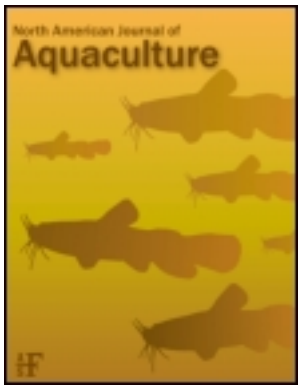

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COMMUNICATIONS

Effect of Fasting on Pituitary Growth Hormone Expression and Circulating Growth Hormone Levels in Striped Bass

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Abstract.—The mechanisms controlling the circulating levels of growth hormone (GH) during periods of starvation in fish are not well defined. In this study, the effect of fasting on GH release and pituitary messenger RNA (mRNA) expression in striped bass *Morone saxatilis* was examined. Over a 4-week period, the body mass of striped bass fed to satiety increased 22.9%, while that of fasted fish decreased 17.3%. The plasma GH concentrations of fed fish remained low throughout the experiment, but those of fasted fish increased significantly ($P < 0.001$) after 2 and 4 weeks. The striped bass that were fasted for 2 weeks demonstrated a 33% increase ($P = 0.10$) in GH mRNA expression compared with those fed to satiety. These data confirm that short-term fasting in striped bass increases their circulating levels of GH in a manner similar to that experienced in other species of fish. This study also demonstrates—for the first time in a fish species—that pituitary GH mRNA expression increases during fasting, which suggests a corresponding increase in pituitary GH synthesis. Further research will be necessary to determine whether a direct correlation exists between increased expression and increased release during starvation.

Growth hormone (GH) research has expanded significantly in recent years, and GH has emerged as a multifunctional hormone involved in numerous physiological processes. In teleosts, GH functions in the regulation of growth (Donaldson et al. 1979), osmoregulation (Komourdjian et al. 1976; Clarke et al. 1977; Miwa and Inui 1985; Borski et al. 1994), and reproduction (Stacey et al. 1984; Singh et al. 1988; Le Gac et al. 1992; Singh and Thomas 1993). Research in recent years has also begun to define the nutritional regulation of GH action.

In many fish species, starvation results in increased plasma GH concentrations. Numerous researchers have reported increased levels of circulating GH among salmonids when they are deprived of food (Wagner and McKeown 1986; Barrett and McKeown 1989; Sumpter et al. 1991; Kakisawa et al. 1995; Rand-Weaver et al. 1995; Johnsson et al. 1996). Despite an increase in circulating GH levels, Duan and Plisetskaya (1993) found no change in pituitary GH content of coho salmon *Oncorhynchus kisutch* after a 28-d fast. Marchelidon et al. (1996), however, not only observed increased levels of circulating GH in European eels *Anguilla anguilla* that were fasted for 3 months but also a significant increase in pituitary GH content.

The relationship between pituitary GH content and GH synthesis and release is not straightforward. More direct observations of the effect of fasting on pituitary GH synthesis in fish have not been previously reported, and the mechanisms that lead to fasting-induced increases in circulating GH levels are not clear. In an effort to better understand these mechanisms, the present paper reports on the effect of fasting on growth, circulating GH levels, and pituitary GH messenger RNA (mRNA) expression (an index of synthesizing capacity) in striped bass *Morone saxatilis*.

Methods

Experimental animals.—Striped bass were obtained from the University of Maryland Crane Aquaculture Facility, USA, and maintained in 2,200-L circular tanks as previously described (Small and Soares 1998). All fish were injected with a passive integrated transponder for individual identification and fed a common commercial feed for 2 weeks prior to experimentation. The striped bass were anesthetized by immersion in 0.2

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g/L MS-222. Blood samples were collected from the caudal vasculature by inserting a 21-gauge needle attached to a disposable syringe coated with heparin (1,000 units/mL). The blood was immediately centrifuged and plasma was collected and stored at −20°C. Pituitaries were collected from anesthetized striped bass following decapitation and flash frozen in liquid nitrogen and stored at−80°C. All animals were used in accordance with guidelines established by the University of Maryland Animal Care and Use Committee.

