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SOCS1 and SOCS3 are the main negative modulators of the somatotrophic axis in liver of homozygous GH-transgenic zebrafish (*Danio rerio*)

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ABSTRACT

Homozygote individuals (HO) of the GH-transgenic zebrafish lineage (F0104), despite expressing double the amount of growth hormone (GH) in relation to the hemizygote (HE) individuals, presented smaller growth in relation to the last, and similar to the non-transgenic (NT) group. Through the analysis of the expression of genes of the somatotrophic axis in the livers of HO and NT individuals, it was verified that GHR, JAK2 and STAT5.1 did not present significant differences among the analyzed genotypes (NT and HO). However, in the IGF-I gene expression, an accentuated decrease was observed in group HO ($p < 0.01$), suggesting a resistance effect to excess GH. This resistance could be related to the insufficient amount of energy for supporting the accelerated metabolic demand caused by excess circulating GH. Analysis of the genes involved in the regulation of GH signalization by dephosphorylation (PTP-H1 and PTP-1B) did not show any significant alteration when comparing groups HO and NT. However, the analysis of the SOCS1 and SOCS3 genes showed an induction in homozygotes of 2.5 times ($p < 0.01$) and 4.3 times ($p < 0.05$), respectively, in relation to non-transgenics. The results of the present work demonstrate that, in homozygotes, GH signaling is reduced by the action of the SOCS1 and SOCS3 proteins.

1. Introduction

The growth hormone (GH) is a polypeptide hormone produced mainly at the anterior portion of the pituitary, and its main function is stimulation of somatic growth in animals. Besides this growth function, GH also affects metabolism of fats, proteins and carbohydrates (Davidson, 1987; Mommsen, 2001; Moller and Norrelund, 2003), immune system maintenance (Jeay et al., 2002), stress and behavior response (Yoshizato et al., 1998). After being synthesized at the pituitary under control of hypothalamic factors, such as GHRH (GH releasing hormone) and somatostatin, GH is released in the blood stream, acting on specific organs through its association with receptors present at the surface of target cells.

Intracellular signalization of GH initiates with its association with its receptor (GHR), which induces phosphorylation and consequent activation of members of an enzymatic family known as Janus Kinases (JAKs), commonly associated with the intracellular portion of the receptor (Argetsinger et al., 1993; VanderKuur et al., 1994, 1995). JAKs activation is apparently the initial step for intracellular unleashing of most signalization systems initiated by GH (Argetsinger et al., 1993; Smit et al., 1997). Due to the fact that GHR does not possess, on its own, any intrinsic kinases activity, the approximation of two JAK molecules through dimerization of receptors results in the transphosphorylation of one JAK by the other, and therefore, the activation of both (Ihle, 1994). Once activated, the JAKs phosphorylate specific regions of the receptor, rich in tyrosines, which will function as anchorage sites for molecules known as Signal Transducers and Activators of Transcription (STATs). Of the seven STATs described for mammals, the one involved specifically with GH signaling is STAT5b which is functionally equivalent to STAT5.1 in zebrafish (*Danio rerio*) (Lewis and Ward, 2004). STATs are also phosphorylated by the JAKs, form dimers and translocate themselves to the nucleus in order to activate specific genes involved in the development of GH biological responses.

In the liver, GH causes the production of growth factors such as insulin-like growth factors (IGFs), which are small polypeptide chains that directly influence animal growth and development (Butler and Le Roith, 2001). The IGFs stimulate, mainly, the chondroitin-sulphate and collagen production by chondrocytes, which is important for cartilage and bone growth (Schindler and Darnell, 1995; Ihle, 1996). IGFs are present in relatively high concentrations in a wide variety of tissues, exhibiting hypertrophic and hyperplas-

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tic properties, being particularly important regulators of myogenesis, exercising an endocrine, autocrine and paracrine role in the integration of tissue-specific regulation and other biological events (Kamegai et al., 2005).

There are various mechanisms that control the GH intracellular signalization pathway in the case of circulating hormone excess. STATs activity can be regulated by inhibitory proteins such as SOCS (Suppressor of Cytokine Signaling), dephosphorylation processes by tyrosine-phosphatases (PTPs), or by degradation of the GH/GHR/JAK complex through ubiquitin action. However, most genetic models utilized nowadays for studying regulation of the expression of genes involved in the GH intracellular signalization pathway are for mammals. Recently, Figueiredo et al. (2007a) produced a genetically modified zebrafish model (*Danio rerio*) which can be utilized for studies of the intracellular signalization of this hormone. This lineage was designated F0104 and is characterized by overexpressing a GH gene of exogenous origin. Hemizygote and homozygote individuals of this lineage were analyzed for growth and expression of genes related to the somatotrophic axis (Figueiredo et al., 2007b). Results obtained in this study demonstrated that, despite the fact that the homozygotes were expressing almost twice as much GH, they presented a lower hepatic IGF-I expression than the hemizygotes, with a consequent decrease in body growth rate. Additionally, it was verified that the homozygotes presented a lower condition factor in relation to the remaining genotypes, as a result of their catabolic state. In this manner, this transgenic lineage can be utilized as a genetic model for the study of expression of genes involved in the GH intracellular signalization pathway regulation, since it was observed hemizygote fish present accelerated growth, while homozygotes, despite presenting higher GH levels, seem to have intracellular signalization blocked in some way. In this context, the objective of the present study was to analyze the expression of genes related to somatotrophic axis (GHR, JAK2, STAT5.1 and IGF-I) and establish if the control of this intracellular signalization pathway in the livers of homozygotic fish of the F0104 lineage occurs through decrease in expression of somatotrophic axis-related genes or by increase in expression of genes that code for inhibitory proteins such as SOCS1 and SOCS3, or phosphatases such as PTP-H1 and PTP-1B.

