Seasonal change in circulating growth hormone (GH) hepatic GH-binding and plasma insulin-like growth factor 1 immunoreactivity in a marine fish gilthead sea bream Sparus aurata

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Seasonal changes in circulating growth hormone (GH), hepatic GH-binding and plasma insulin-like growth factor-I immunoreactivity in a marine fish, gilthead sea bream, *Sparus aurata*

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Abstract

We have studied the seasonal relationship between growth and circulating growth hormone (GH), hepatic GH-binding and plasma insulin-like growth factor-I immunoreactivity in gilthead sea bream, *Sparus aurata*. The seasonal increase in plasma GH levels preceded by several weeks the summer increase in growth rates. In contrast, a marked increase in hepatic GH-binding with a high degree of endogenous GH occupancy was found during the period of maximum growth which suggests an enhanced sensitivity of liver to GH action. Thus, circulating levels of immunoreactive IGF-I, probably derived from the liver in response to GH action, were positively correlated with growth throughout the experimental period although a consistent relationship between growth and circulating GH was not found. In spite of this, we consider that, in gilthead sea bream, as in several other teleosts, the availability of endogenous GH can limit growth. Thus, under environmental conditions of suboptimal growth, a single intraperitoneal injection of recombinant rainbow trout GH (rtGH) induced over the dose range tested (0.75, 1.5, 3 μg g BW^{-1}) an increase in plasma IGF-I-like immunoreactivity comparable to that seen during the period of maximum growth.

Introduction

Pituitary growth hormone (GH) is part of the hormonal cascade that has a major influence on somatic growth. Its growth-promoting action is indirect and largely mediated through the generation of the insulin-like growth factor-I (IGF-I), which may act as an endocrine, paracrine and autocrine hormone (Isaksson *et al.* 1987; Holly and Wass 1989).

The feasibility of GH treatment to improve somatic growth has been demonstrated in a wide variety of vertebrates, including teleostean fish (Donaldson *et al.* 1979; Weatherley and Gill 1987; Danzmann *et al.* 1990). However, it cannot be concluded that GH treatment stimulates growth in all intact fish, as the administration of exogenous GH in gilthead sea bream, *Sparus aurata*, does not significantly accelerate growth under the conditions tested to date (Cavari *et al.* 1993; Donaldson, personal communication). A further complication is that frequently circulating levels of GH are not positively correlated with the growth rate of growing fish (Clarke *et al.* 1989; Marchant and Peter 1986; Stefansson *et al.* 1991; Sumpter *et al.* 1991; Sumpter 1992) and in long-term fasted fish, the loss of weight is accompanied by a marked increase in GH levels (Farbridge and Leatherland 1992). Nevertheless, this rise in GH levels promotes insulin

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resistance and lipolysis (Minick and Chavin 1970; Leatherland and Nuti 1981; Sheridan 1986; Sheridan *et al.* 1986), both being protective metabolic changes during catabolic states. Similarly, the accompanying fall in hepatic IGF-I mRNA and circulating IGF-I activity (Komourdjian and Idler 1978; Duan and Hirano 1992; Gray *et al.* 1992; Pérez-Sánchez *et al.* 1994) seems to be an essential permissive event to allow muscle catabolism and to yield metabolic fuels during acute and prolonged dietary restriction.

The aim of this work is to gain better understanding about the role of GH in growing fish. For this purpose, in gilthead sea bream, we have examined the seasonal relationship between growth and circulating GH, hepatic GH-binding and plasma IGF-I-like immunoreactivity. Additionally, we have determined the effect of GH treatment on plasma IGF-I-like immunoreactivity to evaluate whether the elevation of circulating GH could be effective in increasing gilthead sea bream growth under environmental conditions of suboptimal growth.

