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Review

Somatotropic regulation of fish growth and adiposity: growth hormone (GH) and somatolactin (SL) relationship ☆

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Abstract

Growth hormone (GH) and insulin-like growth factors (IGFs) play a major role in fish development and metabolism, and several studies have allowed discernment of a complex and tissue-specific collection of salmonid IGF-I transcripts (Ea-4, Ea-3, Ea-2, Ea-1), which are the result of the alternative splicing of the E-domain region. However, the pattern of IGF-I expression is different in non-salmonid fish, and only one or two transcripts (Ea-4, Ea-2) have been detected in hepatic and extrahepatic tissues of common carp, barramundi, black sea bream and gilthead sea bream. Despite this, when comparisons are made within Mediterranean fish species (European sea bass, common dentex and gilthead sea bream), plasma IGF-I levels are consistent with fish species differences in growth rates. Changes of growth rates, and plasma IGF-I and GH levels are also found in response to changes in diet composition and ration size, which may serve to assess the suitability of feeding regimes in aquaculture practice. Regulation of plasma somatolactin (SL) levels is also examined in gilthead sea bream, and the resulting plasma SL profile differs from that of GH. Thus, in contrast to GH, plasma SL levels augment with the increase of ration size and fish size (advancement of age). A transient increase in plasma SL levels is also found in short-term fasted fish, and this fish peptide may act as an anti-obesity hormone helping to expedite growth–reproductive processes following replenishment of fat stores, and/or mediate the adaptation to fasting until the lipolytic action of GH and/or other endocrine factors is fully accomplished. This agrees with the known increase of plasma SL levels during acute stress and exhaustive exercise. However, a causal link between SL and energy mobilisation (lipid metabolism) remains to be established, and further research is needed to determine the extent to which SL and GH act in a complementary manner to make available metabolic fuels and to regulate body fat mass and feeding behaviour.

Keywords: Growth; Feed intake; Adiposity; Insulin-like growth factor-I; Growth hormone; Leptin; Somatolactin; European sea bass; Common dentex; Gilthead sea bream; Perciform fish

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1. Introduction

Information from both internal and external stimuli is processed and integrated for the appropriate regulation of growth through hormonally mediated pathways, the central circuit being the growth hormone (GH) and insulin-like growth factor-I (IGF-I) axis. Thus, transfer and overexpression of piscine GH transgenes has resulted in a marked enhancement of salmonid growth, with a 3–10-fold increase of body weight gain (Devlin, 1997). However, more modest effects (1.2–1.8-fold increase) have been reported in other fish species, such as common carp (Chen et al., 1993), crucian carp (Zhu, 1992), zebrafish (Zhao et al., 1993) and tilapia (Martínez et al., 1996). These different growth responses may be attributed to the source and structure of GH construct (e.g. exclusion of signal peptide), and changes in the copy number and site of integration. In addition, the biology of fish dramatically differs from one species to another, and it is likely that GH acts to different degrees as a growth limiting factor. This is consistent with fish species differences in plasma GH levels (see Pérez-Sánchez and Le Bail, 1999), although in fish and most higher vertebrate species nutritional deprivation leads to increased plasma GH levels, which suggests that the primary cause of growth arrest is the tissue resistance to GH action rather than a limited availability of endogenous GH (Duan et al., 1994; Dickhoff et al., 1997). This is also true for overfed fish, and some increase of plasma GH levels in association with a diminished feed conversion efficiency and a high degree of adiposity has been reported in juvenile gilthead sea bream fed to visual satiety with high energy diets (see Pérez-Sánchez, 2000). Major changes in plasma somatolactin (SL) levels also occur with the increase of ration size, and this mini-review focuses on the nutritional regulation of SL and GH/IGF-I axis in Mediterranean fish species, exploring the physiological significance of the opposing patterns of plasma SL and GH levels.

