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ORIGINAL ARTICLE

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Molecular characterization, tissue distribution, and expression regulation from fasting and re-feeding of two growth hormone receptors in mandarin fish *Siniperca chuatsi*

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Abstract Growth hormone receptors (*GHRs*) are especially expressed in the muscle and liver tissues of mandarin fish *Siniperca chuatsi*. cDNAs of two *GHR* types (*scGHR1* and *scGHR2*: 1999 and 1963 bp, encoding 638 and 578 amino acids, respectively) have been isolated and characterized in mandarin fish. Both receptors, formed through fish-specific genome duplication, have conserved FGEFS and box1 and 2 motifs. Partial compensatory growth was noted following 10-day re-feeding after 4-week fasting. After 1-week fasting, pituitary *GH* and muscle *scGHR1* and *scGHR2* mRNA levels and plasma *GH* concentrations increased ($p < 0.05$), while hepatic *scGHR1* and *scGHR2* mRNA expressions reduced rapidly ($p < 0.05$). Upon re-feeding, muscle *scGHR1* and *scGHR2* mRNA expressions decreased, whereas they increased rapidly in the liver, eventually normalizing. Pituitary *GH* mRNA expression increased to 5.1 times higher than that in the controls 3 days after re-feeding ($p < 0.01$), and normalized 7 days after re-feeding. The varying expression of *scGHR1* and *scGHR2* in different tissues suggests their distinct functions. Further, changes in *GH*, *scGHR1*, and *scGHR2* mRNA expressions suggest their important roles in the growth of mandarin fish. Our results provide comparative insights into evolutionary origins and roles of *GHR* genes in teleost growth.

Keywords Growth hormone receptor · Growth hormone · Fasting · Re-feeding · *Siniperca chuatsi*

Introduction

Environmental factors such as temperature, photoperiod, and food availability strongly influence growth in fish, which is primarily controlled by the growth hormone (*GH*)/insulin-like growth factor (*IGF*) system [1–3]. The classical view holds that pituitary *GH* stimulates the synthesis and secretion of *IGF-1* from the liver and other tissues (e.g.,

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muscle) through growth hormone receptor (*GHR*). Further, *IGF-1* stimulates cell proliferation and differentiation mediated by *IGF-1* receptors (*IGFR1*) [4–6]. In mammals, it has long been proposed that most of the effects of *GH* are indirect, with *IGF1* being the final effector produced primarily in the liver [7]. However, *GH* also enhances muscle growth directly through receptors on the muscle cells, and this growth is not mediated by liver-derived or muscle-derived *IGF1* [8, 9]. Transgenic zebrafish with constitutive overexpression of *GH* and/or *GHR1* display high growth rates [9]. *GH* affects many physiological functions, including growth, reproduction, immunity, lipid and protein metabolism, osmoregulation, and feeding behavior in fish via *GHR* signaling pathways [10, 11]. *GHRs* have been cloned from several teleost species and show differential expression in various tissues [12–17]. Different isoforms of *GHR* have been identified in gilthead seabream [18], rainbow trout [13], Nile tilapia [14], orange-spotted grouper [15], black seabream [16], and Japanese seabass [19]. *GHRs* are members of the class I cytokine receptor superfamily composed of an extracellular domain containing a conserved Y/FGEFS motif, a single transmembrane domain, and an intracellular domain containing proline-rich box1 and box2 motifs [1, 4]. The evolution and nomenclature of *GHRs* is a hot topic of debate [1, 4, 20, 21]. It is reported that fish *GHRs* are differentially regulated by fasting [21, 22], salinity [22–24], temperature [25], pathogens [26], stress [27], and cortisol and insulin [28, 29]. However, it is still unknown whether the *GHRs* have similar or distinct functions [4]. Data of the molecular mechanisms by which the *GH* system modulates skeletal muscle growth in teleosts is limited [2].

The principal environmental regulator of growth is nutritional status [3, 30]. Compensatory growth (CG) has been well studied in teleosts [31] and could be a useful model for studying endocrine regulation during negative, normal, and accelerated growths. The different expression patterns of *GH* and *GHRs* have been reported in a few fish species under various nutritional conditions. In catfish, plasma GH concentrations and pituitary *GH* mRNA increased after 4 weeks of fasting [32]; and in hybrid striped bass, 3 weeks of food deprivation depressed hepatic *GHR1/2* mRNA levels, enhanced skeletal muscle expression of *GHR2* mRNA, and increased pituitary *GH* mRNA and plasma *GH* [33]. In Mozambique tilapia, plasma *GH* reduced slightly by 4 weeks of fasting, and transcript levels of *GHR1* and *GHR2* increased in the muscle, but those in the liver were unaffected [22]. Upon re-feeding, fish gained weight at an accelerated rate [31]. Re-feeding for 2 weeks after 4 weeks of fasting in rainbow trout resulted in decreased plasma *GH* concentrations and increased hepatic *GHR1* and *GHR2* mRNA levels [34]. In hybrid striped bass, re-feeding resulted in increased expression of *GHR* in the liver and a return to pre-fasting levels of *GHR1* mRNA in muscle [33]. The results were different and somewhat confusing [35]. However, in the fish studied, the sampling frequencies were mostly low. In addition, the time course of the response of *GH* and *GHRs* to fasting and re-feeding has been described in detail in few fish species. In mandarin fish, there has been no study of endocrine regulation during variable growth phases, including negative, normal, and accelerated growth.

Siniperca chuatsi is an economically important species for freshwater farming in China and is favored for its large size, meat texture, flavor, and high nutritive value [36, 37]. We have previously studied the sperm ultrastructure of three species in *Siniperca* [38], the expression of two *HSP70* isoforms in mandarin fish [39], and insulin-like growth factor I gene polymorphisms and their effects on growth traits in sinipercid species [40]. In this study, to better understand the roles of *GH* and *GHRs* in the growth of mandarin fish, the cDNAs of two *GHR* subtypes were cloned and characterized. Further, we designed an experiment with different frequencies of sampling and assessed the time course variations in plasma *GH*, pituitary *GH* mRNA, and GHR1/2 mRNA levels in the liver and muscle.

Materials and methods

Animals

Mandarin fish was maintained in an indoor circulating water system containing a series of 400-L tanks at Sun Yat-sen University. The fish were allowed to acclimate to the tanks for 1 month prior to the beginning of experiments. Fish were fed twice a day (09:00 and 16:00) to satiety with live fingerlings. The water temperature was maintained at 25–26 °C. The fish used in this study were 5 months old, weighed 145.5 ± 38 g, and were offspring of the same pair. All experiments were conducted in accordance with the guidelines and procedures approved by the Institutional Animal Care and Use Committee at Sun Yat-Sen University.

Sampling

Body weight and length were measured under anesthesia with tricaine methanesulfonate (MS-222, 0.5 g/l) at each sampling time point from the start of the experiment to the end of the experiment. The specific growth rates were calculated as $(\ln Wf - \ln Wi)/t \times 100$, where Wf is the final weight (g), Wi is the initial weight (g) at each time interval, and t is the growth time (days). At the time of sampling, six fish from the fed group and six fish from the treated group were netted and anesthetized. Blood was collected from the

caudal vasculature using 1-ml heparinized syringes. Plasma was separated by centrifugation at 1000×*g* for 30 min at 4 °C, and stored at −80 °C for later *GH* analysis by ELISA. Livers, muscles, and pituitaries were excised, flash-frozen in liquid nitrogen, and stored at −80 °C.

RNA extraction and cDNA preparation

Total RNAs from different mandarin fish tissues were extracted using Trizol Reagent (Invitrogen, USA) according to the manufacturer's instructions. The integrity of the total RNA was verified by electrophoresis on 1.0 % agarose gel. The concentration and purity of total RNA were measured by a UV spectrophotometer (Nanodrop 2000c, Thermo, USA). Total RNA of 1 μg from each sample was digested with 2 μg RNase-free DNase I (New England Biolabs, USA) and then used for synthesis of the first-strand cDNA with M-MLV Reverse Transcriptase (Invitrogen, USA) according to the manufacturer's instructions.