2. Materials and methods

2.1. Obtainment of transgenic fish

Transgenic fish of lineage F0104 utilized in the present work carried two transgenes, constructed from the β-actin promoter of carp (*Cyprinus carpio*), directing gene expression of the green fluorescent protein (GFP) and cDNA of growth hormone of marine silverside fish *Odonthestes argentinensis* (msGH). Strategy for simultaneous transfer of these to constructions permits the development of a fish lineage transgenic for GH gene, which presents fluorescence under ultraviolet light, making transgene identification quite easy (Figueiredo et al., 2007a). In order to obtain different genotypes, crossing between hemizygote individuals of lineage F0104 were performed, through which a mendelian proportion of 25% homozygote (HO), 50% hemizygote (HE) and 25% non-transgenic (NT) individuals was obtained. HO individuals were identified by presenting a higher level of fluorescence under UV light and a higher expression of exogenous GH gene than HE. For the growth experiment, the three classes of obtained genotypes were utilized. However, for gene expression analyses, only HO and NT individuals were utilized.

2.2. Growth experiments

For the growth experiment, 5-week-old juveniles were utilized. HO, HE and NT groups were reared for a 23-week period in aquariums with capacity of 25 L, with water temperature around 28 °C and photoperiod of 14 h light/10 h dark. Fish were fed daily with 5% total biomass, with commercial feed (ColorBits, Tetra). In each group, at least 30 individuals were utilized, in triplicate. Biometry was performed every 2 weeks until the 15th week, with final biometry performed 8 weeks after that. For biometry, individuals were anesthetized with tricaine (0.1 mg/ml), weighed and returned to the aquariums.

2.3. RNA extraction and semiquantative RT-PCR

After 23 weeks of rearing, NT and HO individuals were sacrificed and their livers removed. Total RNA was extracted using TRIzol Reagent (Invitrogen, Brazil), according to manufacturer instructions. Approximately 5 μg total RNA extracted from the liver were utilized for complementary DNA (cDNA) synthesis with reverse transcriptase enzyme (Superscript III RT, Invitrogen, Brazil) and primer AP (5′-GGCCACGCGTCGACTAGTAC$(T)_{17}$-3′, Invitrogen, Brazil), according to protocol suggested by the manufacturer. Synthesized cDNA was utilized as template for gene-specific PCRs. All reactions were performed in a 12.5 μL volume, containing 1.25 μL of 10X PCR buffer (Invitrogen, Brazil), 0.375 μL $MgCl_2$ (50 mM), 0.25 μL DNTP (10 mM), 0.25 μL each primer (0.2 μM), 0.5 μL cDNA (dilution 1:10), 0.1 μL Platinum Taq polymerase (5 U/μL) (Invitrogen, Brazil) and 9.525 μL pure water (Invitrogen, Brazil). Protocol consisted of 1 min at 94 °C, 30 s at 94 °C, 30 s at 60 °C, 1 min at 72 °C, with a final extension step of 5 min at 72 °C. The number of cycles utilized for each gene was established in order to avoid the reaction's plateau phase. Analyzed genes, GenBank accession number, number of utilized cycles and primer sequences are described in Table 1. For amplification of the IGF-I gene, it was necessary a nested PCR protocol. In this case, the first PCR was performed utilizing 35 cycles in a reaction containing the forward primer described in Table 1 and primer AUAP (5′-GGCCACGCGTCGACTAGTAC-3′, Invitrogen, Brazil), which anneals in an anchorage site placed in the poli(T) tail of cDNA. Approximately 1 μl of first PCR product was utilized as template in the second PCR reaction, which utilized the same conditions of the first, but with a larger number of cycles (37) and the more internal reverse primer de-

Table 1
Gene-specific primers used for RT-PCR analysis, PCR amplicon size, GenBank accession numbers and number of PCR cycles used for each pair of primers (for more detail see Section 2).

