# 産卵回帰シロサケの肝臓における脂質代謝


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# Lipid Metabolism in the Liver of Chum Salmon during Spawning Migration

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The lipid biosynthetic ability in the liver using [1-$^{14}$C] sodium acetate and [1-$^{14}$C] oleic acid as precursors and the *in vitro* hepatic lipase activity were examined to clarify the lipid metabolism of chum salmon. The decrease of triglyceride in the muscle and liver, and the increase of free fatty acid in the liver were found in spawning salmon. The lipase activity increased markedly during spawning migration. The high levels of sterol and triglyceride biosynthetic activities were found in the pre-spawning salmon, whereas phospholipid biosynthesis was almost the same level during spawning migration. The lipid metabolism in the liver was presumed to affect the lipid in the muscle of spawning salmon.

Pacific salmon are typically anadromous fish, developing in the ocean and spawning in the freshwater. This biological feature governs the pattern of changes in their chemical composition.[1] The lipids in muscle,[2,3] liver[2,4] and serum[5,6] are known to decrease markedly during the spawning migration and the depletion of lipid is probably associated with the energy utilization for migration and gonadal maturation.[2,3]

It has been understood that the liver plays an important role in the lipid metabolism of fish as well as mammals,[7] however, there are very few reports which describe the lipid metabolism in the liver of Pacific salmon. The biosynthetic ability of triglyceride has been lost in the liver of post-spawning pink salmon.[8] The hepatic enzyme activities on lipogenesis such as fatty acid synthetase (EC 2.3.1.38), glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.43) have markedly decreased during the starvation of coho salmon.[9–11] Since these studies are limited to the lipid biosynthesis in the liver, both biosynthetic and catabolic approaches are considered to be necessary to obtain a better understanding of the lipid metabolism in the liver of Pacific salmon during the spawning migration.

The aim of the present study was to investigate the lipid biosynthetic ability in the liver using [1-$^{14}$C] sodium acetate and [1-$^{14}$C] oleic acid as precursors and the *in vitro* hepatic lipase activity during the spawning migration of chum salmon.

## Materials and Methods

*Fish*

A list of chum salmon *Oncorhynchus keta* specimens collected at two different migratory stages is given in Table 1. Each of five male and female fish was used for the following analysis.

*Hepatic Lipase Assay*

Livers were excised from living fish. The liver was thoroughly washed with 0.9% NaCl solution and homogenized in 4 volumes of ice-cold 30 mM phosphate buffer (pH 7.4). The homogenate was centrifuged at 12,000×*g* for 20 min, and the resulting supernatant used for the hepatic lipase activity.[12]

The reaction mixture for the hepatic lipase activity consisted of 0.9 m*l* of 30 mM phosphate buffer (pH 7.4) containing 5% bovine serum albumin, 0.1 m*l* of Intralipos (Green Cross Corporation) as substrate, and 1.0 m*l* of crude enzyme solution. The mixture was incubated at 37°C for 1 h. Blanks were incubated in the absence of Intralipos. The free fatty acid liberated was assayed using NEFA-Test *Wako* (Wako Pure Chemical Industries).

*Incubation Conditions for Hepatic Biosynthetic Activity*

[1-$^{14}$C] Sodium acetate (New England Nuclear Co., Ltd.) was used as the precursor of lipid biosynthesis. The liver was thinly sliced (0.2–

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**Table 1.** Characteristics of chum salmon specimens

| Stage | Date and locality of collection | Sex | Age | Fork length (cm) | Body weight (g) | GSI*1 | HSI*2 |
|---|---|---|---|---|---|---|---|
| Pre-spawning | Sept. 30-Oct. 13, 1987 | Male | 3 | 65.2±5.1 | 3040±690 | 4.97±1.19 | 1.28±0.14 |
| | Moheji coast of Hakodate Bay, Hokkaido | Female | 3 | 64.9±2.8 | 2970±430 | 16.15±2.53 | 2.37±0.28 |
| Spawning | Nov. 10–17, 1987 | Male | 2–4 | 74.2±4.8 | 4470±1100 | 4.77±1.19 | 1.66±0.15 |
| | Lower reaches (0.2 km) of Moheji River, Hokkaido | Female | 3–4 | 67.9±3.3 | 4080*3±290 | 21.33*3±3.92 | 1.08±0.09 |

*1 Gonadosomatic index: (Gonad weight/Body weight) × 100.
*2 Hepatosomatic index: (Liver weight/Body weight) × 100.
*3 The GSI value and body weight became lower because two of spawning female fish eliminated parts of roes after caught.
Values represent the mean±S.D. of five fish.

0.5 mm), weighed and placed in Erlenmeyer flask with glass-stopper. The reaction mixture contained 20 μmol of sodium acetate (0.18 μCi) and 125 μmol glucose in 4.5 m*l* of Krebs-Ringer-bicarbonate buffer (pH 7.4). The reaction was run at 30°C for 2 h.