2. Alternative IGF-I splicing

Genomic analyses reveal that two non-allelic IGF-I genes exist in chum salmon (Kavsan et al., 1993, 1994) and chinook salmon (Wallis and Devlin, 1993), which is in agreement with the tetraploid nature of salmonid genome (Allendorf and Thorgaard, 1984). A major IGF-I mRNA with lengths of 3.9–4.2 kb has been detected in Northern blots, but more sensitive methods (RT-PCR assays) have allowed discernment of a complex and tissue-specific collection of salmonid IGF-I transcripts, designated now as Ea-1, Ea-2, Ea-3 and Ea-4 (Duguay et al., 1992; Shamblott and Chen, 1993; Wallis and Devlin, 1993). This IGF-I mRNA heterogeneity is the result of the differential splicing of the E-domain region, which is finally cleaved during the post-transcriptional processing. Thus, IGF-IEa-1 codes for a prohormone with a 35-amino acid E-domain; IGF-IEa-2 contains a 36-bp insertion and codes for a prohormone with a 47-amino acid E-domain; IGF-IEa-3 and IGF-IEa-4 contain 81- and 117-bp insertions, respectively. Several studies evidence that Ea-1 and Ea-3 transcripts primarily occur in the liver under the systemic regulation of GH, insulin and nutritional state, and it has been hypothesised that the resulting mature protein mainly acts as an endocrine factor. In contrast, Ea-4 transcripts take place in a wide variety of tissues, and they may have the potential for an autocrine–paracrine function not mediated by short term changes in systemic factors (see Duan, 1998). However, the pattern of E-domain transcripts is different in non-salmonid fish, and further studies are needed to reveal whether the E-domain of proIGF-I has its own specific function in fish species. Thus, only Ea-2 transcripts have been detected in the liver of common carp (Liang et al., 1996; Hashimoto et al., 1997), whereas the Ea-4 transcript seems to be the most abundant form in the liver of black sea bream (Chen et al., 1998). Ea-4 transcripts are also present in a large extent in hepatic and non-hepatic tissues of barramundi, whereas Ea-2 transcripts are expressed at a low level in liver and are hardly detectable in non-hepatic tissues (Ståhlbom et al. 1999).

The above findings are in agreement with our gilthead sea bream studies, in which two sets of primers were employed to amplify and fully resolve small size differences of IGF-I mRNAs at the 5′ (from 16 bp upstream the predicted start codon to the C-domain) and 3′ (from the C-domain to 10 bp upstream the predicted stop codon) end regions (Fig. 1). PCR products were resolved on 8% polyacrilamide gels, and no detectable band was found by silver staining in the absence

