Comparative Biochemistry and Physiology, Part A 170 (2014) 38–45

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Glucose metabolic gene expression in growth hormone transgenic coho salmon

Stéphane Panserat [a,*], Biju Sam Kamalam [a], Jeanne Fournier [a], Elisabeth Plagnes-Juan [a], Krista Woodward [b], Robert H. Devlin [b]

[a] INRA, UR1067 Nutrition Metabolism and Aquaculture, Aquapôle, F-64310 Saint-Pée-sur-Nivelle, France
[b] Fisheries and Oceans Canada, Centre for Aquaculture and Environmental Research, 4160 Marine Drive, West Vancouver, BC V7V 1N6, Canada

ARTICLE INFO

Article history:
Received 31 October 2013
Received in revised form 17 January 2014
Accepted 23 January 2014
Available online 29 January 2014

Keywords:
Glucose metabolism
Salmon
Transgenic fish
Growth hormone

ABSTRACT

Salmonids are generally known to be glucose intolerant. However, previous studies have shown that growth hormone (GH) transgenic coho salmon display modified nutritional regulation of glycolysis and lipogenesis compared to non-transgenic fish, suggesting the potential for better use of glucose in GH transgenic fish. To examine this in detail, GH transgenic and non-transgenic coho salmon were subjected to glucose tolerance test and subsequent metabolic assessments. After intra-peritoneal injection of 250 mg/kg glucose, we analysed post-injection kinetics of glycaemia and expression of several key target genes highly involved in glucose homeostasis in muscle and liver tissues. Our data show no significant differences in plasma glucose levels during peak hyperglycaemia (3–6 h after injection), demonstrating a similar glucose tolerance between transgenic and non transgenic. However, and unrelated to the hyperglycaemic episode, GH transgenic fish return to a slightly lower basal glycaemia values 24 h after injection. Correspondingly, GH transgenic fish exhibited higher mRNA levels of glucokinase (GK) and glucose-6-phosphate dehydrogenase (G6PDH) in liver, and glucose transporter (GLUT4) in muscle. These data suggest that these metabolic actors may be involved in different glucose use in GH transgenic fish, which would be expected to influence the glucose challenge response. Overall, our data demonstrate that GH transgenic coho salmon may be a pertinent animal model for further study of glucose metabolism in carnivorous fish.

1. Introduction

Fish do not have a specific requirement for exogenous glucose, because they are able to survive and grow when fed diets devoid of carbohydrates (NRC, 2011). However, it is theoretically a good strategy for aquaculture to incorporate digestible carbohydrates in the diet, to decrease the levels of expensive fishmeal (rich in proteins) and fish oil (rich in lipids) in new aquafeeds (Panserat, 2009). The maximum tolerable dietary level of digestible carbohydrates has been extensively tested in salmonids, and was found to be 20% (20 g of carbohydrates per 100 g of diet) or lower (Hemre, 2002; NRC, 2011). Therefore, carbohydrate inclusion in salmonid feeds is often limited to avoid a significant decrease in growth and associated persistent postprandial hyperglycaemia (up to 20 mM) that can arise when salmonids are fed with more than 20% carbohydrates (Panserat, 2009; NRC, 2011). Further, a detrimental effect on liver ("fatty liver") can also be observed (Hemre, 2002). These data suggest a problem in metabolic use of dietary glucose in salmonids which can be considered as a glucose intolerant phenotype, confirmed by application of glucose tolerance tests (Moon, 2001). Main mechanisms potentially involved in poor glucose use in salmonids include low induction of glycolytic genes/enzymes in liver and muscle, low induction of lipogenesis, absence of inhibition of hepatic gluconeogenic genes, and low glucose transport in muscle (Polakof et al., 2012).

Growth hormone (GH) gene transgenesis has been used in several fish species to enhance growth, in some case dramatically (Du et al., 1992; Devlin et al., 1994, 2001; Nam et al., 2001). GH transgenic coho salmon show elevated growth rates (e.g. 2–3 fold enhancement in daily weight gain) which can result in large differences in size from control animals (Devlin et al., 1995, 2004). To achieve this growth rate, transgenic salmon have enhanced appetite, feeding motivation and feed conversion efficiencies (Devlin et al., 1999; Raven et al., 2006; Higgs et al., 2009). Concerning the last point (i.e., feed efficiency), this can be related to the global but controversial effect of growth hormone on protein, carbohydrate and lipid metabolism (Sangiao-Alvarellos

