Bull. Fac. Fish. Hokkaido Univ.
37(3), 246-251. 1986.

Influence of 17α-Methyltestosterone on the Level and Composition of Lipid in Adult Chum Salmon Muscle

Seiichi ANDO*, Fumio YAMAZAKI** and Mutsuo HATANO*

Abstract

A chum salmon was treated with 17α-methyltestosterone (MT) to clarify the role of muscle lipid in gonadal development. MT caused a marked decrease in total lipid content of muscle (in particular, the triglyceride fraction), while the phospholipid fraction was maintained at a constant level. MT treatment induced a relative increase in polyenoic fatty acids to total lipid. Increased gonadal growth was found in the MT-treated salmon. These results suggest that muscle lipid (in particular, the triglyceride fraction) was utilized as an energy source for the development of the gonads of chum salmon.

Introduction

Chum salmon spend the majority of their life in the ocean where they appear in the North Pacific Ocean at certain times of the year. At the end of their life cycle they enter their natal rivers for spawning and later die there. This biological cycle governs the patterns of change in their chemical composition. Pentegoff *et al.* (1928) reported that as the fish move from the estuary to the spawning grounds, the moisture content in the muscle increases and the lipid and protein contents decrease. They have not discussed in detail, however, the physiological and biochemical role of muscle lipid and protein during the spawning migration of chum salmon. Recently, it has been suggested that muscle lipid is a primary energy source utilized in gonadal development and muscle protein is utilized as a secondary energy source for upstream migration (Ando *et al.*, 1985a). Also, it has been demonstrated that the changes in muscle proximate composition during the spawning migration of chum salmon are closely related to the blood androgen levels (Ando *et al.*, 1985a, 1986a, b).

To clarify the role of muscle lipid as a source of energy for gonadal maturation as opposed to an energy source for ascending the river, a chum salmon was treated with 17α-methyltestosterone and subsequent changes in muscle lipid content and composition were observed.

* Laboratory of Food Chemistry I, Faculty of Fisheries, Hokkaido University (北海道大学水産学部食品化学第一講座)
** Laboratory of Embryology and Genetics, Faculty of Fisheries, Hokkaido University (北海道大学水産学部発生学遺伝学講座)

Materials and Methods

Sample

Injection of 17α-methyltestosterone (MT) into chum salmon (*Oncorhynchus keta*) was accomplished as follows: Immediately after capture in the North Pacific Ocean (lat. 47°30′N, long. 170°01′E) on July 17, 1983, the fish (fork length 54 cm, body weight 1,460 g) was injected intraperitoneally with MT (1.5 mg) dissolved in olive oil. The fish was then transferred to a 700 liter aquarium supplied with running seawater where it was retained unfed for 5 days on board the training ship Hokusei Maru, of the Faculty of Fisheries, Hokkaido University. The control fish (fork length 51 cm, body weight 1,480 g) received an injection of olive oil alone. These samples were kept at −20°C until use. Dorsal muscle was collected for the following analysis.

Lipid analysis

Lipid extraction from the dorsal muscle was carried out using the Bligh and Dyer method (1959).

The total lipid was analyzed quantitatively by thin-layer chromatography (TLC). The TLC plates (Kieselgel 60, ready-made plate from Merck) were developed using *n*-hexane/diethyl ether/acetic acid (85 : 15 : 1, v/v/v) for the non-phospholipid fraction. The TLC plate was then sprayed with a 3% copper acetate-8% phosphoric acid solution, heated on a hot plate, and quantitated using an Ozumor 82 densitometer. The amount of phospholipid was calculated from lipid-phosphorus assayed using the Fiske and Subbarow method (1925).

A portion of the extracted lipid was also applied to preparative TLC (Wakogel B-0). The plate was sprayed with a Rhodamine 6G reagent, and non-phospholipid and phospholipid were detected under ultraviolet light. The fatty acid composition of non-phospholipid and phospholipid fractions was analyzed by gas-liquid chromatography (GLC) after methylation with methanol containing anhydrous hydrogen chloride (Prevot and Mordret, 1976). GLC analyses were performed with a Hitachi 063 gas chromatograph equipped with a hydrogen flame ionization detector and by using a glass column (3 mm i.d. and 3 m length) packed with Unisol 3000 on a Uniport C (80-100 mesh).

