The role of growth hormone in growth, lipid homeostasis, energy utilization and partitioning in rainbow trout: Interactions with leptin, ghrelin and insulin-like growth factor I

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Abstract
The growth-promoting effects of in vivo growth hormone (GH) treatment were studied in relation to size and lipid content of energy stores including liver, mesentery, white muscle and belly flap in rainbow trout. In order to elucidate endocrine interactions and links to regulation of growth, adiposity and energy metabolism, plasma levels of GH, insulin-like growth factor I (IGF-I), leptin (Lep) and ghrelin, were assessed and correlated to growth and energy status. In addition tissue-specific expression of lepa1 mRNA was examined. Juvenile rainbow trout were implanted with sustained-release bovine GH implants and terminally sub-sampled at 1, 3 and 6 weeks. GH increased specific growth rate, reduced condition factor (CF) and increased feed conversion efficiency resulting in a redistribution of energy stores. Thus, GH decreased mesenteric (MSI) and liver somatic index (LSI). Lipid content of the belly flap increased following GH-treatment while liver and muscle lipid content decreased. Independent of GH substantial growth was accompanied by an increase in muscle lipids and a decrease in belly flap lipids. The data suggest that the belly flap may function as an energy buffering tissue during episodes of feeding and lean growth. Liver and muscle lipids were positively correlated to body weight, indicating a size-dependent change in adiposity. Hepatic lepa1 mRNA positively correlated to MSI and CF and its expression decreased following GH treatment, coinciding with decreased hepatic lipid content. Plasma Lep was positively correlated to MSI and belly flap lipid content, suggesting that Lep may communicate energy status. In summary, the observed GH tissue-specific effects on lipid metabolism in rainbow trout highlight the complex physiolo-

gy of the energy reserves and their endocrine control.

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1. Introduction
In fish as in other vertebrates, ingested and accumulated energy is the key to survival, growth and reproduction, with body fat normally being the most important energy reserve. Such reserves allow fish, especially temperate and cold-water species to survive prolonged (seasonal and episodic) periods of limited feed availability. The size of the body fat reserve is also thought to be a key parameter for initiation of puberty in fish [31,72] well illustrated by the increased incidence of early maturation of farmed fish fed large amounts of high-energy diets [68]. For anadromous salmonids, sufficient energy reserves are also essential for the long and strenuous upstream migration they undertake to the spawning grounds without active foraging. Concomitant to the migration, the female salmonid sexual maturation demands significant amounts of lipids to be mobilized and incorporated into the oocytes.

While energy storage is an important function of adipose tissue, it is becoming increasingly evident through mammalian research that adipose tissue has multiple and probably often, tissue-specific roles. Thus, the complex endocrine function of adipose tissue is becoming increasingly evident [14], and in humans, the tissue-specific distribution of fat has important health-related implications [17]. Teleost fish have various strategies to accumulate lipid reserves, but broadly, fish are categorized as “lean” and “fatty”, referring to their muscle fat content. “Lean” fish such as e.g., the Atlantic cod, have a low muscle fat content of 1–2% [25], while the liver somatic index (LSI) is 7–9%, representing the major energy
Sweden. The fish were distributed into 12 0.5 m3 fiberglass tanks at the Department of Zoology, University of Gothenburg, Antens Laxodling AB, Sweden, and transported to the experimental facilities.

2. Materials and methods

2.1. Fish, holding conditions, experimental design, GH treatment and sampling

Juvenile rainbow trout were purchased from a local hatchery, Antens Laxodling AB, Sweden, and transported to the experimental facilities at the Department of Zoology, University of Gothenburg, Sweden. The fish were distributed into 12 0.5 m3 fiberglass tanks (14 fish per tank) supplied with recirculating, aerated fresh water at 12°C and exposed to a 12:12 LD photoperiod. During the acclimation period, fish received commercial dry pellets containing 23% lipids (Skretting, Norway) at 2% body weight (BW) day−1. At the start of the experiment, this was adjusted to 1.5% BW day−1, a ration which was fully consumed by the sham-treated group. From week 3, with rapidly increasing fish size, the ration was further adjusted to 1.2% BW day−1. After a 1-week acclimation period, the experiment was initiated by randomly designating six tanks to be GH-treated and six tanks to be sham-treated. Animals were treated in agreement with the European regulations of animal welfare (ETS No. 123, 01/01/91) and according to Swedish legislations (1988:534).