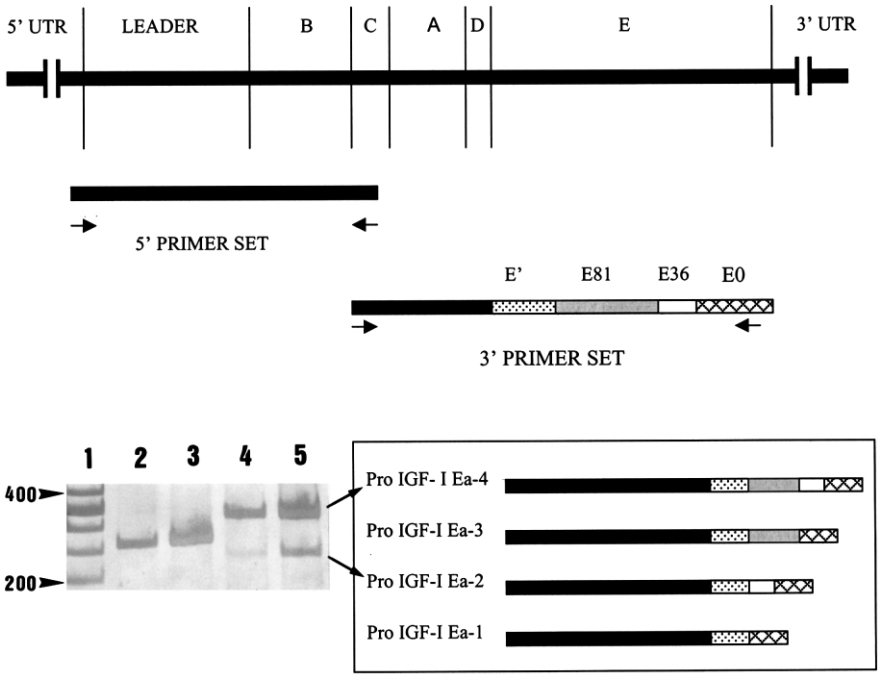

Fig. 1. Diagrammatic representation of PCR strategy to amplify gilthead sea bream cDNAs coding for IGF-I. Total RNA (2–5 μg) was reverse transcribed with 200 units of Superscript II (GIBCOBRL), using oligo $(dT)_{17}$ as an anchor primer. The resulting product was treated with RNAse H (2 units) prior to PCR amplification (35 cycles with denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min) of target cDNA in a final volume of 50 μl. The reaction mix included 0.1% Triton X-100, 50 mM KCl, 10 mM Tris–HCl (pH 9), 0.5 mM of each dNTP and DNA primer, and 2.5 units of a hot start DNA polymerase (Promega). PCR (1/5) products were resolved on 8% polyacrilamide gels, and visualised by silver staining. Lane 1: 50-bp molecular marker; lanes 2 and 3: PCR amplification with the 5′ primer set of skeletal muscle and liver cDNAs; lanes 4 and 5: PCR amplification with the 3′ primer set of skeletal muscle and liver cDNAs.

of reverse transcriptase in the RT-PCR procedure. Only one size transcript was detected with the 5′ primer set (sense: 5′ TTC GCC GGG CTT TGT CTT; antisense: 5′ CGT GCA TTG GGG CCG TAG), but two size forms corresponding to Ea-4 and Ea-2 trancripts were generated with the 3′ primer set (sense: 5′ GCT ACG GCC CCA ATG CA; antisense: 5′ CCC GCG TTG CCT CGA CTT). Ea-4 transcripts seem to be the constitutive form, and they were largely expressed in liver and skeletal muscles of juvenile and adult fish. In contrast, Ea-2 transcripts were expressed at a relatively high level in the liver, but they were hardly detectable in the skeletal muscle, which remains both in salmonids and gilthead sea bream non-responsive to GH treatment (Duguay et al., 1996).

3. Systemic regulation of GH and IGF-I levels

The key role of IGFs in fish growth and development is now recognised, and the expression of IGFs and IGF receptors has been reported during embryonic and larval development (Funkenstein et al., 1997; Perrot and Funkenstein, 1999). From available data, it is difficult to determine whether fish species differences in growth rates also reflect a different activity of the GH–IGF axis. However, it is now accepted that hyperinsulinemia induced either by arginine or insulin injection is accompanied by increases in plasma IGF-I levels (Baños et al., 1999). Unlike insulin, most circulating IGFs bind to specific binding proteins (IGFBPs), which prolong half-life of IGFs, prevent their insulin-like activity and control their availability to target tissues. In mammals, most IGFs exist as a 150-kDa complex containing IGF-ligand, binding protein (BP-3) and an acid-labile subunit (ALS) (Jones and Clemons, 1995). The presence of this large complex in the non-mammalian circulation is under discussion, but different binding forms (40–20 kDa) have been detected in salmonid (Niu and Le Bail,

1993) and non-salmonid fish (Fukazawa et al., 1995; Siharath et al., 1996; Duan et al., 1999; Park et al., 2000), and a clear understanding of the putative interference of IGFBPs in plasma/serum IGF assays becomes a necessity. In some RIA procedures currently used in salmonids, IGF-II quantification in fasted rainbow trout is lower in extracted than in non-extracted plasma (Gentil et al., 1996), whereas other authors indicate that IGF-I measures before and after extraction are equal (Moriyama et al., 1994). When sea bream IGF-I and barramundi IGF-I antiserum (GroPep, Australia) are used to asses IGF-I levels in Mediterranean perciform fish, IGF-I measures before and after acid–Sephadex C-25 extraction are of the same order of magnitude. However, the outcome of the assay is sometimes compromised in unrestricted fed fish (serial dilutions of plasma samples does not parallel well with the standard curve), and IGFs are currently extracted prior the RIA procedure. In this way, when juvenile European sea bass, gilthead sea bream and common dentex were fed to visual satiety with a 47% protein and 17% lipid diet (see Company et al., 1999b), plasma IGF-I levels (70–150 ng/ml) were consistent with fish species differences in growth rates (Fig. 2). Furthermore, in gilthead sea bream, concurrent changes in growth rates and plasma IGF-I levels were achieved as a result of the partial replacement in the diet of fish meal by vegetable proteins. Using fish meal diets, a twofold increase of plasma IGF-I levels was also observed with the increase of ration size. The lowest IGF-I level (50–70 ng/ml) was found in fish fed at the lowest ration size (30% full ration size), but a plateau was achieved in fish fed at the intermediate ration size (60% full ration size) in coincidence with the best feed conversion ratio (1.05). This suggests that circulating IGF-I is a function not only of growth rates but also of feed conversion. However, in agreement with Plisetskaya (1998), it would be of interest to standardise piscine IGF-I assays, and to determine the amount of IGF-I that circulates as a free form. In a recent study in coho salmon, Shimizu et al. (1999) estimated free IGF-I as approximately 0.3% of the total amount of IGF-I. These authors failed to demonstrate a significant change in the ratio of free-to-total IGF by either GH treatment or fasting, but more detailed experiments are needed to reveal how free IGF-I is regulated by additional physiological states and treatments.