Materials and methods

Experiment 1: Seasonal growth

On October 10, 1991, four hundred gilthead sea bream ranging between 50 and 100 g were transferred to two experimental tanks with a volume of approximately 4,000 l. The seawater flow was 20 l min^{-1} in each tank. Photoperiod followed the natural daylength (40° 5′ N; 0° 10′ E). Water temperature decreased from 18°C in November, 1991, to 10°C in February 1992, and then increased progressively achieving a maximum level of 25°C in August, 1992. Fish were fed by hand, twice daily with dry pellets (55% proteins, 12% lipids, 15% carbohydrates). Feeding ratio (0.7–1.9% BW day^{-1}) was adjusted to temperature and fish size, maintaining a level near to satiety.

On November 13, 1991, and January 14, March 16, May 20, June 28, August 8, September 18, 1992, ten fish from each tank were rapidly removed and quickly anesthetized (MS-222, Sigma). Blood was collected from the caudal vessels into heparinized tubes. The samples were centrifuged at 3,000 × g for 20 min; plasma was stored at −20°C for subsequent analyses. The livers were removed and immediately processed to obtain suitable membrane preparations for GH-binding assays (Pérez-Sánchez *et al.* 1994). The remaining fish were measured for fork length to the nearest 0.1 cm, and weighed to the nearest 0.1 g. Specific growth rate (SGR) was calculated as $\ln(Wt_1/Wt_0) \times 100 \times day^{-1}$, where Wt_0 and Wt_1 are initial and final mean weights for each tank. Protein efficiency ratio (PER) was calculated as live weight gain/protein intake.

Experiment 2: GH treatment

Juvenile gilthead sea bream, weighing about 100 g, were allocated in 8 circulating seawater tanks under natural conditions of temperature (11–13°C) and photoperiod (February-March). Recombinant rainbow trout (*Oncorhynchus mykiss*) GH ($rtGH_{II}$ lot BP 19), generously provided by Eurogentec (Liège, Belgium), was the test substance. Just before administration, rtGH was dissolved in 0.9% NaCl containing 0.1% bovine serum albumin (BSA). Three treatments corresponding to three different doses (0.75, 1.5, 3 μg g BW^{-1}) of injected hormone (200 μl) were assayed. Each treatment was replicated by randomly assigning 2 tanks of 30 animals per tank. Control fish (2 tanks) received an equal volume of saline containing 0.1% BSA. All fish from a given tank were lightly anaesthetized with MS-222, and intraperitoneally injected in less than 10 min with a repeater dispenser fitted to a 23 G needle. The first five injected fish from each tank provided plasma samples for the zero time, and the remaining fish were returned to their respective aquaria for subsequent sampling over a period of 24 h. Blood was collected from the caudal vessels into heparinized tubes and centrifuged at 3,000 × g for 20 min; plasma was stored at −20°C until analyses.

GH radioimmunoassays

Plasma levels of endogenous GH were determined by a homologous radioimmunoassay (RIA) under disequilibrium conditions. The protein used for iodination and for standards was a gilthead sea bream GH (sbGH) purified from pituitary extracts. The

usual sensitivity of the assay was 0.2 ng ml^{-1} and the ED_{50} 2.5 ng ml^{-1} (Le Bail *et al.* 1993).
Plasma levels of rtGH were determined by a double antibody RIA under disequilibrium conditions. The protein used for iodination and for standards was the same as that injected into experimental fish. The first antiserum was raised in New Zealand rabbits against chinook salmon, *Oncorhynchus tshawytscha*, GH (Le Bail *et al.* 1991b). The usual sensitivity of the assay was 0.2 ng ml^{-1} and the ED_{50} 3.1 ng ml^{-1}. Undiluted plasma from non-treated gilthead sea bream caused a low displacement (8–10%) of the antibody-bound labelled rtGH.

GH radioreceptor assay

GH-binding was determined in liver homogenates using ^{125}I-sbGH as a tracer (Pérez-Sánchez *et al.* 1994). Before GH-binding assays, liver membrane preparations were treated with 4 M $MgCl_2$ for 10 min to remove endogenous ligand and to provide a measure of the total GH receptors. Free receptors were assayed simultaneously in water-treated homogenates. The amount of binding sites (B_{max}) was calculated from X-intercepts in Scatchard plots.