Abbreviations: 6PFK, 6-phosphofructo-1-kinase (EC 2.7.1.11); FDPase fructose, 1,6-bisphosphatase (EC 3.1.3.11); G6Pase glucose, 6-phosphatase (EC 3.1.3.9); G6PDH, glucose 6-phosphate dehydrogenase (EC 1.1.1.49); GH, growth hormone; GHR, growth hormone receptor; GK, glucokinase (HK IV, EC 2.7.1.1, EC 2.7.1.2); GLUT, glucose facilitative transporter; HK, hexokinase (EC 2.7.1.1); PBS, phosphate buffered saline; PK, pyruvate kinase (EC 2.7.1.40).

* Corresponding author at: INRA, UR1067 Nutrition Metabolism Aquaculture, Aquapôle, CD918, F-64310 St-Pée-sur-Nivelle, France. Tel.: +33 5 59 51 59 60; fax: +33 5 59 54 51 52.
E-mail address: panserat@st-pee.inra.fr (S. Panserat).

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http://dx.doi.org/10.1016/j.cbpa.2014.01.013

et al., 2005; Vijayakumar et al., 2010). Indeed, the effects of GH on carbohydrate metabolism are more complicated (both short and long term) and may be indirectly linked via the antagonism of insulin action. However, unlike non-transgenic fish, GH transgenic coho salmon fed a diet high in carbohydrates maintained growth rates, had increased capacity for lipid synthesis, and increased potential for biosynthetic roles of amino acids (Higgs et al., 2009). GH transgenesis influences metabolic reactions in coho salmon by emphasizing carbohydrate degradation for energy production and lipid synthesis, and increasing utilization of lipids and proteins for synthetic roles necessary to maintain accelerated growth (Leggatt et al., 2009). The enhanced ability of transgenic coho salmon to store energy under high levels of dietary carbohydrates suggests enzymatic improvements in their anaerobic and aerobic metabolism of glucose (Higgs et al., 2009). The higher capacity of use of glucose was also suggested in GH transgenic Atlantic salmon (*Salmo salar*) by analysing glycolytic and oxidative phosphorylated enzymes in muscle and liver (Levesque et al., 2008). Overall, the GH transgenic coho salmon seems to possess improved glucose use compared to non-transgenic control salmon.

In this context, the present study was undertaken to compare for the first time, the glucose tolerance in GH transgenic and non-transgenic coho salmon, using intraperitoneal glucose injections. Our objective was to examine potential differences in the molecular regulation of glucose metabolism by glucose per se. Glycaemic profile and gene expression of specific metabolic markers were analysed in liver and muscle in order to characterize the changes in glucose metabolism following a glucose tolerance test, and to study new relationships between (chronic) GH elevation and glucose metabolism in fish.

2. Material and methods

2.1. Experimental fish

All fish were reared in compliance with the guidelines of Canadian Council for Animal Care, under permit 12-017 issued by Fisheries and Oceans Canada's Pacific Region Animal Care Committee. The transgenic fish used in this experiment were produced and raised in a biosecure facility at the Centre for Aquaculture and Environmental Research (CAER) in West Vancouver, British Columbia, Canada. The transgenic coho salmon (*Oncorhynchus kisutch*, strain M77) contained a growth hormone gene construct OnMTGH1 from sockeye salmon (*Oncorhynchus nerka*; Devlin et al., 1994) and were generated by crossing homozygous transgenic males with ten wild females obtained from the Chehalis River in south-western British Columbia. Non-transgenic coho salmon were half-sibs of the transgenic salmon and were generated by crossing wild males with the same wild females used above. Experimental fish were randomly selected from these populations. Prior to the experiment, fish were reared in aerated fresh well water (10 ± 0.5 °C) at densities less than 10 kg/m^3 and were fed commercial salmon diets (Skretting Canada) three times per day. Wild-type salmon were fed to satiation, whereas transgenic fish were pair fed the wild-type ration to allow experimental fish to be at the same size (50 ± 3 g) at the same age of analysis.