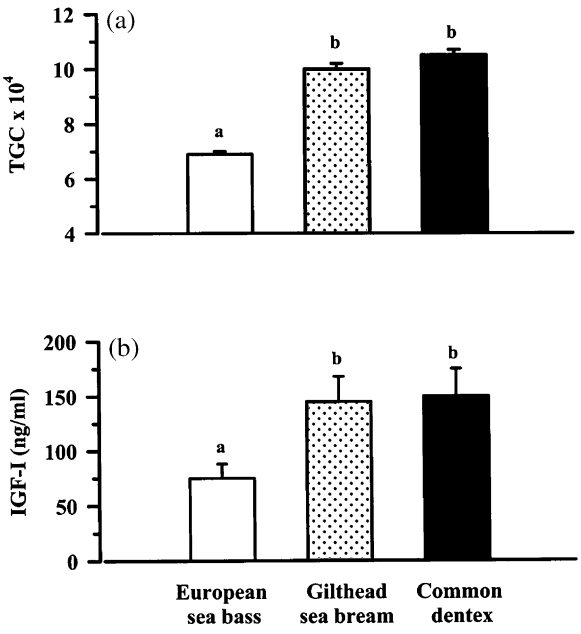

Fig. 2. Fish species differences in thermal-unit growth coefficients (a) and plasma IGF-I levels (b). Thermal-unit growth coefficient was calculated as $TGC = (W_f^{1/3} - W_o^{1/3}) \times (\Sigma D^o)$, where W_o and W_f are the respective initial and final fish weight, and ΣD^o is the thermal sum (experimental days × average temperature measured at 10.00 h). Growth trials (7 weeks) were carried out in summer under natural photoperiod and temperature (22–26°C) conditions. Initial body weight of European sea bass, gilthead sea bream and common dentex was 11.6, 17.5 and 8.5 g, respectively. Growth rates are the mean ± S.E.M. of 2–3 tanks (30 fish per tank). Plasma IGF-I levels are the mean ± S.E.M. of 12–18 animals. Anti-barramundi IGF-I was used as a first antibody (1:8000) in the IGF-I assay. The non-specific binding was lower than 0.5%, and the midrange of the assay (ED_{50}) was 1.2–1.5 ng/ml. Different letters indicate fish species differences at $P < 0.05$ (Student–Newman–Keuls test).

A strong link between energy status and circulating plasma GH levels also exists in fish, and the loss of hepatic GH receptors and circulating IGF-I levels is a characteristic feature of catabolic states, which induces the increase of circulating GH levels probably due to the lack of the negative feedback effect of IGF-I on pituitary GH release (see Pérez-Sánchez, 2000). This state is reversed by feed intake, but in comparison to salmonids, circulating GH concentration in gilthead sea bream varies more gradually and reflects in a more accurate manner changes in diet composition and ration size. Thus, given a fixed ration size (restricted fed fish), juveniles of gilthead sea bream fed with high-energy diets exhibited the lowest plasma GH concentration. However, an

opposite trend was found in unrestricted fed conditions, and fish fed with high energy diets exhibited not only higher plasma GH levels but also a higher energy expenditure, as evidenced by the increased loss of body fat mass following food deprivation (Company et al., 1999a). Taking into account all this, GH (via its lipolytic action) may increase energy supply in a state of negative energy balance, but at the same time protects adipose tissue and other organs and tissues from the excessive lipid deposition when energy is largely available. Thus, most phenotypes of human obesity are associated with reduced plasma GH levels, and there is now increasing evidence that the GH–IGF axis should include leptin feedback. In fact, leptin has a stimulatory action upon the mammalian hypothalamic–GH axis, whereas directly or indirectly GH treatment inhibits the synthesis and release of leptin (Heiman et al., 1998), which has been considered a fuel gauge that broadcasts the level of energy storage to mediate lipid metabolism trough the central nervous system or directly on peripheral tissues (Reidy and Weber, 2000). A recent study revealed that a long-term treatment of immature coho salmon with human leptin has no clear effect on