IGF-I radioimmunoassay

IGF-I-like immunoreactivity was measured by a double antibody RIA under disequilibrium conditions. A recombinant human IGF-I (rhIGF-I), generously provided by Ciba-Geigy (Basel, Switzerland), was used for iodination and for standards. As a first antibody, we used a rabbit antiserum against hIGF-I (UB3-189) which was prepared by Drs. L. Underwood and J.J. Van Wyk (University of North Carolina at Chapel Hill, USA) and made available through the National Hormone and Pituitary Distribution Program (Baltimore, MD, USA). The cross-reactivity of insulin and human IGF-II in the IGF-I assay is lower than 0.5%. The usual sensitivity of the assay was 0.2 ng ml^{-1} and the ED_{50} 2.5 ng ml^{-1}. Plasma samples were prepared for assay by an acid Sephadex C-25 extraction procedure (Niu *et al.* 1993). As it has been described elsewhere, this method removes IGF binding proteins from gilthead sea bream plasma, being the displacement curve of serial dilutions of extracted plasma parallel to rhIGF-I standard curve (Pérez-Sánchez *et al.* 1994).

Statistical analysis

All data were analyzed using one-way analysis of variance. Multiple comparisons among means were made with the Duncan Multiple Range test when the analysis of variance value was significant ($p < 0.05$). Analysis of covariance was used to determine the relationship between growth and IGF-I-like immunoreactivity. Data of circulating GH, hepatic GH-binding and plasma IGF-I-like immunoreactivity from tanks of the same treatment were combined into one group at each sampling time. Preliminary data analysis indicated this to be a valid assumption (one-way analysis of variance, $p < 0.05$).

Results

Experiment 1

Table 1 shows the overall growth characteristics of juvenile gilthead sea bream under natural conditions of photoperiod and water temperature. The lowest SGR was found in fish sampled in March. At this moment, the SGR was significantly different from SGRs at all other times of the year. The SGRs measured in January and May were similar and were also significantly different from the SGRs recorded at all other times of the year. The highest SGR was found in fish sampled in early August. Similar values were attained in fish sampled in September and late June. Feeding rates were low in fish sampled in January, March and May. At these three times, the feeding rates were sinificantly different from those attained in fish sampled in late June, August and September. The lowest PER was found in fish sampled in March. A significant in-

Table 1. Seasonal changes in weight, length, feeding ratio (FR, % BW day^{-1}), specific growth rate (SGR, % BW day^{-1}), and protein efficiency ratio (PER, weight gain/protein intake). Initial weight (W_o) and initial length (L_o) for each sampling period. Each value is the mean of two replicates. For SGR and PER, values with the same letter are not significantly different ($p < 0.05$)

Sampling period	W_o(g)	L_o(cm)	FR	SGR	PER
11/13/19–1/14/92	80.83	13.27	1.00	0.25[a]	0.45[a]
1/14/92–3/16/92	94.25	14.50	0.72	0.11[b]	0.27[b]
3/16/92–5/20/92	101.39	14.97	0.66	0.25[a]	0.68[c]
5/20/92–6/28/92	121.40	15.81	1.60	0.73[c]	0.82[d]
6/28/92–8/ 8/92	165.12	17.13	1.90	0.89[c]	0.85[d]
8/ 8/92–9/18/92	241.15	19.52	1.70	0.77[c]	0.82[d]
9/18/92–	328.10	21.5			