Gene (GenBank Accession No.)	Amplicon size (bp)	Number of cycles	Forward Primer (5′–3′)	Reverse Primer (5′–3′)
GHR (XM683592)	335	35	CTGGTTTCCAGAAGCAGCTC	ACACCTTGAGTTGGGAATGC
JAK2 (MN131093)	486	35	CCACAGCAGTTATAAGTCGTTCC	AGTCTGTAGTAGCCGTCGATCAG
STAT5.1 (AAT95391)	522	35	TTTGTGAATAAACAGCAAGCTCA	CTCTTCTACATGCCTCGCTACAT
IGF-I (AF268051)	345	37	GGCATTGGTGTGATGTCTT	GTGTGTCGTTGTGCTCGTA
SOCS1 (DQ350479)	291	35	CAGAGCGACGTTTTCTTTACACT	GAGGAAGTCTTTGAGGATTGGTT
SOCS3 (DQ333315)	329	32	ATAACCCACAGCAAGTTGGATAA	GTCTGCAGGAAAAACGACTTGT
PTP-H1 (AF097477)	203	35	ATCCACCTGAAGTGAGGGATTAT	TGACTTGACAAAATCCAGGAAGT
PTP-1B (NM130924)	351	35	GAAAATCTGTCGACACAGGAAAC	ACCCTCTATGAAGGCAAGGTAAG

scribed in Table 1. β-actin gene was utilized as control for the relative quantification of gene expression, permitting normalization of results. For amplification of this gene, primers ZFBAC-FOR (5′-CCCTTGTTCACAATAACCT-3′) and ZFBAC-REV (5′-TCTGTTGGCTTTGGGATTCA-3′) were utilized (Pang and Ge, 2002). PCR products were analyzed in 1.5% agarose gels stained with ethidium bromide (500 ng/ml) and photodocumentated. The obtained bands were analyzed by densitometry utilizing 1Dscan Ex software version 3.1 (Scanalytics, USA).

2.4. Statistical analyses

For testing differences in growth between HO, HE and NT individuals and in gene expression between HO and NT individuals, ANOVA was utilized, followed by multiple comparisons of means (Tukey; $p < 0.05$). Presumptions of normality and homogeneity of variance were previously tested, with data being transformed when necessary. Analyses were performed with software Statistica version 6.0 (Statsoft, USA).

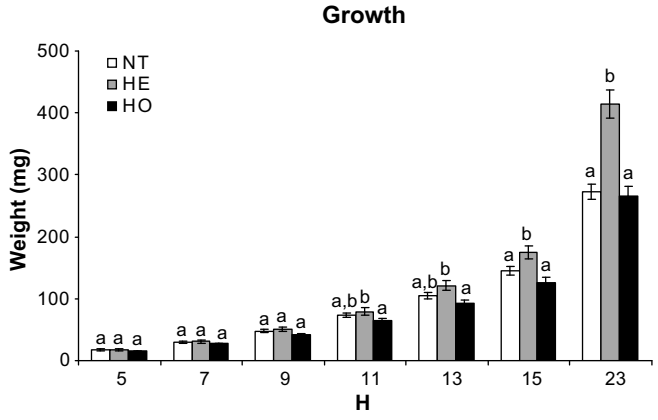

Fig. 1. Growth of different genotypes of GH-transgenic zebrafish (*Danio rerio*). NT: non-transgenic; HE: hemizygous, and HO: homozygous. Different letters indicate significant differences ($p < 0.01$) among fish groups.

3. Results

3.1. Growth

After 23 weeks, HE individuals reached a final mean weight (±SE) of 414.8 mg ± 22.8, significantly higher than the weight attained by individuals of groups HO (266.6 mg ± 15.3; $p < 0.01$) and NT (272.6 mg ± 11.7; $p < 0.05$). Fig. 1 shows that the difference between HE and HO individuals is significant from the eleventh week on, while between HE and NT the difference was only significant from the fifteenth week onward. Between groups HO and NT, no statistically significant differences were observed in any of the performed biometrics.

3.2. Gene expression

Results obtained from the analyses of the somatotrophic axis gene expression (GHR, JAK2, STAT5.1 and IGF-I), comparing individuals from 23-week-old HO and NT groups, are shown in Fig. 2. For GHR, JAK2 and STAT5.1 expression, no significant difference was observed between the analyzed genotypes. However, in the IGF-I gene expression analysis an expressive decrease in group HO ($p < 0.01$; Fig. 2d) was observed. In this case, IGF-I expression in group HO is approximately 30% of the expression level encountered for individuals of group NT.

Expression results for the somatotrophic axis regulator genes (SOCS1, SOCS3, PTP-H1 and PTP-1B) are demonstrated in Fig. 3. SOCS1 and SOCS3 analyses point to a significant increase in the expression of both genes in group HO, as shown in Fig. 3c and d. For SOCS1, an induction of approximately 2.5 times was observed

