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Hepatic *insulin-like growth-factor binding protein* (*igfbp*) responses to food restriction in Atlantic salmon smolts

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ABSTRACT

The growth hormone (Gh)/insulin-like growth-factor (Igf) system plays a central role in the regulation of growth in fishes. However, the roles of Igf binding proteins (Igfbps) in coordinating responses to food availability are unresolved, especially in anadromous fishes preparing for seaward migration. We assayed plasma Gh, Igf1, thyroid hormones and cortisol along with *igfbp* mRNA levels in fasted and fed Atlantic salmon (*Salmo salar*). Fish were fasted for 3 or 10 days near the peak of smoltification (late April to early May). Fasting reduced plasma glucose by 3 days and condition factor by 10 days. Plasma Gh, cortisol, and thyroxine (T_4) were not altered in response to fasting, whereas Igf1 and 3,5,3′-triiodo-L-thyronine (T_3) were slightly higher and lower than controls, respectively. Hepatic *igfbp1b1*, *-1b2*, *-2a*, *-2b1* and *-2b2* mRNA levels were not responsive to fasting, but there were marked increases in *igfbp1a1* following 3 and 10 days of fasting. Fasting did not alter hepatic *igf1* or *igf2*; however, muscle *igf1* was diminished by 10 days of fasting. There were no signs that fasting compromised branchial ionoregulatory functions, as indicated by unchanged Na^+/K^+-ATPase activity and ion pump/transporter mRNA levels. We conclude that dynamic hepatic *igfbp1a1* and muscle *igf1* expression participate in the modulation of Gh/Igf signaling in smolts undergoing catabolism.

1. Introduction

Growth performance of teleost fishes, including salmonids, is principally controlled by the actions of the growth hormone (Gh)/insulin-like growth factor (Igf) system (Björnsson, 1997; Duan et al., 2010; Wood et al., 2005). The Gh/Igf system directs the allocation of acquired nutrients toward anabolic processes such as somatic and linear growth. In anadromous salmonids, the Gh/Igf axis also has a role in increasing salinity tolerance that occurs prior to, and during, the downstream migration of smolts (Hoar, 1988; McCormick, 2013). On the other hand, in conditions of prolonged nutrient restriction, the labile nature of Gh/Igf signaling safeguards survival by shifting energy away from anabolic processes to essential physiological processes (Wood et al., 2005). The Gh/Igf system responds to nutrient conditions through a suite of responses that often vary across the organism, permitting adaptive tissue-specific responses to environmental circumstances (Reindl and Sheridan, 2012). By revealing the molecular mechanisms by which Gh/Igf signaling is modulated in both endocrine and paracrine/autocrine fashions, physiologists are positioned to more precisely infer the growth patterns of wild fish populations and optimize rearing strategies for domesticated stocks (Picha et al., 2008; Beckman, 2011).

Gh acts on target cells via transmembrane receptors that initiate JAK/STAT, PI3K and/or MAPK signaling pathways (Reindl and Sheridan, 2012). Endocrine Gh directly stimulates the growth of target tissues by acting as a mitogen (Butler and LeRoith, 2001). Gh indirectly regulates growth through the synthesis and secretion of Igfs (LeRoith et al., 2001). While plasma Igf levels are primarily determined by their rate of secretion from the liver, local production of Igfs may also be important in regulating local and whole animal growth (LeRoith et al., 2001; Wood et al., 2005; Duan et al., 2010). Upon binding receptors, Igfs stimulate growth of tissues such as muscle and bone by controlling cell differentiation, proliferation, migration, and survival (Wood et al., 2005; Castillo et al., 2004; Codina et al., 2008; Capilla et al., 2011). As in mammals, Igf1 has long been considered a somatomedin in teleosts; growing evidence also implicates Igf2 as a key mediator of Gh-regulated growth in fishes (Shamblott et al., 1995; Chen et al.,

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2000; Codina et al., 2008; Pierce et al., 2010, 2011; Azizi et al., in press). Teleosts, like mammals, exhibit a pattern in which plasma Gh levels and hepatic Igf synthesis become uncoupled during times of nutrient restriction, a situation termed "Gh resistance" (Jenkins and Ross, 1996; Björnsson, 1997). This uncoupling seemingly underlies diminished hepatic *igf1* levels when food is limited in the environment (Duan and Plisetskaya, 1993; Chauvigne et al., 2003; Pierce et al., 2005). Comparatively less is known about *igf1* and *igf2* in extrahepatic tissues, and most notably, in muscle, where paracrine/autocrine activities may be modulated by nutritional status (Chauvigne et al., 2003; Bower et al., 2008; Bower and Johnston, 2010).

Insulin-like growth factors interact with an extensive set of binding proteins, termed Igf binding proteins (Igfbps). These proteins affect hormone availability, transport, and receptor binding, and thus modulate the actions of Igfs (Duan et al., 2010). While many of the physiologically relevant actions have been determined for individual Igfbps in mammals (Firth and Baxter, 2002), this is not the case for most Igfbps in fishes. Salmonids express an especially large suite of Igfbps; for instance, Atlantic salmon (*Salmo salar*) express 19 *igfbp* genes (Macqueen et al., 2013). Initial characterizations of these *igfbps* reveal that several isoforms (expressed in muscle) are sensitive to food conditions (Bower et al., 2008; Macqueen et al., 2013), while the regulatory systems controlling the mRNA levels of the full array of *igfbps* across tissues remain unresolved. As metabolic hormones such as Gh, thyroid hormones, and cortisol seemingly modulate *igfbps* in salmonids (Pierce et al., 2006), they represent potential regulators of *igfbps* in Atlantic salmon. An important step toward functionally characterizing the extensive *igfbp* network now identified in Atlantic salmon by Macqueen et al. (2013) is to consider their dynamic responses, along with putative endocrine regulators, to various nutritional conditions.

