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Effects of fasting on circulating IGF-binding proteins, glucose, and cortisol in channel catfish (*Ictalurus punctatus*)

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Abstract

The effects of fasting on IGF-binding proteins (IGFBPs), glucose, and cortisol in channel catfish were examined. Fed fish (controls) were compared to 14-, 30-, and 45-day fasted fish and 45-day fasted fish refed for 15 additional days. Body length and weight changes, condition factor (CF), hepatosomatic index (HSI), and plasma glucose and cortisol were assessed to determine growth and metabolic status. Body length and growth rates were inhibited ($P < 0.05$) after 14, 30, and 45 days of fasting. The 14-, 30-, and 45-day fasted fish exhibited hypoglycemia and reduced CF and HSI. Cortisol levels were increased (22.8 ± 15.2 ng/ml versus 4.7 ± 3.9 ng/ml) in 30-day fasted fish compared to fed controls ($P < 0.05$). Associated with the increase in cortisol in fasted fish was a concomitant increase in plasma levels of a 20-kDa IGFBP through day 45. A 35- and a 45-kDa IGFBP were also identified but were similar between fed and unfed fish throughout the experiment. At the end of 15 days of refeeding, 20-kDa IGFBP, glucose, and cortisol levels were similar to fed controls. Refeeding also caused an increase in growth rates. These results suggest the existence of a catfish counter part to mammalian IGFBP-1, similar to lower molecular mass IGFBPs reported in other species of fish. These results also suggest that a 20-kDa IGFBP is upregulated during fasting-induced growth inhibition of channel catfish and provide additional evidence of the conserved nature of the IGF-IGFBP-growth axis in fish.

Keywords: Fasting; Insulin-like growth factor binding protein; Cortisol

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1. Introduction

Insulin like growth factor-I (IGF-I) is primarily produced in the liver and mediates many of the growth-promoting actions of growth hormone (GH) [1,2]. It is becoming clear that the GH-IGF-I axis controlling vertebrate growth is highly conserved in fish [3–8]. The activity of IGF-I and IGF-II (IGFs) is regulated not only by GH and other endocrine modulators that enhance or suppress local and systemic IGF levels, but also by the presence of IGF-specific binding proteins (IGFBPs). IGFBPs play a central role in prolonging the half-lives of IGFs and coordinating and transporting IGFs in the blood and across the capillary barrier to target tissues [9–14]. In mammals, the IGFBPs are composed of a family of six proteins (IGFBP-1–6) that bind to IGFs with high affinity and specificity [15]. Less than 5% of the IGFs circulate in the free form; instead most of the IGFs (90%) are complexed with IGFBP-3 and an acid-labile subunit (ALS), forming a 150-kDa ternary complex [10,12,13,16,17]. IGFBPs also exist in several teleost species [18,21–31]. Three IGFBPs, ranging in size from 24 to 50 kDa, are commonly reported in studies in teleosts.

In adult mammals, the levels of IGF-I and IGFBPs are appreciably affected in catabolic or stressful conditions such as fasting [32–34]. IGFBP-1 has been shown to inhibit IGF actions in numerous in vitro and in vivo animal studies [34]. Based on comparable molecular mass and an assumption of endocrine regulation similar to that in mammals, it has been proposed that the ≤31-kDa fish IGFBPs may be counterparts of mammalian IGFBP-1 or -2; and the 40–50-kDa fish IGFBP may correspond with mammalian IGFBP-3. Kelley et al. [45] recently compared the IGF/IGFBP system in teleost and mammalian species. The 40–50-kDa fish IGFBP shows positive regulation by GH and is correlated with somatic growth in the striped bass (*Morone saxatilis*) [26], coho salmon (*Oncorhynchus kisutch*) [19,27], and tilapia (*Oreochromis mossambicus*) [28]. On the other hand, ≤31-kDa fish IGFBPs are upregulated in catabolic states and inversely correlated with somatic growth [7,20,35,36]. Siharath et al. [7] demonstrated that fasting increased levels of a 25-kDa IGFBP (sb-IGFBP-1) in striped bass (*M. saxatilis*). Similarly, in the longjaw mudsucker goby (*Gillichthys mirabilis*) fasting increased levels of the 24- and 30-kDa IGFBPs [20,36]. In addition, Kelley et al. [20] observed increases in levels of cortisol, which is a principal hormone of catabolism, in *G. mirabilis* after 20 days of fasting. This is an interesting finding because IGFBP-1 gene expression in mammals is stimulated by glucocorticoids, resulting in elevations in serum IGFBP-1 levels [34]. IGFBP-1 may play a role in sequestering available IGF peptide through high IGF-binding affinity [34] and curtailing expensive anabolic functions during times of stress or nutritional deficiencies [20]. Kelley et al. [20] proposed that the measurement of lower molecular mass IGFBPs (≤31-kDa fish IGFBP) might provide an assessment of growth status of fishes.