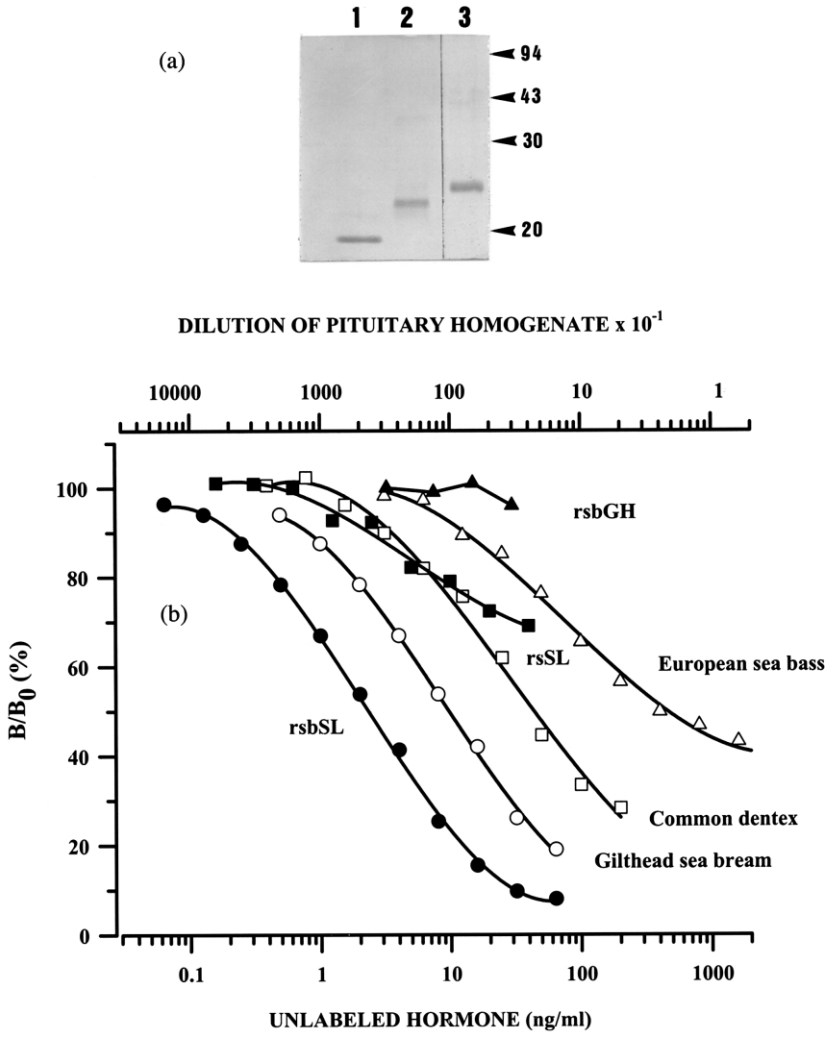

Fig. 3. (a) Silver staining of SL and GH preparations. Lane 1: recombinant gilthead sea bream GH (non-reducing conditions); lane 2: recombinant gilthead sea bream SL (non-reducing conditions); lane 3: recombinant gilthead sea bream SL (reducing conditions). (b) Competitive displacement of ^{125}I-rsbSL bound to rabbit antiserum by serial dilutions of pituitary hormones (recombinant gilthead sea bream SL, rsbSL; recombinant sole SL, rsSL; recombinant gilthead sea bream GH, rsbGH) and pituitary homogenates (1 pituitary/ml) of European sea bass, common dentex and gilthead sea bream.

