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Short-term fasting increases skeletal muscle lipid content in association with enhanced mRNA levels of lipoprotein lipase 1 in lean juvenile red seabream (*Pagrus major*)

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ABSTRACT

Cultured fish are often subjected to short-term fasting for a few days prior to transport or slaughter. This intervention reduces waste and oxygen consumption during transport and improves flesh quality, but little is known about how it affects the muscle lipid content. Even less is known for wild-caught fish that are also sometimes fasted before slaughter. To investigate the effects of short-term fasting on muscle lipid content, we performed three fasting experiments using adult, lean juvenile, and fatty juvenile red seabream (*Pagrus major*). In the first experiment using adult specimens (performed at 20 °C), fasting for 10 days caused up to 2.3-, 7.8-, and 3.7-fold increases in muscle mRNA levels of lipoprotein lipase 1 (*LPL1*), *LPL2*, and peroxisome-proliferator-activated receptor gamma (*PPAR*γ), respectively (n = 6 each). These genes all participate in lipid accumulation. The second experiment was performed on 5 groups of lean juvenile red seabream [considered as wild-caught fish; 19 °C, n = 5 each except for one group (n = 4)]. Fasting for 5 days significantly increased muscle lipid content (percentage of wet weight) and *LPL1* mRNA levels up to 2.2- and 11-fold, respectively. The mRNA levels of *LPL2* and *PPAR*γ could not be determined possibly due to their low expression levels. The lipid amount (mg) in the liver decreased by 88% during fasting, whereas adipose tissue apparently disappeared after fasting for 10 days. These results suggest that lipid mobilization from liver and adipose tissue anticipated the muscular utilization of lipid, resulting in the counterintuitive increase in muscle lipid content during fasting. The third experiment was conducted on 7 groups of fatty juvenile red seabream [considered as cultured fish; 20 °C, n = 5 each except for one group (n = 4)]. Fasting up to 10 days did not increase muscle lipid content or *LPL1* mRNA levels. The lipid amount in the liver, but not in the adipose tissue, decreased during the experiment. Fasting for 10 days was not enough to induce active lipid mobilization when fish were well fed prior to fasting. Contrary to our expectations, regression analysis using data from juvenile specimens found a significant inverse relationship between *LPL1* mRNA levels and lipid amount in the liver, which might indicate that *LPL1* mRNA levels are a reflection of temporal tissue demand for lipids. Overall, these results suggest that short-term fasting for ~5 days increases muscle lipid content of wild-caught lean fish possibly by an LPL1-dependent lipid transport mechanism.

Statement of relevance: We showed that short-term fasting improves flesh quality.

1. Introduction

Fasting fish prior to transport or slaughter is a common procedure in aquaculture. This intervention, sometimes referred to as "purging," was originally used to empty fish gut in order to reduce waste and oxygen consumption during transport (Lines and Spence, 2012). However, it is now increasingly recognized that pre-slaughter fasting also improves flesh quality by decreasing off-flavors (Einen and Thomassen, 1998; Palmeri et al., 2009), increasing firmness (Sáez et al., 2013) and freshness during ice storage (Ginés et al., 2002). Long-term fasting more than 2 weeks is required to eliminate off-flavors in intensively cultured fish (Einen and Thomassen, 1998; Palmeri et al., 2009), while it poses the risk of weight loss and uncontrolled decrease in flesh lipid content. Short-term fasting up to ~10 days is sometimes beneficial to improve flesh quality, but usually has marginal effects (López-Luna et al., 2014).

Wild-caught fish are also occasionally kept in fish cages without feeding before transport or slaughter. The major purpose of this cultivation is the recovery from fishing stress, and fish are fasted to avoid

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intensive exercise upon feeding. For this purpose, durations of the pre-slaughter fasting are generally shorter than those for cultured fish mentioned above. In wild-caught spotted mackerel (*Scomber australasicus*), fasting up to 3 days improved compressive elasticity of the skeletal muscle, increased ATP, and decreased lactate levels (Tamotsu et al., 2012), while in wild-caught snapper (*Pagrus auratus*), even short resting for 10 h was sufficient to delay the onset of rigor mortis (Lowe et al., 1993). However, probably due to the short-time frame and inconvenience to use wild-caught fish, very few studies have addressed the effects of short-term fasting on flesh quality, except for the indicators of freshness (e.g., ATP content and K-value). Wild fish are generally lean and have less odorous substances in their body; therefore, short-term fasting probably produces a more severe metabolic response compared to cultured fish.

One of the common metabolic responses to fasting in fish is lipid mobilization from storage organs to peripheral tissues, which is associated with a shift of primary energy sources. Lipids in the liver and adipose tissue, as well as glycogen in the liver and skeletal muscle, are preferentially utilized as energy sources in the early phase of fasting, while the order of precedence differs among species (Black and Love, 1986; Einen et al., 1998; Han et al., 2011). Active utilization of skeletal muscle proteins usually occurs in the later phase of fasting, although some species use considerable amounts of protein to conserve glycogen (Gillis and Ballantyne, 1996; Navarro and Gutierrez, 1995). Therefore, it is likely that short-term fasting affects the whole-body distribution of lipids and glycogen, while it has less effect on skeletal muscle protein content.

In the present study, we investigated the effect of short-term fasting on lipid metabolism in red seabream (*Pagrus major*). The lipid content and relative mRNA levels of some genes involved in lipid incorporation and accumulation were measured with a special emphasis on lipoprotein lipase (*LPL*; E.C. 3.1.1.34), which is considered to promote skeletal muscle lipid accumulation this species (Oku et al., 2006a; Oku and Umino, 2008; Kaneko et al., 2013).