Results

Changes in muscle lipid

Table 1 shows the effect of MT on muscle lipid content. MT caused a marked decrease in the total lipid content of muscle from a level of 4.29% in the control fish to 0.94% in the treated fish. The decline in muscle lipid content following treatment with MT was mainly due to a decrease of the triglyceride fraction from 3.31% to 0.45%. The phospholipid fraction was maintained at a constant level.

Changes in fatty acid composition of muscle lipid

Following treatment with MT (Table 2), the relative ratio of saturated fatty acids to total lipid markedly decreased while that of polyenoic fatty acids markedly

Table 1. Effect of 17α-methyltestosterone (MT) on lipid content of chum salmon muscle.

Lipid class	Lipid contents (g/100 g muscle)	
	Control	MT
Phospholipid	0.35 (8.2)	0.26 (27.6)
Partial glyceride	0.31 (7.2)	0.02 (2.1)
Sterol	0.25 (5.8)	0.04 (4.2)
Free fatty acid	0.07 (1.6)	0.17 (18.1)
Triglyceride	3.31 (77.2)	0.45 (47.9)
Total lipid	4.29 (100)	0.94 (100)

Values in parentheses represent percentages, with total lipid being 100%.

Table 2. Fatty acid compositions of total lipid, non-phospholipid, and phospholipid in chum salmon muscle (weight %).

Fatty acids	Total lipid		Non-phospholipid		Phospholipid	
	Control	MT	Control	MT	Control	MT
14 : 0	8.2	6.7	7.0	9.7	2.3	1.7
15 : 0	1.4	1.6	0.5	Tr	0.4	0.4
16 : 0	19.4	11.6	17.5	12.2	26.0	21.6
17 : 0	1.5	2.2	1.2	1.6	1.1	1.0
18 : 0	3.7	4.8	3.4	3.1	4.7	5.7
20 : 0	1.3	2.5	1.2	2.9	0.4	0.2
22 : 0	0.7	1.0	0.8	1.9	0.5	0.4
Saturated	36.2	30.4	31.6	31.4	35.4	31.0
16 : 1	7.5	5.3	7.4	5.8	1.2	0.5
17 : 1	0.8	2.8	0.8	Tr	0.1	0.2
18 : 1	23.8	22.2	25.5	23.9	10.5	9.0
19 : 1	1.0	2.1	1.0	1.8	0.3	0.3
20 : 1	5.2	7.9	6.1	11.1	2.0	2.0
22 : 1	5.2	5.1	7.3	5.1	Tr	Tr
Monoenoic	43.5	45.4	48.1	47.7	14.1	12.0
18 : 2	2.0	2.5	1.2	2.4	0.4	0.3
20 : 4	0.4	0.3	0.5	Tr	0.9	0.9
20 : 5	6.8	4.4	7.1	5.3	7.8	6.2
22 : 5	0.8	1.0	1.0	1.9	1.9	2.1
22 : 6	10.3	16.0	10.5	11.3	39.5	47.5
Polyenoic	20.3	24.2	20.3	20.9	50.5	57.0

increased. Similar changes in fatty acid composition were observed in phospholipid fraction, however, no significant differences were found in the fatty acid composition of non-phospholipid fraction following treatment with MT. It was observed that MT induced a relative increase in polyenoic fatty acids to total lipid.

Changes in gonad

A marked increase in gonadosomatic index value was found in MT-treated fish. The gonadosomatic index value of control fish was 1.2 and that of MT-treated fish was 10.3. The mean oocyte size of control fish was 1.40 mm in diameter while that of MT-treated fish was 3.85 mm in diameter (Fig. 1). Thus, it appeared that the administration of MT stimulated gonadal growth.

