Postprandial expression of growth-related genes in Atlantic salmon (Salmo salar L.) juveniles fasted for 1 week and fed a single meal to satiation

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Abstract
We investigated postprandial changes in transcript abundance following a single satiating meal in juvenile Atlantic salmon (Salmo salar L.) (about 70 g body mass) following fasting for 1 week at 12°C. The expression of twenty-three growth-related genes was determined in fast myotomal muscle using quantitative real-time PCR at the following postprandial time points: −12, 0, 1, 3, 6, 12, 24, 48 and 96 h. The gut was fullest 1–6 h after feeding and emptied within 48–96 h. IGF-I, MyoD1c, MRF4 and myf5 transcripts were sharply up-regulated within 1 h of refeeding and are promising candidate genes involved in a fast-response signalling system that regulates fish myotomal muscle growth. These genes clustered together with MyoD1b and suggest a coordinated regulation to favour resumption of myogenesis as an early response to feeding. Insulin-like growth factor (IGF)-II and the ubiquitin ligase MAFbx/atrogin-1 were initially down-regulated but restored to initial values after 12 h. It is also suggested that local production of IGF-I within the muscle might suppress catabolic pathways depressing MAFbx/atrogin-1.

Key words: Salmo salar L.; Myogenesis; Refeeding and fasting; Nutrient availability; Insulin-like growth factor system

The natural life cycle of many fish species often includes long periods of low winter temperatures and restricted feeding opportunities or prey availability that lead to a depletion of energy reserves and a reduction in growth rate(1). The restoration of adequate nutrition or favourable environmental conditions results in rapid weight gain and compensatory growth relative to continuously fed controls. Compensatory growth occurs at various stages in salmonid fish and is an important adaptation that allows fish to remain on target in a fluctuating and unpredictable environment(2–5). From a practical point of view, the compensatory strategy is of great interest to the aquaculture industry because feeding programmes can be designed to improve food conversion and growth rates, thereby minimising production costs(1).

Periods of nutrient restriction are associated with changes in metabolism to provide cellular energy via catabolic processes(3). In carnivorous fish, nutrient restriction enhances the release of amino acids from muscle fibres which are used by hepatocytes as the main gluconeogenic precursors(6). During refeeding and compensatory growth an accelerated turnover takes place resulting in an increased protein synthesis: degradation ratio(3,7). In salmon, the main mechanism underlying compensatory growth after a nutrient-restriction period is an increase of feed intake rates(2). Food contains ligands for retinoic acid receptors, PPAR, vitamin D receptors and other nuclear transcription factor receptors and can directly affect signal transduction pathways(8,9). Branched-chain amino acids, particularly leucine, have a major role in stimulating protein synthesis(10). These phenomena seem to be the result of endocrine alterations, although in most species it is difficult to detect differences in the endocrine status between animals undergoing compensatory growth and control animals(11). In fish, recent molecular tools enable us to gain deeper insight into how growth responses are regulated by dietary factors(12,13). In Atlantic salmon(14) and rainbow trout(15) a genomic approach was used to identify nutritionally regulated genes involved in muscle growth and revealed a complex response. The principal groups of up-regulated transcripts post-refeeding were genes involved in transcription, ribosomal biogenesis, translation, chaperone activity,
ATP production, cell division and muscle remodelling. These genes possibly play a role in the stimulation of myogenesis during the transition from a catabolic to anabolic state in skeletal muscle.

