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Short communication

Fatty acid content in muscles of amago salmon homozygous or heterozygous for a growth hormone transgene

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ABSTRACT

Fatty acids are a vital energy source in fish and are of significant importance to their physiological wellbeing. Amago salmon (*Oncorhynchus masou ishikawae*) transgenic for a growth hormone (GH) show both accelerated growth and altered fatty acid composition and content in liver tissues. In particular, they show a decrease in saturated fatty acids and monounsaturated fatty acids, and an increase in polyunsaturated fatty acids except for docosahexaenoic acid (22:6n−3) and eicosapentaenoic acid (20:5n−3). Furthermore, transgenic fish have decreased levels of serum glucose, triacylglycerol and an increase in 3-hydroxybutyric acid, generally considered a starvation marker. As liver tissue is physiologically connected to muscle tissue, here we examined the effects of GH transgenesis on fatty acid contents in muscles of homozygous and heterozygous GH transgenic fish. The major monounsaturated fatty acids oleic acid (18:1n−9) and palmitoleic acid (16:1n−7) were slightly higher in the control, whereas polyunsaturated fatty acids, except 22:6n−3 and 20:5n−3, were significantly greater in the transgenic fish ($P < 0.05$), similar to the results from the liver. However, by contrast to the liver, the major saturated fatty acids palmitic acid (16:0) and stearic acid (18:0) and polyunsaturated fatty acids (22:6n−3) and (20:5n−3) were significantly higher ($P < 0.05$) in the transgenic fish than in the controls.

1. Introduction

GH transgenesis in fish almost invariably causes increased growth performance, for example, GH transgenic salmon typically show a 6 to 11 fold increase in body weight and occasionally a 40 fold or more increase has been reported (Devlin et al., 1994; Rahman et al., 1998). We have also generated fast-growing GH transgenic amago salmon, and showed that the fish had down-regulation of Δ-6 fatty acyl desaturase (Δ6FAD) expression using functional microarray analysis (Mori et al., 2007). This enzyme is important for the modification of polyunsaturated fatty acids (PUFAs) in many vertebrates (Zheng et al., 2004), and a decrease in its expression causes changes to the levels of various PUFAs. In a subsequent study of the effect of GH transgenesis on metabolic processes, we produced homozygous (Tg/Tg) and heterozygous (Tg/+) GH transgenic amago salmon (Kurata et al., 2012). Analysis of these fish showed that serum IGF-I concentrations were significantly higher in the transgenic fish than in the controls. The highest serum GH1 concentrations occurred in the Tg homozygotes, with a significantly lower level in heterozygotes and the lowest level in controls. Moreover, an iTRAQ-MS/MS proteome and microarray analysis showed that these GH transgenic amago salmon had a drastic decrease in the amount of fat tissue that accumulated around the pyloric caeca compared to the controls, and also had down-regulation of fatty acid synthase (FAS) in the pituitary (Kurata et al., 2012).

We examined metabolic processes in the liver tissue of GH transgenic amago salmon and found an enhanced catabolic reaction of fatty acids compared to controls. This change in catabolism caused an increase in β-oxidation of saturated (SFAs) and monounsaturated fatty acids (MUFAs) in homozygous (Tg/Tg) and heterozygous (Tg/+) amago salmon compared to the controls (Sugiyama et al., 2012). Expression of the Mid1 interacting protein 1 gene (*Mid1ip1*), which is important in enhancing de novo fatty acid synthesis, was down-regulated, and an increase in 3-hydroxybutyric acid (a ketone body) was observed in the livers of the GH transgenic fish. These results indicate that the liver tissue from GH transgenic fish is in a state of starvation. The amounts of SFAs and MUFAs in the livers were found to decrease in the order homozygous (Tg/Tg) and heterozygous (Tg/+) GH transgenic, and control fish. By contrast, the amounts of n−3 PUFA rose in this order.

Fish lipids are rich in PUFAs, and these have important roles in regulation of inflammation (Arts and Kohler, 2009) and the immune system (Rowley et al., 1995). Therefore, analysis of fatty acid composition in lipids is a valuable means of understanding physiological changes and the health condition of both mammals and fish. Therefore, analysis of the effect of GH transgenesis on lipid metabolism in muscle tissue will

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provide valuable insights into the health and physiological condition of the fish.