At the start of the experiment the fish were anaesthetized (2-phenoxyethanol using 0.3 ml l−1) and, a passive integrated transponder (a PIT-tag) was inserted through the opening into the body cavity using a PIT-tag injector to allow for individual identification. Then a small incision was made in the body wall. Long-term, sustained-releasing implants of recombinant bovine GH (bGH) (Posilac®; Monsanto Co., St. Louis, MO, USA) or vehicle (sesame seed oil) was injected using a 250 μl positive displacement pipette (Microman, Eppendorf). GH-treated fish received 0.6 mg bGH g−1 (1.4 μg g−1), while sham-treated fish received 1.4 μg g−1 of vehicle. Similar GH-implant (1 mg bGH g−1) has been found to release bGH into the circulation of juvenile Atlantic salmon and stimulate growth for at least 3 months [52].

At each of the three samplings (after 1, 3 and 6 weeks), duplicate tanks of GH-treated and sham-treated fish were sampled, 12 fish from each tank. During treatment (week 0) as well at each sampling, fish were weighed (BW) to the nearest g and fork length (FL) to the nearest 0.1 cm. At each sampling, the fish were sacrificed with a blow to the head, measured and blood collected from the caudal vein using heparinized syringes and centrifuged at 3000g and 4°C for 5 min in order to obtain plasma. Plasma was aliquoted for different assays, with one aliquot being acidized with HCl to a final concentration of 0.1 M for analyses of active (octanoylated) plasma ghrelin [26]. All mesenteric fat, the liver, as well as samples from white muscle (mid ventral part) and belly flap (ventral part) were dissected out and weighed. Plasma and tissues (for lipid analysis) were quickly frozen on dry ice, while tissues for Lep mRNA analysis were snap frozen in liquid nitrogen. Samples were stored at –80°C until analyzed.

2.2. RNA extraction, cDNA synthesis and qPCR analysis of lepa1 gene expression

Total RNA was isolated from liver, muscle and belly flap by phenol–chloroform extraction using TRI Reagent® (Sigma, St. Louis, MO, USA) as outlined by Chomczynski [10]. The RNA quantity and integrity was determined using a ND-1000 spectrophotometer (NanoDrop Technologies, NC, USA) and the Agilent 2100 expert bioanalyzer (Agilent Technologies, USA), respectively. Total RNA was treated with TURBO DNA-free™ kit (Ambion, Austin, TX, USA) and cDNA synthesized using 1.2 μg total RNA and oligo d(T15) in conjunction the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, USA) as outlined by the manufacturer.

