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Seawater adaptability and hormone levels in growth-enhanced transgenic coho salmon, *Oncorhynchus kisutch*

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Abstract

Transgenic coho salmon containing a growth hormone (GH) gene construct have been examined for their hormone levels and ability to osmoregulate in sea water. Relative to their smaller nontransgenic siblings (age controls), GH-transgenic coho precociously develop external phenotypes and hypo-osmoregulatory ability typical of smolts. Specific growth rates of the transgenic coho were approximately 2.7-fold higher than older nontransgenic animals of similar size, and 1.7-fold higher than their nontransgenic siblings. GH levels were increased dramatically (19.3- to 32.1-fold) relative to size control salmon, but IGF-I levels were only modestly affected, being slightly enhanced in one experiment and slightly reduced in another. Insulin levels in transgenic animals did not differ from size controls, but were higher than nontransgenic siblings, and thyroxine levels in transgenic animals were intermediate between levels found in size and age controls. The homeostatic controls of, and interactions among, these hormones are discussed with respect to their effects on growth and osmoregulation. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Transgenic; Growth; Smolt; Osmoregulation; Growth hormone; Salmon

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1. Introduction

In the past decade, genetic engineering of fish has been explored to develop model systems for studying physiological processes, and to produce enhanced strains for improving production efficiency in aquaculture. Growth enhancement using growth hormone (GH) genes has been the aim of many experiments, with variable effects achieved in several species (Iyengar et al., 1996; Devlin, 1997). For salmonids, Du et al. (1992) were the first to demonstrate a dramatic increase in growth of transgenic Atlantic salmon (*Salmo salar*) using a gene construct, opAFPGHc, which contains a chinook salmon GH1 cDNA under the control of the ocean pout antifreeze promoter. This gene construct also causes rapid growth when introduced into coho salmon (*Oncorhynchus kisutch*), chinook salmon (*O. tshawytscha*), rainbow trout (*O. mykiss*) and cutthroat trout (*O. clarki*) (Devlin et al., 1995a,b). Another gene construct, OnMTGH1, comprised of the sockeye salmon (*O. nerka*) metallothionein promoter and GH1 gene, has also been shown to have dramatic effects on growth in salmonids (Devlin et al., 1994).

In addition to growth enhancement, elevation of circulating GH in salmon by transgenesis is anticipated to have pronounced effects on other physiological processes. Considerable data exists demonstrating the effects of somatotropins in promoting the parr-smolt transformation and seawater adaptability, increasing body size, chloride-cell function and number, and enhancing cortisol-mediated activation of hypo-osmoregulation (McCormick, 1995). GH is also known to influence other hormones, including direct stimulation of IGF-I gene activity (Duan et al., 1993) and enhancing conversion of thyroxine to T₃ (3,3',5-triiodo-L-thyronine) by increasing thyroxine 5'-monodeiodinase activity (de Luze and Leloup, 1984; MacLatchy et al., 1992). All above effects are correlated with elevated anabolic actions on cell and tissue growth that are directly involved in growth stimulation. Other hormones, such as insulin, also can have growth-stimulatory effects in fish (Mommsen and Plisetskaya, 1991). However, it is not known how elevated production of GH in transgenic fish over a sustained period influences circulating levels of these hormones and their effects on related physiological processes.

The OnMTGH1 construct has been shown to elevate plasma GH levels approximately 40-fold (Devlin, 1997; Devlin et al., 1994) in coho salmon, but interestingly, the opAFPGHc construct used in the present study did not increase GH levels significantly in Atlantic salmon (Du et al., 1992). This difference in hormone level is intriguing, and suggests that either these two gene constructs differ significantly in their abilities to produce elevated GH, or that the responses of coho and Atlantic salmon to GH transgenesis are distinct. In the present report, we examine the effects of the opAFPGHc gene construct on GH, IGF-I, thyroxine, and insulin levels, and on hypo-osmoregulatory ability in coho salmon.

2. Materials and methods

Coho salmon eggs and milt were obtained from a winter-spawning population from the Chehalis River, B.C., Canada. Founder transgenic animals (G₀) were generated by

microinjecting eggs (Devlin et al., 1994, 1995a) with the GH gene construct opAFPGHc (Du et al., 1992). F_1 animals were produced by crossing a single verified transgenic male with nontransgenic wild females from the Chehalis river. Transgenic individuals were identified by PCR using transgene-specific primers as described in Devlin et al. (1995a). Size-matched control animals (size controls) were selected to match the size of transgenic animals as close as possible with available fish, and were reared from previous year's broods under the same conditions as transgenic animals and their nontransgenic siblings (age controls). F_1 and G_0 populations of transgenic coho salmon used in this study are unrelated (except by stream of origin), and were generated in different years and from different founder fish. All transgenic fish were reared in a secure aquarium facility designed to prevent accidental escape from the laboratory.