The mechanisms regulating these physiological changes in IGFBPs appear to be conserved across species, but there is no information on anabolic and catabolic roles of IGFBPs as they relate to growth in the channel catfish (*Ictalurus punctatus*). The objectives of this study were to examine the effects of fasting on plasma levels of IGFBPs, glucose, and cortisol in channel catfish, which represent a major aquaculture 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, Stoneville, MS aquaculture facility. Prior to experimentation, approximately 25 fish from each of five different families were placed into a 120-l holding tank. The following day, 72 fish (mean initial size 17.7 ± 0.5 g) were randomly assigned to eight 76-l tanks (nine fishes per tank), and allowed to acclimate for 10 days. The fish were fed once per day to apparent satiation and reared in 26.0 °C flow-through well water under a 14:10 h light/dark photoperiod. A commercial floating catfish feed (36% crude protein; Land O'Lakes Farmland Feed LLC, Fort Dodge, IA) was used throughout the study. Water quality (pH ∼ 8.5; dissolved oxygen levels >5.0 mg/l) and flow rates were similar between tanks.

The fish were separated into two groups. One group served as controls ($n = 36$) and was fed once daily to apparent satiation. The other group of 36 fish was subjected to a 14-day fast ($n = 9$), a 30-day fast ($n = 9$), a 45-day fast ($n = 9$), or a 45-day fast followed by 15 additional days of refeeding ($n = 9$). At 14, 30, 45, and 60 days, unfed fish ($n = 9$) and control fish ($n = 9$) were sampled as described below.

2.2. Sampling and plasma preparation

On sampling days (14, 30, 45, and 60), unfed and fed fish from each group were euthanized with an overdose (0.3 g/l) of tricainemethane sulfonate (Finquil; Argent Chemical Laboratories, Redmond, WA). Fish were euthanized at one time to prevent potential stress due to prolonged anesthesia. Each fish was weighed and measured for length. The fish were then bled from the caudal vasculature into syringes coated with heparin and livers were removed and weighed to the nearest 0.1 g. Plasma was separated, frozen at −80 °C, and later analyzed for cortisol, glucose, and insulin-like growth factor binding proteins (IGFBPs).

2.3. Western ligand blotting using digoxigenin-labeled IGF-I

Recombinant barramundi IGF-I (rbIGF-I; GroPep Pty Ltd., Adelaide, SA, Australia) was labeled with digoxigenin-3-*o*-methylcarbonyl-ε-aminocaproic acid-*N*-hydroxysuccimimide ester (DIG; Roche, Indianapolis, IN) according to the method of Shimizu et al. [37]. Digoxigenin-labeled rbIGF-I (DIG-rbIGF-I) was stored at −20 °C until use.

Western ligand blotting was carried out according to the method of Hossenlopp et al. [38] with modifications. A 5-μl plasma sample was diluted with 20 μl 0.01 M PBS and 25 μl Laemmli sample buffer (BioRad, Hercules, CA). Samples were heated for 5 min at 95 °C and then vortexed. A 25-μl sample was electrophoresed for 1.0 h at 160 V in a discontinuous 4% stacking/12% separating polyacrylamide gel. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) [39] was performed under nonreducing conditions. Proteins were then electrotransferred (Trans Blot; BioRad) to nitrocellulose paper (BioRad) after SDS–PAGE. The nitrocellulose membrane was blocked with Blotto (Pierce, Rockford, IL) for 1 h and then incubated with 0.4 μg DIG-rbIGF-I in SuperBlock (Pierce) for 1.5 h.