Cloning of scGHR1 and scGHR2

Degenerated PCR primers (Table 1) were designed based on the alignment of known *GHR1* or *GHR2* cDNA sequences and highly conserved regions of amino acid sequences in bony fish (EF052273.1, EF052274.1, AF438176.2, AY573601.2, NM_001279601.1, AY973233.1, KF601961.1, KF770840.1, AB621336.1, AB621337.1). First-strand cDNA synthesis from liver was treated with RNase-free DNase I (New England Biolabs, USA) and used as the template to amplify the fragment cDNAs of *scGHR1* and *scGHR2*. Gene-specific primers (Table 1) were designed based on the partial cDNA sequences identified by RT-PCR, and were used for rapid amplification of cDNA ends with SMARTer RACE cDNA Amplification Kit (Takara, China) according to the user manual. To confirm the composite open reading frame (ORF) sequences, RT-PCR was performed using two pairs of gene-specific primers upstream and downstream of the protein coding sequences. The PCR reactions were as follows: denaturation for 3 min at 94 °C; followed by 40 cycles of 94 °C for 30 s, 55–62 °C for 30 s, and 72 °C for 0.5–3 min; and a final extension step at 72 °C for 10 min. All PCR products were examined by agarose gel electrophoresis, purified using an E.Z.N.A. gel extraction kit (Omega BioTek, USA), and then sub-cloned into a pCR2.1 vector (Invitrogen, USA).

The deduced protein sequence alignments were performed using Clustal X 1.81 software (ClustalWeb: http://www.clustal.org/). The phylogenetic trees of *GHR1* and *GHR2* were constructed with MEGA 4 by the neighbor-joining method with 1000 replications (MegaWeb: http://www.megasoftware.net/). The transmembrane domains of both *GHRs* were predicted with the TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/). Signal peptides were analyzed with SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/) and the domains were searched with the Pfam database (PfamWeb: http://pfam.xfam.org/).

Real-time PCR

Real-time PCR was used to quantify the expression of *scGH* and *scGHRs* at the transcriptional level. The primers were designed based on the known *GH*, *GHR1* and *GHR2* cDNA sequences of *S. chuatsi* (EF205280.1, KJ477038.1, KJ477039.1). Those used in real-time PCR after screening are shown in Table 1. Real-time PCR was performed with Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen, USA) in a total volume of 10 μl on the Roche Light Cycler 480 Real-Time PCR detection system in 384-well plates according to the manufacturer's instructions. Each reaction used 1 μl of the cDNA product (20 ng/μl), 0.4 μl of each gene-specific primer (10 μM), 5 μl of Platinum SYBR Green qPCR SuperMix, and 3.2 μl of nuclease-free H_2O. Three replicates were set for all reactions, from which mean threshold cycle (Ct) values were calculated. Based on the T_m value of primer pairs, the real-time PCR reaction conditions were as follows: UDG incubation at 50 °C for 2 min; initial denaturation at 95 °C for 2 min; and then 40 cycles of 95 °C for 15 s, 57 °C for 15 s, and 72 °C for 30 s. Fluorescence data were collected in the third step of each cycle. When amplification was complete, the melting curves were generated by raising the temperature from 60 to 95 °C in increments of 0.5 °C per 5 s to anneal, and a final denaturation at 95 °C for 10 s.

In a pilot study, expression levels of *GAPDH* and *β-actin* in different tissues were not as stable as *18S*. The expression levels of *18S* were correlated with input RNA concentration, indicating that *18S* is a valid reference gene for the experiment. The standard curve for each gene was generated through a tenfold dilution series of plasmids PEASY-T1 (TransGen Biotec, China) containing the target or reference genes. The relative transcript amounts of *scGHRs* in fish were normalized to the level of *18S* rRNA in the same sample by the $2^{-\Delta Ct}$ method. The relative expressions of *scGHRs* in liver and muscle after exposure to fasting and re-feeding were calculated by the comparative CT method ($2^{-\Delta\Delta Ct}$) using the lightcycler 480 Software 1.5 (ROCHE, CH) and indicated as n-fold differences compared to the control group.

Melting curve analyses for all new qPCR assays showed a single peak, confirming the specificity of PCRs. PCR efficiency varied between assays and between runs of a single assay, but was within a reasonable range (90–110 %). No bias was found across the PCR plate.

Table 1 Primers used in cloning and real-time PCR

Primer	5′–3′ Sequences	Purpose
GHR1-F1	TGWMTYTAAACTGGACYCTKCTGAAT	GHR1 partial cDNA cloning
GHR1-R1	TAGSCRCTKCCTTCWGGATC	GHR1 partial cDNA cloning
GHR1-F2	CRAGGGASCWGGAGACRTTC	GHR1 partial cDNA cloning
GHR1-R2	KTGTCATTTCAYGGTGAGAGGT	GHR1 partial cDNA cloning
GHR1-F3	CAGATGACCAASTTSTGARAAGT	GHR1 partial cDNA cloning
GHR1-R3	GAGAYGMCWATKAGCAWGATGAGT	GHR1 partial cDNA cloning
GHR1-F4	GATCCTGAAGGAAGCGGCTA	3′-RACE for GHR1
GHR1-R4	TCGGGACACTCTTTCCACTCACT	5′-RACE for GHR1
GHR2-F1	ACATGRWGACTTTCCGCTGC	GHR2 partial cDNA cloning
GHR2-R1	GATRCTTCCCARARGGTCAG	GHR2 partial cDNA cloning
GHR2-F2	TTACCACGAATCGGATACAACC	GHR2 partial cDNA cloning
GHR2-R2	TGTGCTCTTCCTCCCTCATACC	GHR2 partial cDNA cloning
GHR2-F3	ATGCCCTTCTTCTTCCTTC	GHR2 partial cDNA cloning
GHR2-R3	CCTGTCGGTGCTGTATTG	GHR2 partial cDNA cloning
GHR2-F4	CATGGCTGCCKCTYTCAC	GHR2 partial cDNA cloning
GHR2-R4	GACCCGAACCTCGTGATTG	GHR2 partial cDNA cloning
GHR2-R5	CACATTCAGCAGCGTCCAGTT	5′-RACE for GHR2
GHR2-F5	AGCTTGGGAACGGTCTTGGAG	3′-RACE for GHR2
GHR2-qF1	CATCACTGGCTGTGTCTCC	Real-time PCR
GHR2-qR1	CCTGTCGGTGCTGTATTG	Real-time PCR
GHR1-qF1	AGTCCACTTTCCCGCTCACA	Real-time PCR
GHR1-qR1	CACCCATACCTCCACCACTCAG	Real-time PCR
GH-qF	GGCGGAGACCTACCTGAC	Real-time PCR
GH-qR	CCAAAACACTTGACTTGCTTC	Real-time PCR
18S-F	GACTCGGGGAGGTAGTGACG	Real-time PCR
18S-R	GGGCTGGGACAGACGGTAG	Real-time PCR
GAPDH-F	CGCATCGGTCGTCTGGT	Real-time PCR
GAPDH-R	GCCGTGGGTGGAGTCATAC	Real-time PCR
β-actin-F	TGTCCCTGTATGCCTCTGGTC	Real-time PCR
β-actin-R	TCCTTGATGTCACGCACGAT	Real-time PCR
AP	GGCCACGCGTCGACTAGTAC$(T)_{18}$	3′-RACE
AAP	GGCCACGCGTCGACTAGTAC$(G)_{10}$	5′-RACE
AUAP	GGCCACGCGTCGACTAGTAC	3′- and 5′- RACE

Degenerate bases: V = A + C + G; R = G + A; Y = C + T; W = A + T; M = A + C; D = A + T + G; K = G + T

Tissue distribution of GHR1 and GHR2 transcripts

Three 5-month-old *S. chuatsi* fish with body weights of 80–120 g were anesthetized using MS-222 and killed by decapitation. Tissue samples of muscle, kidney, gonad, liver, stomach, heart, intestine, brain, gill, and pituitary were collected quickly and flash-frozen in liquid nitrogen and stored at −80 °C until RNA extraction.