Several in vivo and in vitro studies evaluated the impact of feeding protocols on muscle growth. Using a candidate gene approach, it was observed that switching onto fast growth induced by a fasting–refeeding schedule involves the local up-regulation of several components of the insulin-like growth factor (IGF) system, a major hormone axis regulating the cellular dynamics of muscle growth(16–19). The role of plasma IGF-I during compensatory growth is not clear and must be explained in connection with changes of its binding proteins, which act on the phosphoinositide-3-kinase/Akt/mammalian-target-of-rapamycin (PI3K/Akt/mTOR) pathway(20,21). The autocrine IGF-II transcription required for skeletal myocyte differentiation is regulated by mTOR and the availability of amino acids(22,23). Thus the mTOR–IGF axis provides a molecular link between nutritional levels and protein synthesis leading to muscle fibre hypertrophy. In salmonids, fasting decreased the expression and plasma levels of IGF, and up-regulated IGF-I binding, whereas the plasma level of growth hormone was shown to increase(24,25). Switching to fast growth in Atlantic salmon muscle involved the up-regulation of IGF-I, IGF binding protein (IGFBP)-5,2 and IGFBP-4(19), whereas 1d of refeeding completely restored plasma growth hormone levels in rainbow trout(26). IGF-I induced the activation of the PI3K/Akt pathway, which causes an increase in protein translation via activation of p70S6K and inhibition of 4E-BP (also known as PHAS-1)(24). In cell lines, it was recently shown that in addition to its hyperactive effects, Akt can dominantly inhibit induction of the atrophy genes the muscle-specific E3 ubiquitin ligases, MuRF1 (muscle RING finger protein 1) and MAFbx/atrogin-1 (muscle atrophy F box), by phosphorylating and thereby inhibiting the function of the forkhead box O (FOXO) family of transcription factors(26,27).

The genetic networks mobilised in muscle following recovery from fasting are likely to be dependent on nutrient availability. The experimental protocols employed to investigate compensatory growth often involve prolonged fasting followed by continuous refeeding with transcript abundance monitored over several days or weeks(14,17,26,29). In contrast, a single satiating meal design allows the evolution and decay of transcriptional responses to nutrient input to be studied with relatively high temporal resolution. Using this approach the aim of the present study on Atlantic salmon was to investigate the transcript abundance of muscle growth-related genes to changes in nutrient supply. The expression of genes involved in myogenesis (muscle regulatory factors (MRF) and paired box protein 7 (Pax7)), growth signalling (the IGF system), myofibrillar protein degradation and synthesis pathways (the PI3K/Akt/mTOR pathway and the muscle-specific E3 ubiquitin ligases) and metabolic genes (Creb4) shown to be critical modulators of fish myotomal muscle growth were analysed using quantitative real-time PCR (qPCR).

**Materials and methods**

**Experimental conditions and sampling**

All experiments were approved by the Animal Welfare Committee of the University of St Andrews and fish were humanely killed following Schedule 1 of the Animals (Scientific Procedures) Act 1986 (Home Office Code of Practice, H. M. Stationery Office: London, January 1997).

Two homogeneous groups of Atlantic salmon (Salmo salar L.) juveniles (average body weight 70 g) were reared in duplicate tanks (500 litres; thirty fish per tank). Each tank was supplied with fresh water with an average water temperature of 12°C and dissolved oxygen >80%. Fish were exposed to an artificial photoperiod of 12 h light–12 h dark (08.00 to 20.00 hours) and provided a commercial salmon feed (EWOS Innovation) during 3 weeks (1.5% body weight). Fish were then fasted for 1 week, and fed a single meal distributed to all fish to visual satiation.

Sampling of fish occurred at −12 and 0 h (before the meal), and at 1, 3, 6, 12, 24, 48 and 96 h (following the single meal), with seven fish sampled at each time point and individual mass and fork lengths measured. The intestine and stomach content was determined for each fish and photographs were taken. Fast muscle was then dissected from the dorsal myotome and snap-frozen in liquid N2. Samples were kept at −80°C until analysed.

**RNA extraction and cDNA synthesis**

Total RNA was extracted by the addition of 100 mg of salmon muscle to Lysis matrix D (Qbiogene) with 1 ml TRI reagent (Sigma) and homogenised for 40 s using a Fast Prep instrument (ThermoFisher Scientific) and its integrity was confirmed by agarose gel electrophoresis on a 1·2 % gel (w/v) after 3–5 min denaturation at 65°C. Genomic DNA contamination was removed and first-strand cDNA was synthesised from RNA extraction and cDNA synthesis with the Minimum Information for Publication of Quantitative Real-Time PCR experiments MIQE guidelines(30).