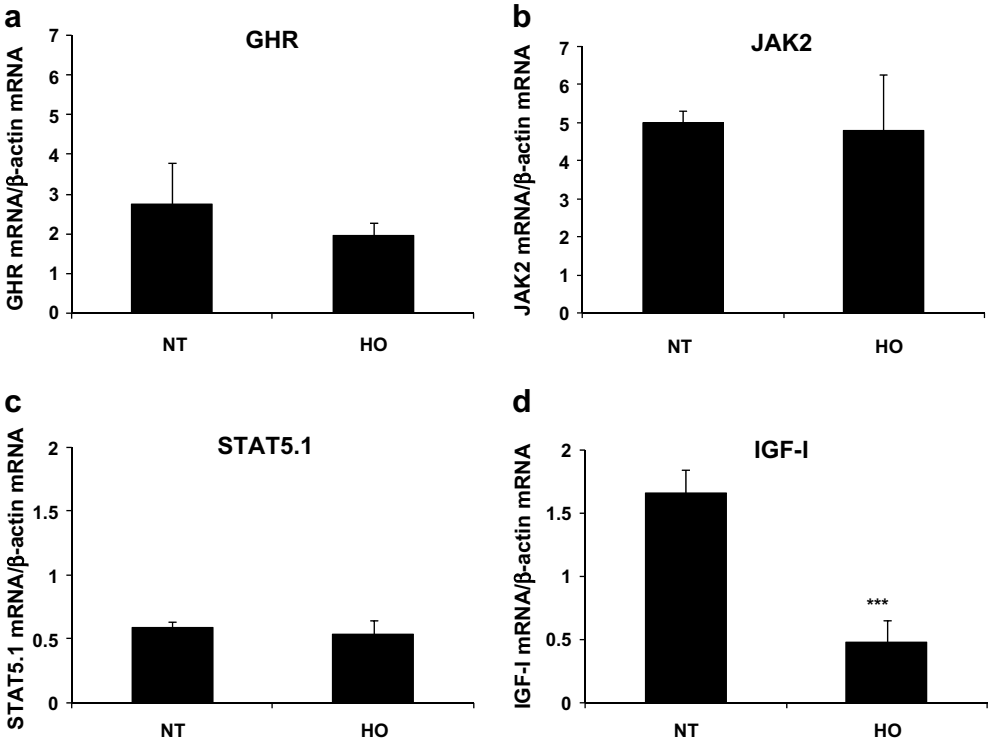

Fig. 2. Gene expression in liver of non-transgenic (NT) and homozygous (HO) GH-transgenic zebrafish (*Danio rerio*). (a) GHR, (b) JAK2, (c) STAT5.1 and (d) IGF-I. Data are expressed as mean ± SE ($n = 3$). Asteristic (***) indicate significant differences ($p < 0.01$).

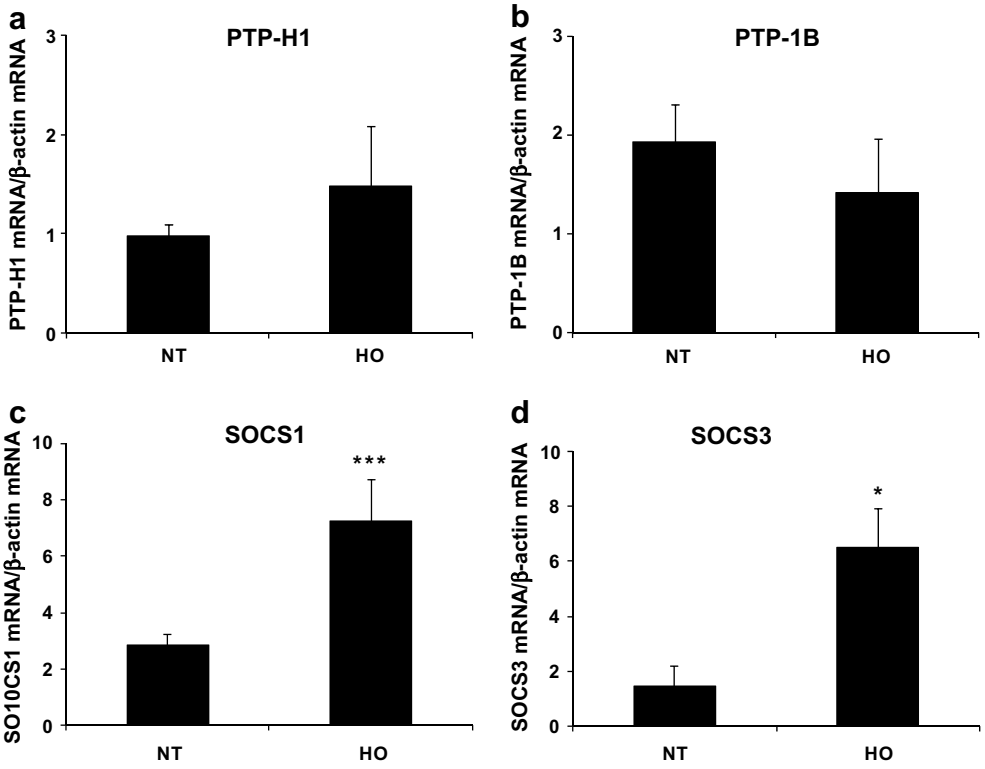

Fig. 3. The expression levels of the (a) PTP-H1, (b) PTP-1B, (c) SOCS1 and (d) SOCS3 genes in zebrafish (*Danio rerio*) liver. HO: homozygous and NT: non-transgenic. Data are expressed as mean ± SE ($n = 3$). Asteristic (∗) and (∗∗∗) indicate significant differences ($p < 0.05$) and ($p < 0.01$), respectively.

for group HO compared to group NT ($p < 0.01$), while for SOCS3 an induction of 4.3 times was observed in the same comparison ($p < 0.05$). For genes related to regulation of signalization of the somatotrophic axis, through dephosphorylation, both PTP-H1 and PTP-1B did not show any significant alteration when comparing groups HO and NT (Fig. 3a and b).