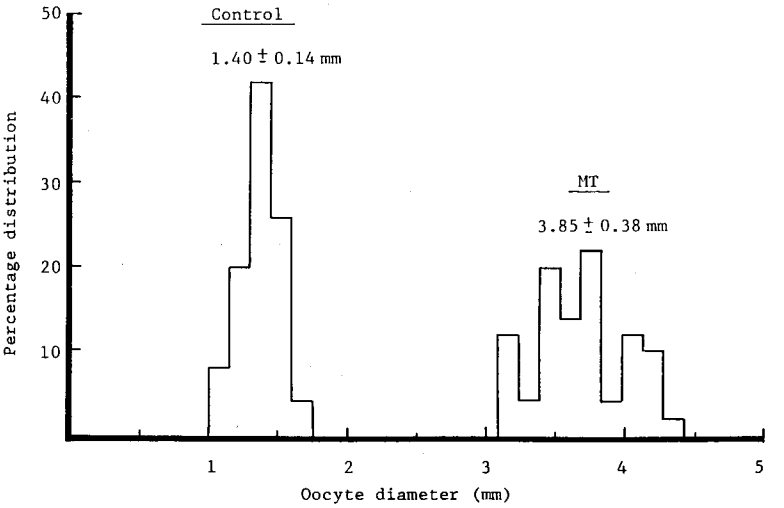

Fig. 1. Effect of 17α-methyltestosterone (MT) on oocyte diameter of chum salmon ovary.

Discussion

In a previous paper (Ando *et al.*, 1985a), changes in proximate composition of chum salmon muscle during spawning migration were reported and a relationship between muscle composition and physiological state was suggested. Total lipid content in muscle, particularly the triglyceride fraction, markedly decreased while the phospholipid fraction was maintained at a constant level during spawning migration. A close correlation between gonadal maturation and changes in muscle triglyceride content was suggested by the marked increase in gonadosomatic index values during spawning migration of chum salmon. Also, the relative ratio of polyenoic fatty acids to total lipid gradually increased during spawning migration (Table 3, Ando *et al.*, 1985b).

In the present study, a chum salmon was treated with MT, a synthetic androgen, in an attempt to clarify the role of muscle lipid in gonadal maturation because previous studies have suggested that the level of androgen, but not estrogen, was closely related to the changes in muscle composition during spawning migration (Ando *et al.*, 1985a, 1986a, b). The results of the present study clearly show that MT treatment caused a marked decrease in total lipid of muscle (in particular, the triglyceride fraction, Table 1) and an increase in the relative ratio of polyenoic fatty

Table 3. Fatty acid composition of total lipid in chum salmon muscle during spawning migration (weight %)*.

Fatty acids	Stage	
	Feeding migration	Spawning migration
14 : 0	8.3	4.5
15 : 0	1.6	1.3
16 : 0	15.7	12.3
17 : 0	1.6	2.9
18 : 0	3.3	4.8
20 : 0	2.7	1.4
22 : 0	1.3	0.6
Saturated	34.5	27.8
16 : 1	6.4	5.5
17 : 1	1.0	Tr
18 : 1	21.0	24.3
19 : 1	1.5	1.3
20 : 1	8.1	8.1
22 : 1	7.7	4.5
Monoenoic	45.7	43.7
18 : 2	2.3	2.7
20 : 4	0.4	0.7
20 : 5	5.3	3.2
22 : 5	0.7	2.0
22 : 6	11.1	19.9
Polyenoic	19.8	28.5

* Data from Ando *et al.* (1985b).

acids to total lipid (Table 2). MT administration was assumed to have induced the gonadal growth of chum salmon because of a marked increase in the gonadosomatic index values and the mean oocyte size (Fig. 1). The foregoing changes found in the chum salmon were caused by MT directly or indirectly, but not by individual differences in the fish themselves, since the mean GSI value of 25 fish (which were captured at the same time and location as those used in the present study) was less than 1 (Data Rec. Oceanogr. Obs. Expl. Fish., 1984).

The biosynthesis of estradiol-17β and 11-ketotestosterone from testosterone has been demonstrated in teleost gonads (Nagahama, 1982). The role of MT in muscle lipid mobilization and gonadal maturation was suggested by the results of the present study, although it is unknown whether MT itself or metabolites such as estradiol-17β and 11-ketotestosterone affected the muscle lipid and gonadal growth of chum salmon.

The possibility of the transfer of muscle lipid to the gonads has been suggested for sockeye salmon (Idler and Bitners, 1960) and rainbow trout (Takashima *et al.*, 1971). Our results suggest that muscle lipid (in particular, the triglyceride fraction) was utilized as an energy source in the development of the gonads of chum salmon.

Acknowledgments

The authors wish to thank Captain S. YAMAMOTO, Chief Officer K. ISHII, and the other crews of training ship Hokusei Maru for their kind help in obtaining samples.

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