Several muscle growth-related genes (Table 1) (IGF-I, IGF-II, IGF-IRa, IGF-IRb, IGF-IR, IGFBP-2,1, IGFBP-4, IGFBP-5,2, IGFBP-5,6, IGFBP-6, Myogenin, MyoD, MyoD1b, MyoD1c, myf5 (myogenic factor 5), MRF4 (also known as myogenic factor 6; myf6), the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1, fibroblast growth factor 2 (FGF2), CrebA, MEPZ2A, Pax7 and five reference genes, HPRT1 (hypoxanthine phosphoribosyltransferase 1), elongation factor 1-α (EF1-α), 680 ribosomal protein L13 (rpl13), 408 ribosomal protein S29 (rps29), beta-actin and RNA polymerase II) were selected, and primers were designed to have a melting temperature (Tm) of 60°C as previously...
<table>
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<th>Gene</th>
<th>Primer 5′-3′</th>
<th>Product size (bp)</th>
<th>Temperature (°C)</th>
<th>E (%)</th>
<th>Accession number</th>
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<td>IGF-II</td>
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<td>f: GGGGCCTCCCTTGTGCTCTA r: AGAGATAGACGGCCTCTTA</td>
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<td>97·2</td>
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<td>IGF-IrB</td>
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<td>f: CTTCTCCAGGTGGTGCAAGAC r: ACGCTGGCGGGCTGTCCTAC</td>
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<td>94·6</td>
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E, PCR efficiency; f, forward; r, reverse; IGF-I, insulin-like growth factor I; IGF-II, insulin-like growth factor II; IGF-IrA, insulin-like growth factor I receptor a; IGF-IrB, insulin-like growth factor I receptor b; IGF-Ir, insulin-like growth factor II receptor; IGFBP-2.1, insulin-like growth factor binding protein 2 paralogue 1; IGFBP-4, insulin-like growth factor binding protein 4; IGFBP-5.1, insulin-like growth factor binding protein 5 paralogue 1; IGFBP-5.2, insulin-like growth factor binding protein 2 paralogue 2; IGFBP-6, insulin-like growth factor binding protein 6; IGFBP-P1, insulin-like growth factor binding protein-related protein 1; CrebA, cyclic AMP response element binding protein; FGF2, fibroblast growth factor 2; MAFbx, muscle atrophy F box; ME2FA, myocyte enhancer factor 2A; MuRF1, muscle RING finger protein 1; myf5, myogenic factor 5; MyoD1a, myoblast determination factor 1a; MyoD1b, myoblast determination factor 1b; MyoD1c, myoblast determination factor 1c; Pax7, paired box gene 7; MRF4, myogenic factor 6; EF1-α, elongation factor 1-α; Rp13, paired box gene 7; Rps29, myogenic factor 6; beta-actin, actin; RNA pol II, RNA polymerase II; HPRT1, hypoxanthine phosphoribosyltransferase 1.
reported by Bower et al.\(^{(14,19)}\). Quantification of gene expression was performed by qPCR using a Stratagene MX3005P QPCR system (Stratagene) with SYBR Green chemistry (Brilliant II SYBR green, Stratagene). The cDNA were diluted 80× before using them as templates for the qPCR reactions. Each qPCR reaction mixture contained 7.5 μl 2× Brilliant II SYBR green master mix, 6 μl cDNA (80-fold dilution), 500 nm each primer and RNase-free water to a final volume of 15 μl. Amplification was performed in duplicate in ninety-six-well plates with the following thermal cycling conditions: initial activation 95°C for 10 min, followed by forty cycles of 15 s at 95°C, 30 s at 60°C and 30 s at 72°C. Dissociation analysis of the PCR products was performed by running a gradient from 60 to 95°C to confirm the presence of a single PCR product. The PCR amplification efficiency of each primer pair was calculated using LinRegPCR 2009 (http://LinRegPCR.HFRC.nl)\(^{(31)}\).