Juveniles were reared in 10°C well water, initially indoors in 200-l tanks under simulated photoperiod, and then subsequently (for transgenic and size control animals) in 5000-l outdoor tanks. Animals in the experiment involving F_1 transgenic coho salmon were grown in fresh water for their entire life span until the time of sampling (October 6, 1993). For the experiment involving G_0 transgenics, rearing was also in fresh water except that seawater adaptability was assessed in the fall (November 28, 1994) by means of a 24-h seawater challenge test (Blackburn and Clarke, 1987). Fish were fed to satiation until the day before challenge, but not during the challenge period. After challenge, fish were anesthetized in 100 mg/l MS-222 buffered with 100 mg/l sodium bicarbonate, and blood was collected in capillary tubes coated with ammonium heparin. After centrifugation for 5 min at 4°C, plasma was collected for measurement of sodium concentrations, and separate aliquots were frozen for measurement by radioimmunoassay of GH (Swanson, 1994), total IGF-I (both bound with binding proteins and free) (Moriyama et al., 1994; Plisetskaya, 1998), insulin (Plisetskaya, 1994), and thyroxine (Dickhoff, 1993). Statistical tests were performed using the SigmaStat statistical package, using Kruskal–Wallis ANOVA on Ranks followed by Dunn's Multiple Comparison test, and correlation coefficients were determined using Pearson Product Moment correlations.

3. Results

Hypo-osmoregulatory ability and levels of several hormones were examined in growth-enhanced transgenic salmon containing the GH gene construct opAFPGHc. Because of the rapid growth of GH transgenic salmonids, nontransgenic controls for these experiments are either of a different age or different size. Thus, we routinely employ two groups of controls: nontransgenic siblings (age controls) and size-matched nontransgenic coho from the previous year's brood (size controls). Two experiments were performed, one using F_1 progeny derived from a single G_0 transgenic male crossed to regular females, and a second experiment using founder transgenic animals (G_0) that were produced from eggs microinjected with the gene construct. In both cases, transgenic animals were growing faster than nontransgenic siblings (specific growth rate (SGR) averaging 1.7-fold higher) and size controls (SGR averaging 2.7-fold higher), and

Table 1

Growth and plasma sodium levels of control and transgenic coho salmon containing the opAFPGHc gene construct (mean \pm S.E.)

Experiment	Group	<i>n</i>	Weight (g)	Length (cm)	Condition factor	Growth rate (%W/day)	Plasma Na (mM)
I. 1992 F ₁ coho	Nontransgenic	40	15.1 \pm 0.4a	10.4 \pm 0.1a	1.33 \pm 0.01a	1.88 \pm 0.01a	ND
1992 F ₁ coho	Transgenic	10	241.1 \pm 22.4b	27.4 \pm 0.7b	1.15 \pm 0.03b	3.09 \pm 0.04b	ND
1991 coho	Size controls	10	156.3 \pm 8.3b	23.1 \pm 0.3b	1.26 \pm 0.03c	1.09 \pm 0.01c ¹	ND
II. 1993 G ₀ coho	Nontransgenic	18	10.9 \pm 0.6a	9.8 \pm 0.2a	1.14 \pm 0.01a	1.50 \pm 0.02a	196.8 \pm 3.8a
1993 G ₀ coho	Transgenic	16	266.5 \pm 22.1b	28.7 \pm 0.8b	1.09 \pm 0.03b	2.72 \pm 0.04b	171.3 \pm 2.4b
1992 coho	Size controls	16	194.7 \pm 8.3b	24.4 \pm 0.2b	1.33 \pm 0.03c	1.09 \pm 0.02c ¹	167.2 \pm 0.9b

Lower case letters within each column and experiment indicate groups which differ significantly ($P < 0.05$). ND = Not determined. Nontransgenic and Transgenic individuals within each experiment are siblings, whereas size controls are nontransgenic individuals that are 1 year older.

¹Since specific growth rates (SGRs) are calculated from first feeding until sampling time, SGRs for size controls are not determined over the same interval as for transgenic and nontransgenic siblings.

had significantly lower condition factors than both control groups (Table 1). Nontransgenic sibling age controls displayed a dark coloration and prominent parr marks typical of underyearling coho salmon, whereas transgenic salmon and size controls had an appearance typical of seawater-adapted smolts, with silvery scales and dark fin margins (Fig. 1).

The transgenic animals also displayed head, jaw and opercular abnormalities typical of the effects of this gene construct in coho salmon (Devlin et al., 1995b), indicating that some imbalance in growth processes has been induced. Excessive cartilage deposition was apparent (particularly along posterior opercular margins) analogous to acromegaly in transgenic mammals (Costa et al., 1998).

