Dynamics of Increase in Insulin-like Growth Factor-I mRNA Expression in Nile Tilapia, Oreochromis niloticus, in Response to Elevated Temperature

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ABSTRACT

Insulin-like Growth Factor-I (IGF-I) is a physiological mediator and a potentially important growth indicator candidate in teleost fishes. In this study, the effects of increased temperature on the growth and hepatic IGF-I gene expression in Oreochromis niloticus were evaluated. Twenty all-male fish were reared separately at temperatures below 24°C for 12 days and then water temperature in 15 aquaria was gradually raised to 30°C within a day. Growth and hepatic IGF-I gene expression in five fish were obtained before the temperature change and after 2, 5 and 7 days of increasing the water temperature. The growth rate of the fish reared in the warmer temperature for 2, 5 and 7 days was significantly increased in a time dependent manner (r = 0.93). Mean hepatic IGF-I mRNA levels in fish reared at warm temperature for 2, 5 and 7 days were elevated 1.6-, 2.5-, and 3.6-fold, respectively compared to that of fish reared at cold temperature (<24°C). The IGF-I levels were significantly elevated after at least 5 days of exposure to warm temperature, which is consistent with the idea that hepatic IGF-I gene expression can be used as a short-term growth rate indicator for O. niloticus. A significant positive correlation was observed between days of rearing at warm temperature and hepatic IGF-I levels (r = 0.92); between specific growth rate (length) and IGF-I levels (r =0.92); and between condition factor and IGF-I levels (r = 0.55). The high positive association between IGF-I mRNA and growth rate validated the assertion that hepatic IGF-I levels are sufficiently sensitive to be used as instantaneous growth rate indicator in this species of fish.

Keywords: Growth indicator; Hepatic IGF-I; Real time qRT-PCR

Abbreviations: CF, condition factor; DNA, deoxyribonucleic acid; GH, growth hormone; IGF-I, Insulin-like Growth Factor-I; mRNA, messenger ribonucleic acid; SGR, specific growth rate

INTRODUCTION

Growth of fish is affected by several factors which include their genetic constitution, nutritional and environmental factors. In teleost fishes, developmental and growth processes are triggered by temperature, photoperiod and food availability. The most important environmental factor is temperature. As temperature increases, feed consumption increases to a maximum level and then decreases rapidly prior to the upper limit that the fish can tolerate (Jobling 1994). These environmental cues and internal information are processed and integrated in the brain for appropriate modification of growth through hormonally mediated pathways (Duan 1998). Central in the hormonal control of growth is the growth hormone (GH) – Insulin-like Growth Factor –I (IGF-I) axis. Pituitary GH stimulates the production of hepatic IGF-I which in turn is the primary source of circulating IGF-I (Duan 1998; Kajimura et al. 2001; Pierce et al. 2004), and which mediates the growth promoting actions of GH. Growth hormone increases the abundance of IGF-I mRNA by enhancing the transcription of the IGF-I gene as well as affecting the processing of IGF-I mRNA (Daughaday and Rotwein 1989).

Several studies have indicated that physiological and biochemical markers can be used as instantaneous indices of growth rate (Ali and Wootton 2003; Beckman et al. 2001; Vera Cruz et al. 2006; Vera Cruz and Brown 2007, 2009). Buckley et al. (1999) and Ali and Wootton (2003) have used of biochemical markers such as RNA: DNA ratio and lipid concentration as indices of growth rate. Circulating levels of hormones such as growth hormone (GH), thyroxine, insulin and IGF-I, on the other hand, have been proposed as growth indices in several species of fish (Pérez-Sánchez and Le Bail 1999; Shimizu et al. 2000; Larsen et al. 2001; Beckman et al. 2004; Vera Cruz et al. 2006; Vera Cruz and Brown 2007, 2009). Of these hormones, however, IGF-I is the most promising candidate in fish since this hormone acts proximally in the regulation of growth. This is evidenced by several studies indicating a significant association between IGF-I and growth rates (Uchida et al. 2003; Beckman et al. 2004; Ueda 2004; Vera Cruz et al. 2006). In addition, the association between IGF-I levels and growth rate is more consistent than that of GH with growth rate. Growth hormone levels can become dissociated with growth rate under some conditions, such as starvation, while the positive correlation between IGF-I and growth rate persists (Duan and Plisetskaya 1993; Duan 1997, 1998). The increase in circulating GH level during fasting is due to the significant decrease in the binding capacity of hepatic receptors to GH (Gray et al. 1992). This leads to reduced hepatic responsiveness to GH, in which hepatocytes become resistant to the effects of GH, thus decreasing IGF-I production in spite of high levels of GH (Thissen et al. 1999; Pierce et al. 2005a, 2005b). For these reasons, the detection
of IGF-I is gaining more appeal as an index of growth in several species of fish.