After washing, the membrane was incubated with antibody against DIG conjugated with horseradish peroxidase (Pierce) at a dilution of 1:1200 for 1 h. The membrane was then washed and incubated in SuperSignal West Pico Substrate (Pierce) for 30 min. Finally, the membrane was exposed to CL-XPosure film (Pierce) for approximately 45 s. All steps were carried out at room temperature. GelExpert software version 3.5 (Nucleotech, Westport, CT) was used to calculate peak intensities of IGFBPs. Peak intensity values are reported as mean arbitrary densitometric units (ADU) ±S.E.

2.4. Plasma cortisol and glucose determination

Cortisol was measured using a DELFIA® time-resolved fluroimmunoassay kit (Perkin-Elmer Life Sciences, Boston, MA). This kit has been validated for the quantification of plasma cortisol in channel catfish [40]. Plasma glucose was quantified with an Accucheck Advantage glucometer (Boehringer Mannheim Diagnostics, Indianapolis, IN).

2.5. Hepatosomatic index, condition factor, and percent length and weight change/day

Hepatosomatic index (HSI) was determined by dividing the liver weight (g) by total body weight (g) and multiplying by 100. Condition factor (CF) was calculated by dividing the weight (g) of a fish by its length^3 (cm^3) and multiplying by 100. Instantaneous length rate was calculated as $(\ln l_2 - \ln l_1)/(t_2 - t_1)$, where l_2 and t_2 are length (cm) and time (day) at sacrifice and l_1 and t_1 are initial length and time. Instantaneous growth rate was similarly calculated by substituting weight (g) for length.

2.6. Statistical analysis

The statistical analyses were conducted as a one-way ANOVA using separate ANOVAs for each sampling interval (days 14, 30, 45, and 60) using Statistical Analysis System Version 8.0 software (SAS Institute, Inc., Cary, NC, USA) and are presented as means ± standard error (S.E.). A significance level of $P < 0.05$ was used. The response variables included weight, CF, HSI, ADU of IGFBPs, growth, and length rates. HSI and CF were calculated as percentages and log transformed prior to statistical analysis. Arbitrary density units of IGFBPs were analyzed from nine plasma samples.

3. Results

Three specific IGFBPs (20, 35, and 45 kDa) were identified in the plasma of channel catfish (Fig. 1a). Adding a 50-fold excess of unlabeled rbIGF-I and comparing it to DIG-rbIGF-I demonstrated specificity of the assay. The addition of unlabeled rbIGF-I eliminated or significantly reduced the appearance of the 20, 35, and 45 kDa bands (Table 1).

Weight and CF of the fasted fish were lower ($P < 0.05$) than the fed controls throughout the 60-day study (Table 2). Hepatosomatic index was also decreased ($P < 0.05$) in the unfed fish but was increased ($P < 0.05$) after refeeding compared to fed controls. By day 14, fasting decreased ($P < 0.05$) growth, assessed by the instantaneous growth and length