Seawater adaptability was examined in the experiment involving G_0 transgenic animals. Nontransgenic siblings of approximately 10 g in size had significantly higher plasma sodium levels than nontransgenic animals, which were 1 year older (size controls), consistent with the known natural acquisition of hypo-osmoregulatory ability by coho salmon in their second year in fresh water. Transgenic salmon exhibited an

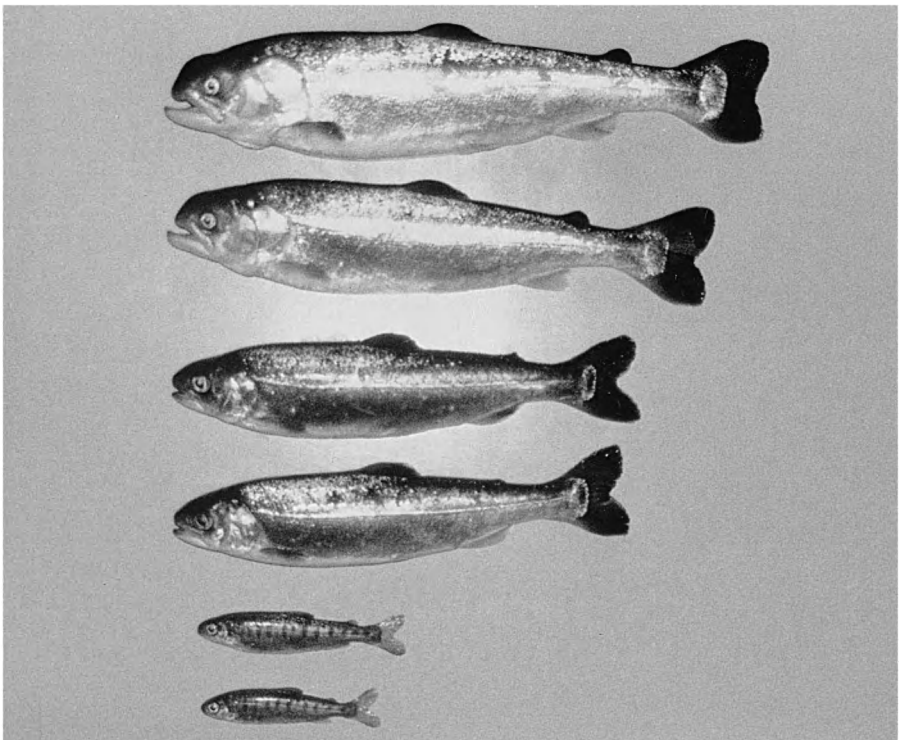


Fig. 1. Phenotypes of G_0 transgenic coho salmon (top pair), size controls (middle pair) and age controls (nontransgenic siblings) (bottom pair). Note that transgenic animals have acquired a smolt phenotype whereas nontransgenic siblings of the same age have not. Cranial abnormalities are also apparent in the transgenic animals.

ability to regulate plasma sodium levels in the seawater challenge test indicating they had acquired hypo-osmoregulatory ability in their first year in fresh water; their plasma sodium levels were not different from those of the size controls.

Levels of GH and IGF-I were examined in both experiments, and, additionally, insulin and thyroxine were examined in the experiment involving F₁ transgenic salmon (Fig. 2). Within an experiment, GH levels did not differ between nontransgenic siblings and size controls, although both groups had some individuals with GH levels below the detection threshold (0.2 ng/ml) of the assay (in the case of nontransgenic G₀ siblings, only two individuals had levels of GH above the detection limit). In contrast, GH could be detected in all transgenic animals, with average levels much higher than either type of control: For G₀ and F₁ transgenic coho salmon, GH levels were respectively, 19.3-fold and 32.1-fold higher than size controls. The levels of GH observed in transgenic animals between the two experiments also differed, with G₀ levels approximately 6-fold higher