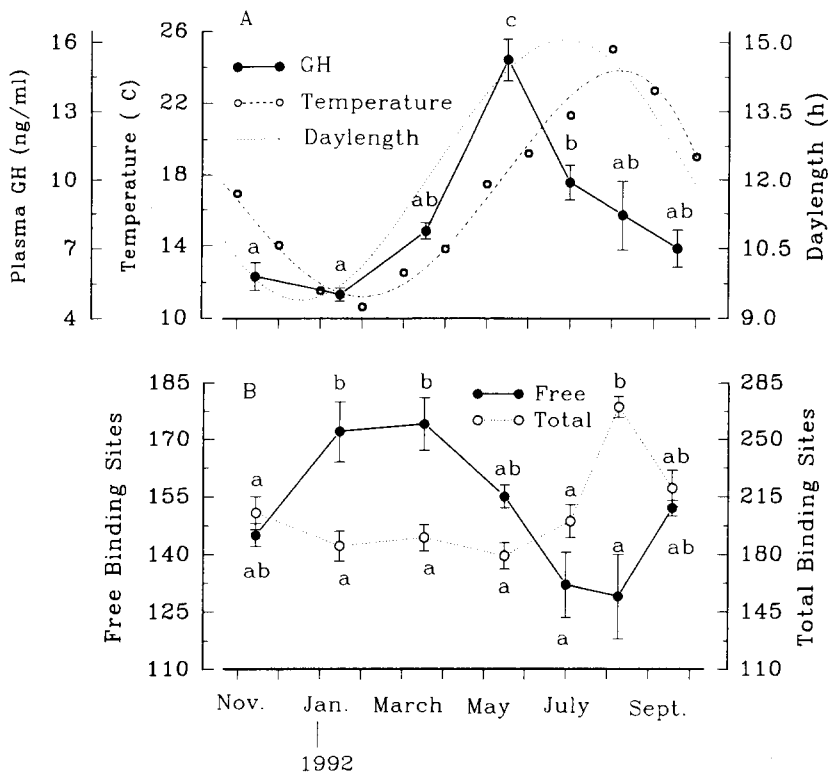

Fig. 1. Seasonal variations in daylength, water temperature and plasma GH levels (A). Seasonal variations in free and total hepatic GH-binding sites (fmol mg protein^{-1}) (B). Each value (plasma GH and GH-binding) is the mean ± SEM of 14–19 separate determinations. Values with the same letter are not significantly different ($p < 0.05$).

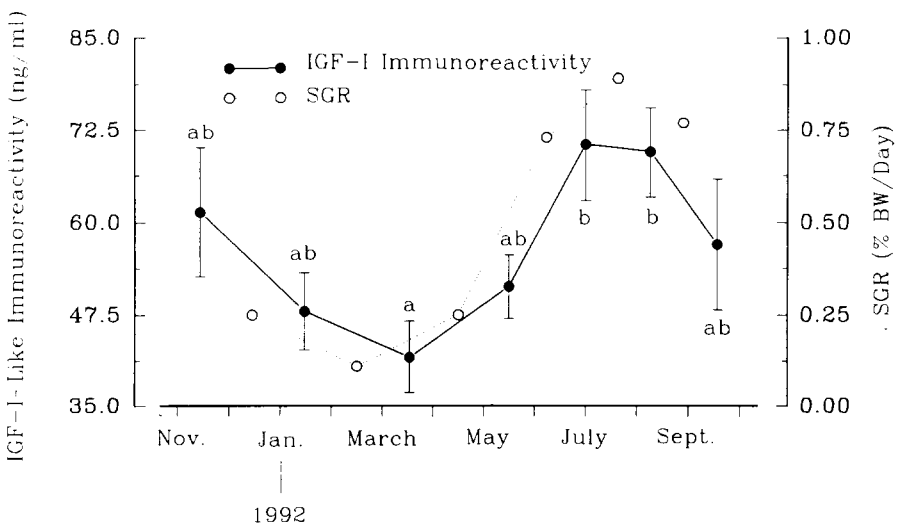

Fig. 2. Seasonal variations in plasma IGF-I-like immunoreactivity. Each value is the mean ± SEM of 13–18 separate determinations. Values with the same letter are not significantly different ($p < 0.05$). Specific growth rates (SGR) are plotted in the same graph through the sampling period, instead of the sampling date.

crease in PER was observed in May, peaking in the summer period.