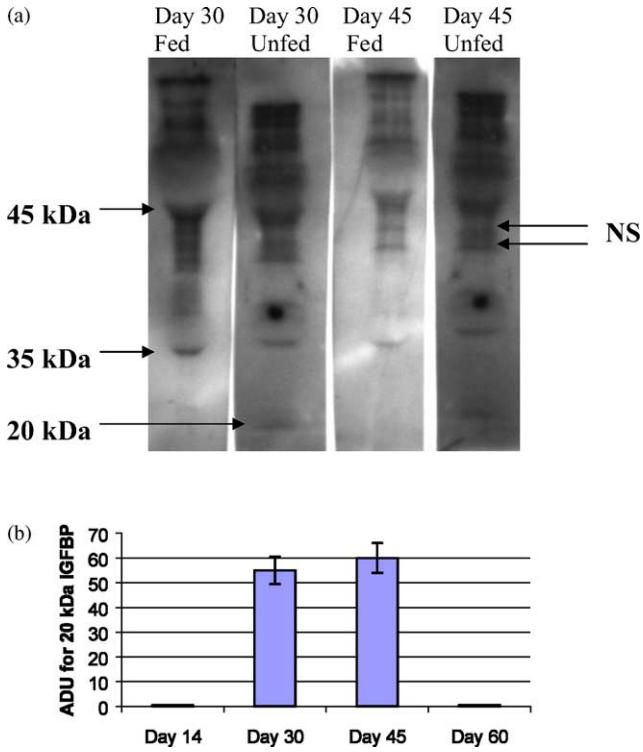

Fig. 1. (a) Representative western ligand blot for IGFBPs in days 30 and 45 fed and unfed channel catfish. Note the occurrence of the 20-kDa bands in days 30 and 45 unfed fish. Also note the 35 and 45 kDa bands present in fed and unfed fish on days 30 and 45. The 35 and 45 kDa bands were also present on day 14 and 60 but were not different between fed and unfed fish throughout the study (western blot not shown). NS, nonspecific bands. (b) Levels of a 20-kDa insulin like growth factor binding protein in unfed fish on days 14, 30, 45, and 60. Levels of the 20 kDa insulin like growth factor binding protein were not detected at day 14 in unfed fish, in control fed fish at any time throughout the study, or in fish refed for 15 days. Western blots were quantified using GelExpert software and values are expressed as arbitrary densitometric unit (ADU) means ± S.E. ($n = 9$).

Table 1
Band specificity by competitive displacement of digoxigenin-labeled recombinant barramundi IGF-I (DIG-rbIGF-I) with 50-fold excess of unlabeled rbIGF-I (rbIGF-I) using plasma from 30-day fasted catfish

Treatment	20 kDa	35 kDa	45 kDa
DIG-rbIGF-I	55.5 ± 2.3[a] x	45.2 ± 5.6 x	47.0 ± 3.8 x
(DIG-rbIGF-I) + rbIGF-I (50×)	1.5 ± 0.9 y	1.7 ± 0.5 y	1.9 ± 0.8 y
No rbIGF-I	0.0 z	0.0 z	0.0 z

Mean values having different letters (x, y, and z) within columns are significantly ($P < 0.05$) different.
[a] Peak intensity values are represented as arbitrary density units (ADU) ± S.E.

Table 2
Mean (±S.E.) weight, hepatosomatic index, and condition factor in fed and fasted catfish ($n = 9$)

Day	Treatments[a]	Weight (g)	HSI	CF
14	Fed vs. fasted	28.70 ± 0.43 vs. 18.57 ± 0.45*	1.29[a] ± 0.08 vs. 0.79 ± 0.06*	0.78 ± 0.02 vs. 0.69 ± 0.02*
30	Fed vs. fasted	38.48 ± 0.61 vs. 17.92 ± 0.58*	1.58 ± 0.09 vs. 0.66 ± 1.01*	0.71 ± 0.02 vs. 0.58 ± 0.02*
45	Fed vs. fasted	55.55 ± 0.56 vs. 15.62 ± 0.55*	1.86 ± 0.15 vs. 0.80 ± 0.14*	0.69 ± 0.01 vs. 0.45 ± 0.01*
60	Fed vs. refed	76.06 ± 0.53 vs. 23.60 ± 0.54*	1.19 ± 0.19 vs. 2.31 ± 0.20*	0.69 ± 0.02 vs. 0.54 ± 0.02*

[a] Treatments were fed controls, 14-day fasted fish, 30-day fasted fish, 45-day fasted fish, and 45-day fasted fish followed by 15 days of refeeding.
* Values are different from controls ($P < 0.05$).

rate (Fig. 2). A decrease ($P < 0.05$) in skeletal and body growth was observed through day 45. After 15 days of refeeding, growth and length rates were increased but were still lower than fed controls.