**Data analysis**

Evaluation of expression stability of several potential housekeeping genes including elongation factor-1α (EF1-α), 60S ribosomal protein L13, rpl13, 40S ribosomal protein S13 and S29 (rps13 and rps29), beta-actin and RNA polymerase II was done using the statistical application called geNorm (http://medgen.ugent.be/~jvdesomp/genorm/)\(^{(32)}\). Analysis revealed both rpl13 and RNA polymerase II as the most stable genes in this experiment. Hence, the reference gene rpl13 was used for normalisation of qPCR data and QGene was used for normalisation and calculation of relative expression data\(^{(33)}\).

All data were tested for normality and homogeneity of variances by Kolmogorov–Smirnov and Bartlett tests, and then submitted to a one-way ANOVA using STATISTICA software (version 10; StatSoft, Inc.). When the data did not meet the normality and/or equal variance requirements, a Kruskal–Wallis one-way ANOVA was performed instead. When these tests showed significance, individual means were compared using Tukey's honestly significant different test or Dunn's test. Correlation of gene expression was analysed by the Spearman rank order correlation test. Hierarchical clustering was performed using Cluster3 software\(^{(34)}\).

**Results**

Fish mass and length ranged between 90–117 g and 20–23 cm, respectively, and were similar among sampling points \((P<0.05)\). The gut content of the salmon increased significantly 1 h after distributing the meal and remained high for 6 h (Fig. 1). These results confirm that all sampled fish had ingested food, and the intestine was fully evacuated between 48 and 96 h after the meal.

An up-regulation of several components of the IGF system was observed immediately after the meal. IGF-I transcripts increased significantly 1 h after the single meal \((P<0.05)\), decreasing thereafter (Fig. 2). Expression of IGF-II was significantly reduced in response to feeding \((P<0.05)\) with significant down-regulation observed at 3 and 6 h after the single meal, returning to initial values after 12 h.

Expression levels of IGF-IRb (Fig. 2) and IGF-IRa did not vary significantly in response to feeding \((P>0.05)\), but IGF-IRa was minimal 12 h after the single meal. Several IGFBP were detected in fast muscle of Atlantic salmon (Fig. 3). IGFBP-related protein 1 (IGFBP-rP1), IGFBP-2-1 and IGFBP-5-2 expression did not vary significantly with the single meal. IGFBP-4 and IGFBP-5-1 had increased expression 1 h after the meal administration, but then decreased. IGFBP-6 expression decreased 12 h after the meal, reaching maximal expression levels 48 h postprandially. IGFBP-6 was significantly correlated with IGF-II \((P<0.05)\). A positive correlation was found between IGF-I, IGFBP-rP1 and IGFBP-4 expression.

Expression of MRF revealed that MRF4 and myf5 expression were significantly correlated with the gut content of the fish \((P<0.05)\). These two genes were up-regulated until 3 h after the single meal, returning to initial values 6 h after feeding (Fig. 4), and their expression clustered together when compared with other genes (Fig. 5). The three paralogues of MyoD1 responded differently to the meal distribution. MyoD1a showed no variation following the meal, whereas MyoD1b and MyoD1c peaked 1 h after feeding a single meal, although without statistical significance in the case of MyoD1b. MyoD1c mRNA expression correlated with gut content \((P<0.05)\) and clustered together with IGF-I (Fig. 5). Myogenin expression was not significantly affected by feeding, although a slight decrease was observed after feeding.

The ubiquitin ligase MAFbx/atrogen-1 mRNA levels were significantly down-regulated until 12 h after feeding, returning...
MuRF1 expression was initially up-regulated at 1 h, before being down-regulated at 3 and 12 h postprandially. MAFbx/atrogin-1 expression was positively correlated ($R = 0.66$) with IGF-II. FGF2, CrebA and Pax7 expression was not significantly affected by the single meal distribution.