Variations throughout the year in plasma GH levels and hepatic GH-binding are shown in Figure 1. The lowest level of plasma GH was observed in January and a significant and progressive increase was found in March and May, following the day-length cycle rather than the water temperature cycle. A progressive and significant decrease in plasma GH levels was observed in late June, August and September (Fig. 1A). Significant variations in hepatic GH-binding sites were also observed (Fig. 1B). The highest amount of free binding sites was found in fish sampled in January and March, and the lowest level in late June and August. Conversely, the highest amount of total binding sites was observed in August.

Figure 2 shows the seasonal variations in plasma IGF-I-like immunoreactivity. The lowest level was found in fish sampled in March. A significant and progressive increase was observed in May and late June, tending to decrease in September. Analysis of covariance revealed a significant and consistent parallelism ($p < 0.05$) between plasma IGF-I-like immunoreactivity and SGR, taking into account that the latter are calculated from the sampling periods, instead of the sampling dates.

Experiment 2

Figure 3 shows the plasma decrease of exogenous GH after a single intraperitoneal injection of rtGH over the dose range tested (0.75, 1.5 and 3 μg rtGH g BW^{-1}). The disappearance curve followed a exponential function, which can be approximated by a two-compartment model (data not shown). The four-fold increase in the rtGH dose resulted in a proportional increase of plasma rtGH at each time after injection. This direct relationship between the injected dose and the resulting plasma concentration was defined as follows:

$$C = (234.3 \times R) / T^{1.11}$$

where C is the plasma concentration of rtGH (ng ml^{-1}), R is the injected dose (μg g body $weight^{-1}$, and T is the time after injection (min).

Figure 4 depicts the effect of GH treatment on the profile of plasma IGF-I-like immunoreactivity. The increase in the rtGH dose resulted in a proportional increase in plasma IGF-I-like immunoreactivity. In all cases, plasma IGF-I-like immunoreactivity increased significantly at 1.5 h postinjection, peaked at 3 h, and decreased progressively at 6, 10 and 24 h postinjection following the decrease in circulating levels of rtGH.

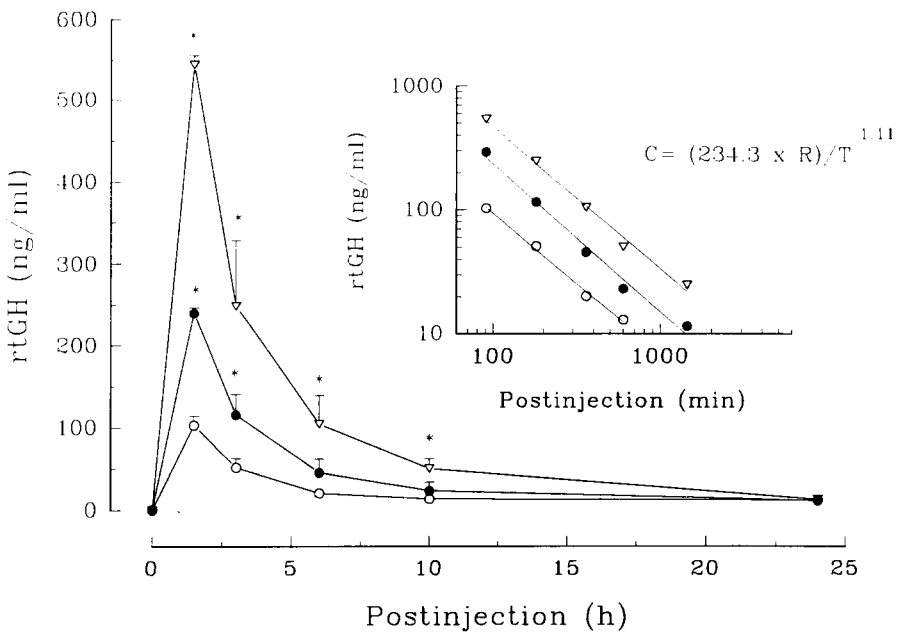

Fig. 3. Plasma levels of exogenous GH after a single intraperitoneal injection of recombinant trout GH (rtGH); (o—o) 0.75 μg g BW^{-1}, (●—●) 1;5 ,μg g BW^{-1}, (▽—▽) 3 μg g BW^{-1}. Each value is the mean ± SEM of 8–10 separate determinations. *Significantly ($p < 0.05$) different from the low dose ($p < 0.05$). The insert shows the relationship between the injected dose and the resulting plasma concentration; C is the plasma concentration of rtGH (ng ml^{-1}), R is the injected dose (μg g BW^{-1}), and T is the time after injection (min).