Plasma glucose levels were reduced ($P < 0.05$) by 75% in the 14-day unfed fish but were not different than fed controls at day 30 (Table 3). However, at day 45, glucose levels were again reduced ($P < 0.05$) in the 45-day unfed fish compared to fed controls. Plasma glucose concentrations were similar to fed control fish after 15 days of refeeding. Cortisol levels increased ($P < 0.05$) in the 30-day unfed fish but levels were similar to fed controls throughout the rest of the study. Associated with the increase in cortisol in unfed fish on day 30 was an increase in plasma levels of a 20-kDa IGFBP (Fig. 1b). Elevated levels of the 20-kDa IGFBP were observed through day 45. The 20-kDa IGFBP was not detected in fed control fish at any time during the study and was not detectable in fasted fish after

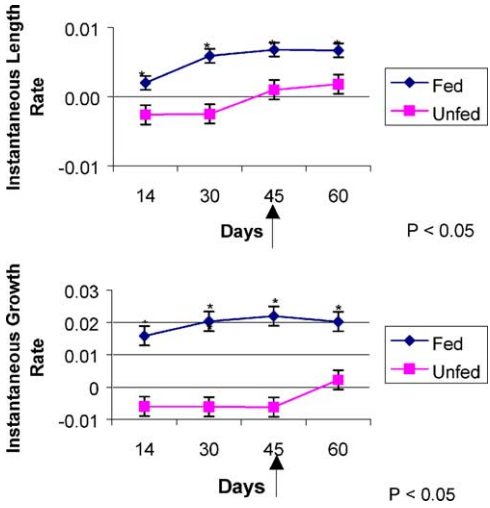

Fig. 2. Instantaneous length and growth rate of fed and fasted channel catfish. Instantaneous length rate was calculated as $(\ln l_2 - \ln l_1)/(t_2 - t_1)$, where l_2 and t_2 are length (cm) and time (day) at sacrifice and l_1 and t_1 are initial length and time. Instantaneous growth rate was calculated similarly. Arrow indicates day fish were refed. Values are expressed as mean length and growth rate ± S.E. ($n = 9$). Asterisk indicate a significant ($P < 0.05$) difference between control and fasted fish.

Table 3
Mean (±S.E.) plasma glucose (mmol/l) and cortisol (ng/ml) in fed and fasted catfish ($n = 9$)

Day	Treatments[a]	Glucose	Cortisol
14	Fed vs. fasted	5.8 ± 0.7 vs. 1.4 ± 0.8*	2.9 ± 0.2 vs. 2.7 ± 0.2
30	Fed vs. fasted	3.1 ± 0.7 vs. 2.7 ± 0.8	4.7 ± 3.3 vs. 22.8 ± 4.1*
45	Fed vs. fasted	2.5 ± 0.3 vs. 1.5 ± 0.3*	9.5 ± 3.3 vs. 13.1 ± 4.1
60	Fed vs. refed	1.7 ± 0.3 vs. 1.8 ± 0.4	22.3 ± 5.1 vs. 12.3 ± 6.2

[a] Treatments were fed controls, 14-day fasted fish, 30-day fasted fish, 45-day fasted fish, and 45-day fasted fish followed by 15 days of refeeding.

* Values are different from controls ($P < 0.05$).

15 days of refeeding (Fig. 1b). A 35- and a 45-kDa IGFBP were also identified, but were similar between fed and unfed fish throughout the experiment (Fig. 1a).

4. Discussion

Fasting of channel catfish caused a cessation of body and skeletal growth and a reduction in HSI and CF. Decreased HSI and CF have also been previously noted in 28-day fasted catfish [41]. HSI was dramatically increased after refeeding compared to fed controls. Gaylord and Gatlin [41] also observed an increase in HSI after refeeding fasted catfish. Although the fat content of the livers was not measured directly in the present study, liver lipid has been shown to increase in food-deprived catfish after refeeding [41]. An increase in lipid content of the liver would increase HSI.