growth, energy stores or plasma hormone levels (Baker et al., 2000), and it is likely that the function of this endocrine factor in fish species may not be characterised until the issue of its structural variation in vertebrate phylogeny is resolved, and homologous proteins are made available. Despite extensive efforts with degenerated primers, we and other authors failed to amplify by RT-PCR a fish leptin-like sequence. However, screening of fish tissues with murine antisera against leptin revealed that an immunoreactive band is present in blood, brain, heart and liver of several fish species, and their intensities are threefold higher in fed fish than in fasted fish, which is consistent with mammalian models of leptin function (Johnson et al., 2000). Leptin-like immunoreactivity has also been detected in the gastric mucosa of rainbow trout (Muruzábal et al., 2000), but major efforts are needed to fully corroborate the existence of fish leptin.

4. GH and SL relationship

Fish SL is expressed in the pars intermedia of the pituitary gland of all species studied to date. This new member of GH and prolactin (PRL) family does not show any significant homology to any other known mammalian peptide (Ishibashi and Imai, 1999), but it is related in structure to the wide family of helical cytokines, which includes, among others, mammalian and avian leptins. The number of SL amino acid residues is within a narrow range (204–209) with a strict conservation of seven Cys residues, six of which will be involved in disulfide bonding. The overall amino acid identity among perciform, pleuronectiform, scorpaeniform, salmoniform and gadiform SLs is higher than 70%, and it decreases to 60% when comparisons are made with SLs of primitive fish. It appears therefore that fish SL is highly conserved among distant fish orders and the reconstruction of the phylogenetic tree, based on SL nucleotide sequences, shows the same clustering as the presently proposed hierarchy of fish species (Company et al., 2000).

The physiological function of this protein remains, however, a matter of discussion, although it is likely that SL is involved in multiple and even overlapping functions with the other members of the GH/PRL family (e.g. immune function). Thus, there is now increasing evidence that GH and PRL exert an immunostimulatory action both in fish and higher vertebrate species, which may serve to counteract negative immunoregulatory signals as a result of stress, illness and/or energy imbalance (Dorshkind and Horseman, 2000). The presence of specific GH receptors has been demonstrated in erythroid, lymphoid and myeloid

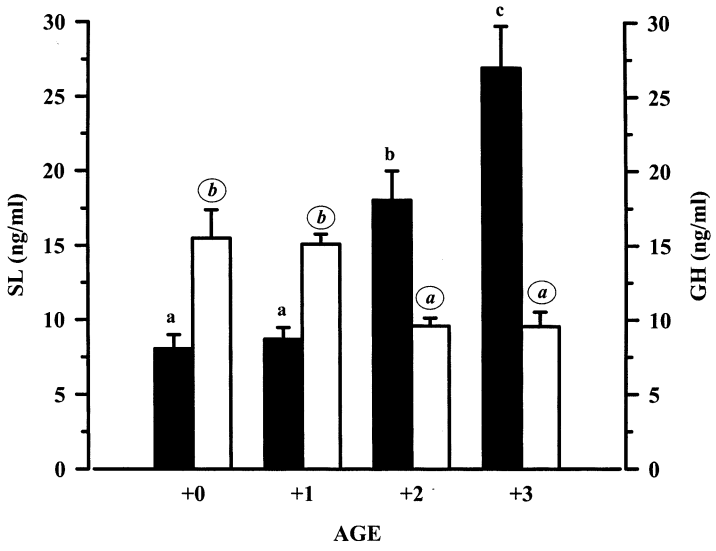

Fig. 4. Concurrent changes in plasma SL (black bars) and GH (white bars) levels with the advancement of age (years). Each value is the mean ± S.E.M. of 12–15 animals. Correlation analyses on individuals were made by Pearson Product Moment correlations ($r = -0.77$, $P < 0.05$). Different letters indicate significant differences in plasma SL or GH levels ($P < 0.05$, Student–Newman–Keuls test).

cells of the head kidney of gilthead sea bream (Calduch-Giner et al., 1995). GH exerts a proliferative action in primary cultures of gilthead sea bream leukocytes, acting as well this protein as a fish phagocyte-activating factor (Calduch-Giner et al., 1997). SL is also able to prime freshly isolated gilthead sea bream phagocytes, although the minimum effective dose is 5–10-fold higher than GH (Calduch-Giner et al., 1998).