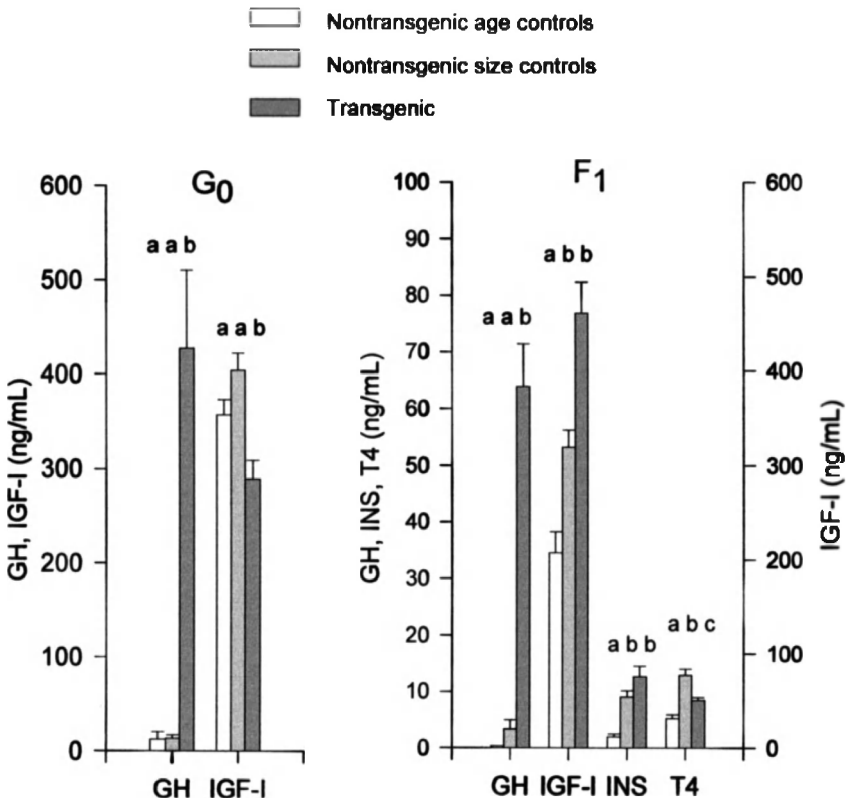


Fig. 2. Hormone levels in transgenic, size control and nontransgenic age control coho salmon. GH and IGF I levels were examined in the experiment involving G₀ transgenic animals (left), whereas GH, IGF-I, insulin (INS), and thyroxine (T4) levels were examined with F₁ transgenic and control animals (right). Different letters above bars indicates a significant difference was detected ($P < 0.05$) within an experiment. Note that the Y-axis scale for GH levels differs between the two graphs.

Table 2

A. Correlation coefficients for variables measured in G_0 opAFPGHc transgenic coho salmon (above the diagonal) and similarly-sized age controls (below the diagonal). NA indicates dependent correlations

	Weight	Length	Condition factor	GH	IGF-I	Plasma Na
Weight		0.97 * *	NA	0.32	0.06	0.41
Length	0.85 * *		NA	0.44	−0.02	0.46
Condition factor	NA	NA		−0.65 * *	0.58 * *	−0.17
GH	−0.09	0.26	−0.47		−0.52 *	0.27
IGF-I	−0.11	−0.18	0.04	0.17		−0.16
Plasma Na	0.41	0.64 * *	−0.12	0.48	−0.43	

B. Correlation coefficients for variables measured in F_1 opAFPGHc transgenic coho salmon (above the diagonal) and similarly-sized age controls (below the diagonal). NA indicates dependent correlations

	Weight	Length	Condition factor	GH	IGF-I	Insulin	Thyroxine
Weight		0.97 * *	NA	−0.03	0.27	0.36	−0.10
Length	0.91 * *		NA	0.00	0.43	0.21	−0.10
Condition factor	NA	NA		−0.39	−0.66 *	0.46	−0.15
GH	0.22	0.54	0.69		0.51	0.17	0.45
IGF-I	0.27	0.21	0.22	−0.19		−0.18	−0.02
Insulin	0.46	0.54	0.12	0.84 *	0.08		0.19
Thyroxine	0.79 * *	0.82 * *	0.33	0.41	0.16	0.59	

* $P < 0.05$.

** $P < 0.01$.

than was observed in F_1 animals (note difference in scale on Y -axis for these two groups in Fig. 2).

In contrast to the large increase observed for GH, IGF-I levels were not raised proportionately in transgenic animals. In the experiment with G_0 transgenic coho, IGF-I levels did not differ between nontransgenic siblings and size controls, whereas transgenic animals had slightly but significantly lower levels. F_1 transgenic animals had levels of IGF-I that were not different from size controls, but were significantly higher than nontransgenic age controls.

Levels of insulin did not differ between transgenic and size controls (i.e., large fish), but were significantly higher than smaller nontransgenic sibling age controls (Fig. 2). Levels of thyroxine differed among all groups examined, with transgenic values intermediate between the two control values.

Relationships among the variables were separately examined in transgenic and size control groups, and correlation coefficients are presented in Table 2A and B for the experiment involving G_0 and F_1 transgenics, respectively. Both GH and IGF-I levels did not correlate significantly with length or weight in control or transgenic animals, and GH did not correlate with IGF-I except negatively in G_0 transgenic animals. GH was also negatively correlated with condition factor in G_0 transgenics. CF correlated with IGF-I in both transgenic groups, but was inversely related in F_1 transgenics where mean IGF-I levels were higher than controls and was positively correlated in G_0 transgenics where mean IGF-I levels were lower than controls (Fig. 2).