Discussion

Nutrient availability is amongst the most important environmental variable altering muscle growth. The genetic network that is mobilised in the stimulation of myogenesis during the transition from a catabolic to anabolic state in skeletal muscle has not been exhaustively described, but seems to be a nutrient-sensing pathway(12,13,15,35). The present study was designed to explore the postprandial regulation of growth-related genes shortly after feeding a single meal. The effects of fasting and subsequent continuous refeeding protocols following transcript abundance over time have been studied in several fish species such as Atlantic salmon(14,36), rainbow trout(17,25), Atlantic halibut(29), sea bass(28) and seabream(37). However, the only study describing early transcriptional changes during the postprandial period was recently performed in zebrafish(38). The present results indicated that a single meal affects the expression of several growth-related genes in Atlantic salmon juveniles shortly after ingestion, confirming data in zebrafish(38). A 3.5-fold increase of IGF-I mRNA expression was observed 1 h after refeeding, indicating a fast response to nutrient availability. An increased expression of IGF-I in fast skeletal muscle was registered 3–4 d after refeeding in Atlantic salmon (19) and rainbow trout (17), and after 1 week in sea bass(28), although no earlier point was analysed. Duan, Plisetskaya (36) also reported a significant increase in hepatic IGF-I mRNA levels in salmon after refeeding, suggesting an endocrine/autocrine/paracrine growth stimulation of myotomal muscle induced by food intake. In myogenic cell culture, Atlantic salmon IGF-I mRNA levels increase in response to IGF and amino acid stimulation. Seiliez et al. (21) showed that in rainbow trout insulin levels peak 0.5 h after feeding, whereas increased amino acid levels were observed after 2.5 h. Based on this, the increased IGF-I mRNA levels we observed 1 h postprandially are likely to be in response to hormonal stimulation. IGF-I regulates many anabolic pathways in skeletal muscle, stimulating cell proliferation and differentiation(39) and myocyte hypertrophy(40) through the subsequent activation of the PI3K/AKT/mTOR pathway and prevention of atrophy mediators(41).

IGF-I exerts its effects on cells through binding to IGF-IR. The expression of IGF-IRα was minimal 12 h after refeeding, whereas IGF-IRβ was not significantly affected by feeding.
which is consistent with previous results in Atlantic salmon\textsuperscript{(19)}. In trout, IGF-IR\textsubscript{a} was shown to be maximal in fasted fish and declined after refeeding, but no changes were reported in IGF-IR\textsubscript{b}\textsuperscript{(17)}. Montserrat \textit{et al.}\textsuperscript{(25)} pointed towards a different regulation of these two genes by nutritional status, with isoform a responding to refeeding and isoform b responding to fasting. During periods of nutrient restriction, sensitivity to IGF-I seems to be increased in muscle by increasing the abundance of IGF-IR\textsubscript{a}.

The expression of several IGFBP in response to a single meal followed distinct patterns. IGFBP-rP\textsubscript{1}, IGFBP-2\textsubscript{1} and IGFBP-5\textsubscript{2} expression did not vary significantly up to 96 h after feeding the single meal. Although IGFBP-rP\textsubscript{1} did not seem to be modulated by feeding, its expression was positively correlated with that of IGF-I, confirming previous results in Atlantic salmon\textsuperscript{(19)} and trout\textsuperscript{(24)}. In a previous study with salmon starved for 22 d and refed to satiation thereafter, IGFBP-2\textsubscript{1} was significantly down-regulated from 14 d onwards, which was attributed to an increased availability of IGF-I to the IGF-I receptor\textsuperscript{(19)}. IGFBP-4 showed maximal expression 1 h after feeding and like IGFBP-rP\textsubscript{1}, was significantly correlated with IGF-I expression, suggesting a coordinated regulation of these genes towards resumption of myogenesis soon after refeeding. IGFBP-6 was significantly correlated with IGF-II, but not with IGF-I. As mammalian IGFBP-6 has a 10- to 100-fold higher affinity for IGF-II than IGF-I\textsuperscript{(42)}, the present results suggest a role for Atlantic salmon IGFBP-6 in IGF-II regulation. Amaral \& Johnston\textsuperscript{(38)} pointed to lineage-specific differences in IGFBP function and regulation among teleosts, suggested by the apparent lack of IGFBP-4 in zebrafish, so caution is needed when comparing results between different fish species.