The liver enzyme for *in vitro* lipid biosynthesis was prepared by the method of Iijima *et al.*[13] The liver was homogenized in 4 volumes of ice-cold 10 mM Tris-maleate buffer (pH 7.0) containing 0.278 M mannitol. The crude homogenate was centrifuged at 1,200×*g* for 10 min to remove cell debris. The supernatant was recentrifuged at 5,900×*g* for 10 min, and the resulting supernatant was used as the liver enzyme preparation.

[1-$^{14}$C] Oleic acid (5 μCi, New England Nuclear Co., Ltd.) was diluted with 61 μmol of cold oleic acid, and warmed at 40°C for 5 min in the presence of 61 μ*l* of 1 N KOH solution and 39 μ*l* of 0.9% NaCl solution. Then, the obtained [1-$^{14}$C] potassium oleate was mixed thoroughly with 39 volumes of 2.5% bovine serum albumin (0.9% NaCl solution), and the specific activity of albumin complex became 0.125 μCi/100 μ*l* of the resulting solution.

Albumin complex of [1-$^{14}$C] oleic acid was used as the precursor of *in vitro* lipid biosynthesis. The reaction mixture contained 1.5 μmol of oleic acid-albumin complex (0.125 μCi), 0.5 μmol of Co-enzyme A, 30 μmol of ATP, 25 μmol of $MgCl_2$, 25 μmol of glutathione and a suitable amount of the crude enzyme solution in 2.5 m*l* of 10 mM Tris-maleate buffer (pH 7.0). The reaction was run at 37°C for 1 h.

*Analytical Procedure for Lipid Biosynthetic Activity*

After incubation with [1-$^{14}$C] sodium acetate or [1-$^{14}$C] oleic acid, the reaction was stopped by the addition of 5 m*l* of chloroform-methanol (2: 1, by volume) containing 0.01% 2, 6-di-*tert*-butyl-*p*-cresol. The lipids were extracted from the incubated mixtures according to the method of Bligh and Dyer.[14] The extracted lipids were dissolved in 220 μ*l* of chloroform and developed on the preparative TLC plate (Kieselgel 60, ready made plate from Merck). As the internal standards monoglyceride, diglyceride, triglyceride, free fatty acid, cholesterol, phosphatidylcholine and phosphatidylethanolamine were used. The TLC plate was developed with *n*-hexane-diethyl ether-acetic acid (70: 30: 1, by volume). After developing, the TLC plate was sprayed with 0.005% Rhodamine 6G in ethanol and then viewed under ultraviolet light. The separated bands were scraped and transferred directly into the scintillation vials and suspended in 10 m*l* of ACS II (Aqueous Counting Scintillant, Amersham Corporation) containing CAB-O-Sil (Thixotropic Gel Powder, Packard) for radioactive assay.

*Other Analyses*

The lipid classes of muscle and liver were analyzed as previously reported.[8] Blood was collected from the caudal vasculature of live salmon, and left at room temperature for several hours. The clotted blood was centrifuged to obtain the serum. The levels of triglyceride, total cholesterol and phospholipid in the serum were measured using the enzyme kits (Wako Pure Chemical Industries).

*Statistics*

Data were analyzed using one way analysis of variance, followed by Student's *t* test.

## Results and Discussion

*Changes of Lipid Classes in Muscle, Liver, and Serum*

The total lipid in the muscle of male salmon decreased during the spawning migration, particularly in triglyceride ($P<0.05$), whereas phospholipid and free fatty acid were maintained at a constant level. The increase of free fatty acid ($P<0.01$) and the decrease of triglyceride ($P<0.05$) were found in the liver of male salmon during the spawning migration (Table 2). Similar results were obtained from the muscle and liver of female salmon. The levels of phospholipid, triglyceride and total cholesterol in the serum of male decreased markedly during the spawning migration ($P<0.05$), whereas almost the same levels of lipid classes were found in the serum of female (Table 3). The serum lipid levels of pink salmon[5] and chum salmon[6] have been known to decrease markedly during the spawning migration irrespective of male and female fish. The captured station of spawning salmon, only 200 m uptsream from the mouth of the Moheji River (Table 1), might relate to the sexual differences of the serum lipid contents.

*Lipase Activity in the Liver*

The levels of lipase activity in the liver increased markedly during the spawning migration (Fig. 1). This was consistent with the result of Table 2 that the increase of free fatty acid and the decrease of triglyceride were found in the liver of spawning salmon. The high levels of lipase activity might be responsible for the increase of free fatty acid in the liver.