Experimental fasting.—To verify the effect of starvation on plasma GH concentrations, 36 striped bass of approximately 270 g were equally distributed and acclimated to two tanks. At the start of the experiment, all the fish were individually weighed and blood was collected. Over the next 4 weeks, fish in one tank were fed to satiety twice daily and the remaining fish were fasted. In an effort to avoid the stressful effects of repeated sampling, nine fish from both treatment groups were randomly selected for weighing and blood collection after 2 weeks. The remaining nine fish were similarly sampled after 4 weeks. To determine the effect of short-term starvation on pituitary GH expression, eight striped bass (distributed and acclimated to two tanks such that four were fed to satiety and four were fasted) were sacrificed for pituitary collection and RNA isolation after 2 weeks.

Growth hormone assay.—The plasma GH levels were analyzed using a heterologous radioimmunoassay (RIA) validated for striped bass blood plasma. Recombinant growth hormone (*Acanthopagrus butcheri*, DSL-R01203) was purchased from GroPep Pty., Ltd. (Adelaide, Australia) for use as the standard, and anti-GH polyclonal antibody (PAG1) was used as the primary antibody at the manufacturer's recommended dilution of 1: 20,000. The RIA of serially diluted striped bass plasma pooled from 4-week fasted fish (n = 9) resulted in a displacement curve that was parallel to the recombinant GH standard curve (Figure 1). Nonspecific binding represented 3% of total radioactivity. Intraassay (n = 4) and interassay (n = 6) coefficients of variation were less than 10%. The accuracy of the RIA, estimated as the percent recovery of exogenous *A. butcheri* GH added at varying levels to striped bass plasma, was greater than 90% (n = 4). Assay sensitivity was calculated as the concentration of GH equal to B_0 minus two standard deviations when interpolated from the standard curve. The minimum detectable level in striped bass plasma was 1.15 ng/mL.

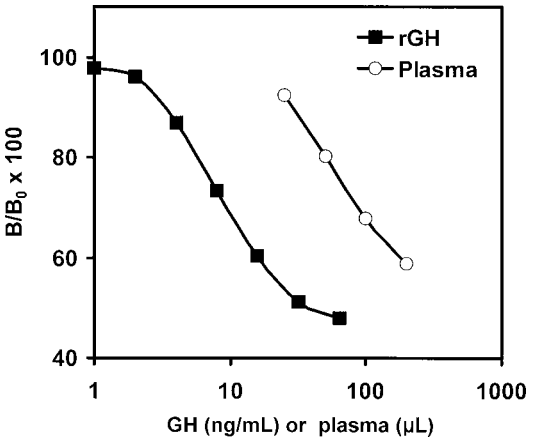

FIGURE 1.—Displacement curves for growth hormone standards (rGH) and serially diluted striped bass plasma. Each point is the mean of duplicate determinations. B/B_0 represents the percent of labeled GH bound.

RNA extraction and Northern blot hybridization.—Total RNA was prepared from individual, whole striped bass pituitaries using the RNeasy total RNA kit (Quiagen, USA). Ten micrograms of total RNA were used for gel electrophoresis. The electrophoresis and Northern blotting were conducted according to Kevil et al. (1997). A 341-bp partial complementary DNA (cDNA) for striped bass growth hormone was generated by reverse transcriptase polymerase chain reaction (RT-PCR) using primers designed from the nucleotide sequence of striped bass GH (172–512 bp; Cheng et al. 1995) and utilized as a probe for hybridization. The β-actin probe was composed of a 306-bp partial cDNA generated by RT-PCR using primers designed from the nucleotide sequence of striped bass β-actin (310–615 bp; GenBank L36342). Both probes were synthesized to a specific activity of at least 1×10^9 cpm/μg using the Ready-to-Go Random Primer Oligo kit (Amersham Pharmacia, Inc., USA) and purified using Sephadex G50 spin columns. Denatured probes were added to Rapid-hyb buffer (Amersham Pharmacia, Inc., USA) and hybridized at 65°C for 2 h. After hybridization, membranes were washed two times successively in 0.5× SSC (sodium chloride–sodium citrate)/0.1% SDS (sodium dodecyl sulfate) at 15-min intervals. Membranes were then wrapped in plastic wrap and exposed to film overnight at room temperature. The resultant bands after autoradiography were quantified by densitometry (Molecular Dynamics, USA) and expressed as arbitrary densitometric units (ADU).