Fasting and re-feeding experiments

Eighty-four fish weighing 145.5 ± 38 g, all offspring of the same pair, were randomly divided into 14 tanks representing two groups. The water temperature was maintained between 25 and 26 °C using an air conditioner. The fish were allowed to acclimate to the tanks for 1 month prior to the beginning of the experiment. Following the acclimation period, food was withheld from seven tanks for 4 weeks, while the remaining seven tanks were fed continuously throughout the experiment. After the 4 weeks, fasted and fed fish were fed continuously for the remaining 10 days. Twelve fish, specifically six fed and six fasted/re-fed (from two groups), were terminally sampled in buckets containing MS-222 (0.5 g/l) at seven time points (1–7). Time point 1 (or 2–4) represents fish that had been fasted for 1 (or 2–4) week(s), while 5, 6, and 7 represent 3,

7, and 10 days after re-feeding, respectively. To minimize the effects of crowding on the growth rate, stocking densities were maintained at approximately 3 g/l as fish were removed for sampling. The fish were sampled every week 18:00 after exposure to fasting and every 3 days 19:00 3 h after re-feeding. The liver, pituitary, muscle, and plasma samples of the fish were collected.

Plasma levels of GH by ELISA

Plasma was separated by centrifugation at $1000\times g$ for 30 min at 4 °C, and stored at −20 °C until *GH* analysis. Plasma *GH* levels were measured by a *GH* ELISA Kit (Cusabio, WuHan, China) according to the manufacturer's instructions. Three replicates were set for all reactions. The standard curve was generated through a dilution series of standard substance ($r = 0.99$).

Data analysis

Data are expressed as the mean ± SEM. Statistical analysis was performed with SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Significant differences were identified by a one-way ANOVA followed by Fisher's least significance difference (LSD) test. A probability of less than 0.05 ($p < 0.05$) was considered significant.

Results

scGHR1 and scGHR2 sequences

Sequences covering the complete coding regions for *GHR1* and *GHR2* cDNAs from the liver of mandarin fish were designated as *scGHR1* and *scGHR2* (GenBank Accession No. KJ477038.1, KJ477039.1). The open reading frame of *GHR1* contains 1914 bp and encodes a putative protein of 638 amino acids (Fig. 1), and shares 92 % identity with that of *Epinephelus coioides* [9] (Fig. 1). *GHR1* contains a putative 28 amino acid signal peptide, an erythropoietin receptor (ligand-binding domain) of 104 amino acids, a transmembrane domain of 23 amino acids, and a *GHR* receptor-binding domain of 296 amino acids (Fig. 1). The open reading frame of *GHR2* contains 1734 bp and encodes a putative protein of 578 amino acids (Fig. 2) that has 86 % identity to *E. coioides* [9]. *GHR2* contains a putative 19 amino acid signal peptide, an erythropoietin receptor (ligand-binding domain) of 104 amino acids, a transmembrane domain of 23 amino acids, and a GHR-binding domain of 296 amino acids (Fig. 1). They both contain an extracellular binding FGEFS motif and a cytoplasmic box1 and box2 motif, which are also found in other fish such as *E. coioides*, *Sparus aurata*, and *Oreochromis niloticus*. Several characteristic landmarks of *scGHRs* have also been identified, including conserved extracellular cysteine residues and the potential *N*-glycosylation sites. A BLAST search using amino acid sequences of the *GHRs* revealed that several other conserved domains are also present, such as TFRCRW (at amino acid 56), WKECPEY (89), and PDPP (142) (Fig. 1). *scGHR1* has a box1 motif of LLPPVPVP, while *scGHR2* has a motif with a V to G replacement (Fig. 1). *scGHR1* has a box2 motif of EPWVEFIEVD, while *scGHR2* has a box2 motif of DPWVEFIDLD (Fig. 1). *scGHR1* has seven extracellular cysteine residues, while *scGHR2* has six (Fig. 1). The amino acid sequences of *GHR1* and *GHR2* in mandarin fish share 40 % identity.

Based on amino acid sequences, the neighbor-joining phylogenetic tree of the fish was constructed to illustrate the phylogenetic relationships of *scGHR1* and *scGHR2* to their counterparts in other known fish (Fig. 2). This tree places *scGHR1* in the *GHR1* clade and *scGHR2* in the *GHR2* clade.

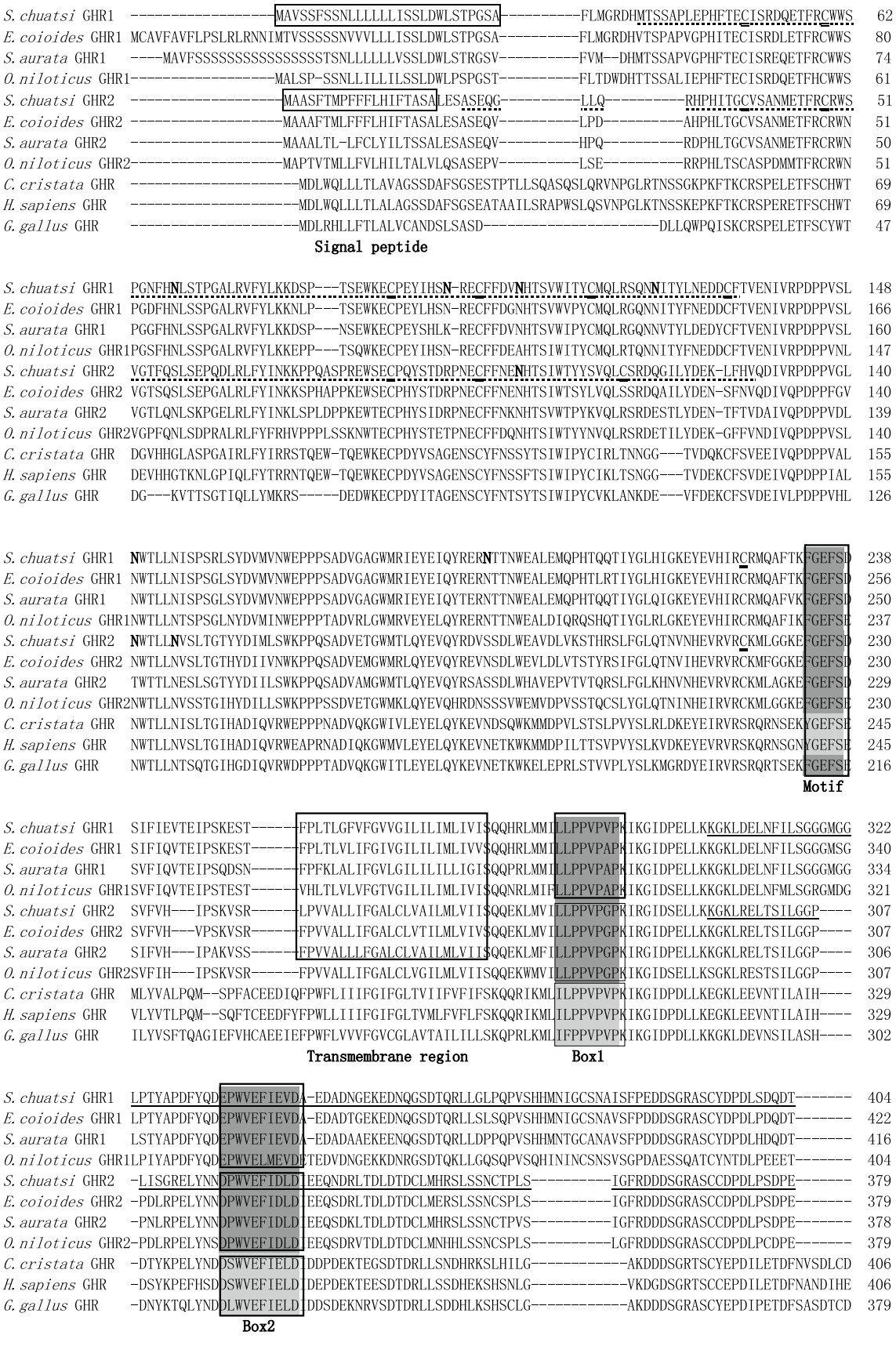
A comparison of the amino acid sequences of mandarin fish *GHRs* with those from other fish (Table 2) shows that generally the sequence identities between *GHR1* and *GHR2* within a given species were low (below 39 %), with the exception of *Oncorhynchus mykiss GHR1* (*omGHR1*) and *omGHR2*, which share 85.9 % identity, while the sequence identities with *GHRs* of other species were higher. *S. chuatsi*, *E. coioides*, *Oreochromis urolepis*, and *S. aurata* all belong to the Perciformes, and the identities of *GHR1* and *GHR2* between these species were all above 70 %.

