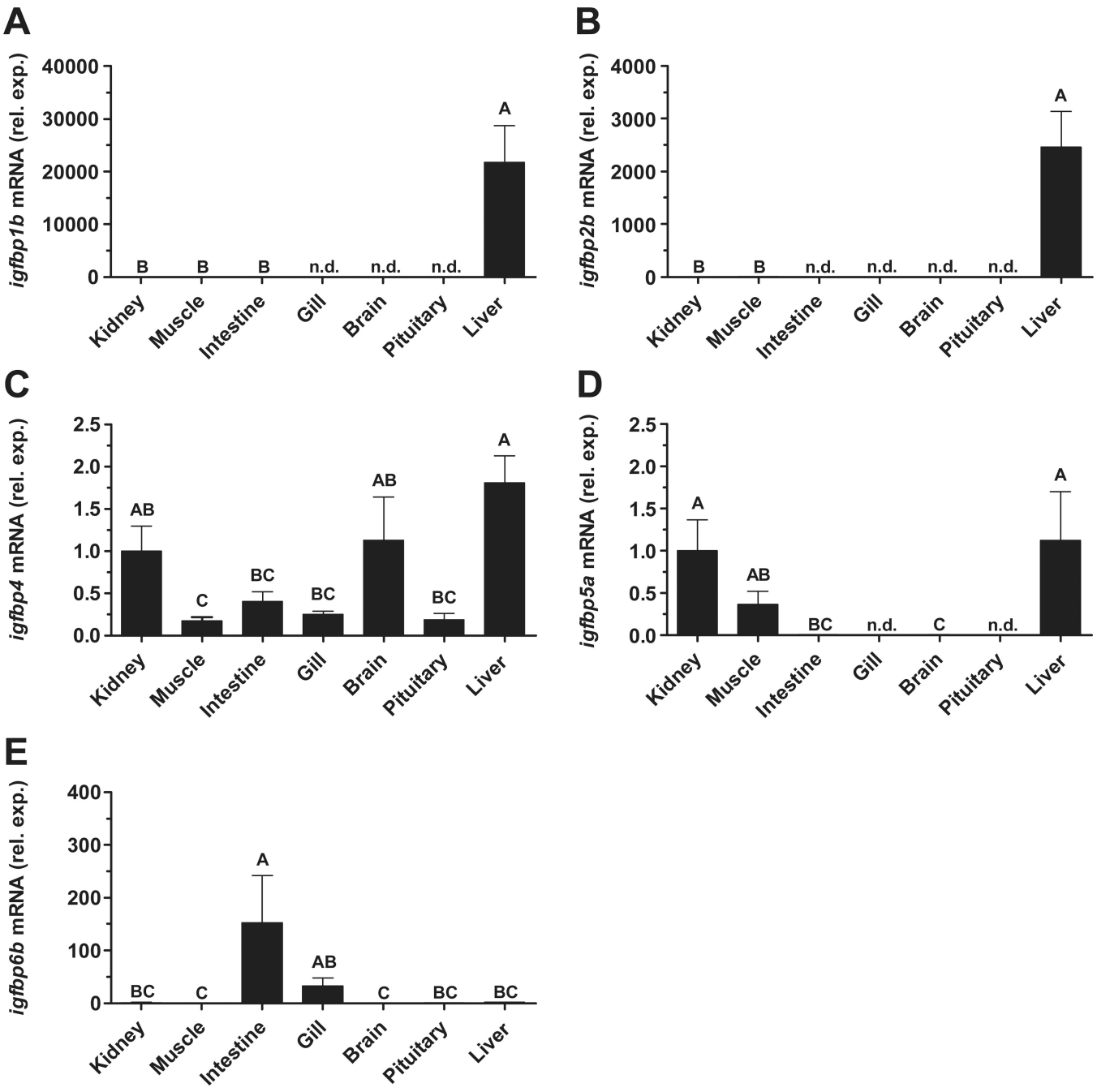

Fig. 1.