Leptin A1 (lepa1) and elongation factor 1 alpha (ef1α) mRNA levels were measured using the Chromo4 Continuous Fluorescence Detector (Bio-Rad) and MJ Opticon Monitor Analysis Software platform (version 3.1, Bio-Rad). The following lepa1 and ef1α forward and reverse primers were designed based on known sequences for lepa1 (GI: 225543426) in rainbow trout and ef1α (GI: 185132715): lepa1 forward TIGCTCAAAACCATCGTGATTAGGA and reverse GTCCATGCCTGATCAGGTTA and ef1α forward CCCCTCAAGGATCAAGGTTA and reverse CACACGCCCCACCGGTACT. Both q-PCR assays were performed using 5 μl cDNA (300 ng RNA), 300 nM of each primer and SYBR Green Universal Master mix (Applied Biosystems) in a total reaction volume of 25 μl. The
thermal cycling protocol consisted of 2 min at 50 °C, 10 min at 95 °C followed by 45 cycles at 95 °C for 15 s and 60 °C for 1 min. Melt curve analysis verified that the primer sets for each q-PCR assay generated one single product and no primer–dimer artifacts. In addition, representative PCR products were analyzed by gel electrophoresis, cloned and sequenced to verify specificity. Omission of reverse transcriptase in the RT reaction resulted in no signal in both assays, which demonstrated that interference from residual DNA in RNA samples after DNase treatment were not significant. Non-template controls were included on all plates. For both assays, twofold cDNA dilution series made from total RNA from liver, muscle and belly flap were used to determine amplification efficiencies \( E \) calculated as the slope from the plot of log RNA concentration versus threshold cycle \( C_t \) values using the following formula: \( E = 10^{(-1/\text{slope})} \). This efficiency was used to correct for difference in amplification efficiency when calculating mRNA levels according to Pfaffl [57]. Lepa1 mRNA levels are presented as relative to the endogenous reference gene, elf2, according to Olsvik and coworkers [55]. Expression of elf2 did not change over time or differ between treatments. Trout lepa2 mRNA (Angotzi et al., unpublished) levels could not be quantified in a reliable and reproducible manner due to very low abundance of this transcript in the present study.

2.3. Plasma hormone radioimmunoassay (RIA) analyses

Plasma GH levels were measured in a salmon GH RIA [8], modified by Björnsson and co-workers [6]. Plasma IGF-1 were measured in extracted plasma [65] prior to RIA assay procedure established for coho salmon [47]. Lep analyses were performed according to a homologous salmonid RIA protocol [36]. Plasma ghrelin was analyzed according to the N-ghrelin-RIA (active ghrelin) protocol as previously described [26,33].

2.4. Lipid analyses

Lipid content of liver, white muscle and belly flap was determined gravimetrically according to Bligh and Dyer [7]. Briefly, homogenization of approximately 1 g tissue was followed by methanol/chloroform extraction. The chloroform phase was then transferred to preweighed tubes. The chloroform was allowed to evaporate under \( N_2 \) (g) at 42 °C, and the tubes were reweighed. Lipid content was calculated as % weight of the tissue analyzed.

2.5. Calculations and statistical analysis

Condition factor (CF) was calculated as \( (\text{BW} \times \text{FL}^{-3}) \times 100 \). Specific growth rates for body weight (SGRW) and fork length (SGRL) were determined as \( (\ln(\text{BW}_2 - \text{BW}_1) \text{D}^{-1}) \times 100 \) or as \( (\ln(\text{FL}_2 - \text{FL}_1) \text{D}^{-1}) \times 100 \), where \( \text{BW}_2 \) and \( \text{FL}_2 \) represents final body weight and fork length, respectively, and \( \text{BW}_1 \) and \( \text{FL}_1 \) are initial body weight and fork length. \( D \) is the number of days between measurements [60]. Feed conversion efficiency (FCE) was assessed at the tank level (two tanks per treatment group) over three periods; 0–1 week, 1–3 weeks and 3–6 weeks, respectively. FCE was calculated as \( (\text{total tank BW}_2 - \text{total tank BW}_1) \times (\text{total feed given to tank})^{-1} \text{D}^{-1} \). Liver somatic index (LSI) and mesenteric somatic index (MSI) were calculated as % of BW. Differences in dependent variables were analyzed in a two-way ANOVA according to general linear modeling using treatment and time as fixed factors. Equal variances were confirmed by Levene’s test. When necessary, data were log-transformed to obtain homogenous variances. The effect of time was confirmed by Levene’s test. Differences in dependent variables were analyzed in a two-way ANOVA. Linear correlation analyses of endocrine and biometric data were carried out at the individual level using Pearson correlation analysis. Numerical values were expressed as the mean ± SE. In all statistical tests, differences were accepted as statistically different at the level of \( p < 0.05 \).