2.2. Glucose tolerance test

Fish were tagged with Passive Integrated Transponder tags, weighed and length measured, and randomly assigned to four treated and untreated groups per genotype. All fish to be treated and/or sampled at a particular time were kept together in a single tank to ensure a common environment and avoid handling stress for fish to be assessed at other time points. Lighting followed the natural photoperiod and utilized artificial lighting with wavelengths to stimulate daylight. Fish were feed deprived for 48 h before the start of the experiment, in order to ensure empty digestive tracts without initiating body protein degradation. At 09:00 h on the day of the experiment, each fish from each of the four tanks (i.e. one tank for 0, 3, 6, and 24 h time point assessments) was withdrawn randomly from its tank, had its tag read, and appropriate treatment applied. One group of GH transgenic and non-transgenic fish received an intraperitoneal injection of glucose (250 mg/kg body mass), whereas another group was sham-treated with the vehicle (saline, PBS), as previously described (Polakof et al., 2010). Prior to treatment and sampling, fish were anaesthetized for approximately 60–90 s in 100 mg/L tricaine methanesulphonate (MS-222, Syndel Laboratories Ltd., Vancouver, BC, Canada) buffered with 200 mg/L sodium bicarbonate. At 0 h (before injection), 3 h, 6 h and 24 h after treatment, fish (n = between 7 and 10 per analysis group) were killed by cervical concussion, and the caudal peduncle was severed and blood glucose levels immediately analysed with a clinical blood glucometer (Accu-Chek Aviva Nano, Roche) as described (Iwama et al., 1995). Liver and muscle samples were rapidly collected, snap frozen in liquid nitrogen, and then stored at −80°C until further mRNA analyses.

2.3. Measure of gene expression by qRT-PCR

Analyses of mRNA levels were performed on samples from the liver and white muscle of fish sampled at 0 h (basal values), 6 h (hyperglycaemia plateau) and 24 h (return to basal values) after glucose injection. Tissue samples from six individual fish per experimental condition were used as biological replicates. Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations, quantified by spectrophotometry (absorbance at 260 nm) and integrity was confirmed by agarose gel electrophoresis. One microgram of the resulting total RNA was reverse transcribed into cDNA using the SuperScript III RNaseH-reverse transcriptase kit (Invitrogen) and random primers (Promega, Charbonniéres, France), according to the instructions of each manufacturer. Quantification of target gene expression levels were carried out in an iCycler iQ real-time PCR detection system (Bio-Rad) using iQ SYBR green supermix and specific primers. PCR was performed using 5 μL of the diluted cDNA (1:50) mixed with 200 nM of each primer in a final volume of 15 μL. The PCR protocol was initiated at 95 °C for 90 s for initial denaturation of the cDNA and activation of the hot-start iTaq TM DNA polymerase, followed by a two-step amplification programme (20 s at 95 °C and 30 s at specific primer hybridization temperature) repeated 40 times. At the end of the last amplification cycle, melting curves (temperature gradient at 0.5 °C/10 s from 55 to 94 °C) were systematically monitored to confirm the specificity of the amplification reaction. Each PCR run included replicate samples (duplicate of reverse transcription and PCR amplification, respectively) and negative controls (reverse transcriptase and RNA free samples, respectively). The qPCR assay was optimized with a linear standard curve ($R^2 > 0.985$) and checked for consistency across replicates. PCR reaction efficiency for each run was estimated based on the slope of the 5 points standard curve obtained with serial dilution of pooled sample cDNAs. Efficiency values ranging from 1.85 to 2.05 were considered to be acceptable.

The following transcripts were analysed: growth hormone (GH), and growth hormone receptor (GHR) as controls of transgenesis (lines) (Raven et al., 2008); glucose facilitative transporter type 2 and 4 (GLUT2, GLUT4) for glucose transport/uptake; glucokinase (GK; EC 2.7.1.2), hexokinase (HK; EC 2.7.1.1), 6-phosphofructo-1-kinase (6PFK; EC 2.7.1.11) and pyruvate kinase (PK; EC 2.7.1.40) for glycolysis; glucose 6-phosphatase (G6Pase; EC 3.1.3.9; two isoforms 1 and 2) and fructose-1.6-bisphosphatase (FDPase; 3.1.3.11) for gluconeogenesis; and glucose 6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) for pentose-phosphate pathway (Panserat et al., 2009; Skiba-Cassy et al., 2009; Kamalam et al., 2012). Since the sequence similarity between rainbow trout and coho salmon genes is high (McClelland and Naish, 2008), we used primers designed for rainbow trout (Table 1) to analyse the gene expression of conserved actors involved in metabolism. The exceptions were those primers previously used for GH and GHR mRNA quantification (Raven et al., 2008). Coho salmon PCR amplicons