Expression of *igfbp1b* (A), *igfbp2b* (B), *igfbp4* (C), *igfbp5a* (D) and *igfbp6b* (E) mRNA in kidney, skeletal muscle, anterior intestine, gill, brain, pituitary and liver of fed, freshwater-acclimated, tilapia. Data were normalized using *ef1*αas a reference gene and are presented relative to the amount of mRNA in kidney. n.d.; no detection. Groups not sharing the same letter are significantly different (ANOVA, Tukey's HSD, $P < 0.05$). Means ± SEM ($n = 6–8$).

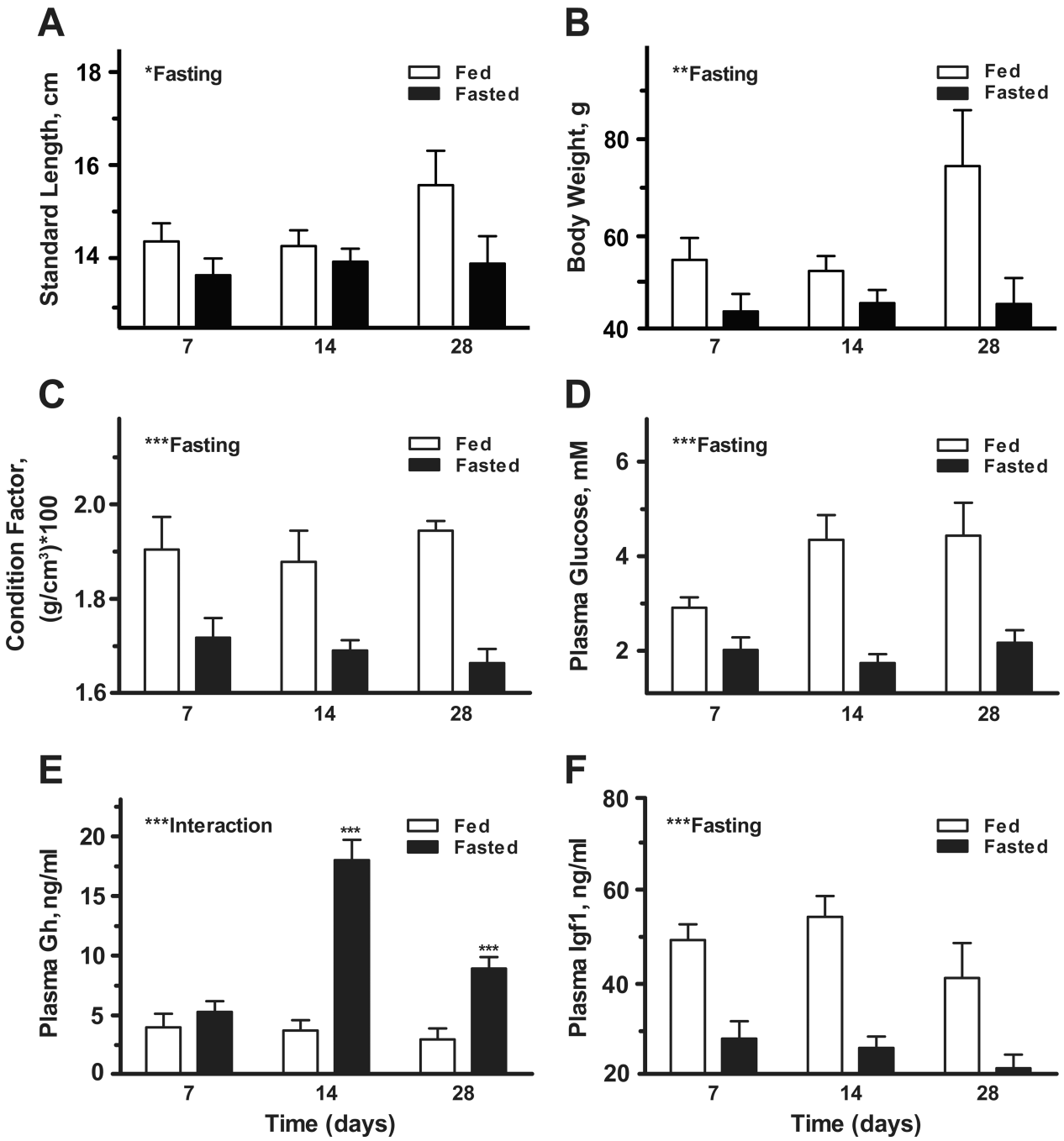

Fig. 2.