3. Results

3.1. Size, growth, feed conversion and condition factor

At the start of experiment, the average BW (306 ± 5 g), FL (285 ± 1 mm) and CF (1.31 ± 0.01) did not differ among tanks. Both sham- and GH-treated fish increased in BW and FL during the study, and CF of the GH-treated fish decreased (Fig. 1). GH-treated fish had higher BW (Fig. 1A) and FL (Fig. 1B) than sham-treated fish from 1 week onwards, and the difference in BW and FL between groups increased throughout the experimental period. Thus, the BW of GH-treated fish increased from 311 ± 5 to 581 ± 14 g (87% increase) during the experimental period, while sham-treated fish increased from 300 ± 5 to 440 ± 13 g (47% increase). The FL of GH-treated fish increased from 286 ± 2 to 366 ± 4 mm during the experimental period (28% increase), while sham-treated fish increased from 284 ± 1 to 318 ± 4 mm (12% increase).

Specific growth rates for BW (SGRW) and FL (SGRL) were significantly elevated for GH-treated fish (Table 1) over all three measuring periods. For SGRW, the increase compared with sham-treated fish was 70% (0–1 week), 84% (1–3 weeks) and 29% (3–6 weeks), and corresponding values for SGRL, were 103%, 130% and 80%.

Feed conversion efficiency (FCE) was calculated only at the tank level, and while the feeding regime (fed by hand twice daily) and ration was designed to result in little or no uneaten pellets, there were no pellet traps in place to verify this. Thus, caution is needed in the interpretation of the data, as any uneaten feed would result in an underestimation of FCE. The GH-treated fish had higher FCE than sham-treated fish throughout the study (Table 1), this being 58.1% higher over week 0–1, 90.8% higher over weeks 1–3 and 33.7% higher over weeks 3–6. CF did not differ significantly between the groups after 1 week, but after 3 and 6 weeks, CF was lower for GH-treated fish than sham-treated fish (Fig. 1C).

3.2. Size and lipid content of energy-storing tissues

3.2.1. Mesenteric fat

There was a main effect of GH-treatment on the relative amount of mesenteric fat expressed as mesenteric somatic index, MSI (Fig. 2A), being lower in GH-treated fish throughout the study.

3.2.2. Liver

There was a trend \( (p = 0.09) \) towards a decreased relative liver size (LSI) following GH treatment (Fig. 2B). There was a main effect of time on LSI. In both sham- and GH-treated fish, there was a time-dependent decrease in LSI at 6 weeks compared with LSI at 1 and 3 weeks. There was no overall effect of treatment on liver lipid content, although a tendency \( (p = 0.11) \) of GH to decrease liver lipid content. GH significantly decreased liver lipid content at 3 weeks. There was a time-dependent increase in liver lipid content in both experimental groups peaking at 6 weeks.

3.2.3. Muscle

Muscle lipid content was analyzed in white muscle as well as the ventral belly flap (Fig. 2B). Although there was no main effect of GH treatment on white muscle lipid content \( (p = 0.059) \), there was a tendency of decreased muscle lipid content following GH-treatment at all time points. There was a main effect of time on muscle lipid content, with muscle lipid content increasing time-dependently in both sham- and GH-treated fish, peaking at 6 weeks.

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3.2.4. Belly flap

The belly flap lipid content increased in both groups at 3 weeks while it was reduced to initial levels after 6 weeks. In addition, GH initially promoted enhanced lipid deposition in the belly flap. At 1 week, there was a 39% higher belly flap lipid content in GH-treated fish (23.7 ± 1.2%) compared with sham-treated fish (17.1 ± 1.4%). The difference in belly flap lipid content decreased thereafter to 10.6% (GH treated 37.4 ± 3.3% versus control 33.8 ± 2.7%) at 3 weeks, and at 6 weeks, the treatment-dependent difference was abolished.

3.3. Plasma hormone levels

3.3.1. Plasma growth hormone levels

Endogenous plasma GH levels were strongly suppressed by GH-treatment already after 1 week of treatment, and the suppression persisted throughout the study (Fig. 1A).