*Lipid Biosynthetic Activity in the Liver*

The incorporation of [1-$^{14}$C] sodium acetate into the lipid classes was examined using liver slices

**Table 2.** Changes in lipid classes of muscle and liver during spawning migration of chum salmon

| | | Lipid content (g/100 g tissue) | | | | | |
|---|---|---|---|---|---|---|---|
| | | PL | PG | S | FFA | TG | SE |
| Muscle | | | | | | | |
| Male | Pre-spawning | 0.34±0.14 | 0.27±0.21 | 0.22±0.09 | 0.82±0.28 | 3.45±1.08 | 0.07±0.06 |
| | Spawning | 0.22±0.03 | 0.17±0.05 | 0.18±0.03 | 0.62±0.06 | 1.43±0.60[b] | 0.04±0.02 |
| Female | Pre-spawning | 0.25±0.04 | 0.16±0.07 | 0.15±0.03 | 0.64±0.08 | 2.31±0.60 | 0.03±0.03 |
| | Spawning | 0.22±0.04 | 0.14±0.04 | 0.16±0.03 | 0.70±0.13 | 0.79±0.13[b] | 0.04±0.02 |
| Liver | | | | | | | |
| Male | Pre-spawning | 0.74±0.17 | 0.19±0.05 | 0.46±0.07 | 0.35±0.12 | 0.78±0.28 | 0.34±0.27 |
| | Spawning | 0.99±0.13 | 0.18±0.17 | 0.53±0.11 | 1.44±0.28[a] | 0.10±0.11[b] | 0.20±0.10 |
| Female | Pre-spawning | 1.09±0.27 | 0.12±0.03 | 0.51±0.15 | 0.35±0.06 | 1.04±0.42 | 0.14±0.11 |
| | Spawning | 0.58±0.07 | 0.05±0.01 | 0.43±0.08 | 1.55±0.02[a] | 0.03±0.02[a] | 0.22±0.16 |

Values represent the mean±S.D. of five fish. Abbreviations: PL, phospholipid; PG, partial glyceride; S, sterol; FFA, free fatty acid; TG, triglyceride; SE, sterol ester.
[a,b] These values are significantly different from those of pre-spawning salmon, $P<0.01$, $P<0.05$, respectively.

**Table 3.** Changes in serum lipid classes during spawning migration of chum salmon

| | | Lipid content (g/100 m*l* serum) | | |
|---|---|---|---|---|
| | | PL | TG | TC |
| Male | Pre-spawning | 1.75±0.33 | 0.38±0.11 | 0.48±0.10 |
| | Spawning | 0.76±0.52[a] | 0.18±0.11[b] | 0.29±0.14[b] |
| Female | Pre-spawning | 1.38±0.25 | 0.51±0.16 | 0.27±0.04 |
| | Spawning | 1.30±0.16 | 0.41±0.01 | 0.32±0.07 |

Values represent the mean±S.D. of five fish. Abbreviations except TC (total cholesterol) are referred to the legend in Table 2.
[a,b] These values are significantly different from those of pre-spawning salmon, $P<0.01$, $P<0.05$, respectively.

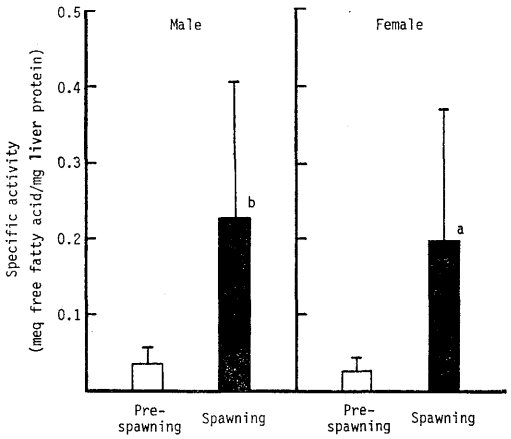


**Fig. 1.** Changes in liver lipase activities during spawning migration of chum salmon. The vertical bars represent the mean±S.D. of five fish. [a,b]These values are significantly different from those of pre-spawning salmon, $P<0.01$, $P<0.05$, respectively.