Discussion

Seasonal variations in the rate of body growth appear to be universal in teleostean species found in temperate climatic zones (Ricker 1979). Early studies in Atlantic salmon (*Salmo salar*) indicate that major changes in the growth rate are positively correlated with histological changes in somatotrop cells (Komourdjian *et al.* 1976, 1989). However, subsequent studies have evidenced a complex and some times controversial relationship between growth and circulating GH. Thus, a concurrent increase in plasma GH, hypoosmoregulatory ability and growth rates has been reported in smoltifying Atlantic salmon (Björnsson *et al.* 1989; Prunet *et al.* 1989) and coho salmon (*Oncorhynchus kisutch*) (Young *et al.* 1989). However, an obvious fail of the positive correlation between growth and GH has been reported in the seawater postsmolts (Stefansson *et al.* 1991;), with concurrent high growth rates and low GH levels. A notable inverse situation was found in stunted Atlantic and coho salmon, in which growth is negligible whereas GH levels are greatly increased (Bolton *et al.* 1987; Björnsson *et al.* 1988). In addition, in goldfish (*Carassius auratus*), the seasonal peak in circulating GH levels occurred in spring to early summer whereas the maximum growth was found in July (Marchant and Peter 1986). Similarly, in gilthead sea bream, we observed that the highest circulating level of GH was attained in May whereas the highest growth rate was achieved during the summer period.

Further, when growth selected lines of rainbow trout are compared, the slower growing line displays higher circulating GH concentrations (Sumpter 1992). The paradox of lower circulating GH in association with faster growth or larger body weight has been described in other vertebrate species, including those for which a large growth-promoting effect of GH has been demonstrated (Bacon *et al.* 1987; Goddard *et al.* 1988; Norton *et al.* 1989). A possible explanation may be that the growth-promoting action of GH is dependent on factors other than circulating GH, such as clear-