The role of IGF-II in fish metabolism is unclear, but it seems to be implicated in the autocrine/paracrine regulation of growth\textsuperscript{(23,25)}. In the present study, IGF-II mRNA expression showed a dramatic decrease in muscle of refed salmon until 6 h after feeding, but levels were restored 12 h postprandially. Likewise, Bower \textit{et al.}\textsuperscript{(19)} reported a significant decrease of IGF-II expression in a time-dependent fashion after at least 7 d continuous feeding. Hevrøy \textit{et al.}\textsuperscript{(43)} reported an up-regulation of IGF-II in the muscle of fish fed high lysine levels, suggesting a role as an anabolic stimulatory agent. In juvenile rainbow trout, IGF-II mRNA levels in myotomal muscle tissue increased 34 d after refeeding\textsuperscript{(17)}, but in a
different study Montserrat et al. (25) could not observe any effect of fasting or refeeding on IGF-II mRNA expression. These distinct responses could be due to distinct developmental stages and/or nutritional status of the fish.

The expression of several MRF has been reported to be modulated by the nutritional status of the fish. The three paralogues of MyoD1 responded differently to the meal distribution. MyoD1a showed no variation following the meal, whereas MyoD1b and MyoD1c peaked 1h after feeding a single meal. The up-regulation of MyoD1c following the single meal was positively correlated with IGF-I. No clear change in either MyoD isoform could be observed during compensatory growth in trout (25), but amino acid withdrawal led to a down-regulation of both MyoD1b and MyoD1c in salmon myogenic cells culture, and increased levels of Pax7 mRNA, suggesting that serum and amino acid withdrawal

![Graphs showing expression of muscle-regulatory factors (MRF) and atrophy genes](https://www.cambridge.org/core/)

Fig. 4. Normalised expression of muscle-regulatory factors (MRF) (MyoD1c (A), Myogenin (B), myogenic factor 5 (myf5) (C) and MRF4 (D)) and the atrophy 
genes (MAFbx/atrogin-1 (E) and muscle RING finger protein 1 (MuRF1) (F)) at different times postprandially. Values are means (seven fish per sample), with their standard errors represented by vertical bars. a,b,c Mean values with unlike letters were significantly different (P< 0.05).
Fig. 5. Heat map summary and unsupervised hierarchical clustering analysis of the genes in muscle according to the similarity in their expression across different postprandial times (−24 to 96 h). Yellow and blue shading represents the maximal (Max) and lowest (Min) transcript abundance, respectively. IGFBP-2, insulin-like growth factor binding protein 2 paralogue 1; IGFBP-5, insulin-like growth factor binding protein 5 paralogue 1; IGF-II, insulin-like growth factor II; MEF2A, myocyte enhancer factor 2A; FGF2, fibroblast growth factor 2; CREB3A, cyclic AMP response element binding protein; IGFBP-6, insulin-like growth factor binding protein 6; IGF-IR, insulin-like growth factor II receptor; IGF-IRa, insulin-like growth factor I receptor α; MuRF1, muscle RING finger protein 1; IGF-IRb, insulin-like growth factor I receptor β; IGFBP-rP1, insulin-like growth factor binding protein-related protein 1; IGFBP-4, insulin-like growth factor binding protein 4; Pax7, paired box protein 7; MyoD1b, myoblast determination factor 1b; IGF-I, insulin-like growth factor I; MyoD1c, myoblast determination factor 1c; MRF4, myogenic factor 4; myf5, myogenic factor 5; myog, myogenin; MAFbx, muscle atrophy F box; IGFBP-6-2, insulin-like growth factor binding protein 2 paralogue 2; MyoD1a, myoblast determination factor 1a.

leads to cell cycle arrest and the production of quiescent cells(44).