3.3.2. Plasma insulin-like growth factor I levels

GH treatment significantly elevated IGF-I throughout the study (Fig. 1B). There was a time-dependent decrease in IGF-I levels in GH-treated fish from 1 to 6 weeks, suggesting a gradual decrease in exogenous GH release during the experiment. Plasma IGF-I levels in sham-treated fish remained unchanged during the experiment.

3.3.3. Plasma leptin levels

Plasma Lep levels were not affected by GH treatment (Fig. 3C). However, there was a time-dependent increase in plasma Lep levels in sham-treated fish after 3 weeks, with an increase from 910 ± 84 pM (1 week) to 1331 ± 175 pM (3 weeks).

3.3.4. Plasma ghrelin levels

Plasma ghrelin levels were not affected by GH treatment and did not change during the course of the experiment (Fig. 3D).

3.4. QPCR analysis of lepa1 mRNA expression in energy storing tissue

There was a main effect of GH treatment on hepatic lepa1 mRNA expression (Fig. 4A). Lepa1 levels in GH-treated fish were decreased to 50%, 63% and 29% of that of hepatic lepa1 expression in sham-treated fish, at 1, 3 and 6 weeks, respectively. In addition to the observed GH-induced decrease in hepatic lepa1 expression, GH also caused a decrease in liver lipid content and LSI (Fig. 2). There were no effects of time on hepatic lepa1 expression. Although no effect of GH treatment on lepa1 expression in white muscle was observed, there was a time-dependent decrease in lepa1 expression in both GH- and sham-treated groups (Fig. 4B), concomitant with a time-dependent increase in muscle total lipid levels (Fig. 2B). There was no significant effect of time or treatment on lepa1 expression in the belly flap (Fig. 4C).

3.5. Correlations between hormone levels, growth and tissue-specific lipids

Correlation analysis was carried out on endocrine parameters (IGF-I, Lep, ghrelin), growth (BW, FL, SGRL) and factors related to energy and nutritional status (CF, MSI, LSI, tissue-specific lipid content). In total, 38 significant correlations were identified (Table 2). Plasma Lep levels correlated positively to both MSI and belly flap lipid content. In addition there was a trend towards a positive correlation between plasma Lep levels and endogenous GH (data not shown). Correlation of tissue-specific lepa1 expression revealed that hepatic levels was positively correlated to energy-related factors (CF and MSI), while negatively correlated to growth-related factors (IGF-I, SGR, and FL). In contrast, lepa1 expression in white muscle was positively correlated to growth (SGRwL) and IGF-I, while negatively correlated to white muscle lipid content.

Fig. 1. (A) Body weight, (B) fork length and (C) condition factor of sham-treated (•) and GH-treated (○) rainbow trout over a 6-week study. Data were obtained from 24 fish from each treatment and sampling point and presented as means ± SE. **Indicate statistical differences between groups at the level of p < 0.01.

3.2.4. Belly flap

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Belly flap lipid content was positively correlated to both IGF-I and SGRL, while negatively related to white muscle lipid content. White muscle lipid content was positively correlated to growth (BW, FL) and hepatic lipid content, while negatively correlated to IGF-I, LSI and belly flap lipids. In addition, there was a positive correlation between liver lipid content and BW. In addition, several other parameters correlated to growth (BW, FL, SGR_{BW}) were identified, as seen in Table 2.

4. Discussion

4.1. Effectiveness of the GH-treatment protocol

The significant growth-promoting effects of the sustained-releasing bGH treatment seen throughout the present study are in line with previous studies on several salmonid species including Atlantic salmon [23,43,52], coho salmon [46] and brown trout [30,51]. In addition, the sustained suppression of endogenous GH plasma levels by the exogenous GH treatment confirms feed-back inhibition of GH on GH secretion, seen previously both in vivo [30,67] and in vitro [2]. Furthermore, the GH-treatment resulted in significant elevation of plasma IGF-I levels, in line with the regulatory mechanism of GH-induced stimulation of hepatic IGF-I secretion, as demonstrated earlier in shorter-term experiments on rainbow trout by Moriyama et al. [47]. Taken together, the sustained-release bGH implants appear to have been functional throughout the 6-week study.