Tissue distribution of receptors

The mRNA expression profiles of *GHR1* and *GHR2* in various tissues of mandarin fish were examined by real-time PCR (Fig. 3). Both *GHR1* and *GHR2* were detected in all tissues tested. For both *GHR1* and *GHR2*, the highest mRNA abundance was observed in skeletal muscle, liver, and heart. When comparing *GHR1* and *GHR2* mRNA abundance, *GHR1* was expressed at higher levels in muscle, brain, and gill than *GHR2* ($p < 0.05$), while *GHR2* mRNA was more abundant in liver, heart, gonad, pituitary gland, and kidney than *GHR1* ($P < 0.05$). The lowest *GHR1* and *GHR2* mRNA abundance was found in the stomach and pituitary gland.

Effects of fasting and re-feeding

Throughout the experiment, the body weight of fish in the fed group increased gradually, while that in the fasting group decreased over the 4-week fasting period (Fig. 4a). When re-feeding over the following 10 days, the body weight of previously fasted fish increased quickly,


S. chuatsi GHR1
E. coioides GHR1
S. aurata GHR1
O. niloticus GHR1
S. chuatsi GHR2
E. coioides GHR2
S. aurata GHR2
O. niloticus GHR2
C. cristata GHR
H. sapiens GHR
G. gallus GHR
Signal peptide
Motif
Transmembrane region
Box1
Box2

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S. chuatsi GHR1  ------------------------------LMLMATLLPGQPEDGEASFDVVERAPAPESGERPLVQTQTGGPQTWVN---TDFYAQVSNVM  463
E. coioides GHR1 ------------------------------LMLMATLLPGQPEDGEASFDVVERAPAPERGERPLVQTQTGGPQTWVN---TDFYAQVSNVM  481
S. aurata GHR1   ------------------------------LMLMATLLPGQPEDGEDSFDVVERAPVIERSERPLVQTQTGGPQTWLN---TDFYAQVSNVM  475
O. niloticus GHR1------------------------------LMLMATLLPGQPDEEETSLDTVERSSASETGERQLIQTQTRGPQTWVN---TDFYAQVTNVM  463
S. chuatsi GHR2  ------------------------------ASPFHPLIP-----TQSSFPAACEPSSP-------VQSPTAGEPSFVAPGREALYTQVSEVR  429
E. coioides GHR2 ------------------------------PSPFIPLIPNQIHSKEPACLTPCEPNSP-------AQSPTAGEPFSVAPGREAMYTKVSEVR  434
S. aurata GHR2   ------------------------------ASPFHPLIPNQTLSKEVSCQTASEPSSP-------VQSPASGEPPFAALGREAMYTQVSEVR  433
O. niloticus GHR2------------------------------PSPFLPLVPNLALSQETLCAATSEPSSP-------IQSCNSGELPSFVTGRDTLYTQVTEVR  434
C. cristata GHR  GTSEVAQPQRLKG-DADLLCLDQKNQNNSPSIDASLSTQQ-HSVSPTQENKSRPILIGGTESTPQASHSQLSNLSSLANIDFYAQVSDIT  494
H. sapiens GHR   GTSEVAQPQRLKG-EADLLCLDQKNQNNSPYHDACPATQQ-PSVIQAEKNKPQPLPTEGAESTHQAAHIQLSNPSSLSNIDFYAQVSDIT  494
G. gallus GHR    AISDIDQFKKVTEKEEDLLCLHRKDDVEALQSLANTDTQQPHTSTQSESRESWPPFADSTDSANPSVQTQLSNQNSLTNTDFYAQVSDIT  469

S. chuatsi GHR1  PSGGVVLSPGQQLRIQESTSATEEETQQKGKEHKSNEDTEEKKQKELQFQLLVVDPEGSGYTTESNARQISTPP-SSPMPGEGYQTIHPQ  552
E. coioides GHR1 PSGGVVLSPGQQLRIQENTLATEEETQKKGKEHKGNEDTEENKQKELQFQLLVVDPEGSGYTTESNARQISTPP-SSPMPGEGYQTIHPQ  570
S. aurata GHR1   PSGGVVLSPGQQLRFQESTSAAEDEAQKKGK---GSEDSEEKTQKELQFQLLVVDPEGSGYTTESNARQISTPP-STPMPGSGYQTIHPQ  561
O. niloticus GHR1PTGGVVLSPGQQLRIQESISAAEKETKKKRKESEDSEESEERKQKEPQFQLLVVDPEGSAYSTESSIQQISTPPPSSPMPGEGYHIIHPQ  553
S. chuatsi GHR2  SSGKVLLSPEEQTEVEKTTSKDTEKDI----------IVEKEKEKKEFQLLVVNLDHGGYTSELNAGKTSLKL----STGD-----TSE  499
E. coioides GHR2 SSGKVLLSPEEQTEEP--TSKDTEKEK----------MAEKEKEKKEFQLLVVNPEHGGYTSELNAGKMSPRS----SSGD-----MSE  502
S. aurata GHR2   SSGKVLLSPEEQTEVEKTTGKDTEKDI----------MAEKEKAKKEFQLLVVNADHGGYTSELNAGKMSPRL----SIGD-----QSE  503
O. niloticus GHR2SSGKVLLLPEEQTEREKMSSKEKEDEI----------VVEKEK--KEFQLLVMNADHRDYTSELNAGKTSPEL----STGD-----LTE  502
C. cristata GHR  PAGSVVLSPGQKNKAGTARCDAHPEAV----------SRCQANFIMDNAYFCEADAKKCITMAPHMQMETRG----------EPSFNQ  562
H. sapiens GHR   PAGSVVLSPGQKNKAGMSQCDMHPEMV----------SLCQENFLMDNAYFCEADAKKCIPVAPHIKVESHI----------QPSLNQ  562
G. gallus GHR    PAGSVVLSPGQKSKVGRAQCES----------CTEQNFTMDNAYFCEADVKKCIAVISQEEDEPRV----------QEQSCN  531