Insulin did not correlate with other variables in transgenic animals, but was positively correlated with GH in size controls. Thyroxine was strongly correlated with size (both weight and length) in control animals, but this was not the case in transgenic coho. Plasma sodium levels were positively correlated with length in size controls but not transgenics (Table 2), and there was no significant relationship between sodium levels and plasma GH or IGF-I levels.

4. Discussion

4.1. GH levels in transgenic coho salmon

It has been well demonstrated that administration of exogenous GH and IGF-I to salmonids stimulates appetite and growth, acting by increasing feed-conversion efficiencies, muscle protein synthesis rates, organ growth, and cartilage growth (Higgs et al., 1975; Duan and Hirano, 1990; McCormick et al., 1992a; McLean and Donaldson, 1993; Fauconneau et al., 1996; Devlin et al., 1999). Previous work has shown that the transfer of GH gene constructs into salmonids enhances growth (Du et al., 1992; Devlin, 1997; Devlin et al., 1994, 1995a), and the present experiments also reveal that SGR of transgenic animals was approximately double that of controls. The same gene construct as used in the present study (opAFPGHc) did not elevate plasma GH levels significantly in Atlantic salmon (Du et al., 1992), but nevertheless did increase growth rate dramatically. In contrast, another gene construct (OnMTGH1, comprised of the sockeye salmon

MT-B promoter and GH1 gene) did result in a significant elevation of plasma GH levels in transgenic coho salmon relative to controls (Devlin, 1997; Devlin et al., 1994). In the current study, we have found that the opAFPGHc gene construct also elevates plasma GH levels in transgenic coho salmon, suggesting that the differences in GH levels observed between transgenic Atlantic and coho salmon are not due to the transgene used, but rather probably arise from differences in the biology of the two species or from differences in seasons when hormone levels were measured. Salmonids have very low growth rates and correspondingly low plasma GH levels (Prunet et al., 1989; Young et al., 1989a; Yada et al., 1991) during the fall and winter months (when our experiments were conducted). This characteristic may accentuate the apparent difference in GH levels relative to our transgenic animals, which have a seasonally uncoupled growth pattern (Devlin et al., 1994). Consequently, to better understand the role of GH, it will be important to compare levels during the entire lifespan of transgenic and control animals, particularly when control animals are in a rapid growth phase.

Differences in GH level were also observed between G_0 (founder) and F_1 transgenic animals used in the present study, which may have arisen if the selected F_1 line (derived from a single G_0 individual) was expressing the GH transgene at lower than average levels. Despite having a lower mean GH level, individuals from the F_1 line displayed a higher SGR than G_0 individuals, which had a 7-fold greater GH level. It is possible that levels of GH in both transgenic groups may be close to saturating GH receptors, and that direct effects of GH may be detrimental at excessively high levels. Previous observations have shown that transgenic individuals with very high growth rates as juveniles often develop morphological abnormalities (Devlin et al., 1995b), and ultimately such fish are not the largest obtained at sexual maturity (Devlin et al., 1995a). These results imply that GH transgenesis can elevate GH concentrations to pathological levels in salmonids, and that more modest increases may be most appropriate to obtain optimal stimulation of growth without deleterious side effects (Devlin, 1997).

4.2. Insulin-like growth factor-I

While GH may act directly on some tissues to enhance growth or stimulate reproductive tissue function (Van Der Kraak et al., 1990; Cheng and Chen, 1995; Peter and Marchant, 1995), its primary action appears to be mediated through the stimulation of IGF-I mRNA and circulating IGF-I protein levels (Funkenstein et al., 1989; Duan et al., 1993; Sakamoto and Hirano, 1993; Niu et al., 1993). Correlations between IGF-I levels and growth rate have been observed previously (Beckman et al., 1998), although another study failed to find any correlation between IGF-I levels and growth rate (Silverstein et al., 1998). In the current experiments, a significant positive correlation between GH and IGF-I was not apparent either in control or in transgenic animals (in one case, G_0 transgenic animals, a negative correlation was actually observed). In the two experiments of the present study, mean IGF-I levels relative to size controls were slightly depressed in G_0 transgenic animals (with high mean GH levels), but were slightly enhanced in F_1 transgenics (with more modest GH stimulation). These results differ from effects in transgenic mammals expressing GH transgenes where a consistent (up to a 3-fold) elevation of IGF-I is observed (Mathews et al., 1988; Miller et al., 1989;

Chow et al., 1994). A major difference between mammals and salmon is that the former cease growing at the onset of adulthood, whereas fish continue to grow throughout their lives and growth rate may vary such that levels of directly-acting growth factors (e.g., IGF-I) are within the normal physiological range.