A role of fish SL in background adaptation has also been suggested, and recently Zhu and Thomas (1998) demonstrated in red drum that plasma SL levels are under the inhibitory control of light received by photoreceptive organs. Nevertheless, background adaptation does not affect plasma SL levels in rainbow trout (Kakizawa et al., 1995a), and seasonal changes in SL secretion appear more closely linked to water temperature than to photoperiod (Rand-Weaver et al., 1995b). The stress-induced increase in circulating SL levels is also markedly different in two strains of rainbow trout (Rand-Weaver et al., 1993), which suggests that SL levels attained in response to a particular stimuli could be different between and within fish species. Synthesis and release of SL increase during the gonadal development of salmonids, reaching a peak at final maturation and spawning (Rand-Weaver et al., 1992; Olivereau and Rand-Weaver, 1994; Kakizawa et al., 1995b; Taniyama et al., 1999). Morphological studies reveal that GnRH-immunoreactive fibres are associated with GH and SL cells of the pituitary gland of rainbow trout (Parhar and Iwata, 1994). In addition, in Atlantic salmon, gonadectomy is able to reduce the increased SL availability of mature fish, but steroid replacement fails to abolish in a consistent manner this castration effect (Mayer et al., 1998), which suggests that other factors than gonadal development alone mediate the increased SL activity of older fish. In fact, SL producing cells are activated during the sexual maturation of *Mugil cephalus* (Mousa and Mousa, 2000), but experimental evidence does not support a reproductive function of SL in other non-salmonid fish, such as red drum and Atlantic croaker (Zhu and Thomas, 1995), or Atlantic halibut and English sole (Johnson et al., 1997).

Little is known about the regulation of SL in Mediterranean perciform fish, and bacterial expression and purification of gilthead sea bream SL has been carried out in the pET-3a vector system as previously reported for sole SL (Calduch-Giner et al., 1998). The expressed protein (24–25 kDa) was purified to homogeneity by gel filtration on a Superdex 200 column, and the resulting protein appeared as a monomer form when it was analysed by SDS-PAGE under reducing and non-reducing conditions (Fig. 3a). A rabbit antiserum against recombinant gilthead sea bream SL (rsbSL) was raised, and it was used (1:60 000) to develop a specific and sensitive RIA with rsbSL as tracer and standard (ED_{50} = 1.8–2.2 ng/ml). Recombinant gilthead sea bream GH did not cross-react, whereas a non-parallel displacement was achieved with recombinant sole SL (81% identity with gilthead sea bream SL) and pituitary

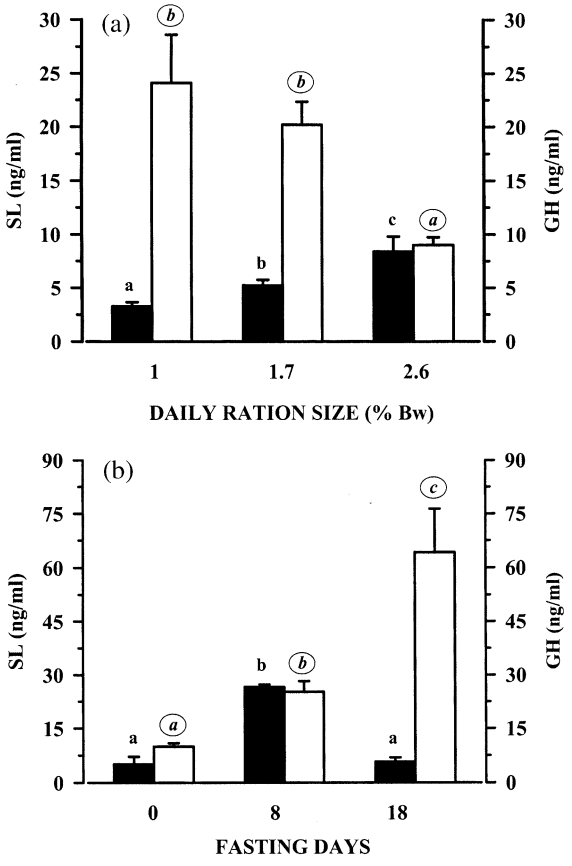

Fig. 5. Effect of ration size (a) and fasting (b) in plasma SL (black bars) and GH (white bars) levels of juvenile gilthead sea bream. Growth trials were carried in summer under natural photoperiod and temperature (22–26°C) conditions. Each value is the mean ± S.E.M. of 13–16 animals (30–80 g). In the fasting experiment, plasma SL and GH levels of continuously-fed fish did not differ from those found at the zero time (data are not included to simplify the figure). Different letters indicate significant differences in plasma SL or GH levels ($P < 0.05$, Student–Newman–Keuls test).