Effects of fasting on standard length (A), body weight (B), condition factor (C), and plasma glucose (D), Gh (E) and Igf1 (F). Tilapia were exposed to continuous feeding (open bars) or fasting (solid bars) and sampled at 7, 14 and 28 days. Significant effects of fasting or interaction between time and fasting are indicated in respective panels. When there was a significant interaction between time and fasting, post-hoc comparisons were made between fed and fasted groups at each time point. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. Means ± SEM (n = 9–10).

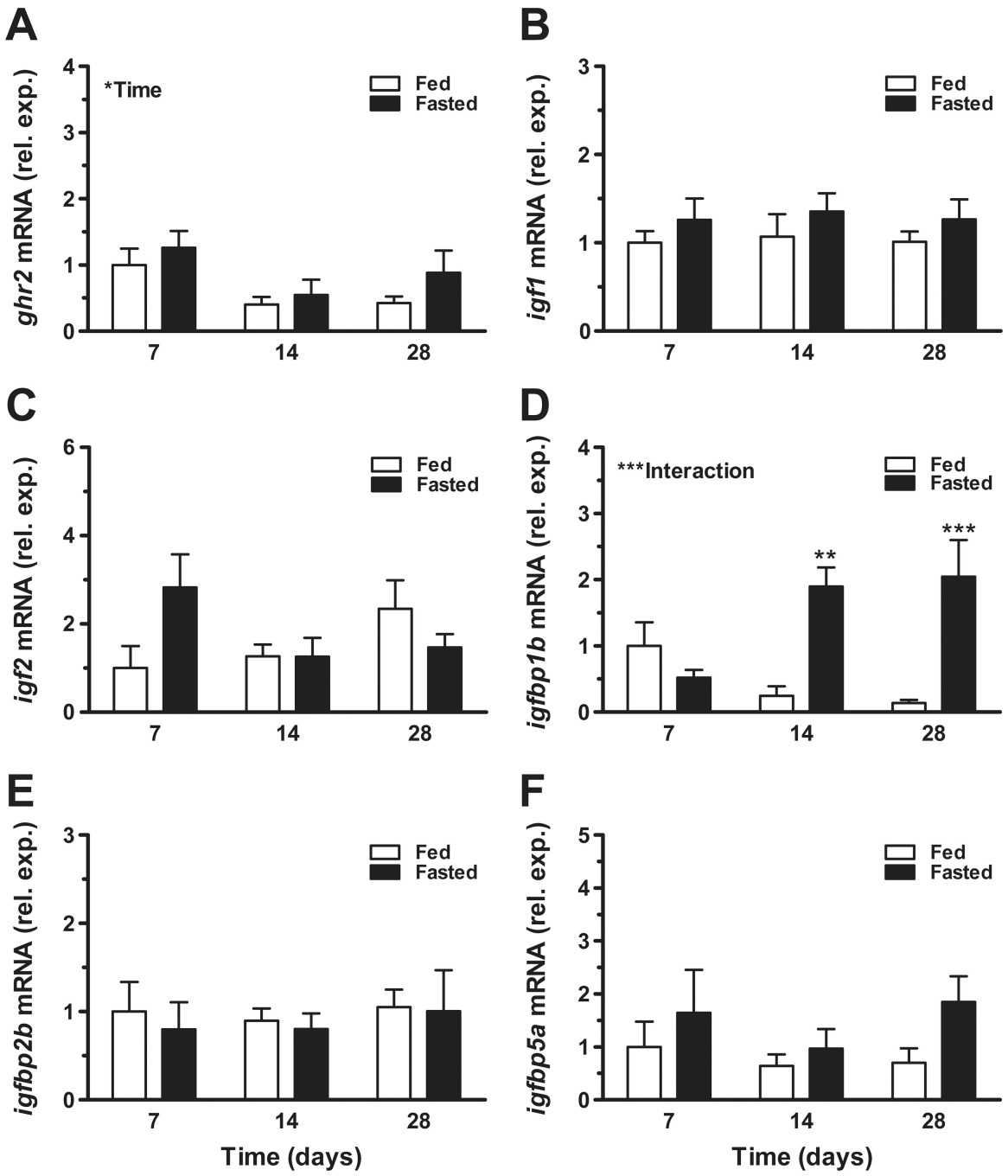

Fig. 3.