2. Material and methods

2.1. Experimental animals

GH transgenic amago salmon were generated by injecting *OnMTGH1* gene construct into fertilized eggs (Devlin et al., 1994). In this experiment, we used heterozygous (Tg/+) GH transgenic amago which were produced by fertilizing domestic-type sperms with eggs collected from transgenic fish containing the *OnMTGH1*. Homozygous (Tg/Tg) GH transgenic fish were produced by mixing eggs and sperm obtained from heterozygous (Tg/+) fish. The fish were reared in equal densities in circulating tanks under a natural light cycle, and fed to satiation with a stage-specific commercial diet for juvenile fish (1-4CDX and Masu 5-8p from Nippon Formula Feed Mfg. Co., Ltd) until the end of the experiment (about 6 months). The mean weights of the homozygous (Tg/Tg), heterozygous (Tg/+) (note that all the heterozygotes were produced using eggs from transgenic fish and sperm from wild type), and age control (+/+) fish used in this experiment were 131 g, 109 g, and 85 g, respectively. Details of the production and detection of the transgenic fish using PCR were described in a previous study (Sugiyama et al., 2012).

Muscle tissues were obtained from 5–6 specimens of homozygous (Tg/Tg), heterozygous (Tg/+), and control (+/+) amago, frozen in liquid nitrogen, and stored at −80 °C until analysis. Extraction and purification of total lipids were performed following the method of Folch et al.

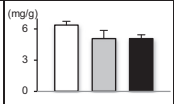
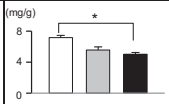
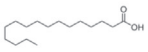
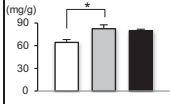
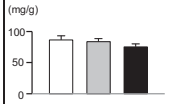
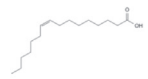
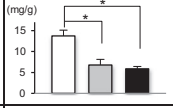
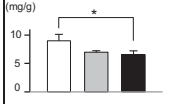
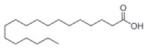
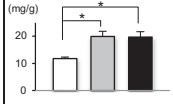
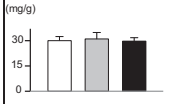
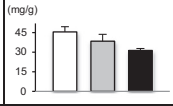
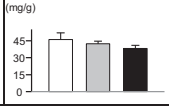
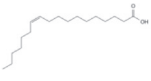
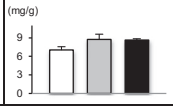
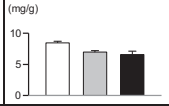
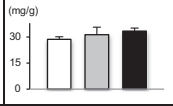
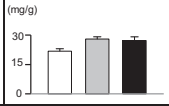
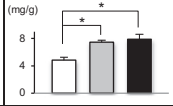
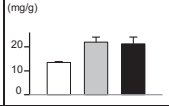
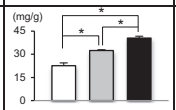
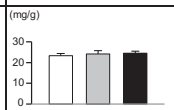
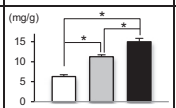
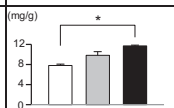
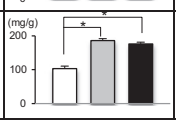
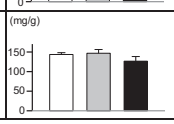
Tissue		Muscle		Liver	
Common name Numerical symbol (Content of diet)	Structure	Content (mg/g)	Pattern	Content (mg/g)	Pattern
Myristic acid 14:0 (2.1 %)			Down		Down
Palmitic acid 16:0 (21.3 %)			Up		No alteration
Palmitoleic acid 16:1n-7 (3.4 %)			Down		Down
Stearic acid 18:0 (5.6 %)			Up		No alteration
Oleic acid 18:1n-9 (17.4 %)			Down		Down
cis-Vaccenic acid 18:1n-7			Up		Down
Linoleic acid 18:2n-6 (24.3 %)			Up		Up
Arachidonic acid 20:4n-6 (1.0 %)			Up		Up
Eicosapentaenoic acid 20:5n-3 (4.2 %)			Up		No alteration
Docosapentaenoic acid 22:5n-3 (1.1 %)			Up		Up
Docosahexaenoic acid 22:6n-3 (10.8 %)			Up		Down

Fig. 1. Fatty acid contents (mg/g) in muscle tissue from homozygous (black), heterozygous (gray) of GH transgenic amago salmon, and from controls (white), compared with those in liver tissue (Sugiyama et al., 2012). Contents of fatty acids were calculated using heptadecanoic acid (17:0) as an internal standard. The changes in content are shown as Up or Down in comparison to the control. Data are presented as means ± standard error. Asterisk "*" indicates a significant difference ($P < 0.05$). Figure in parentheses shows fatty acid content (%: W/W) of the fish diet.