S. chuatsi GHR1  PVETKPAATAEDNQSPYILPDSPQSQFFAPVADYTVVQEVDSQHSLLLNPPPRQSPPPCLPQHPLKALPAMPVGYITPDLLGNLSP  638
E. coioides GHR1 PVETKPTPTAEDNQSPYILPDSPQSQFFAPVADYTVVQEVDSQHSLLLNPPPRQSPPPCVPQHPLKALPAMPVGYITPDLLGNLSP  656
S. aurata GHR1   PVETKPAATAENNQSPYILPDSPQSQFFAPVADYTVVQEVDSQHSLLLNPPPRQSPPPCLPHHPTKALAAMPVGYVTPDLLGNLSP  647
O. niloticus GHR1PVEPRPAATMELNQSPYIIPDSPQ--FFAPVADYTVVQELDSHHSLLLNPPCHQTPPPCLPQHPLKAP--MPVGYITPDLLGNLSQ  635
S. chuatsi GHR2  SCQTAGDLSSL--PSPYHESDTTPMSPLPPAPVYTVVEGVDRENSLLLTPNSTPAPQLIIPKT-----TPTPDGYLTPDPLGSITP  578
E. coioides GHR2 PCQTGGD-------SPYHESDPTPMSPLSPAPVYTVVEGVDMQNSLLLTPNSTPAPQLIIPKT-----VPTPDGYLTPDPLGSITP  576
S. aurata GHR2   PGLTGDLSPLPP-ASPYHESDTTAVSPLPPAPVYTVVEGVDRQNSLLLTPNSTPAPQLIIPKT-----MPTPGGYLTPDLLGSITP  583
O. niloticus GHR2PCKTFPS--------PHHESNTTTPPPIP-APVYTVVEGVDRQNSLLLSPNSAPAPHLIIPKN-----MPTPDGYLTADLLGSITP  574
C. cristata GHR  EDIYITTESLTTT-VGRSGAAERAPGSEMPVPDYTSIHIVQSPQGLVLNATALPLPDKEFLSS---------CGYVSTDQLNKIMP  638
H. sapiens GHR   EDIYITTESLTTA-AGRPGTGEHVPGSEMPVPDYTSIHIVQSPQGLILNATALPLPDKEFLSS---------CGYVSTDQLNKIMP  638
G. gallus GHR    EDTYFTTESLTTTGINLGASMAETPSMEMPVPDYTSIHIVHSPQGLVLNATALPVPEKEFNMS---------CGYVSTDQLNKIMP  608
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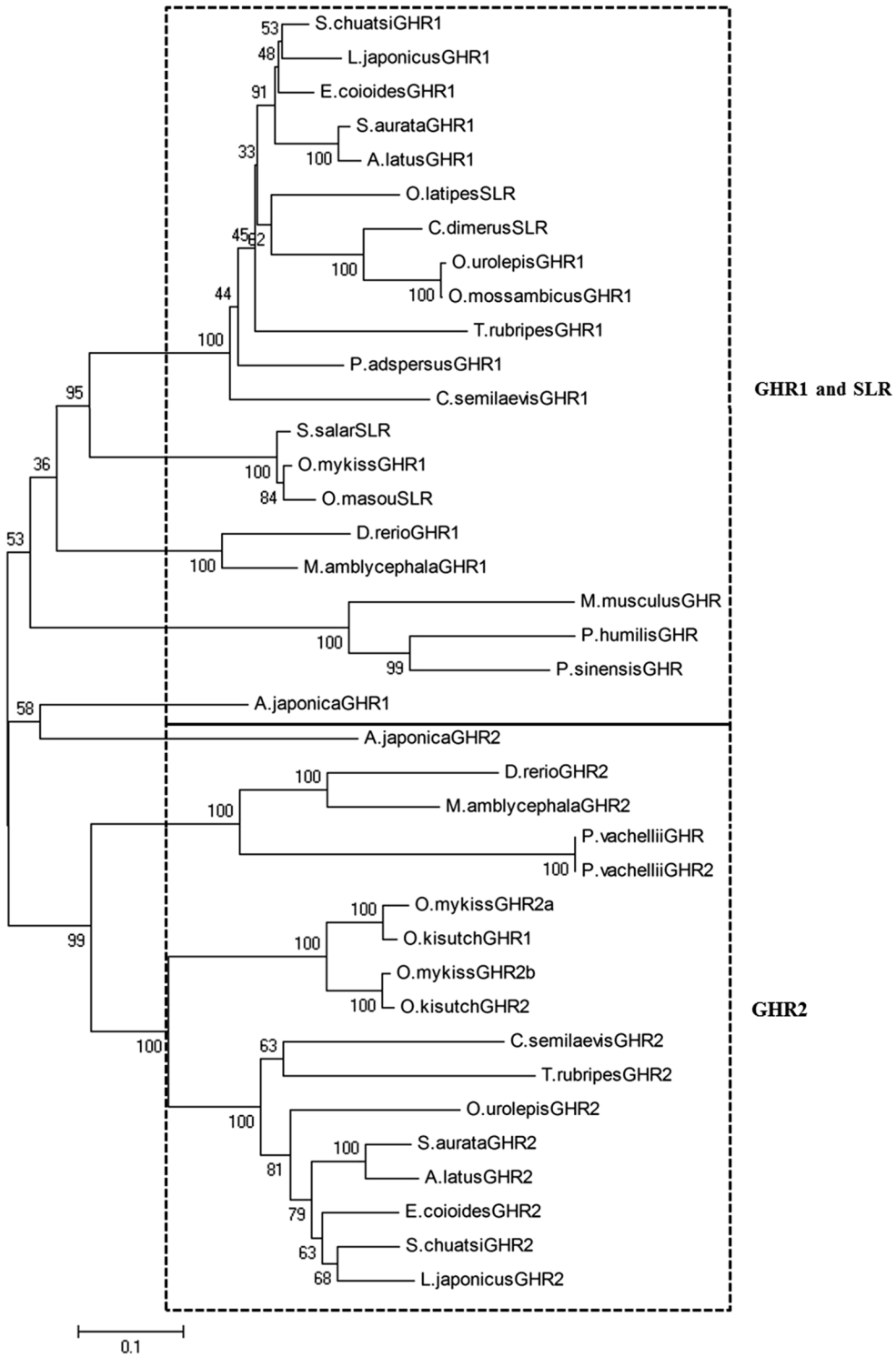
Fig. 1 continued

◀**Fig. 1** Alignment of amino acid sequences of *scGHRs* and other vertebrate *GHRs*. Missed amino acids are marked by *dashes*. Three *GHR* protein family signature sequences—FGEFS motif, and conserved box1 and box2—are indicated by *shaded* regions. The putative signal peptide is in an *open rectangle*. Transmembrane region is in *bold*. Extracellular cysteines are *double underlined*. Potential N-glycosylation sites are in *bold*. Putative erythropoietin receptor (ligand binding) is *underlined* with *broken line*. Growth hormone receptor binding is *underlined*. The stop codon is denoted by *asterisk*. The GenBank accession numbers of the *GHRs* are as follows: *Siniperca chuatsi GHR1* (KJ477038.1), *Siniperca chuatsi GHR2* (KJ477039.1), *Epinephelus coioides GHR1* (EF052273.1), *Epinephelus coioides GHR2* (EF052274.1), *Sparus aurata GHR1* (AF438176.2) *Sparus aurata GHR2* (AY573601.2), *Oreochromis niloticus GHR1* (NM_001279601.1), *Oreochromis niloticus GHR2* (AY973233.1), *Condylura cristata GHR* (XM_004678346.1), *Gallus gallus GHR* (NM_001001293.1), Homo sapiens *GHR* (NP_000154.1)

although it was still less than the mean body weight of the fed group. The specific growth rate (SGR) decreased from -0.33 ± 0.07 to -0.44 ± 0.06 in the fasted group over the 4 weeks of fasting. When re-fed, growth rate increased significantly to 1.57 ± 0.16, which was much higher than the continuously fed controls (0.8 ± 0.12, Fig. 4b).

In this experiment, we investigated the mRNA expression levels of *GH* in pituitary and *GHR1* and *GHR2* in liver and muscle of *S. chuatsi* during feeding, fasting, and re-feeding.

Expression of hepatic *scGHR1* and *scGHR2* mRNA initially decreased significantly to a lower level than that of the controls after 1 week of fasting ($p < 0.05$). Over the following 3 weeks of fasting, the levels of hepatic *scGHR1* and *scGHR2* mRNA were still lower than those of the controls ($p < 0.05$) (Fig. 5a, b). In muscle, *scGHR1* mRNA levels increased to 1.22 times that of the controls after 1 week of fasting and significantly increased to 2.67 times after 4 weeks of fasting ($p < 0.01$, Fig. 5c). *scGHR2* mRNA expression levels in the muscle were always more than those in the continuously fed controls during fasting ($p < 0.05$, Fig. 5d). *GH* mRNA in the pituitary of mandarin fish increased significantly during the first 3 weeks of



fasting ($p < 0.05$) and decreased to a normal level after 4 weeks of fasting (Fig. 5e).

During re-feeding, *scGHR1* mRNA expression levels in the liver increased rapidly and were not significantly different from those of the continuously fed controls. *scGHR2* initially increased to a level higher than in the controls after 3 days of re-feeding ($p < 0.05$), and then recovered to a normal level (Fig. 5a, b). There are no significant differences in muscle *scGHR1* and *scGHR2* mRNA expression during periods 5, 6, and 7 (re-fed period) between fed and re-fed fish (Fig. 5c, d). In addition, no significant differences were observed in *scGHR1* and *scGHR2* mRNA expression