It is important to note that because of the dramatic differences in growth rate between transgenic and control animals, circulating plasma levels may not show a relationship with actual synthesis rates and activities of the different hormones examined. For example, to maintain IGF-I levels at normal physiological levels, rapidly-growing transgenic individuals (with more rapidly-increasing body volumes) either have synthesized more hormone per animal per unit time or, if they are synthesizing the same amount of IGF-I per animal, have reduced turnover rates relative to controls. Higher rates of IGF-I production in transgenic fish may be achieved by increasing the number of cells secreting the same level of IGF-I, or by increasing IGF-I production from fewer cells responsible for the majority of IGF-I synthesis. In either case, the faster increase in body volume achieved by transgenic animals may arise from this elevated IGF-I production and indirectly serve to reduce circulating concentrations of this hormone. Unfortunately, we do not currently have sufficient understanding of IGF-I metabolism in transgenic fish to determine the importance of receptor binding or other mechanisms for affecting IGF turnover rates. Elevation of IGF-I in fish injected with GH (see above for references) may reflect a temporary response and imbalance, whereas in transgenic animals with chronically-elevated GH, this imbalance can be compensated for by homeostatic endocrine controls (i.e., feed-back mechanism) or by adjusting growth rate. Other examples exist where GH and IGF-I levels or growth rate are not correlated, notably in stunted or starved animals which have very high GH, few unbound GH receptors, and low IGF-I mRNA and circulating IGF-I protein levels (Fryer and Bern, 1979; Björnsson et al., 1988; Young et al., 1989b; Sumpter et al., 1991; Gray et al., 1992; Duan et al., 1993, 1995; Niu et al., 1993; Sakamoto et al., 1994; Perez-Sanchez et al., 1994). Clarke et al. (1989) found significant correlations between fork length and plasma GH levels in only four out of twenty groups of juvenile Pacific salmon; there were no significant differences in plasma GH levels in coho or stream-type chinook exposed to long- and short-day photoperiods despite major differences in growth rate. In transgenic coho salmon with elevated GH, it is possible that GH receptors are saturated with ligand and downregulated such that IGF-I gene transcription and circulating IGF-I levels are normalized. Without such control mechanisms, excessive levels of IGF-I could have very detrimental effects: for example, IGF-I has insulin-like effects at higher doses that can result in hypoglycemia and death (McCormick et al., 1992a) suggesting that only a fairly narrow range of IGF-I levels can be physiologically tolerated by salmonids. In this regard, it is of interest that we have not been able to produce transgenic salmonids containing IGF-I gene constructs using the same salmonid expression vector which successfully produced GH transgenic animals (Devlin et al., 1994), presumably due to the detrimental overproduction of IGF-I in the absence of sufficient binding protein (unpublished observations). Similarly, in cases where GH transgenes increase IGF-I levels disproportionately from IGF binding proteins levels, such animals may not be recovered in transgenesis experiments, leaving only viable animals where IGF-I levels were maintained within the normal physiological range.

4.3. Interactions between GH and IGF-I

As is seen in mammals, GH levels in fish are controlled by a negative feedback loop involving IGF-I acting at the level of the pituitary gland (Perez-Sanchez et al., 1992; Blaise et al., 1995; Weil et al., 1999). In primary pituitary cell cultures, IGF-I but not GH reduces GH release, and in vivo, IGF-I treatment rapidly inhibits GH levels whereas GH treatment had a slower effect consistent with indirect action via IGF-I. In transgenic animals, dramatic feed-back control of pituitary GH gene expression and pituitary size is apparent (Mori and Devlin, 1999), but this effect is not anticipated to affect overall GH levels dramatically due to the high level of production of this hormone in nonpituitary tissues, which is uncoupled from normal pituitary control systems. GH levels have been found to be stimulated by increased feeding levels in unstarved fish (Farbridge et al., 1992; Reddy and Leatherland, 1995), but these effects are more subtle when compared to the high levels of ectopic GH production occurring from expression of GH transgenes.

In transgenic coho, condition factor was correlated with IGF-I levels, but curiously, this relationship was positive in F_1 animals with lower GH levels and was negative in G_0 transgenic animals with high GH levels. Condition factor has been observed to be negatively correlated with GH in nontransgenic fish (Stefansson et al., 1991), suggesting that IGF-I stimulation of bone and cartilage growth (represented by length) can be more effective than for muscle mass in some cases. In this regard, Tsai et al. (1994) have shown that in vivo treatment of fish with GH can sensitize branchial cartilage sulfation by IGF-I, and Cheng and Chen (1995) observed that GH could stimulate sulfation in a dose dependent fashion in the presence of IGF-I. Thus, although GH does not appear to act directly in growth assays (Duan and Hirano, 1990; McCormick et al., 1992b), high levels of this hormone in transgenic animals might accentuate growth by interacting with other growth factors, for example by stimulating local synthesis and paracrine action of IGF-I, or by enhancing the production of IGF-I receptors or GH receptors. An alternative hypothesis for why transgenic coho salmon display a reduced condition factor relative to control fish is suggested by the lipolytic action of GH (Sheridan, 1994) which could act to reduce fat stores relative to total body mass.