Effects of fasting on hepatic gene expression of *ghr2* (A), *igf1* (B), *igf2* (C), *igfbp1b* (D), *igfbp2b* (E) and *igfbp5a* (F). Tilapia were exposed to continuous feeding (open bars) or fasting (solid bars) and sampled at 7, 14 and 28 days. Significant effects of time or interaction between time and fasting are indicated in respective panels. When there was a significant interaction between time and fasting, post-hoc comparisons were made between fed and fasted groups at each time point. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001. Means ± SEM (*n* = 7–8).

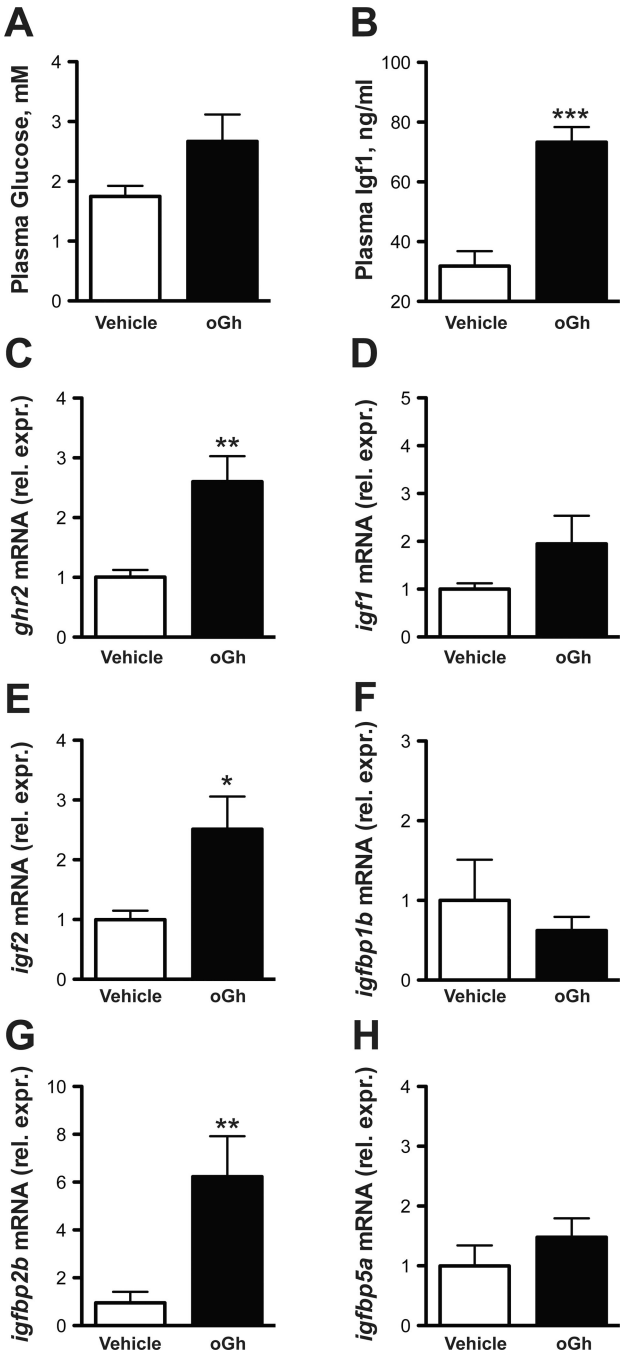

Fig. 4.

Effects of ovine Gh (oGh) on plasma glucose (A) and Igf1 (B) and hepatic gene expression of *ghr2* (C), *igf1* (D), *igf2* (E), *igfbp1b* (F), *igfbp2b* (G) and *igfbp5a* (H). Hypophysectomized animals were sampled 12 h after a single IP-injection of oGh (5 μg/g body weight). Group differences were analyzed by Student's t-test. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001. Means ± SEM (*n* = 6–9).