◀**Fig. 2** Phylogenetic analysis of teleost *GHR1*, *GHR2* and somatolactin receptor (*SLR*). The phylogenetic tree was constructed by MEGA 4.0 using neighbor-joining method. Phylogeny test was performed by the bootstrap method with 1000 replicates. The number shown at each branch indicates the bootstrap value (%). GenBank Accession Nos. of fish *GHR* and *SLR* used are as follows: *Siniperca chuatsi GHR1* (KJ477038.1); *Siniperca chuatsi GHR2* (KJ477039.1); *Epinephelus coioides GHR2* EF052274.1; *Epinephelus coioides GHR1* EF052273.1; *Oreochromis urolepis GHR1* EF371466.1; *Oreochromis urolepis GHR2* EF371467.1; *Sparus aurata GHR1* AF438176.2; *Sparus aurata GHR2* AY573601.2; *Oncorhynchus mykiss GHR1* NM_001124535.1; *Oncorhynchus mykiss GHR2a* AAW56611.1; *Oncorhynchus mykiss GHR2b* AAW27914.1; *Danio rerio GHR1* NM_001083578.1; *Danio rerio GHR2* NM_001111081.1; *Acanthopagrus latus GHR1* JN399057.1; *Acanthopagrus latus GHR2* JN399058.1; *Anguilla japonica GHR1* AB180476.1; *Anguilla japonica GHR2* AB180477.1; *Lateolabrax japonicus GHR1* KF601961.1; *Lateolabrax japonicus GHR2* KF770840.1; *Megalobrama amblycephala GHR1* JN896373.1; *Megalobrama amblycephala GHR2* JN896374.1; *Pelteobagrus vachellii GHR1* JN392017.1; *Pelteobagrus vachellii GHR2* JN392016.1; *Takifugu rubripes GHR1* AB621336.1; *Takifugu rubripes GHR2* AB621337.1; *Oryzias latipes SLR* XM011478846.1; *Cichlasoma dimerus SLR* FJ208943.1; *Oreochromis mossambicus GHR1* AB115179.1; *Oryzias latipes SLR* XP011477148.1; *Cichlasoma dimerus SLR* ACI42879.1; *Oreochromis mossambicus GHR1* BAD83668.1; *Paralichthys adspersus GHR1* ABS29325.1; *Cynoglossus semilaevis GHR1* AEO97318.1; *Cynoglossus semilaevis GHR2* AEO97315.1; *Salmo salar SLR* ABY76179.1; *Oncorhynchus masou SLR* BAD51998.1; *Mus musculus GHR* EDL03404.1; *Pseudopodoces humilis GHR* XP_005533357.1; *Pelodiscus sinensis GHR* XP_006139558.1; *Oncorhynchus kisutch GHR1* AAK95624.1; *Oncorhynchus kisutch GHR2* AAK95625.1

in the fed controls during re-feeding. The mRNA expression of *GH* in the pituitary increased to a level 5.1 times higher than the controls 3 days after re-feeding ($p < 0.01$) and decreased to a normal level 7 days after re-feeding (Fig. 5e).

Plasma levels of GH

Plasma *GH* concentrations in fasted fish over the 4 weeks were significantly higher than in the continuously fed controls ($p > 0.05$), but when re-fed, plasma concentration of *GH* decreased to a normal level similar to that of the continuously fed controls (Fig. 6).

Discussion

In this study, the cloning, characterization, and expression analysis of two distinct mandarin fish *GHR* isoforms, namely *scGHR1* and *scGHR2*, are reported for the first time. According to the deduced amino acid sequences, *scGHR1* and *scGHR2* both have a single short transmembrane domain, an extracellular erythropoietin receptor (ligand-binding) domain located at the C-terminus of the protein, and an intracellular GHR-binding domain, including an FGEFS motif and a box1 and box2 motif, as are characteristic of *GHRs* also found in *Lateolabrax japonicus*,

Table 2 Amino acid sequence percent identity of mandarin fish *GHRs* compared to other fishes

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
100	36.4	40.4	35.4	37.3	74.4	44.3	43.4	32.6	38.1	35.6	51.5	33.1	40.5	36	39.2	1	drGHR2
	100	38.8	89.5	46.2	37.8	38.8	39.7	77.6	37.1	81.6	35.7	87	39.2	92.6	38	2	ecGHR1
		100	37.7	43	42.7	59	59.5	35.6	72.1	38.1	40	38.5	80.6	38.1	84.5	3	ecGHR2
			100	45.8	36.5	38.8	40.1	76.5	35.6	80.7	34.9	85.3	38.6	91.5	37.2	4	ljGHR1
				100	39.5	43.3	44.8	43.6	40.2	46.4	37.4	46.2	41.4	46.4	41.7	5	maGHR1
					100	46.3	45.7	34.3	39.5	36.5	58.1	35.9	41.5	37.2	42.2	6	maGHR2
						100	85.9	36.1	55.7	39.2	42.1	37.7	59.4	38.8	60.5	7	omGHR1
							100	36.9	55.1	39.7	42.1	39.4	60	40.1	60.8	8	omGHR2
								100	34.5	72.5	32.1	73.9	34.9	78.6	35.8	9	ouGHR1
									100	35.9	37.8	37.5	71.8	36.7	73.7	10	ouGHR2
										100	34	78	38.4	83.8	37.6	11	paGHR1
											100	34.5	38.4	34.3	39.4	12	pvGHR1
												100	38.8	88.3	38.4	13	saGHR1
													100	39	80	14	saGHR2
														100	37.4	15	scGHR1
															100	16	scGHR2

The amino acid sequence identity between mandarin fish *GHRs* and other fishes *GHRs* was calculated with MegAlign of DNAStar software. Data are expressed as percentage amino acid identity. The GenBank accession numbers of the *GHRs* are seen in Fig. 1

dr, *Danio rerio*; ec, *Epinephelus coioides*; lj, *Lateolabrax japonicus*; ma, *Megalobrama amblycephala*; om, *Oncorhynchus mykiss*; ou, *Oreochromis urolepis*; pa, *Paralichthys adspersus*; pv, *Pelteobagrus vachellii*; sa, *Sparus aurata*; sc, *Siniperca chuatasi*

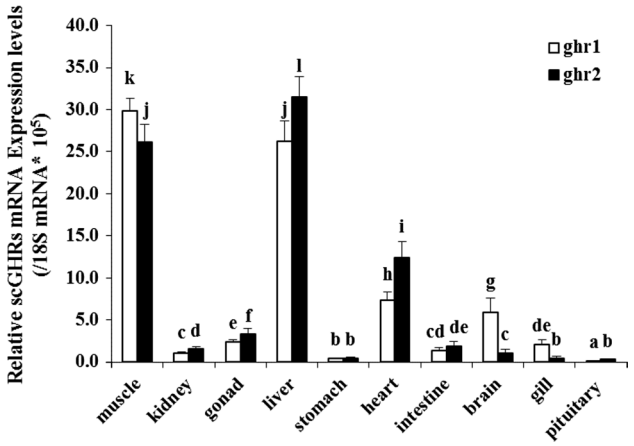


Fig. 3 Relative expression of *GHR1* and *GHR2* mRNA in various tissues of *Siniperca chuatsi*. mRNA of *18S*, *GHR1*, and *GHR2* were quantified by real-time PCR (SYBR Green I), using *18S* as a housekeeping gene. All data are represented as the mean ± SEM ($n = 3$). Different *letters* indicate significant differences at $p < 0.05$

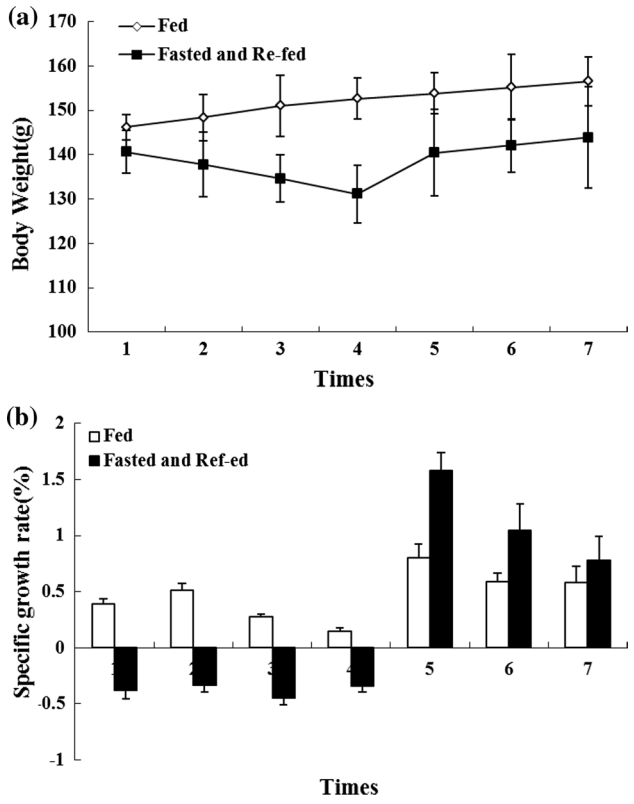


Fig. 4 Changes in body weight (**a**), specific growth rate (**b**), during 4 weeks of fasting and 10 days of re-feeding in *Siniperca chuatsi*. *Vertical bars* indicate ± SEM (body weight, specific growth rate, $n = 6$). Times of sampling: times 1, 2, 3, 4 mean 1, 2, 3, 4 weeks after fasting, respectively, times 5–7 means 3, 7, 10 days after re-feeding