4.2. Adiposity and tissue-specific GH effects

The study reveals complex dynamics among the energy/lipid-storing tissues. In the course of the 6-week feeding trial, the sham-treated rainbow trout gained 47% in weight, the belly flap lipid content increased transiently at 3 weeks, there was a steady increase in muscle lipid content, and a decrease in LSI. To some extent, it is likely that these changes are size-related, as muscle lipid content may increase in salmonids with size/age as seen for Atlantic salmon in aquaculture [22]. GH appears to rapidly mobilize lipids from mesentery and to lesser extent from white muscle and liver, suggesting increased lipolysis and reallocation of energy, most likely for skeletal and muscle growth. It is notable, however, that some of the GH-induced mobilized lipids appear to be reallocated to the belly flap. The belly flap is the major lipid-depositing tissue in Atlantic salmon [75] and is therefore likely to serve as an important energy reserve, although the energy dynamics of this tissue have not received much attention. The lipid content profile of the belly flap in the present study, with increased lipid content during the first 3 weeks, and a decrease over the subsequent 3 weeks, suggests that the belly flap may function as an energy-buffering tissue. During good feeding conditions, as during the first part of this study (ration of 1.5% BW day^{-1}), energy is rapidly accumulated in the belly flap, most of which is then mobilized during the latter half of the study. This may be due to the slightly lower ration (1.2% BW day^{-1}) during the last 3 weeks, but it also appears plausible that the belly flap functions as an energy buffer in order to sustain long-term growth during and after natural feeding episodes, when energy intake can initially increase faster than lean growth. In mammals, fat metabolic effects of GH preferentially include lipolysis in visceral adipose tissue [4], with a simultaneous increase in triglyceride uptake in liver and skeletal muscle, through
the stimulation of lipoprotein lipase and hepatic lipase [56]. The lipolytic action of GH in fish was first demonstrated in adipose tissue and liver in juvenile coho salmon in vivo activating lipolytic triacylglycerol lipase [64]. GH-induced lipolysis in isolated gilthead sea bream (Sparus aurata) adipocytes suggesting that GH can reallocate energy through direct action at the cellular level [3], but some of the growth-promoting actions of GH are considered to be indirect via IGF-I production [20]. The observed GH-dependent decrease in adipose stores in the present study is in agreement with the lipolytic action of GH in fish. In addition, the present study provides the first demonstration of an apparent GH-induced shift of lipid allocation strategy among energy-storing tissues, with site-specific lipolysis in visceral adipose tissue, liver and white muscle, coinciding with lipogenesis in belly flap. Although some data exist, the mechanisms underlying such differentiated GH regulation of different lipid stores remain largely unclear. Fasting is known to elevate plasma GH levels in salmonids as other vertebrate species, and this is thought to be due to hepatic GH resistance and the subsequent lack of negative feedback by circulating IGF-I. It has been observed in chinook salmon and rainbow trout, and taken to explain growth retardation during fasting [5,19,53,59]. It has been proposed that differential tissue-specific GH receptor (GHR) gene expression may lead to differences in GH sensitivity, such that lowered hepatic expression may underlie impaired growth, while increased expression in visceral adipose tissue may explain GH sensitivity and the lipolytic effects of fasting [53]. Hence, the differential effects of GH on energy-storing tissues in the present study may be due to differences in GH sensitivity. Further studies are needed to quantify and characterize GHR and IGF-I receptor expression as well as elucidating tissue-specific activities of lipolytic and lipogenic enzymes.