Statistical analysis.—All statistical analyses

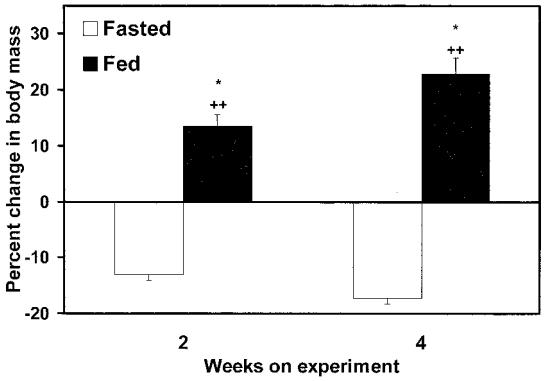

FIGURE 2.—Percent change in body mass of striped bass fed or fasted for 2 and 4 weeks (mean ± SE; n = 9). Two plus signs indicate significant ($P < 0.0001$) differences compared with fasted fish, and asterisks indicate significant ($P < 0.0001$) interactions between fasting and time.

were conducted using the SAS system (SAS Institute 1996). Plasma GH concentrations and ADU were subjected to analysis of variance mixed-model procedures. For statistical analysis, plasma GH levels determined to be below the sensitivity of the assay were conservatively set at the minimum detectable level (1.15 ng/mL). The assumptions of homogeneity of variance and normality of the data were tested by an examination of correlation and the Shapiro–Wilk W test of normality.

Results

Striped bass fasted for 4 weeks lost an average of 17.3% body mass, whereas fed fish gained 22.9% body mass in the same time period (Figure 2). After 2 weeks of fasting, significant differences in plasma GH concentrations ($P \leq 0.0001$) were detected between fasted and fed striped bass (Tables 1, 2). Plasma GH levels continued to increase and nearly doubled over the next 2 weeks, with 4-week concentrations averaging 4.21 ng/mL in fasted fish. Increased circulating GH concentrations corresponded with increased pituitary GH mRNA expression. The results of Northern blot hybridization demonstrated a 33% increase ($P = 0.10$) in pituitary GH mRNA expression of fasted fish when compared with fish fed to satiety for 2 weeks (Figure 3).

TABLE 1.—Mean (±SE) plasma growth hormone (GH) concentrations of striped bass fed or fasted for 2 and 4 weeks (n = 9). ND = below assay sensitivity (<1.15 ng/mL).

Condition	Plasma GH (ng/mL)
Initial concentration	
Fed	ND
Fasted	ND
2-week concentration	
Fed	ND
Fasted	2.24 ± 0.27
4-week concentration	
Fed	ND
Fasted	4.21 ± 0.45

TABLE 2.—Results of analysis-of-variance mixed-model procedures for plasma growth hormone concentrations of striped bass fasted for 2 and 4 weeks.

Effect	P
Fasting	0.0001
Time	0.0006
Fasting × time	0.0006

Discussion

After 2 weeks of fasting, striped bass plasma GH concentrations increased to 2.24 ng/mL, whereas plasma levels in fed striped bass remained below assay sensitivity. By 4 weeks of fasting, plasma GH concentrations of fasted bass averaged 4.21 ng/mL. In rainbow trout *O. mykiss* Kakisawa et al. (1995) and Johnsson et al. (1996) also observed significant increases in the plasma GH concentrations as a function of fasting duration, demonstrating a two- to threefold increase above plasma GH levels of fed fish at 2 weeks. In the present