homogenates of European sea bass (perciform fish of Moronidae family). In contrast, serial dilutions of plasma and pituitary extracts of gilthead sea bream and common dentex (two sparid fish) parallel well with the standard rsbSL (Fig. 3b). This parallelism was also accomplished with serial dilutions of plasma samples (data non-shown), and repeated SL determinations in a plasma pool (1,9 ng/ml) gave intra- and interassay coefficients of variation of 3.1% ($n = 4$) and 5% ($n = 4$), respectively. The recovery of rsbSL added to plasma ranged between 94 and 98%, which indicates that our assay represents an useful tool to asses SL regulation in perciform fish of the Sparidae family. With these means, consistent changes in plasma SL levels were found as a function of nutritional state and fish size in a protandrous fish. Thus, in the non-reproductive season, plasma SL levels of females (3–4-year-old fish) were 3–4-fold higher than those found in males (1–2-year-old fish), whereas a marked decrease of plasma GH levels occurred with the advancement of age and sex reversal (Fig. 4). This opposite pattern of plasma GH and SL levels was also found in juvenile fish, and a 2–3-fold increase of plasma SL levels occurred with the increase of ration size in fish fed (7 weeks) with a practical diet containing 55% protein and 9% lipid (Fig. 5a). In addition, following 8 days of food deprivation, juvenile fish previously fed (4 weeks) to visual satiety showed a marked increase of plasma SL levels, with the recovery of control values at 18 days of fasting in concurrence with an 8–10-fold increase of plasma GH levels (Fig. 5b). Through 21 days of fasting, Kakizawa et al. (1995a) failed to demonstrate in rainbow trout consistent changes in plasma SL levels. However, coincident with an 18-fold increase of plasma GH concentration, plasma SL levels were twofold decreased at 16 weeks of fasting (Rand-Weaver et al., 1995a). Similarly, SL cells were hypoactive in long-term starved eels, whereas GH cells remained overstimulated (Olivereau and Olivereau 1997), which confirms that SL and GH are under a complex and perhaps different regulation in short and long-term fasted fish.

In summary, all the above findings show a complex and sometimes controversial regulation of fish SL. However, taking into account the coincident augmentation in gilthead sea bream of adiposity and plasma SL levels (as a result of the increase of ration size and fish size), it is tempting to suggest a role of SL as an anti-obesity hormone, helping to expedite growth and/or reproductive process. This is consistent with our current research on a seasonal basis, according to which a pronounced peak of SL occurs at autumn following replenishment of body fat stores and cessation of growth. Furthermore, when comparing juveniles of gilthead sea and common dentex, the latter exhibits a higher plasma SL (17 ± 1.5 vs. 8.9 ± 0.75 ng/ml) concentration in association with a lower degree of adiposity (see Company et al., 1999b). A transient increase of plasma SL levels also occurs in short-term fasted sparid fish, which may serve to mediate the adaptation to fasting until the lipolytic action of GH and/or other endocrine factors is fully accomplished. In contrast, there is evidence of an increased deposition of visceral fat in the 'cobalto' variant of rainbow trout, lacking most of the pituitary pars intermedia, which results in a drastic reduction of plasma SL levels in association with a slight but significant decrease of circulating GH and thyroid hormone levels as well (Kaneko et al., 1993). All this agrees with the known increase of plasma SL levels during acute stress and exhaustive exercise in salmonids. However, a causal link between SL and energy mobilisation (lipid metabolism) remains to be established, and future studies are needed to examine the extent to which SL and GH act in a complementary manner to make available metabolic fuels and to regulate body fat mass and feeding behaviour regardless of their involvement in reproductive and immune functions. It appears, therefore, that an integrative study of the GH/PRL family with leptins and/or other lipostat signals is an exciting and important area of research for a sustainable aquaculture practice.

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