Our previous study on *O. niloticus* indicates that three weeks exposure to warm temperature (≥28°C) dramatically increased the fish’s hepatic IGF-I level compared to that of fasted fish and fish reared at a lower (≤24°C) temperature (Vera Cruz et al. 2006). To evaluate the possibility of using hepatic IGF-I as instantaneous or rapid growth indicator in this species of fish we evaluated the effect of increased temperature at shorter duration of exposure on the rates of growth and hepatic IGF-I mRNA expression.

**MATERIALS AND METHODS**

**Fish**

All male Nile tilapia (*O. niloticus*) obtained from Aquasafra, Inc., Bradenton, Florida were maintained in 1700-l circular fiberglass tanks at the Marine Biology Laboratory, Florida International University (FIU). Fish were reared in continuously aerated fresh water under natural photoperiod (14 h light: 10 h dark) and were fed a commercial (AquaMax) pelleted diet once a day. All fish care and experimental procedures were reviewed and approved by FIU’s institutional Animal Care and Use Committee (protocol number 02-018).

**Experimental procedure**

Fish were randomly distributed into 20 aquaria of 64-liter volume at one fish per aquarium. Fish were fed to satiation once a day. Water temperature was maintained below 24°C for a period of 12 days (Fig. 1), and then the water temperature was gradually increased to 30°C within a day in 15 aquaria. The water temperature was maintained at 30 ± 1°C by a thermostatically controlled heater. During the same period, five 64-liter aquaria were kept at a water temperature of < 24°C. Standard length and body weight of 20 fish were recorded at the beginning of the experiment and prior to temperature change. Afterwards, standard length and body weight of 5 fish were recorded 2, 5 and 7 days after the water temperature changes. Two, five and seven days after exposure to warm temperature, five fish were anesthetized with tricaine methanesulfonate (MS-222) and the hepatic tissue was rapidly removed from each fish and frozen in liquid nitrogen prior to RNA extraction. During the same collection time liver samples were also collected from fish maintained at < 24°C. The fish’s specific growth rate (SGR), in terms of weight and length, and condition factor (CF) were calculated as:

\[
SGR (weight; \%\text{-day}^{-1}) = \left[ \frac{\ln W_f - \ln W_i}{t} \right] \times 100
\]

where \( W_i \) is the initial body weight (g), \( W_f \) is the final body weight (g) and \( t \) is the growth time (days);

\[
SGR (length; \%\text{-day}^{-1}) = \left[ \frac{\ln L_f - \ln L_i}{t} \right] \times 100
\]

where \( L_i \) is the initial standard length (cm), \( L_f \) is the final standard length (cm) and \( t \) is the growth time (days);

\[
CF = \frac{BW}{L^3} \times 100
\]

where \( BW \) is the body weight (g) and \( L \) is the standard length (cm).

**RNA isolation and cDNA synthesis**

Total RNA from each liver sample was isolated using Trizol® (Invitrogen™, Carlsbad, California, USA) according to the manufacturer’s protocol. Sodium chloride (Sigma-Aldrich, St. Louis, Missouri, USA) and sodium citrate (Sigma-Aldrich, St. Louis, Missouri, USA) solutions were used for precipitation of glycogen in the samples. The RNA samples were treated with a DNase I kit (DNA-free™, Ambion®, Austin, Texas, USA) in two separate reactions to remove any possible genomic DNA contamination. The total RNA was quantified using the NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, Delaware, USA), and the purity was assessed by determining the ratio of the absorbance at 260 and 280 nm. The A260/A280 values of all samples ranged from 1.9–2.0. The first strand of cDNA was synthesized using the Omniscript® reverse transcriptase kit (Promega®, Madison, Wisconsin, USA). Briefly, 1 μg of total RNA was reverse transcribed in 20-μl reaction volume [10X RT buffer, 5 μM dNTP, 10 μM oligo-dT primer plus RNase inhibitor (RNasin®, Promega®)]. The reaction was carried out for 60 min at 37°C.