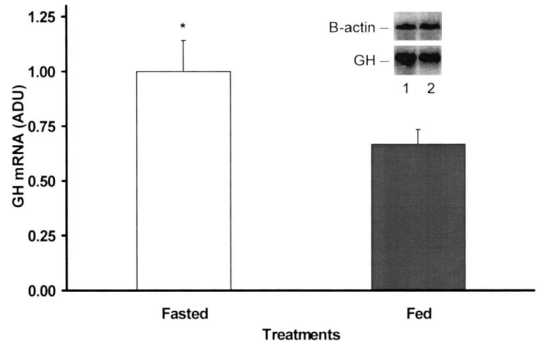

FIGURE 3.—Growth hormone messenger RNA (GH mRNA) in the pituitaries of striped bass fed or fasted for 2 weeks. The upper panel shows a representative Northern blot hybridization signal (lane 1 = fasted fish; lane 2 = fed fish). The lower panel shows the mean ± SE of arbitrary densitometric units (ADU) of all observations (n = 4). Values obtained for GH were normalized to the values obtained for β-actin in the same lane. The asterisk indicates a significant ($P = 0.10$) difference compared with fed fish.

study, plasma GH concentrations nearly doubled again between 2 and 4 weeks after fasting was induced. Duan and Plisetskaya (1993) reported a fourfold increase in the plasma GH levels of coho salmon after 4 weeks of fasting, and in European eels starved for 3 months, plasma and pituitary GH levels were found to increase by 160% and 38%, respectively (Marchelidon et al. 1996). Thus the fasting induced increase in circulating GH observed in striped bass is very similar to that reported for other fish.

In the present study, increased levels of circulating GH were found to correspond to increased pituitary GH synthesis, as indicated by increased mRNA expression. GH mRNA expression in the pituitaries of striped bass fed to satiety was 67% of the levels found in the pituitaries of fasted fish. Thomas et al. (1990) reported a similar effect following a period of restricted feeding in sheep. In addition to increased plasma GH concentrations and GH pulse amplitude, sheep fed half the normal ration for 20 weeks exhibited a three- to four-fold increase in pituitary GH mRNA content compared to levels in normally fed animals. In rats, however, pituitary GH expression decreases as a result of feeding below 50% of ad libitum intake (Rodríguez et al. 1995). The rat differs from most vertebrates in its response to fasting as starvation also decreases its plasma GH concentrations (Cambell et al. 1977). By contrast, the fasting of striped bass resulted in increased GH gene expression, similar to sheep (Thomas et al. 1990), and increased circulating GH concentrations, similar to the majority of species studied, including rabbits (McIntyne and Odell 1974), pigs (Antinmo et al. 1978), sheep (Driver and Forbes 1981), cows (Blum et al. 1985), and man (Ho et al. 1988).

The mechanism controlling GH synthesis and release in starved fish is likely very similar to that of most vertebrates. The fasting and dietary protein restriction of sea bream *Archosargus rhomboidalis* resulted in increased circulating GH levels and decreases in hepatic GH-receptors (GHRs; Pérez-Sánchez et al. 1994, 1995). In coho salmon, a decrease in hepatic GHRs (Gray et al. 1992) followed by a decrease in insulin-like growth factor I (IGF-I) mRNA expression (Duan et al. 1995) is thought to decrease the negative feedback inhibition of GH circulation by circulating IGF-I (Björnsson 1997); IGF-I has been shown to directly inhibit GH release while stimulating somatostatin (SRIF) secretion (Clemmons and Van Wyk 1984). Somatostatin is a potent antagonist for the release of GH, whereas growth hormone-releasing hormone (GHRH) stimulates GH release and synthesis from the anterior pituitary (see Harvey et al. 1995). The complete mechanism underlying fasting-induced increases in circulating GH concentrations is not fully understood; it may be due, in part, to an increase in pituitary sensitivity to GHRH, perhaps through a reduction in inhibitory SRIF tone, or to increased GHRH release and GHRH receptor synthesis. Increased pituitary sensitivity to GHRH would, in turn, stimulate an increase in GH synthesis (as indicated by the fasting-induced increase in pituitary GH mRNA expression observed in this study) and could lead to a corresponding increase in GH release into circulation.

Conclusions

In this study, it was demonstrated that striped bass, like other teleosts, exhibited a significant increase in circulating GH levels in a time-dependent manner. While several factors may be involved in the elevation of plasma GH levels associated with fasting, the corresponding increase in pituitary GH mRNA expression, as observed in this study for the first time in a fish species, indicates a potential correlation with increased pituitary GH synthesis. Further research—both in vitro and in vivo—is needed to address the relationships between gene expression and protein synthesis and release under various physiological conditions in fish.

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