4. Discussion

The growth experiment performed in the present study demonstrated that the different genotypes of the F0104 lineage present conflicting characteristics when referring to effective growth. Individuals homozygotic (HO) for exogenous GH gene, which expressed double the hormone quantity in relation to hemizygotes (HE), demonstrated a similar growth to the non-transgenic fish group (NT). On the other hand, HE transgenic fish presented a significant growth increase in relation to the other analyzed genotypes. In the comparison with the HO group, difference was significant already on the eleventh week, while in the comparison with the NT group, difference was only observed on the 15th week. These results provide evidence for existence of an optimum level of circulating GH, above which a mechanism of decrease of somatotrophic axis signalization probably initiates. This hypothesis was recently raised by Figueiredo et al. (2007b), while working with the same lineage utilized in the present study, demonstrated that excess GH does not increase, proportionally, GHR and IGF-I expression, presenting a condition of resistance to excess GH.

Due to the fact that GH is a multiple-function hormone, it is evident that its excess can cause a wide variety of collateral effects which could lead the organism to a stressful situation. Physiological and behavioral alterations produced in a situation of excess GH in the animal's metabolism are not yet fully understood. Studies utilizing transgenic models overexpressing the GH gene have already demonstrated some of these important alterations, such as: decrease in fertility (Cecim et al., 1995a,b), increase in oxygen consumption (Cook et al., 2000; Mckenzie et al., 2003) premature aging (Wolf et al., 1993; Bartke et al., 1998), hyperinsulemia and resistance to insulin (Dominici et al., 2005), and decrease in learning and memory (Meliska et al., 1997). Recently, Rosa et al. (2008) identified a significant increase in oxygen consumption and production of reactive oxygen species (ROS) in homozygote individuals of the F0104 lineage utilized in the present study. These authors verified a significant decrease in the expression of the gene which codifies for glutamate cysteine ligase (GCL) enzyme, responsible for tripeptide glutathione synthesis, a key element of the antioxidant defense system. In this same sense, Leggatt et al. (2007), working with GH-transgenic coho salmon (*Oncorhynchus kisutch*), also observed a reduction in GCL activity in relation to non-transgenic individuals. This same fact has been observed in GH-transgenic mice, where an increment in GH levels was demonstrated to be associated with an increased metabolic demand (Carlson et al., 1999), increasing ROS production by mitochondria (Muradian et al., 2002; Fridovich, 2004) as well as superoxide production and lipids and proteins oxidation, which indicates a situation of elevated oxidative damage (Rollo et al., 1996; Brown-Borg et al., 2001). These evidences indicate that GH excess has the potential of generating oxidative stress in fish and mammals, possibly compromising growth by the imposition of an additional energetic cost that does not occur when a favorable relation exists between available energy and circulating GH level.

In the case of the animal being in an unfavorable energetic situation, the necessity for decreasing signalization of the somatotrophic axis exists, with consequent decrease in IGF-I production, which is the main effecter of the somatotrophic actions induced by GH. The first available mechanism to the animal is the utilization of a negative feedback system on the GH production in the pituitary gland. Although this system is efficient in controlling the physiological effects of GH, not only GH itself as well as IGF-I

can negatively modulate the synthesis and liberation of the pituitary hormone. However, in the case of transgenic fish, this system is inefficient due to the fact that exogenous GH is under transcriptional control of a promoter that does not respond to the negative modulators. This fact has already been demonstrated for the F0104 lineage by Figueiredo et al. (2007b), who demonstrated that the HE and HO transgenic individuals presented a significant decrease in the expression of endogenous GH at the pituitary gland. Therefore, although the transgenic animal is able to decrease endogenous GH production, it cannot affect the exogenous hormone production. In this situation, the organism needs to utilize other available mechanisms for blocking intracellular GH signalization, turning the cells into resistant targets to the excess-circulating hormone. Analysis of IGF-I gene expression in the model utilized in this work showed a very significant decrease of this gene in group HO when compared to group NT. This fact indicates a decrease in intracellular signalization of the somatotrophic axis in homozygote fish of the F0104 lineage, which corroborated the results observed in the growth experiment.

Based on the fact that a negative control of GH signalization in group HO exists, one of the possibilities for explaining this resistance to circulating GH could be the decrease in expressions of genes that codify for the GH receptor (GHR), for the kinase associated to the receptor (JAK2), and for the main transcription factor which leads information to the cell nucleus (STAT5.1). Results obtained based on the expression of these genes did not indicate any significant difference in the comparison between HO and NT individuals. This indicated that the levels of these proteins probably maintain themselves identical in the two analyzes groups. Therefore, it is evident that the growth level observed in group HO is related to the decrease in IGF-I expression and that HO individuals are utilizing a negative control mechanism of this signalization pathway which is not related to the decrease in the main components of the somatotrophic axis, but to regulatory proteins that act on these components.