MRF4 and myf5 showed a dramatic and simultaneous up-regulation immediately after feeding a single meal. MRF4 and myf5 are closely linked genes that clustered together (Fig. 5). Myf5 is the first MRF to be expressed during embryonic development and is considered a specification factor that determines the muscular lineage, whereas MRF4 functions later and can be considered as both a specification and a differentiation factor(45). An early peak in myf5 expression was correlated with increased MyoD1b transcript abundance during the maturation of an Atlantic salmon primary myogenic cell culture(44), and so it is interesting that we see increased Myogenin expression 1 h postprandially for MyoD1b, myogenic factor 6; myf5, myogenic factor 5; myog, myogenin; MAFbx, muscle atrophy F box; IGFBP-6-2, insulin-like growth factor binding protein 2 paralogue 2; MyoD1a, myoblast determination factor 1a.

In the present study no modulation of Pax7 or FGF2 was observed in refeeding, although Chauvigné et al. (17) indicated FGF2 was a critical modulator of trout myotomal muscle growth 4 d after refeeding. Expression of the muscle-specific gene Myogenin decreased soon after a single meal but without statistical significance (P > 0.05). Similarly, Bower et al. (49) described a Myogenin reduction in response to feeding. In rainbow trout, Myogenin mRNA was unchanged 4 d after refeeding, but increased significantly after 12 d (17). However, increased Myogenin expression was reported in trout during feed restriction, suggesting a role in muscle maintenance(25).

The genes regulated by atrophy include the E3 ubiquitin ligase MAFbx/atrogin-1 and MuRF1 that are up-regulated during catabolism and atrophy and down-regulated during fibre hypertrophy. Fasted individuals showed increased expression of MAFbx/atrogin-1 and MuRF1 and both genes were strongly down-regulated after feeding in Atlantic salmon(14,75) and zebrafish(88). The present results showed that a single meal was capable of promoting MAFbx/atrogin-1 expression, though its effect on MuRF1 was less clear. The increased expression observed 1 h postprandially for MuRF1 at first glance is puzzling. However, it is noteworthy that during the fasting period, fish were inactive, but became active once feed was distributed in the tank. This sudden increase in activity could lead to depletions in muscle glucose reserves, leading to metabolic stress in the muscle, and MuRF1 is known to regulate responses to metabolic stress in muscle of mice(40). MAFbx/atrogin-1 is regulated by both IGF signalling and amino acid availability(47), and the expression patterns we observed following a single meal is consistent with this. In trout, circulating amino acids remained high from 2 to 12 h following feeding and returned to those of a fasted state by 24 h(21) and the expression profile we observed for MAFbx/atrogin-1 is inversely proportional to this. The decrease in MAFbx/atrogin-1 expression (within 1 h) suggests that hormonal stimulation of the AKT/mTOR pathway...
via endocrine signalling or through local production of IGF-I is responsible for this rapid transcriptional response that could result in increased protein synthesis. Recent findings demonstrated that MAFbx/atrogen-1 contributed to muscle wasting by down-regulating protein synthesis whereas MuRF1 is mostly involved in the breakdown of myofibrillar proteins. In conclusion, the present results show that the transcription of several growth-related genes in the fast skeletal muscle of Atlantic salmon responds quickly to a single meal. In muscle, our observations indicate that refeeding induced a coordinated regulation of several genes involved in a strong resumption of myogenesis with feeding. IGF-I, MyoD1c, MRF4 and myf5 transcripts in muscle were sharply up-regulated in response to refeeding, being promising candidate genes involved in a cellular-level signalling system that regulates fish myotomal muscle growth. It is also suggested that local production of IGF-I within the muscle might suppress catabolic pathways depressing MAFbx/atrogen-1.

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References