4.3. Growth and feed conversion efficiency

In spite of a large body of research demonstrating the growth-promoting effects of GH in salmonids, as in other vertebrate species, there is a surprising lack of data explaining the mechanisms through which GH induces growth in salmonid fish [5]. Two principal mechanisms are, however, likely to interact; increased appetite and feed intake, and improved food conversion efficiency. FCE represents in itself a complex suite of physiological processes in which ingested food is converted into tissue growth. In the present study, visual made observations during the twice-daily hand-feeding can characterize the much more active foraging
behavior of the GH-treated fish as “feeding frenzy”. However, as both groups were fed the same ration, the present study supports the notion that GH-induces growth also through improved FCE. The daily feed ration, adjusted to the observed feed consumption of the sham-treated group (1.5–1.2% day−1), and feeding method (hand feeding twice daily) used in the present study was designed to minimize the amount of uneaten feed. Although visual observations suggest that all food was consumed, as pellet traps were not in place in the tank outflow, a certain error due to uneaten feed cannot be ruled out. This given, FCE of the GH-treated fish was significantly improved at all time-periods, on average by ~60%. Although FCE may have been somewhat underestimated for the sham-treated fish (due to uneaten food), it appears improbable that this could fully explain the difference in FCE between groups. Rather, it appears that GH-treatment does improve FCE as seen previously for GH-treated [29] and GH transgenic fish [12]. The present data may help explain unexpected results of former studies, such as when GH-implanted juvenile Atlantic salmon in a freshwater stream were found to grow faster than controls without any apparent increase in their foraging range [43]. It was suggested that the fish were able to do so by increasing their foraging efficiency within their territory, but improved FCE provides an alternative explanation. Factors improving FCE in fish, such as GH in the present study, could act at different levels, including digestive and absorptive processes [11]. A second level is the utilization and allocation of nutrient energy which has entered the circulation. Through its effects on protein and lipid metabolism [15,64] GH favors allocation of energy towards muscle and skeletal growth over adipose tissue growth, as indicated by the present data, with the GH-treated fish not only being larger, but also leaner (lower CF) than the sham-treated fish. Given the higher water content of muscle than adipose tissue, together with the greater energy content of fat than protein per unit weight, the nutritional energy deposited during muscle growth is significantly less than that of adipose tissue growth on a wet weight basis. For farm livestock production, improved ratio of lean to fat deposition is seen as key factor in improving feed conversion efficiency [66], and supports the conclusion that the GH-induced shift in energy allocation from adipose tissue to muscle and skeletal growth explains the improved FCE of the GH-treated fish.

4.4. Leptin and adiposity

In the present study, GH did not affect plasma Lep levels, while a GH-dependent decrease in hepatic lepa1 (the lepa2 paralogue was not detectable) mRNA expression was observed throughout the experiment. Paralogue genes are often found in teleosts due to the whole-genome duplication that occurred early in the teleost lineage [73]. In diploid teleosts, such as the Japanese medaka and zebrafish, duplicate genes coding for lepa and lepb have been identified [18,37]. In tetraploid species such as Atlantic salmon and common Carp, the last tetraploidization of the genome most likely gave rise to lepa1 and lepa2 paralogues [27,61]. Although a lepb ortholog yet have to be identified in salmonids, we have confirmed that rainbow trout possess lepa1 and lepa2 paralogues. Previously, a similar pattern of stable Lep levels in plasma concomitant with tissue-specific changes in lepa1 gene expression have been observed in Atlantic salmon [61], indicating a possible paracrine role of Lep in the liver, and/or that plasma Lep is mainly of non-hepatic origin. The decrease in hepatic lepa1 coincides with a GH-dependent decrease in liver lipid content. In addition, hepatic lepa1 expression is positively correlated to MSI and CF. Taken together these observations reveal a link between hepatic adiposity and Lep. The liver is, however, not the primary lipid store in salmonids, but as demonstrated in this study and confirmed previously [18,27,37,38,49,58] is a major Lep expressing tissue in undisturbed fish. Studies in pufferfish indicate Lep expression to be confined to oil droplets in the liver, linking it to hepatocyte energy...
metabolism [38], but in Atlantic salmon, hepatic lepa1 expression is not dependent on nutritional status [61]. Food restriction causes increased hepatic lepa2 gene expression in Atlantic salmon [61], but in the present study, hepatic lepa2 mRNA expression was below detection limits and can therefore not be correlated to nutritional status.