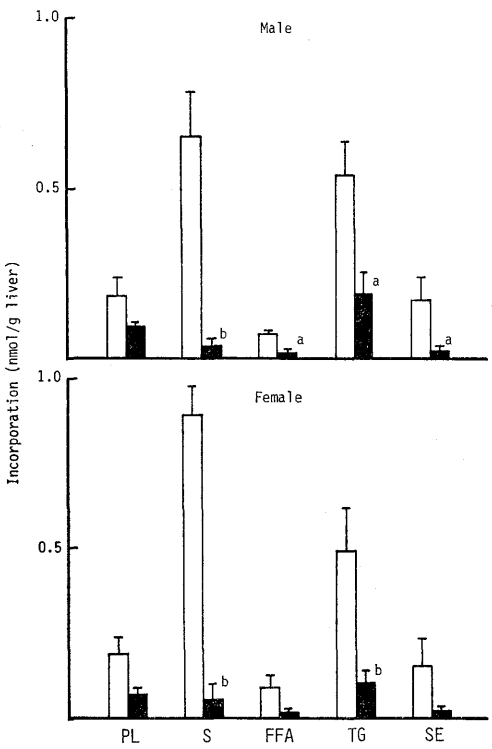


**Fig. 2.** Incorporation of [1-$^{14}$C] sodium acetate into lipid classes of pre-spawning (□) and spawning (■) chum salmon by liver slices. The vertical bars represent the mean±S.D. of five fish. Abbreviations: PL, phospholipid; S, sterol; FFA, free fatty acid; TG, triglyceride; SE, sterol ester. [a,b]These values are significantly different from those of pre-spawning salmon, $P<0.01$, $P<0.05$, respectively.

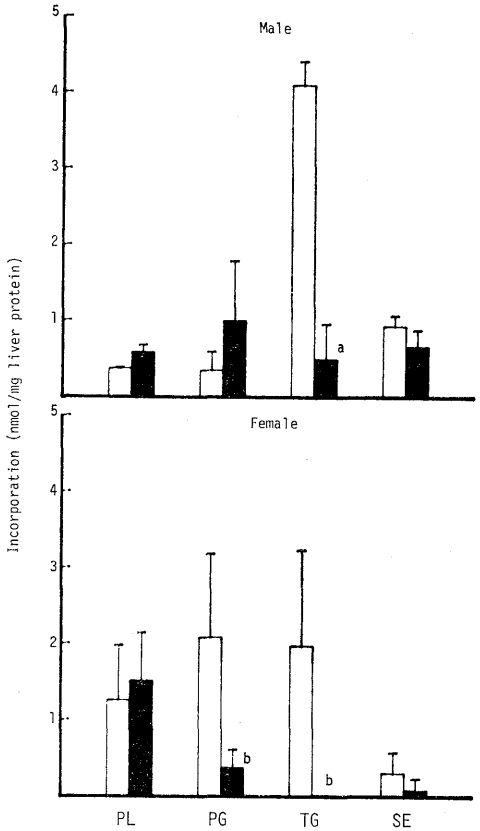


**Fig. 3.** Incorporation of [1-$^{14}$C] oleic acid into lipid classes of pre-spawning (□) and spawning (■) chum salmon by liver enzyme preparation. The vertical bars represent the mean±S.D. of five fish. Abbreviations except PG (partial glyceride) are referred to the legend in Fig. 2. [a,b]These values are significantly different from those of pre-spawning salmon, $P<0.01$, $P<0.05$, respectively.

(Fig. 2). The incorporation of [1-$^{14}$C] sodium acetate into the lipid classes of liver decreased markedly during the spawning migration, suggesting the drop of lipid biosynthesis in the liver. [1-$^{14}$C] Sodium acetate was incorporated into sterol and triglyceride fractions of pre-spawning salmon, in particular, a pronounced incorporation was found in sterol fraction of female. Acetic acid is a precursor of steroid biosyntheses.[15] The biosyntheses of steroids as well as triglyceride were very active in the liver of pre-spawning salmon. High levels of such steroid hormones as testosterone, 11-keto-testosterone and estradiol-17$\beta$ have been found in the sera of pre-spawning chum salmon.[16,17] The high levels of sterol biosynthetic activity found in the liver might relate to the increase of steroid hormone productions in the

endocrine organs of pre-spawning chum salmon. However, the incorporations of acetate into the lipid classes including sterol and triglyceride decreased significantly in the liver of spawning salmon ($P<0.05$). The loss of the biosynthetic ability of triglyceride has been similarly found in the liver of post-spawning pink salmon.[8]

The incorporation of [1-$^{14}$C] oleic acid into the lipid classes was examined using the liver enzyme preparation (Fig. 3). Iijima *et al.*[13] presumed the metabolic pathways of triglyceride and phospholipid biosyntheses *via* intermediates of phosphatidic acid and diglyceride in the intestine of carp. A similar biosynthetic pathway was considered in the liver of salmon, since [1-$^{14}$C] oleic acid was incorporated into phospholipid, partial glyceride, triglyceride and sterol ester fractions. The incorporation of [1-$^{14}$C] oleic acid into triglyceride decreased markedly during the spawning migration ($P<0.05$), whereas that into phospholipid was almost the same level. This suggests that the mobilization of triglyceride but not of phospholipid was regulated in the liver of spawning salmon.

The supply of external lipids from diets is also considered to be restricted in the liver of spawning salmon, since salmon are in a state of fasting during spawning migration. The lipid accumulated in the muscle was consequently presumed to be utilized as the energy source for migration and gonadal maturation, because of low lipid biosynthetic ability and high lipase activity in the liver of fasting salmon. This might be responsible for the depletion of lipid in the muscle of spawning chum salmon.

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