In addition to mediating growth performance, the Gh/Igf system works in concert with other endocrine systems to the drive parr-smolt transformation, the ontogeny of morphological, physiological, and behavioral phenotypes supporting migration from freshwater to pelagic marine environments (Hoar, 1988; Björnsson, 1997). This is especially true with respect to the acquisition of seawater tolerance via the remodeling of branchial epithelium (Sakamoto et al., 1993). Energized by Na^+/K^+-ATPase, seawater-type ionocytes mediate Na^+ and Cl^- extrusion through the coordinated activities of ion cotransporters and channels, including $Na^+/K^+/2Cl^-$ cotransporter 1 (Nkcc1) and cystic fibrosis transmembrane regulator 1 (Cftr1) (Pelis and McCormick, 2001; Singer et al., 2002). Concurrent with elevated *nkcc1* and *cftr1* levels at the peak of smoltification, Atlantic salmon exhibit a "switch" in the relative levels of two *Na^+/K^+-ATPase α1* (*nka-α1*) subunit-encoding genes and their translated proteins. *nka-α1b* levels are enhanced during smoltification while *nka-α1a* is maintained in freshwater but dramatically decreases after seawater exposure (McCormick et al., 2013). Thus, coordinated increases in *nka-α1b*, *nkcc1*, and *cftr1* underlie the development of seawater tolerance (Tipsmark et al., 2002; Nilsen et al., 2007; McCormick et al., 2013). Since Gh/Igf1 signaling supports seawater tolerance, at least in part, by stimulating Nkcc1/*nkcc1* and *nka-α1b* (Pelis and McCormick, 2001; Tipsmark and Madsen, 2009), nutrition-elicited perturbations of the Gh/Igf system may disrupt the development and/or maintenance of ionoregulatory capacities supporting survival in marine environments.

To understand the physiological ecology of smolts, it is especially important to examine the endocrine responses to fasting for two major reasons. First, it has long been recognized that the parr-smolt transformation is a period when large increases in metabolic rate and lipolysis occur (McCormick and Saunders, 1987), leading to the concept that smolts are "energy deficient" during downstream migration (Stefansson et al., 2003). This is not just a seasonal attribute, as the relative condition (condition factor) of parr increases, and smolts decreases, when both are reared under identical *ad libitum* feeding conditions (McCormick et al., 2007). Second, as smolts migrate downstream and enter estuarine/coastal environments they transition from a largely insect-based diet to marine invertebrates and fish (Andreassen et al., 2001; Renkawitz and Sheehan, 2011). This may result in periods of prolonged food restriction and further energy deficiency as individual smolts learn to prey on new food items (Stefansson et al., 2003).

In the current study, we investigated how the Gh/Igf system, thyroid hormones, and cortisol respond to fasting in Atlantic salmon smolts. Leveraging recent molecular characterizations by Bower et al. (2008) and Macqueen et al. (2013) of the extensive *igfbp* gene family in Atlantic salmon, we paid special attention to the dynamics of *igfbp* transcripts that exhibit robust hepatic expression. In this initial investigation of *igfbp* responses to fasting

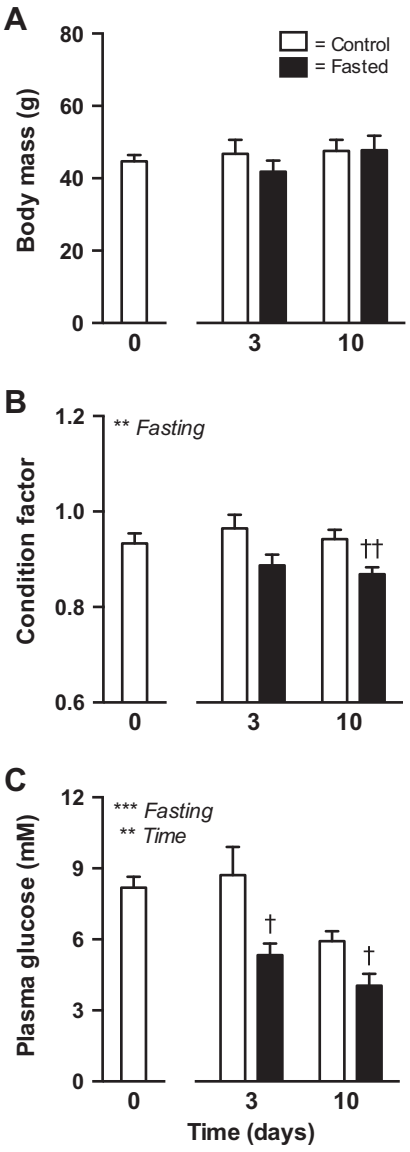

Fig. 1. Effects of fasting on body mass (A), condition factor (B), and plasma glucose (C). Smolts were exposed to continuous feeding (open bars) or fasting (solid bars) and sampled at 3 and 10 days. Significant effects of fasting or time are indicated in respective panels. When there was a significant effect of fasting, post hoc comparisons (Student's t-tests) were made between fed and fasted groups at each time point. $^{\dagger}P < 0.05$ and $^{\dagger\dagger}P < 0.01$. Means ± SEM ($n = 8$).