In the present study, white muscle exhibited the lowest lep1 expression, together with having the lowest lipid content of the tissues studied. Notably, there was a steady increase in muscle lipid content of both groups during the study, leading to a positive correlation between white muscle lipid content and BW. This is in line with previous studies on farmed Atlantic salmon [22]. In addition, the observed positive correlation between white muscle lep1 mRNA expression and plasma IGF-I and SGRW/L suggest that Lep may be linked to cell proliferation and growth rate. This is in support with the findings by Rønnestad and co-workers [61] where full rationed feeding in Atlantic salmon revealed enhanced growth and higher lep1 expression in the major lipid storing tissues (viscera and white muscle). In the present study, GH treatment did not affect plasma Lep levels in the rainbow trout, but in mice, Lep controls somatotroph number and pituitary GH release, thereby indirectly controlling tissue-specific lipolysis and adiposity [9]. In humans, GH-deficiency leads to adiposity and elevated Lep levels [44]. Thus, despite the present data, functional GH–Lep interactions in fish need further attention.

In the present study, there is a positive correlation between energy status and plasma Lep levels. Thus, lipid content of visceral adipose tissue (MSI) as well as of belly flap is positively correlated to Lep levels. This indicates that Lep may have a role in communicating energy reserves in rainbow trout, in line with increased lep1 mRNA expression in major lipid-storing tissues of fully fed Atlantic salmon [61], and mammalian data, where Lep acts as a short-term nutritional as well as a long-term adiposity signal [40,62]. On the other hand, no correlation between hepatic lep mRNA expression and nutritional status has been found in carp [27] and Arctic char [16].

The apparent lack of correlation between tissue expression of the lep gene and plasma Lep levels observed in the present study and in Atlantic salmon [61] underlines the fact that it is currently not known which tissues contribute and to which extent to the circulating peptide levels in fish. Such lack of correlation may suggest that Lep can have paracrine functions, i.e. that the lep gene is expressed in some tissues without affecting plasma Lep levels.

GH treatment did not affect plasma ghrelin levels in the present study, despite the fact that GH treated fish were leaner and had less fat stores. This suggests that neither GH nor alterations in energy stores influence ghrelin, at least not in the long term. It can be speculated that ghrelin has a more acute action in response to energy balance changes, and that plasma levels of ghrelin are then adjusted to their set-point. These results are in contrast to a
previous study on fasting of rainbow trout where plasma ghrelin levels decreased with fasting, correlated positively to CF, liver and muscle lipid content, and negatively with endogenous GH levels [33]. However, in that study, the fasting-induced changes in energy stores were much more severe than in this study. Other studies in goldfish [70] and Atlantic salmon [24] showed that ghrelin- plasma levels vary with time of fasting so that an initial rapid response (days) is followed by a normalization and stabilization of plasma ghrelin levels. This support that ghrelin has a more short- than long-term role in the regulation of energy metabolism during fasting in fish. But further studies are needed to test this hypothesis, and to elucidate the functional relationships between ghrelin, GH, adiposity and energy balance in fish.

5. Conclusions

The GH-induced changes in plasma GH and IGFI-1 levels confer the established endocrine interaction of these hormones. The observed tissue-specific effects on lipid stores suggest that GH affects lipid allocation with a shift from mesentery, liver and white muscle, to the belly flap. The functional significance of this is not clear, but suggests that the belly flap may be of particular importance for energy buffering during episodes of food intake and lean growth. The discrepant GH-effects on plasma Lep levels and hepatic lepa1 mRNA expression may suggest a paracrine action of hepatic Lep, and/or that circulating Lep is not of hepatic origin, but rather secreted from other (adipose?) tissues. Plasma Lep levels correlate positively with adipose stores indicating a role for Lep in energy signaling, but further studies are needed to investigate tissue-specific regulation of Lep secretion and LepR regulation in both brain and periphery. GH treatment or GH-induced changes in energy stores do not seem to influence plasma ghrelin levels in the long term, but further studies are needed to clarify the functional relationship between GH and ghrelin, and the role of ghrelin in growth and energy balance.

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