Fasted fish exhibited reduced plasma glucose concentrations by day 14 and tended to be lower through day 45 of the study. In day 14-fed controls, plasma glucose concentrations were higher compared to fed controls that were sampled later in the study. Measurements of plasma glucose concentrations provide only a snapshot of the existing metabolic status of the fish and do not take into account the turnover of the metabolite [46]. Thus, the higher levels of plasma glucose in the fed controls may represent the metabolic status of the fish at day 14. It is not directly apparent why glucose concentrations on day 30 in unfed fish were similar to fed fish, but could be caused by cortisol-mediated glycogenolysis [46] or gluconeogenesis [20]. Increased plasma cortisol levels were observed in the 30-day unfed catfish.

Kelley et al. [20] reported a six-fold increase in circulating cortisol levels of 20 day fasted gobies and observed that cortisol levels were restored to control levels after 7 days of refeeding. The observed increase in cortisol at day 30 in fasted catfish was associated with an increase in plasma glucose levels, possibly a result of cortisol-induced gluconeogenesis. In a recent review, Mommsen et al. [46] also hypothesized that elevated cortisol in fish may play a role in the production of glucose by increasing glycogenolysis. Thus, one of the metabolic roles of cortisol during stress may be in the glucose-regulation process. In support of this hypothesis, gluconeogenesis has been shown to be directly stimulated by cortisol administration in humans [42].

After 45 days in the present study, cortisol levels in fasted catfish were similar to fed controls. This may suggest a change in metabolic status of the fish or that glucose may be

serving as a signal of nutritional status controlling the cortisol response [43]. In addition, cortisol clearance from the plasma is dependent upon catabolism of cortisol, cortisol binding proteins, and target tissue receptors [46]. Since cortisol did not increase after 14 days of fasting, it is reasonable to speculate that elevated glucose at day 30 may have resulted in suppressed cortisol levels at day 45.

There was an increase in a 20-kDa IGFBP that was associated with an increase in levels of cortisol. This is an interesting correlation but it is too early to speculate that an increase in cortisol caused an increase in a low molecular mass IGFBP. However, in tilapia (*O. mossambicus*), cortisol injected at 2 and 10 μg/g significantly increased IGFBPs of four sizes (24, 28, 30, and 32 kDa) in the plasma within 2 h [47]. This study clearly demonstrated that exogenous cortisol induced a rapid increase in lower mass IGFBPs. A similar inquiry into the effects of cortisol on IGFBPs in channel catfish is warranted.

Based on similar molecular mass, the observed 20-kDa IGFBP may be a counterpart to the mammalian IGFBP-1, which has also been shown to be upregulated during catabolic conditions [34]. Siharath et al. [7] reported an increase in levels of a 25-kDa IGFBP that was observed at day 30 in association with growth inhibition and catabolism caused by fasting striped bass. Kelley et al. [20] reported a five-fold increase in levels of a 30- and 24-kDa IGFBP in fasted gobies. In zebrafish (*Danio rerio*), a 31-kDa IGFBP was upregulated with prolonged fasting [35]. These lower molecular mass IGFBPs may serve to shut down energy-expensive anabolic processes under catabolic circumstances [34,45]. Our study and others suggest that these lower molecular mass IGFBPs may be of general occurrence in teleosts that are metabolically regulated.

The higher molecular mass IGFBPs (35 and 45 kDa; IGFBP-3), which are regulated by GH and associated with positive growth in the striped bass [25,26,44], coho salmon [19,27], and tilapia [28], were not altered by fasting in the present study. In a study similar to ours, Siharath et al. [7] also reported that a 35-kDa IGFBP was not significantly altered after 60 days of fasting in striped bass. In contrast, in mammals levels of IGFBP-3 are strongly reduced during states of catabolic growth inhibition [32,33]. It is not clear from this study or other fish studies the role(s) of higher molecular mass IGFBPs under catabolic conditions.

In summary, an increase in a 20-kDa IGFBP was observed with a concomitant decrease in skeletal and body growth and increase in levels of cortisol after 30 days of fasting. A 35- and a 45-kDa IGFBP were also identified; but were similar between fed and unfed fish throughout the experiment. The role of cortisol as a regulatory hormone stimulating the production of a mammalian IGFBP-1 like protein needs further investigation. These results also provide additional evidence of the conserved nature of the IGF-IGFBP-growth axis in fish.

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