2. Materials and methods

2.1. Fish and experimental design

All experiments were conducted according to the Guidelines for the Care and Use of Laboratory Animals, The University of Tokyo. For experiment 1, adult red seabream specimens [typically >500 g; (Matsuyama et al., 1987)] were purchased from Fish Interior (Tokyo, Japan) and placed in aquarium tanks at The University of Tokyo. Fish were cultured at 20 °C with a commercial diet (Otohime; Marubeni Nisshin Feed Co. Ltd., Tokyo, Japan) for 12 days. After acclimation, red seabream specimens (body weight range; approximately 500–700 g) were placed into two tanks (n = 6 in each tank); specimens in a tank (fasted group) were fasted for 10 days (i.e. 200°-day), whereas those in the other tank (fed group) were fed once a day. All fish were sampled on day 10 (10 days after fasting started), and their skeletal muscle, liver, visceral adipose tissue, and skin were subjected to quantitative real-time PCR (qRT-PCR).

For experiment 2, a cohort of red seabream was purchased from the Kanagawa Prefectural Fisheries Technology Center, Kanagawa, Japan and placed in aquarium tanks at Tokyo University of Marine Science and Technology. Fish were cultured at 19 °C with a commercial feed (Trout Feed No.4, Nippon Formula Feed, Yokohama, Kanagawa) for about 3 months. To mimic natural conditions, juvenile red seabream (about 50 g, total 24 individuals) were fed once in 2–3 days and fasted for 2 days prior to the experiment. Four specimens were sampled at the beginning of experiment (day 0). Then, the remaining 20 specimens were placed into two tanks (each tank contained 10 fish). Specimens in a tank were fasted and sampled after 5 and 10 days (n = 5 each; 95 and 190°-day, respectively). Specimens in the other tank were fed once a day and sampled after 5 and 10 days (n = 5 each). Body weight, total length, standard length, lipid content, and *LPL1* mRNA levels were measured in these samples.

For experiment 3, a cohort of red seabream was purchased from Marua Suisan Co., Ltd., Ehime, Japan and placed in aquarium tanks at The University of Tokyo. Fish were cultured at 20 °C with the same diet as in experiment 2 for 6 months. To mimic cultured conditions, juvenile red seabream specimens (about 60 g, total 34 individuals) were cultured for 2 weeks under satisfactory feeding, where fish were fed twice a day until they stop eating. After confirming that the skeletal muscle lipid content was about 3%, 5 specimens were sampled (day 0). The remaining 29 specimens were placed in two tanks. Fourteen specimens in a tank were fasted, and 5 specimens were sampled from the tank on days 5 and 10 (n = 5 each; 100 and 200°-day respectively). The remaining 4 specimens were re-fed for 10 days and sampled (day 20, n = 4). Fifteen specimens in the other tank were fed once a day and sampled on days 5, 10, and 20 (n = 5 each). Triacylglycerol (TAG) content and *LPL1* mRNA levels were measured in these samples.

2.2. Measurement of lipid content

The lipid content was measured by the conventional Bligh and Dyer method (Bligh and Dyer, 1959). The TAG content was determined using a triglyceride E-test kit (Wako, Osaka, Japan) following the Bligh and Dyer extraction.

2.3. qRT-PCR

Total RNA was extracted from a small tissue sample (about 50 mg) using ISOGEN (Nippon Gene, Japan) or an RNeasy lipid tissue mini kit (Qiagen, Hilden, Germany) according to the manufacturers' instructions. qRT-PCR was performed for peroxisome proliferator-activated receptor gamma (*PPARγ*), *LPL1*, and *LPL2* using ABI PRISM 7300 RT-PCR system (Applied Biosystems, Foster City, CA, USA) as reported previously (Hirano et al., 2011; Teraoka et al., 2014). Primer sequences were obtained from previous studies (Oku et al., 2006b; Kaneko et al., 2013): PPARγ: 5′-ACGCCGTGGACCTGTCA-3′ and 5′-TGTGGACAAATGTTTCATGTCAAG-3′; LPL1: 5′-CTCAAGACCCGCGAGAT-3′ and 5′-AAGCGTCGCTCTGACC-3′; LPL2: 5′-ATTCATTCCTGCTGGTGAC-3′ and 5′-TCAGTGCTTCTCCAGAGTTAC-3′; and β-actin: 5′-GGCACTGCTGCCTCCTC-3′ and 5′-GCCAGGATGGAGCCTCC-3′. The relative mRNA levels of target genes were determined by the comparative Ct method using β-actin as an internal control.

2.4. Data analysis

Outliers were identified by the Smirnov–Grubbs' test using the outlier library of R (version 3.1.1) at the significance level of 0.05. MATLAB R2013 (MathWorks Inc., Boston, MA, USA) was used for the preparation of figures and statistical analysis. Non-linear regression was performed by the least squares method with specified initial values (a = 1, b = 1) in R ver. 3.1.1 using data of 58 individuals from experiments 2 and 3. Akaike information criterion (AIC) was used as a measure of goodness of fit. Since only the total lipid content (%) was measured in experiment 2, TAG content (%) in this experiment was calculated by subtracting 0.6 from the total lipid content, which corresponds to phospholipid content in red seabream of similar size (Mustafa et al., 1995).

3. Results

3.1. Experiment 1: adult red seabream

Fasting for 10 days did not affect fish body weight significantly (579.8 ± 71.8 g in the fasted group vs. 577.0 ± 54.9 in the fed group), suggesting that short-term fasting did not cause severe malnutrition in adult red seabream. Fish were apparently in good health throughout the experimental period.