4.4. Insulin

Insulin has been shown to have direct effects in fish on muscle protein synthesis rates and cartilage sulfate uptake, perhaps mediated in part via the IGF-I receptor (Duan and Hirano, 1992; McCormick et al., 1992b; Marchant and Moroz, 1993; Fauconneau et al., 1996; Plisetskaya, 1998). Plasma insulin levels are positively correlated with fish size, growth rate, and feeding level (Storebakken et al., 1991; Sundby et al., 1991; Duan et al., 1995; Plisetskaya et al., 1988; Silverstein et al., 1998). In the present study, large animals (both transgenic and size controls) had higher insulin levels than smaller nontransgenic siblings (age controls), suggesting that under these conditions (starvation during a 24-h seawater challenge), insulin levels correlate with fish size rather than historical growth rate or ration level. Insulin levels can be dramatically increased (up to

20-fold) in transgenic mice, rats and pigs expressing GH transgenes, but insulin receptors are reduced (Pursel et al., 1990; Balbis et al., 1996; Oberbauer et al., 1997; Ikeda et al., 1998). In transgenic mammals, this effect may result from elevated food intake, but in transgenic fish a similar increase in insulin levels (relative to size controls) was not observed despite substantially-enhanced food intake. GH level was highly correlated with insulin among control coho salmon. A likely reason for the lack of such a relationship in transgenic animals is that GH levels are not subject to feedback regulation and are controlled only by expression of the transgene. McCormick et al. (1992a,b) have shown that elevated IGF-I levels can result in hypoglycemia, presumably due to binding of IGF-I to the insulin receptor (Chan et al., 1997), an effect not apparently occurring in transgenic animals based on observed IGF-I and insulin levels. Hilton et al. (1987) found that T_3 treatment did not affect plasma insulin levels, and we also have found no relationship between thyroid hormones and insulin in transgenic or control animals.

4.5. *Thyroid hormones*

Thyroid hormones play a major role in the maintenance of growth and have been shown to be correlated with growth rate and ration level in salmonids (Eales and Shostak, 1985; McCormick and Saunders, 1990; Farbridge et al., 1992; Kiessling et al., 1994; Gomez et al., 1997). Direct administration of thyroxine and T_3 have also been demonstrated to stimulate growth of salmonids (Higgs et al., 1982). Such effects, however, are variably detected in vitro in branchial cartilage growth assays (e.g., compare McCormick et al., 1992b and Tagaki and Björnsson, 1996) indicating that, in some cases, thyroid hormones may act indirectly to stimulate growth or are cofactors required for growth. In the present study, mean thyroxine levels among groups were not correlated with growth rate, but within nontransgenic animals, thyroxine levels were significantly correlated both with length and weight, an effect previously observed for rainbow trout (Brown et al., 1978). In transgenic individuals, thyroxine was not correlated with size, suggesting that the elevated levels of GH and/or growth rates may be resulting in disruptions to thyroid hormone metabolism. Similarly, growth effects in GH transgenic mice do not correlate with circulating thyroid hormone levels (Oberbauer et al., 1994).

The interrelationship between thyroid hormones and GH appears to be complex: In growth, metabolism and osmoregulation assays, these two hormones do not act independently (Higgs et al., 1977; Miwa and Inui, 1985; Björnsson et al., 1987; Farbridge and Leatherland, 1988; Leloup and Lebel, 1993). GH administration to salmon in vivo has been shown to enhance the thyroxine 5'-monodeiodinase enzyme responsible for conversion of thyroxine to its active form, triiodothyronine (de Luze and Leloup, 1984; MacLachy and Eales, 1990; MacLachy et al., 1992). Consequently, GH administration can enhance T_3 levels in vivo, and this effect occurs in the absence of significant reductions of thyroxine suggesting that sufficient stores of this latter hormone can be released to maintain physiological levels. In the present study, GH transgenic coho salmon had a thyroxine level significantly lower than size-matched controls, but we do

not know if this effect arose from enhanced conversion of thyroxine to T_3 . If so, elevated T_3 levels may be contributing to growth enhancement in transgenic animals, and in the extreme, could result in excessive cartilage growth and cranial abnormalities (Higgs et al., 1982) that resemble the abnormalities observed in GH transgenic coho salmon (Devlin et al., 1995b). It should be noted that transgenic Atlantic salmon containing the same GH gene construct as used in the present study had T_3 levels lower than either age or size controls (Du et al., 1992), again indicating that significant differences in endocrine response are occurring between transgenic coho and Atlantic salmon.