rainbow trout, grouper, Nile tilapia, and seabream. The FGEFS motif is important for ligand-binding and signal transduction, as mutations of this motif in humans result in *GH* insensitivity [41]. The proline-rich box1 takes part in the JAK2–STAT signaling pathway, and internalization of the receptor is mediated by box2 [1]. All of the motifs were highly conserved in mandarin fish compared with the other fish (Fig. 1). Extracellular cysteine residues are involved in disulfide bonds and are structurally important in *GHRs* [7, 21]. In vertebrates, *GHR1* has seven Cys residues with some variability at the last position; *GHR2* mostly has five Cys residues with none in the fifth or sixth positions as *GHR1* [21] (Fig. 1). The extracellular Cys residues are also highly conserved in *scGHR1*, while *scGHR2* has one more Cys residue not found in other teleosts. On the other hand, the sequence identities between *GHR1* and *GHR2* within a given species were generally low (below 39 %), with the exception of *omGHR1* and *omGHR2*, which share 85.9 % identity (Table 2). This suggests that there was a high rate of evolution from an ancestral *GHR* to *GHR1* and *GHR2* in teleosts.

Two distinct phylogenetic isoforms of *GHRs* were also identified in rainbow trout [13], Nile tilapia [14], orange-spotted grouper [15], black seabream [16], gilthead seabream [17], and Japanese seabass [19]. In the phylogenetic tree, *scGHR1* was grouped with *GHR1* and the somatolactin receptor (*SLR*) of other fish, while *scGHR2* was grouped with *GHR2* of other fish (Fig. 2). It is now known that descendants of the early sarcopterygians have only one copy of the *GHR* gene, as seen in the tetrapods, while the early actinopterygians underwent the fish-specific genome duplication (FSGD) and subsequently two distinct clades of *GHRs* within the teleosts appeared [21]. The exception that *omGHR1* and *omGHR2* share 85.9 % identity was caused by a more recent duplication (4R) event in several lineages of teleosts, including the salmonids. The tetraploid salmonids had duplicated *GHR1/2* genes. Recently, a nomenclature system was proposed by Ellens et al. in which different numbers were used for genes derived from the FSGD; then, different letters for paralogs derived from a recent duplication and *SLR* should be classified to *GHR1* [21]. This nomenclature is also confirmed by analysis of the evolution of receptors for *GH* and somatolactin in fish and land vertebrates by Fukamachi and Meyer [20]. Our results also support the nomenclature (Fig. 1, 2). Thus, by applying it, *omGHR1* and *omGHR2* should be designated as *omGHR2a* and *omGHR2b* (Table 2). To avoid confusion, the existing names in several species require revision.

A wide range of *GHR1* and *GHR2* expression levels have been reported in teleost species, including rainbow trout [13], Nile tilapia [14], orange-spotted grouper [15], black seabream [16], gilthead seabream [17], etc. In this experiment, both *scGHR1* and *scGHR2* were detected in all of the tissues tested, which were in agreement with the various functions of *GH* in growth, metabolism, reproduction, immunity, and osmoregulation [11, 42]. However, the

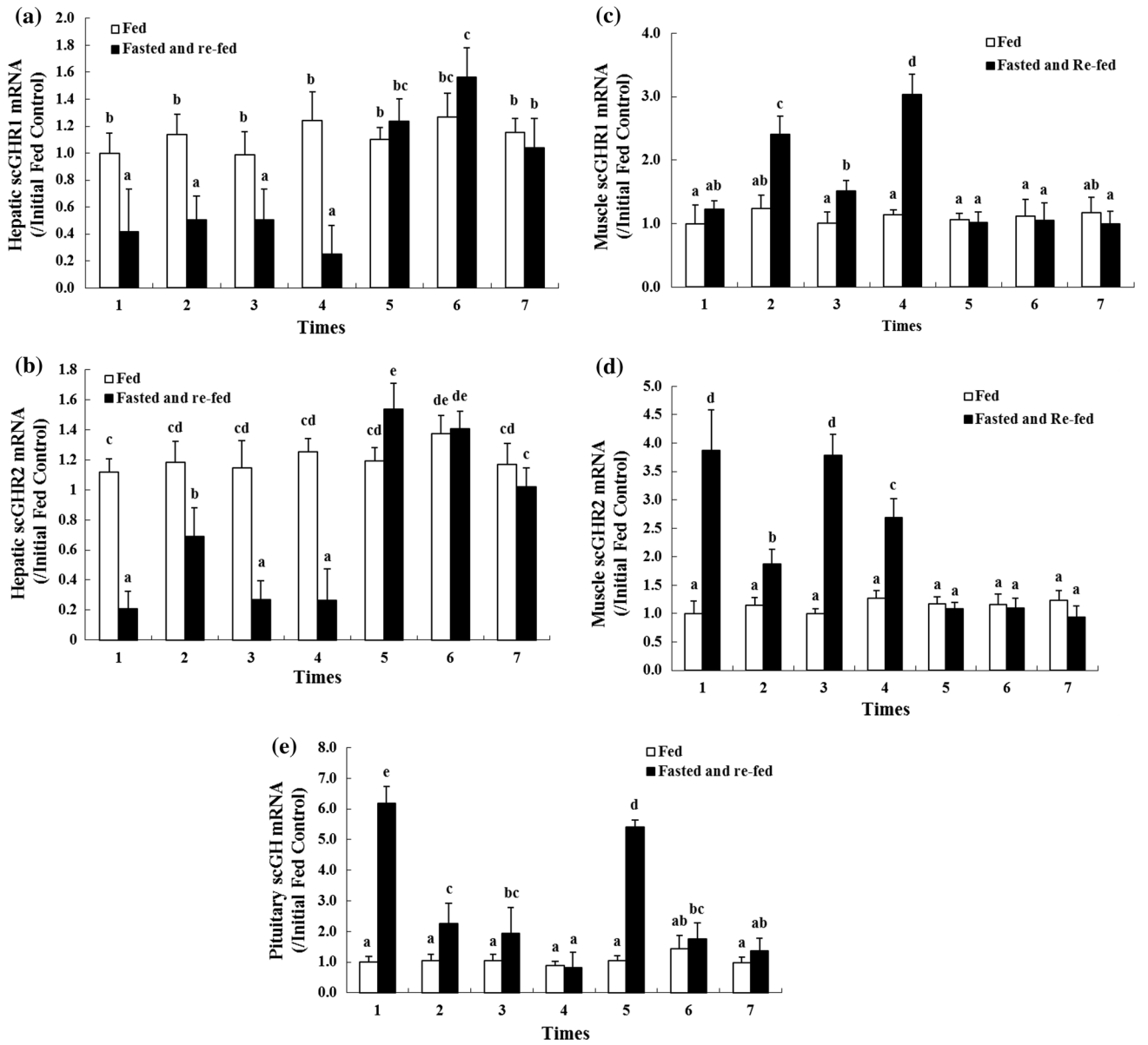


Fig. 5 Effects of fasting and re-feeding on pituitary, liver and muscle expression of *GH*, *GHR1*, *GHR2* in *Siniperca chuatsi*. After acclimated for a month, fish were divided into two groups ($n = 42$/group). As for fed or re-fed group, fish were fed or fasted for 38 days, and at the end of experiment, fish were fed or re-fed for 6 h before sampling; and in the fasted group, fish had been fasted before sampling. The mRNA expression levels of *GHR1*, *GHR2* in liver (**a**, **b**), muscle (**c**, **d**), and *GH* in pituitary (**e**) of mandarin fish were quantified by real-time PCR and normalized against *18S* transcripts. Times of sampling: times 1, 2, 3, 4 mean 1, 2, 3, 4 weeks of fasting, respectively; times 5–7 mean 3, 7, 10 days after re-feeding. *Each bar* represents the mean ± SEM. ($n = 6$). Different *letters* indicate significant differences at $p < 0.05$

amounts of *scGHR* mRNA varied by tissue and the highest expression levels of *scGHR* mRNA were found in liver and muscle (Fig. 3). Similar patterns have been found in rainbow trout [13], Japanese flounder [43], salmon [44], and black seabream [45]. It appears that *GHRs* play important roles in the growth and metabolism of fish [21]. Different expressions of *GHRs* mRNA in tissues have been reported in several teleosts, such as gilthead sea bream [17], Nile tilapia [14], and rainbow trout [13]. In gilthead sea bream, the expression level of *GHR2* was significantly lower than *GHR1* in liver and adipose tissue, but no significant difference was observed in muscle [17]. In Nile tilapia, *GHR2* transcripts were significantly higher than *GHR1* in most tissues but lower in muscle [14]. In mandarin fish, *scGHR1* mRNA was expressed at higher levels than *scGHR2* in muscle, brain, and gill, while the expression of