Regulation of the GH intracellular signalization pathway occurs through a complex mechanism involving the coordinated action of various negative regulators, whose main targets apparently are JAKs and STATs phosphorylated by activated GHR (Herrington and Carter-Su, 2001; Flores-Morales et al., 2006). Two of these mechanisms were tested in the present study, with the first being constituted by proteins known as SOCS (Suppressors of Cytokine Signaling), and the second by phosphatases PTP-H1 and PTP-1B, which are also involved in GH intracellular signalization control.

Many studies have identified three different types of phosphatases involved GH intracellular signalization control: 1) SHP1 (SH2 domain-containing protein-tyrosine phosphatase 1, also known as PTP-1), which bonds to STAT5 phosphorylated at the nucleus, attenuating signalization (Ram and Waxman, 1997); 2) PTP-1B induces GHR dephosphorylation in the endoplasmatic reticule (Frangioni et al., 1992), fact which suggests an endocytosis mechanism of GHR, such as already demonstrated for other receptors of this family (Haj et al., 2002); and 3) PTP-H1, which bonds to phosphorylated GHR (Pasquali et al., 2003).

Analysis of PTP-H1 and PTP-1B genes expression did not show any significant alteration between groups HO and NT, suggesting that the GH intracellular signalization control observed in group HO does not utilize dephosphorylation of GHR bonded to the cellular membrane and neither is related to the receptor internalization process. Differently from phosphatases, the results of SOCS1 and SOCS3 expression analyses demonstrated a significant increase in the expression of both genes, suggesting that HO transgenics utilize this mechanism for controlling excess GH signalization. Of the twelve SOCS identified in fish, only eight are encountered in mammals (Jin et al., 2007). In general, SOCS protein levels are constitutively low in cells, but their expression is rapidly induced by stimulation with different cytokines or growth factors, including GH (Fujimoto and Naka, 2003). Among these members, SOCS1 and SOCS3 bond directly to JAK and inhibit its kinasic activity, while other members of this family act competing with STATs for the anchorage sites presents in the receptor (Kopchick and Andry, 2000).

Although SOCS1 and SOCS3 present structural similarities, their action mechanisms are different. SOCS1 bonds specifically to the JAK2 activation site, which should be phosphorylated in order to permit the enzyme to perform its kinasic activity (Yasukawa et al., 1999) or signalizing for the ubiquitin degradation system. On the other hand, SOCS3 can bond to JAK2 inhibiting its activity or to the receptor (GHR), impeding the STATs from bonding to it, as described by some authors (Hansen et al., 1999; Ram and Waxman, 1999). Taking into account these information, we can conclude that the homozygote individuals (HO) of the F0104 lineage present growth disproportional to the expressed GH level due to the fact that they utilize a negative regulation system for GH intracellular signalization based on the action of SOCS proteins on the kinasic activity of the JAK2 enzyme, which is associated to the GH receptor. It is worth emphasizing that fish utilized in the present study were cultured in food control conditions (5% biomass/day), fact which could have been decisive for the results here obtained. Food quantity offered to homozygote individuals was probably insufficient for supplying the energetic demands of metabolism accelerated by excess circulating GH. In this situation there is not enough energy for growth and the animal is obligated to regulate negatively the somatotrophic axis, culminating in a situation of resistance to the hormone and decrease in hepatic IGF-I production.

To our knowledge, this is the first work to suggest a negative control mechanism of the somatotrophic axis in a GH-transgenic fish model. The utilization of homozygote individuals of the F0104 lineage permitted a comparative model, which demonstrated the existence of an optimal level of hormone action, above which the organism is obligated to turn to a negative regulation system of the somatotrophic axis. This negative regulation permits that the animal save energy that would otherwise be destined to growth, and apply it to systems that counterbalance the collateral effects that excess circulating hormone most certainly provokes on the organism.

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References

Argetsinger, L.S., Campbell, G.S., Yang, X., Witthuhn, B.A., Silvennoinen, O., Ihle, J.N., Carter-Su, C., 1993. Identification of JAK2 as a growth hormone receptor-associated tyrosine kinase. Cell 74, 237–244.

Bartke, A., Brown-Borg, H.M., Bode, A.M., Carlson, J., Hunter, W.S., Bronson, R.T., 1998. Does growth hormone prevent or accelerate aging? Exp. Gerontol. 33, 675–687.

Brown-Borg, H., Johnson, W.T., Rakoczy, S., Romanick, M., 2001. Mitocondrial oxidant generation and oxidative damage in ames dwarf and GH transgenic mice. J. Am. Aging Assoc. 24, 85–100.

Butler, A.A., Le Roith, D., 2001. Control of growth hormone by the somatropic axis: growth hormone and the insulin-like growth factors have related and independent roles. Annu. Rev. Physiol. 63, 141–164.