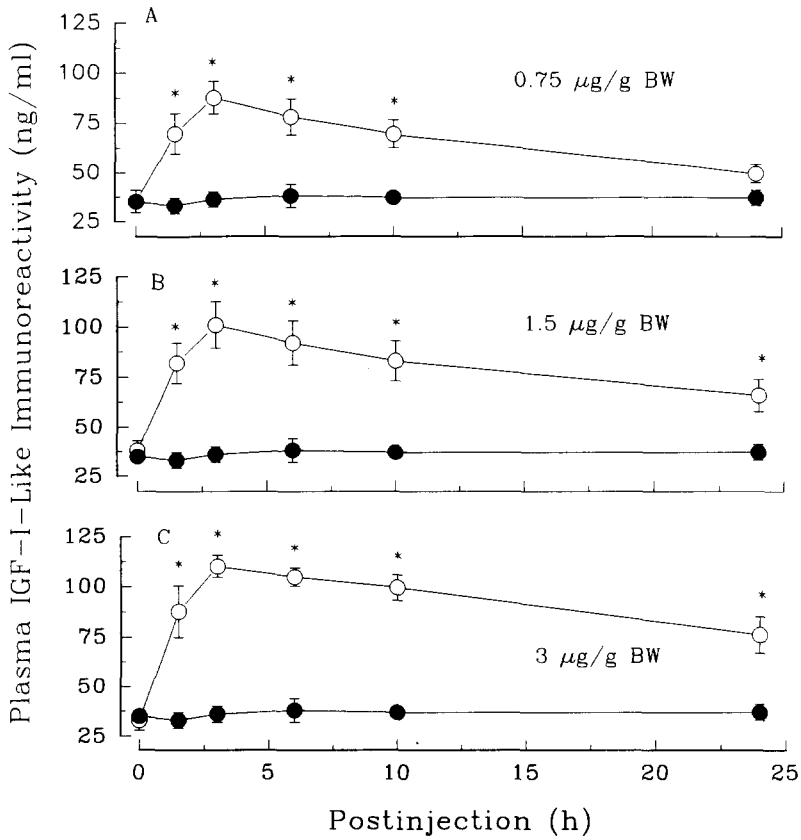

Fig. 4. Effect of a single intraperitoneal injection of recombinant trout GH (rtGH) on plasma IGF-I-like immunoreactivity. Control fish (●—●), GH treated fish (o—o). Each value is the mean ± SEM of 8–10 separate determinations. *Significantly ($p < 0.05$) different from controls.

ance rates, receptor density and interactions with other hormones and growth factors. Thus, in mammals, it is generally accepted that long-term fasting provokes a fall in hepatic GH-binding (Maes *et al.* 1983; Straus and Takemoto 1990), whereas food intake is associated with an increase in the number and affinity of hepatic GH receptors (Breier *et al.* 1988). In agreement with these findings, we have demonstrated in a previous gilthead sea bream work that fasting is associated with a loss of hepatic GH-binding and plasma IGF-I-like immunoreactivity (Pérez-Sánchez *et al.* 1994). In contrast, after several weeks of enhanced food intake, we observed in the present study a marked increase in hepatic GH-binding with a high degree of endogenous GH occupancy (total/free hepatic GH-binding). The concurrent and significant decrease in circulating GH levels presumably reflects and enhanced sensitivity of liver to GH action. This balanced relationship would explain the positive correlationship between circulating immunoreactive IGF-I levels and growth rate values throughout the experimental period, since the former could be mainly derived from the liver in response to GH action. A similar relationship has been reported in mammals and avian species not only on a temporal basis (Merimee *et al.* 1981; Eigenmann *et al.* 1984), but also when comparisons are made between growth selected lines (Blair *et al.* 1988; Scanes *et al.* 1989).

The precise mechanism for the above regulation of GH secretion and hepatic GH-binding remains to be clarified. However, considering the known inhibitory action of IGF-I on pituitary GH release (Pérez-Sánchez *et al.* 1992), it seems likely that the concurrent increase in growth and circulating IGF-I

provokes an enhanced GH feedback inhibition, resulting in lowered plasma GH levels during the summer period. In contrast, according to Marchant and coworkers (1989) the decreased levels of circulating GH during the winter period could reflect an enhanced inhibitory tone of hypothalamic somatostatin. Under these conditions, we have demonstrated that the administration of exogenous GH can induce an increase in plasma IGF-I-like immunoreactivity comparable to that seen during the maximum period of growth. However, other studies do not support such a conclusion, since Cavari and coworkers (1993) failed to demonstrate a consistent effect of GH treatment on gilthead sea bream growth.

This discrepancy may reflect the fact that the amount of GH injected is not adjusted for growth. In salmonids, the lowest effective dose often ranges between 0.1 and 1 μg/g/week (Weatherley and Gill 1987; Le Bail *et al.* 1991a). In this work, it appears conclusive that in gilthead sea bream a single intraperitoneal injection of rtGH (0.75 μg/g) drops below the effective concentration after 2 days. Given the differences in terms of GH source and gilthead sea bream strains, an absolute comparison of results is difficult. However, taking into account that the affinity of rtGH for fish GH receptors is much higher than that reported for mammalian GHs (Pérez-Sánchez *et al.* 1994), it is not surprising that a positive effect of mammalian and avian GH preparations (1 μg/g/2 weeks) was not observed in previous gilthead sea bream studies (Cavari *et al.* 1993).

In summary, this work provides evidence for the converse regulation of GH secretion and hepatic GH receptors which would explain, at least in part, the apparent dissociation between growth and circulating GH. Additionally, we propose that in gilthead sea bream as in several teleosts exogenous supply of GH may improve growth performance.

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