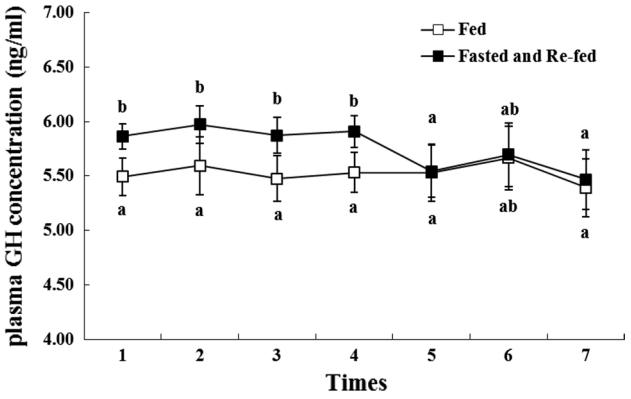


Fig. 6 Effects of fasting and re-feeding on plasma levels of *GH*. Vertical bars indicate mean ± SEM ($n = 6$). Significant differences from the corresponding fed control at the $p < 0.05$ level are indicated by different *letters*. Times of sampling: times 1, 2, 3, 4 mean 1, 2, 3, 4 weeks of fasting, respectively, times 5–7 mean 3, 7, 10 days after re-feeding

scGHR2 mRNA was higher than that of *scGHR1* in liver, heart, gonad, pituitary gland, and kidney. Although the reason why expressions of *GHR1* and *GHR2* between and/or within tissues in different species were different is not known, it might be ascribed to their long evolution history and to environmental and physiological conditions.

Nutritional state has a strong effect on the expression of *GHRs* in teleosts. The influences are different among fish species. In masu salmon, the expression of hepatic *GHR1* mRNA was reduced in fish fasted for 3 weeks [44]. Skeletal muscle expression of *GHR2* mRNA was significantly increased in hybrid striped bass after 3 weeks of complete food restriction, and hepatic *GHR1* and *GHR2* mRNA levels both declined during food deprivation [33], while the liver of fasting tilapia shows no changes in *GHR1* transcript levels [22]. These different results might be due to the different tissues in various fish species and the sampling times. However, decrease of hepatic *GHR1/2* mRNA levels and increase of muscle *GHR1/2* mRNA levels under fasting conditions have been reported in more teleosts, such as black seabream [45], tilapia [22], and channel catfish [46]. The results in mandarin fish are in agreement with these species. In mandarin fish, the expression of *scGHR1* and *scGHR2* mRNA in liver decreased during fasting, and in the muscle, the expression of both mRNAs increased (Fig. 5a, b). The reduced expression of hepatic *GHR* would bring down *GH* sensitivity, which results in reduced *IGF* production and growth inhibition. Moreover, the high expression of muscle *GHR* might promote mobilization of lipids [4]. Thus, as a result of the consumption of lipids, emaciation was observed in mandarin fish during fasting (Fig. 4).

Re-feeding generally restored the fasting-associated changes in fish. Plasma *GH*, hepatic *GHR* mRNAs, and muscle *GHR* mRNAs recovered to pre-fasting levels [1]. In tilapia, muscle *GHR1* mRNA declined to below control levels upon re-feeding for 1 and 2 weeks, but recovered 2 weeks after re-feeding, and there was no effect of re-feeding observed in the expression of *GH* receptor in the liver [47]. In hybrid striped bass, hepatic *GHR1* mRNA increased after 10 days of re-feeding and recovered after 40 days of re-feeding. Further, there were no significant differences in muscle *GHR1* mRNA expression between or within groups [33]. Although the changes in hepatic *GHR* mRNA and muscle *GHR* mRNA during re-feeding in mandarin fish were different, CG was observed and they supported the "restore" mechanism by recovering to normal levels during re-feeding.

GH is the main regulator that controls somatic growth in vertebrates [7, 48]. Changes in *GH* concentrations during fasting and re-feeding have been studied in many fish. In black seabream, serum *GH* levels increased progressively during starvation. After 30 days of starvation, serum *GH* levels were over three times those of the fed controls [45]. In channel catfish, pituitary *GH* mRNA increased while *IGF-I* mRNA and plasma *IGF-I* decreased when fasted for 14 or 28 days [49]. Plasma *GH* and pituitary *GH* mRNA levels were significantly increased in seawater-acclimated tilapia fasting for 4 weeks [50]. Two weeks of fasting produced a significant reduction in plasma *IGF-I*, but not in plasma *GH* in tilapia *Oreochromis mossambicus* acclimated to fresh water [51]. Together, these findings indicate that fasting increases plasma *GH* and pituitary *GH* mRNA levels in fish. It is believed that the starvation-induced increase in plasma *GH* could be due to a reduced feedback on the pituitary from the reduction of *IGF-I* level, in turn resulting from a reduction in hepatic *GHR* [52]. In mandarin fish, *GH* mRNAs in the pituitary and plasma *GH* concentrations both increased after fasting. This result is also in agreement with studies in salmonids [25, 34], tilapia [51], and catfish [49]. The abrupt increase in pituitary *GH* mRNAs at period 5 in mandarin fish might be ascribed to recovery of metabolism after the long-term starvation and 3 days of re-feeding. The different expression patterns of *GH* would be affected by environmental conditions and growth phases of different fish species during fasting and re-feeding [25]. Further studies are needed.

It has also been reported that 1 week of re-feeding can restore the pituitary *GH* mRNA back to its normal levels in *E. coioides* [53]. In rabbitfish, *GH* mRNA levels also returned to normal after 6 days of re-feeding [54]. The mRNA expression of *GH* in the pituitary of mandarin fish returned to a normal level 7 days after re-feeding. These results are in agreement with studies in *E. coioides* and rabbitfish [53, 54]. In mandarin fish, when the fish were re-fed, the plasma concentration of *GH* decreased to the same level as that of the continuously fed controls (Fig. 6). In rainbow

trout, re-feeding for 2 weeks following 4 weeks of fasting also decreased plasma *GH* concentrations [25]. In isolated rainbow trout hepatocytes, *GH* treatment significantly increased the expression of *GHR1* and *GHR2* mRNA [55], while different results in vivo were found in this study (Fig. 5a, b, e). The increase in plasma *GH* concentration was accompanied by a decrease in hepatic *scGHR1* and *scGHR2* mRNA levels. It might be due to the complex regulatory systems in vivo [2, 4].

In summary, cDNAs of two distinct isoforms of *GHRs* (*scGHR1 and scGHR2*) have been isolated and characterized in mandarin fish. Both receptors, which arose through FSGD, have conserved the FGEFS motif and box1 and box2 motifs, as characteristic of *GHRs* also found in other species. They were most highly expressed in muscle and liver. The different expression profiles of *scGHR1* and *scGHR2* suggest distinct functions in different tissues. There was partial CG during 10 days of re-feeding after 4 weeks of fasting in mandarin fish. The response of *GH* and *GHRs* to variable growth phases, including negative, normal, and accelerated growth, has been described in detail with different sampling frequencies along the time course. The changes in *GH*, *scGHR1*, and *scGHR2* mRNA expression during fasting and re-feeding suggest that they play important roles in the growth of mandarin fish. It has been reported that polymorphisms of *GH* and *GHR* genes are associated with growth traits in Boer goat breeds and could be a molecular marker for growth traits [56], so they could also be used as candidate genes for identifying growth-related molecular markers in the selective breeding of mandarin fish. The results provide comparative insights into the roles of these genes in the growth of teleost.

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