Carlson, J.C., Bharadwaj, R., Bartke, A., 1999. Oxidative stress in hypopituitary dwarf mice and in transgenic mice overexpressing human and bovine GH. Age 22, 181–186.

Cecim, M., Kerr, J., Bartke, A., 1995a. Effects of bovine growth hormone (bGH) transgene expression or bGH treatment on reproductive functions in female mice. Biol. Reprod. 52, 1144–1148.

Cecim, M., Fadden, C., Kerr, J., Steger, R.W., Bartke, A., 1995b. Infertility in transgenic mice overexpressing the bovine growth hormone gene, disruption of the neuroendocrine control of prolactin secretion during pregnancy. Biol. Reprod. 52, 1187–1192.

Cook, J.T., Mcniven, M.A., Sutterlin, A.M., 2000. Metabolic rate of pre-smolt growth-enhanced transgenic Atlantic salmon (*Salmo salar*). Aquaculture 188, 33–45.

Davidson, M.B., 1987. Effect of growth hormone on carbohydrate and lipid metabolism. Endocr. Rev. 8, 115–131.

Dominici, F.P., Argentino, D.P., Muñoz, M.C., Miquet, J.G., Sotelo, A.I., Turyn, D., 2005. Influence of the crosstalk between growth hormone and insulin signalling on the modulation of insulin sensitivity. Growth Horm. IGF Res. 15, 324–336.

Figueiredo, M.A., Lanes, C.F.C., Almeida, D.V., Marins, L.F., 2007a. Improving to production of transgenic fish germlines: in vivo evaluation of mosaicism in zebrafish (*Danio rerio*) using a green fluorescent protein (GFP) and growth hormone cDNA transgene co-injection strategy. Genet. Mol. Biol. 30, 31–36.

Figueiredo, M.A., Lanes, C.F.C., Almeida, D.V., Proietti, M.C., Marins, L.F., 2007b. The effect of GH overexpression on GHR and IGF-I gene regulation in different genotypes of GH-transgenic zebrafish. Comp. Biochem. Physiol. D 2, 228–233.

Flores-Morales, A., Greenhalgh, C.J., Norstedt, G., Rico-Bautista, E., 2006. Negative regulation of growth hormone receptor signaling. Mol. Endocrinol. 20, 241–253.

Frangioni, J.V., Beahm, P.H., Shifrin, V., Jost, C.A., Neel, B.G., 1992. The nontransmembrane tyrosine phosphatase PTP-1B localizes to the endoplasmic reticulum via its 35 amino acid C-terminal sequence. Cell 68, 545–560.

Fridovich, I., 2004. Mitocondria, are they the seat of senescence? Aging Cell 3, 13–16.

Fujimoto, M., Naka, T., 2003. Regulation of cytokine signaling by SOCS family molecules. Trends Immunol. 24, 659–666.

Haj, F.G., Verveer, P.J., Squire, A., Neel, B.G., Bastiaens, P.I., 2002. Imaging sites of receptor dephosphorylation by PTP1B on the surface of the endoplasmic reticulum. Science 295, 1708–1711.

Hansen, J.A., Lindberg, K., Hilton, D.J., Nielsen, J.H., Billestrup, N., 1999. Mechanism of inhibition of growth hormone receptor signaling by suppressor of cytokine signaling proteins. Mol. Endocrinol. 13, 1832–1843.

Herrington, J., Carter-Su, C., 2001. Signaling pathways activated by the growth hormone receptor. Trends Endocrinol. Metab. 12, 252–257.

Ihle, J.N., 1994. The janus kinase family and signaling through members of the cytokine receptor superfamily. Proc. Soc. Exp. Biol. Med. 206, 268–272.

Ihle, J.N., 1996. STATs, Signal Transducers and Activators of Transcription. Cell 84, 331–334.

Jeay, S., Sonenshein, G.E., Postel-Vinay, M.C., Nelly, P.A., Baixeras, E., 2002. Growth hormone can act as a cytokine controlling survival and proliferation of immune cells, new insights into signaling pathways. Mol. Cell. Endocrinol. 188, 1–7.

Jin, H.J., Shao, J.Z., Xiang, L.X., 2007. Identification and characterization of suppressor of cytokine signaling 3 (SOCS-3) homologues in teleost fish. Mol. Immu. 44, 1042–1051.

Kamegai, J., Tamura, H., Shimizu, T., Ishii, S., Sugihara, H., Oikawa, S., 2005. Insulin-like growth factor-I down-regulates ghrelin receptor (growth hormone secretagogue receptor) expression in the rat pituitary. Regul. Pept. 127, 203–206.

Kopchick, J.J., Andry, J.M., 2000. Growth hormone (GH), GH receptor, and signal transduction. Mol. Genet. Metab. 71, 293–314.

Leggatt, R.A., Brauner, C.J., Iwama, G.K., Devlin, R.H., 2007. The glutathione antioxidant system is enhanced in growth hormone transgenic coho salmon (*Oncorhynchus kisutch*). J. Comp. Physiol. B, Biochem. Syst. Environ. Physiol. 177, 413–422.