**Quantification of IGF-1**

Hepatic IGF-I levels were determined by TaqMan® quantitative real time polymerase chain reaction (PCR) as described by Vera Cruz et al. (2006). The real time PCR was performed on DNA Engine 2 Opticon® (MJ Research, Inc., Watertown, Massachusetts, USA), using the cycling conditions of 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for 1 min. The sequence of the probe used was 5’-TTTCAATAACCAACAGGCTATGCCC-3’. The reporter and quencher dyes for the probe were 6-FAM and TAMRA, respectively. The forward primer used was 5’-GTCTGTGGAGAGCGAGGCTTT-3’, and the reverse primer was 5’-CACGTGACCGCTTGGCA-3’. Reactions for each sample were done in triplicate with each well containing 25 μl PCR mixture composed of 10 ng cDNA template, 1X TaqMan® universal PCR master mix, 900 nM forward and reverse primers and 250 nM probe. Reactions containing template but not amplified during cDNA synthesis (No Amplification Control) were used to test for any possible genomic DNA contamination in RNA preparations. Reactions without cDNA template (No Template Control) were also included to confirm that reagents were not contaminated with carryover PCR products. A serial dilution of cDNA, with the amount ranging from 0.01 to 100 ng, was run to generate a standard curve (plot of the log of initial target copy number vs. threshold cycle) of IGF-1. The amount of IGF-I mRNA in each experimental sample was calculated by substituting the generated threshold cycle values to the equation derived from the standard curve (Bustin 2002). Values of IGF-I mRNA were then expressed relatively to the lowest sample level measured (assigned an arbitrary value of 1).

**Statistical analyses**

Data were analyzed using one-way analysis of variance (ANOVA) followed by the Fisher Least Significant Difference test to determine significant (P < 0.05) difference between means. In the post hoc test, the Bonferroni corrections of the significant level were
IGF-I mRNA response to temperature in the Nile tilapia. Vera Cruz and Brown

RESULTS

Temperature affects the appetite of the fish

Feed consumption of fish was observed to be influenced by temperature. The amount of feed given to fish reared at warm temperature was more than two times greater than that given to fish reared at low temperature with mean of 0.41 g·day⁻¹. It was observed that fish reared at high temperature had mean feeding rate of 1.25 g·day⁻¹, 1.28 g·day⁻¹ and 1.32 g·day⁻¹ for fish reared for 2, 5 and 7 days, respectively. Since feed was given slightly in excess to ensure satiation feeding, it was also observed that there was greater amount of uneaten food in fish reared at lower temperature.

Temperature effects on growth rate of the fish

At the time that fish were stocked into the experimental aquaria 12 days before the change in temperature, there were no significant differences among body weights and standard lengths among the four treatments. During the 12-day period, there was also no significant difference regarding the SGR of fish in the different treatments. Figs. 2A and 2B show the SGR of the fish at the different periods of rearing at warm temperature. Fish reared for two days at warm temperature (2D-W) had the highest mean SGR, in terms of weight (4.14% ± 0.05), which was significantly different (P < 0.05) from SGR of the other treatments. The next highest SGRs were seen in fish reared for 7 days (7D-W; 3.70% ± 0.20) and 5 days (5D-W; 3.66% ± 0.09) which were not significantly different from each other. Fish reared at cold temperature (CT) had the lowest mean SGR - weight (0.66% ± 0.05) which was significantly different (P < 0.001) from that of the other treatments. In terms of growth in length, 7D-W had the highest mean SGR (0.65% ± 0.02), followed by 5D-W (0.50% ± 0.04), 2D-W (0.32% ± 0.04) and CT (0.26% ± 0.002). All treatments were significantly different (P < 0.01) from each other except 2D-W and CT. The growth rate of fish (SGR) significantly increased with the duration of rearing at warm temperature; both in terms of weight (r = 0.66, P < 0.01) and length (r = 0.93, P < 0.001).

Temperature effects on IGF-I mRNA levels in the liver

Mean hepatic IGF-I mRNA levels in fish reared at warm temperature for 2, 5 and 7 days were elevated 1.6-, 2.5-, and 3.6-fold, respectively compared to that of fish reared at the cooler control temperature (Fig. 3). Statistical analysis showed that hepatic IGF-I levels were significantly elevated after at least 5 days of exposure to warm temperature (P < 0.01). A positive correlation was observed between days of rearing at warm temperature and hepatic IGF-I levels (r = 0.92, P < 0.001; Fig. 4); between SGR (length) and IGF-I levels (r = 0.92, P < 0.001; Fig. 5); and between CF and IGF-I levels (r = 0.55, P < 0.05; Fig. 6).
DISCUSSION

Temperature and nutrition are the two factors most often correlated with fish growth rate. Temperature is a controlling factor that governs the rate of metabolism in poikilothersms, affecting fish appetite, digestion, nutrient absorption and consequently the rate of growth (Fry 1971). Every species of fish has its own high and low temperature tolerance for survival, growth and reproduction. Temperature tolerance range for survival is wider compared to that for growth and the range for reproduction is the narrowest.

