論 文 の 内 容 の 要 旨

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論文題目　Physiological functions of lipoprotein lipase for controlling triacylglycerol levels in medaka
（メダカのトリアシルグリセロールレベル調節におけるリポプロテインリパーゼの機能）

Triacylglycerol (TAG) is the major lipid providing energy required for metabolic process in organisms. It is composed of one glycerol molecule esterified with three fatty acids (FA). In general, glycerol backbone is derived from glucose metabolism, while the three fatty acid components are derived either from dietary lipids or from acetyl CoA via *de novo* FA biosynthesis. Acetyl CoA is well known as one important intermediary metabolite from glucose and amino acid metabolisms. In contrast to mammals, fish utilize TAG as a main energy source, in preference to carbohydrate. They store TAGs in several sites, such as adipose, liver and muscle tissues.

Fasting is known to be a natural occurrence in fish life. During fasting, FA portion of TAG is used for the synthesis of acetyl CoA, a critical precursor for the syntheses of ketone bodies. These derivatives are considered as available energy fuels in muscle and brain. Glycerol portion is generally used as a gluconeogenic substrate, and thus TAG reserves in fish are considered to play an important role in the metabolic adaptation to fasting. The molecular mechanism in the regulation of TAG metabolism under the condition of food

deprivation, however, remains unclear. The aim of this study is to clarify the correlation of changes in TAG reserves and the regulation of related genes in response to fasting and re-feeding.

Four groups of medaka were acclimated to laboratory environment for 3 weeks. One group of medaka was used as the control, and two groups were fasted for 4 and 8 days, while the remaining one group was re-fed for 4 days after the 8-day fasting. Measurement of TAG levels in muscle and liver of medaka under each period was performed using colorimetric method. Fasting for 8 days reduced hepatic TAG levels, followed by their recovery after 4-day re-feeding. In contrast, muscle TAG levels were increased after fasting for 4 days, and the further 4-day fasting did not affect the levels. Re-feeding reduced muscle TAG levels again. These results suggest the tissue-specific effects of fasting and re-feeding on TAG stores in medaka tissues.

Comprehensive assay of gene expression profile was performed using a next generation sequencing (NGS) technique. Overall, eight clusters of gene expressions were identified in both tissues using hierarchical clustering method. Fasting for 4 days resulted in the up-regulations of the genes in four clusters, which were involved mainly in glycolysis and proteolysis. However, most genes related to TAG metabolism and FA oxidation were expressed at low levels. Notably, several up-regulated genes were found to participate in the pathway of TAG synthesis. These findings suggest that medaka reduces TAG metabolism and conserves TAG stores in muscle, while consumes glycogen, glucose and protein as energy fuels for the maintenance of basic cellular functions at the early stage of fasting, in order to adapt to the nutritional restriction. In addition, the induction of TAG synthesis would be associated with the elevation of TAG levels in muscle. During the post period of fasting, the six clusters contained approximately 50 genes showing high expressions in

muscle. These genes were involved in TAG metabolism, FA oxidation, gluconeogenesis and protein/amino acids catabolism. These observations suggest that medaka would acquire the acclimation stage against nutrient restriction with the dynamic metabolic regulations. In contrast, re-feeding reduced most of the genes related to proteolysis and amino acid catabolism, whereas the genes with high expressions in four clusters were related to TAG metabolism and FA oxidation. These findings suggest that re-feeding should promote the metabolic levels of TAG and FA in medaka muscle, accompanied by the conservation of protein stores.

On the other hand, during the early period of fasting, the genes with elevated expressions in liver were involved in lipolysis, FA oxidation, gluconeogenesis, ketone bodies synthesis and proteolysis, while the expressions of the genes in the other clusters were decreased. These observations imply that liver would rapidly respond to fasting and consume its stores for the provision of energy fuels required by peripheral tissues as the center of metabolic control. The post period of fasting induced the down-regulations of most genes, whereas only two clusters containing about 18 genes were expressed at high levels. This complete inversion of gene expression patterns suggests that medaka should acclimate to the low energy status, and thus reduce the metabolic regulation in liver. Re-feeding induced the up-regulations of four groups of genes that were mainly related to TAG synthesis and de novo FA biosynthesis. These observations suggest that re-feeding should reset the state of acclimation to restricted nutritional condition and recover TAG stores in liver.

During the adaptation periods of fasting and re-feeding, the patterns of metabolic regulations in both tissues suggest that TAG transport has a large effect on the maintenance of TAG stores. Especially, the expression patterns of LPL gene that has a critical role in the regulation of TAG transport were found to have close correlations with TAG levels in both

tissues. Therefore, the molecular characteristic and tissue-distribution pattern of medaka LPL gene were further investigated in this study. The regulation of LPL transcripts in response to fasting and re-feeding was also confirmed by quantitative real-time RT-PCR (qRT-PCR) technique.

The cDNA sequence of medaka LPL gene was cloned from liver of adult medaka using rapid amplification of cDNA ends technique. The deduced amino acid sequence of LPL was found to share a high identity (78.3–84.7 %) with LPL1 sequences from several fish species, and therefore was termed LPL1. The 2,259–bp cDNA of medaka LPL1 contained an open reading frame of 1,551 bp encoding 516 amino acids, a 415–bp 5' untranslation region (UTR) and a 563–bp 3' UTR. The deduced amino acid sequence of medaka LPL1 was found to share the common conserved sites with that of LPL1 genes from several fish species and LPL genes from mammals, i.e., one catalytic triad (Ser179, Asp203 and His291), one heparin-binding site (329–332 residues), a putative polypeptide 'lid' (263–289 residues), eight cysteine residues, one lipid-binding site (Trp440, Trp443 and Trp444) and one putative *N*-linked glycosylation site (Asn409).

The tissue-distribution pattern of LPL1 gene indicated that LPL1 transcripts were ubiquitously expressed in various tissues. The highest levels of LPL1 transcripts were found in liver, followed by visceral adipose tissue, whereas the lowest levels were found in the intestine. The observation in the relatively high expression of LPL1 gene in the brain suggests the potential function of LPL1 in this organ.

The expressions of LPL1 gene in muscle and liver modulated by fasting and re-feeding were further analyzed by qRT-PCR assay using three internal reference genes. In general, the relative mRNA levels of LPL1 normalized to each internal reference gene showed a similar tendency in both tissues. The results in muscle confirmed the NGS assay that a higher rate of

the increase in the expression of LPL1 gene was found during the early period of fasting. In contrast, a slight decrease was found during the post period of fasting by qRT-PCR assay. Overall, the continuously high expression of LPL1 gene suggests that this gene has a large effect on the maintenance of TAG levels in muscle during fasting.

In conclusion, the gene expression profile observed in this study suggests that medaka modulates the metabolic patterns of TAG stores in tissue-specific manners for the adaptation to the different stages of fasting. A rapid response of liver to fasting was reflected by the transient lipolysis of its TAG stores for the provision of various energy substrates required by peripheral tissues. However, medaka was likely to prefer to conserve muscle TAG stores for the adaptation to the low energy status. The observations during the post period of fasting indicate that medaka enters the acclimation stage to the poor nutritional condition through the induction of the dynamic metabolic regulations and the reduction of metabolic reactions in muscle and liver, respectively. The potential transport of energy sources among the tissues regulated by LPL gene implied its critical function on the maintenance of muscle TAG stores during fasting.