Lewis, R.S., Ward, A.C., 2004. Conservation, duplication and divergence of the zebrafish stat5 genes. Gene 338, 65–74.

Mckenzie, D.J., Martinez, R., Morales, A., Acosta, J., Morales, R., Taylor, E.W., Steffensen, J.F., Estrada, M.P., 2003. Effects of growth hormone transgenesis on metabolic rate, exercise perfomance and hypoxia tolerance in tilapia hybrids. J. Fish Biol. 63, 398–409.

Meliska, C.J., Burke, P.A., Bartke, A., Jensen, R.A., 1997. Inhibitory avoidance and appetitive learning in aged normal mice, Comparison with transgenic mice having elevated plasma growth hormone levels. Neurobiol. Learn. Mem. 68, 1–12.

Moller, N., Norrelund, H., 2003. The role of growth hormone in the regulation of protein metabolism with particular reference to conditions of fasting. Horm. Res. 59, 62–68.

Mommsen, T.P., 2001. Paradigms of growth in fish. Comp. Biochem. Physiol. 129 B, 207–219.

Muradian, K.K., Utko, N.A., Mozzhukhina, T.G., Litoshenko, A.Y., Pishel, I.N., Bezrukov, V.V., Fraifield, V.E., 2002. Pair-wise linear and 3D nonlinear relationships between the liver antioxidant enzyme activities and the rate of body oxygen consumption in mice. Free Radic. Biol. Med. 33, 1736–1739.

Pang, Y., Ge, W., 2002. Gonadotropin regulation of activin beta and activin type IIA receptor expression in the ovarian follicle cells of the zebrafish, *Danio rerio*. Mol. Cell Endocrinol. 188, 195–205.

Pasquali, C., Curchod, M.L., Walchli, S., Espanel, X., Guerrier, M., Arigoni, F., Strous, G., Van Huijsduijnen, R.H., 2003. Identification of protein tyrosine phosphatases with specificity for the ligand-activated growth hormone receptor. Mol. Endocrinol. 17, 2228–2239.

Ram, P.A., Waxman, D.J., 1997. Interaction of growth hormone-activated STATs with SH2-containing phosphotyrosine phosphatase SHP-1 and nuclear JAK2 tyrosine kinase. J. Biol. Chem. 272, 17694–17702.

Ram, P.A., Waxman, D.J., 1999. SOCS/CIS protein inhibition of growth-hormone stimulated STAT5 signaling by multiple mechanisms. J. Biol. Chem. 274, 35553–35561.

Rollo, C.D., Carlson, J., Sawada, M., 1996. Accelerated aging of giant transgenic mice is associated with elevated free radical processes. Can. J. Zool. 74, 606–620.

Rosa, C.E., Figueiredo, M.A., Lanes, C.F.C., Almeida, D.V., Monserrat, J.M., Marins, L.F., 2008. Metabolic rate and reactive oxygen species productions in different genotypes of GH-transgenic zebrafish. Comp. Biochem. Physiol. B 149, 209–214.

Schindler, C., Darnell, J.E., 1995. Transcriptional responses to polypeptide ligands, the JAK-STAT pathway. Annu. Rev. Biochem. 64, 621–651.

Smit, L.S., Vanderkuur, J.A., Stimage, A., Han, Y., Luo, G., Yu-Lee, L.Y., Schwartz, J., Carter-Su, C., 1997. Growth hormone-induced tyrosyl phosphorylation and deoxyribonucleic acid binding activity of Stat5A and Stat5B. Endocrinology 138, 3426–3434.

Vanderkuur, J.A., Wang, X., Zhang, L., Campbell, G.S., Allevato, G., Billestrup, N., Norstedt, G., Carter-Su, C., 1994. Domains of the growth hormone receptor required for association and activation of JAK2 tyrosine kinase. J. Biol. Chem. 269, 21709–21717.

Vanderkuur, J.A., Wang, X., Zhang, L., Allevato, G., Billestrup, N., Carter-Su, C., 1995. Growth hormone-dependent phosphorylation of tyrosine 333 and/or 338 of the growth hormone receptor. J. Biol. Chem. 270, 21738–21744.

Wolf, E., Kahnt, E., Ehrlein, J., Hermanns, W., Brem, G., Wanke, R., 1993. Effects of long-term elevated serum levels of growth hormone on life expectancy of mice, Lessons from transgenic animal models. Mech. Ageing Dev. 68, 71–87.

Yasukawa, H., Misawa, H., Sakamoto, H., Masuhara, M., Sasaki, A., Wakioka, T., Ohtsuka, S., Imaizumi, T., Matsuda, T., Ihle, J.N., Yoshimura, A., 1999. The JAK bindingprotein JAB inhibits Janus tyrosine kinase activity through binding in the activation loop. Embo J. 18, 1309–1320.

Yoshizato, H., Fujikawa, T., Soya, H., Tanaka, M., Nakashima, K., 1998. The growth hormone (GH) gene is expressed in the lateral hypothalamus, enhancement by GH-releasing hormone and repression by restraint stress. Endocrinology 139, 2545–2551.