Within the optimum temperature range for growth, feed intake increases as temperature increases. This was observed in the study as fish reared at warm temperature (30 ± 1°C), which is within the optimal range for growth of 26 to 32°C, have higher food consumption than fish reared at the cooler temperature (< 24°C). The lower food intake of fish reared at < 24°C was supported by the lower feeding rate and a pattern of greater amounts of uneaten food visible in the aquaria.

The increase in appetite may be attributed to the increased metabolic rate in fish. As temperature increases, metabolic rate increases exponentially and at any given temperature, the difference between the amount of feed consumed and metabolic rate determines the energy available for growth (Jobling 1994). Since temperature influences the metabolic rate and appetite of the fish, growth of fish reared at warm temperature is believed to be a consequence of the combined effects of increased temperature and nutrition.

Water temperature or the direction of change in temperature has been found to influence environmental control of fish growth through an endocrine mechanism. In this study, increased temperature significantly elevated hepatic IGF-I levels and growth rate within 7 days and rearing in warm water produced a significant positive correlation between growth and hepatic IGF-I. In coho salmon, Beckman et al. (2004) similarly observed a significant positive relationship between growth and plasma IGF-I when the fish were reared in warm water but they observed disruption of associations between growth and plasma IGF-I for at least 4 weeks during a temperature decrease from 10 to 7°C. In Atlantic salmon, McCormick et al. (2000) observed a significant increase in plasma IGF-I within 7 days of increased photoperiod at 10°C and IGF-I remained elevated for at least 5 weeks. Similar day length but at a temperature of 2°C, however, resulted in a lower magnitude of increase in IGF-I which lasted for shorter duration. The last two studies provide evidence that low temperature limits the fish’s physiological response to environmental and nutritional changes.

The direct biochemical effect of lowering the temperature might alter the linear association between IGF-I and growth rate (Beckman et al. 2004).

Based on our data, two days exposure to elevated tem-
perature (2D-W) resulted in the highest SGR in terms of weight but 7D-W had the significantly highest SGR in terms of length. This could be explained by the transient differences in weight due to the amount of feed consumed, although this was not necessarily used for basic metabolism or growth. Since feed was given in excess, the fish in the 2D-W treatment might have consumed more food than they actually needed, with gut content changes transiently increasing IGF-I levels. This was hypothesized since similar water temperature presumably leads to an increase in circulating IGF-I. The significant increase in hepatic IGF-I mRNA levels in tilapia (Uchida et al. 2000) in response to temperature change (5 days at 30 ± 1°C). This suggests that mRNA levels in tilapia (Uchida et al. 2000) have a profound effect on circulating and hepatic IGF-I levels observed in coho salmon following a temperature decrease was not observed in response to a temperature increase. Meton et al. (2000) also observed a gradual increase in hepatic IGF-I levels in reared sea bream (Sparus aurata).

CONCLUSION

The significant increase in hepatic IGF-I mRNA level in this study for just 6 days post-initiation of change in water temperature (5 days at 30 ± 1°C), validated the concept that hepatic IGF-I levels can be used as a short-term growth rate indicator for O. niloticus. The rapid and measurable change in IGF-I gene expression and the strong correlation between temperature and the quantity of IGF-I mRNA confirm its validity as an indicator. These results are in agreement with the observation by Larsen et al. (2001) of a significant increase in hepatic-derived plasma IGF-I in coho salmon within a week after a temperature increase from 2.5 to 10°C. The short duration involved in the significant change in IGF-I mRNA levels was also observed by Chauvigne et al. (2003) in rainbow trout muscle, when refeeding increased IGF-I mRNA levels by eightfold in myotomal muscle after 4 days. McCormick et al. (2000), on the other hand, observed elevated plasma IGF-I in Atlantic salmon within 7 days of increased photoperiod at 10°C. These studies indicate that both systemic and local IGF-I synthesis is promoted significantly for a short period by nutrition, and by environmental temperature and photoperiod.

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