4.6. Smolting and osmoregulation

It has been well documented that several hormones are involved in the parr–smolt transformation of salmonids (Plisetskaya et al., 1988; Dickhoff, 1993; Sakamoto et al., 1993; Clarke and Hirano, 1995; McCormick, 1995). Juvenile salmonids display increases in GH, IGF-I mRNA and IGF-I protein secretion, insulin, cortisol, thyroid hormones, and gill Na^+ , K^+ , ATPase activities along with enhanced hypo-osmoregulatory capability during the parr–smolt transformation in the spring (in anticipation of seaward migration), or when artificially transferred from fresh to salt water (Sweeting et al., 1985; Schmitz et al., 1994; and see references above). Further, in vivo treatments of juvenile salmonids with GH or IGF-I protein enhance seawater adaptability by increasing numbers of gill chloride cells and increasing Na^+ , K^+ , ATPase gene and enzyme activities (Komourdjian et al., 1976; McCormick et al., 1991; Madsen et al., 1995). GH-transgenic coho salmon display an appearance typical of smolts, and plasma sodium levels following abrupt transfer to sea water were significantly lower than those found in nontransgenic siblings indicating that the transgenic fish had acquired the ability to hypo-osmoregulate. The rapid growth of GH-transgenic coho salmon allows them to rapidly reach normal smolt size in their first spring post-fertilization (S_0), transforming them from an S_1 strain that normally undergoes smolt transformation during the second spring. In a similar study involving Atlantic salmon, Saunders et al. (1998) observed that transgenic individuals also underwent a precocious smolt transformation (based on survival and Na^+ , K^+ , ATPase activity) and appeared relatively insensitive to photoperiod and temperature manipulations that normally synchronize development of nontransgenic individuals.

Precocious smolt transformation in transgenic coho could result from at least two possible mechanisms: increased body size or GH level. Body size has been shown to have a pronounced effect on hypo-osmoregulatory capability (Parry, 1958, and see correlations within control animals in the current study), and GH has obvious effects on growth. However, GH also possesses hypo-osmoregulatory actions that can operate independently of growth effects (Clarke et al., 1977; Bolton et al., 1987; Collie et al., 1989). GH can act indirectly by stimulating IGF-I synthesis (Sakamoto et al., 1995) and enhancing cortisol receptor number (Shrimpton et al., 1995). GH also has acts to sensitize the responsiveness of gill tissue to IGF-I (Madsen and Bern, 1993; Tsai et al., 1994). There was no correlation of plasma sodium levels with body size, GH, or IGF-I

in the transgenic coho. This is consistent with the observation of Clarke et al. (1977) that the reduction of plasma sodium levels in juvenile sockeye salmon caused by exogenous GH was not dose-related whereas increases in length and weight were linearly related to the same range of doses. The effects of GH on osmoregulation have a lower dose threshold compared with its effects on growth; thus, despite very high levels of plasma GH, transgenic coho transferred to sea water in the present study did not have plasma sodium levels lower than similarly-sized controls. Interestingly, high levels of GH are also found in growth-stunted salmonids that have been prematurely transferred from FW to SW (Björnsson et al., 1988; Young et al., 1989b; Sumpter et al., 1991), but in this case, high GH is not associated with enhanced osmoregulation due to disruptions in GH receptor and IGF-I levels (see above for references). Thyroxine and T_3 when given alone do not appear to have marked direct effects on salmonid osmoregulation (Miwa and Inui, 1985; McCormick et al., 1991; Shelbourn et al., 1992) but have been reported to do so in some long-term studies (Madsen, 1990; Madsen and Korsgaard, 1989). However, T_3 can act synergistically with GH to promote hypo-osmoregulation in salmonids (Björnsson et al., 1987; Leloup and Lebel, 1993), an effect which also could be operating in transgenic animals.

The present study has revealed that coho salmon transgenic for a GH gene construct possess markedly-enhanced GH levels, whereas only smaller effects are observed on plasma IGF-I, insulin and T_4 . These observations suggest that homeostatic physiological controls may be capable of adjusting endocrine responses to this abnormal condition to allow viability and precocious hypo-osmoregulatory ability under the enhanced growth conditions. However, the morphological abnormalities observed in these transgenic animals indicate that the compensatory action of such controls is not completely successful, warranting further studies to elucidate the mechanisms and limits of these regulatory mechanisms.

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