Bull. Fac. Fish. Hokkaido Univ.
28(4), 193–201. 1977.

Lipid Metabolism in Fish

II. Changes of lipids and fatty acids in the liver of puffer, *Fugu vermiculare porphyreum*, during starvation

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Abstract

The puffer, *Fugu vermiculare porphyreum*, was subjected to starvation, and the changes of lipid and fatty acid contents were studied.

During the early period of starvation, the tendency of the total lipid contents in the liver to fluctuate was analogous to fluctuations of the ratio of liver to body weight. It was mainly due to the variations of the triglyceride contents in the liver. Iodine and acid values of non-polar lipids fluctuated in the early period. After prolonged starvation, the total lipids and non-polar lipids were decreased significantly when compared with those of the non-starved fish.

In the percentage composition, monoenoic acids of the non-polar lipids tended to increase in general, but saturated and polyeonic acids decreased during the early period of starvation. The fatty acid contents of non-polar lipids or triglycerides fluctuated during the early period. All of the acids decreased remarkably after prolonged starvation. At early stages without feed, saturated and monoenoic acid contents of non-polar lipids or triglcerides tended to reduce slightly prior to a decrease of polyenoic acids. This suggests that polyenoic acids were utillzed by the fish approaching starvation.

Introduction

It is well known that marine fish lipids are characterized by relatively high levels of polyenoic acids. The preponderance of polyenoic acids in marine organisms might reflect an adaptation to a condition of north temperate marine life, *i.e.*, exposure to relatively low and constant temperature[1]. However, the authors have observed that the fatty acid composition of the non-polar lipids in the liver of a number of deep-sea fish differed from those of the epipelagic fish with relatively higher amounts of monoenoic acids[2–4]. The reasons for this phenomenon's appearing in the deep-sea species were discussed in previous papers[2–4]. Another reason might be that the relatively impoverished environment for the deep-sea species would subject them to chronic starvation. It might be inferred that the fatty acid metabolism in deep-sea species was affected by starvation.

In the case of starvation, the diminution of the stored lipids as an energy sources was observed in marine[5,6] and freshwater[7–9] fish. The utilization of the energy released by oxidation of fatty acids in fish is most evident[10]. Kaneko *et al.*[11] have observed that the proportion of polyenoic acids in the muscle of rainbow trout, *Salmo gairdnerii*, increased during starvation, presumably

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indicating utilization of the saturated acids. The authors have also observed that the flesh of low lipid contents during the season of harvest for sardine, *Sardinops melanosticta*, consisted of high levels of polyenoic acids, and suggested that more saturated acids were preferentially utilized[12]. However, the selectivity in utilizing fatty acids of lipids by fish is not yet well understood.

In this study, an attempt was made to observe the changes in the fatty acids in the liver of a puffer, *Fugu vermiculare porphyreum*, subjected to various levels of starvation.

Materials

The puffer used in this study were collected from a set net located at Kamiiso near Hakodate, Japan, in 1970 and 1972. The experimental animals were placed in a plastic bucket (25-liter capacity) with an air pump and immediately transported to the laboratory.

The fish for each experiment were kept alive without food in two retangular glass aquaria (60×30×36 cm, 50-liter capacity) prepared with commercial artificial seawater. The seawater was continuously filtered through a sand and gravel bed, and aerated with unglazed porcelain diffusers. Temperature of the seawater was kept at ambient temperature by passing water from a water cooler (Coolnics-circulator, Komatsu-Yamato Co.,) through a glass spiral tube fixed at the bottom of the tank.

Non-starved fish (control) were frozen with dry-ice immediately after collection. The period of starvation, number of fish, and measurements of the examined fish are given in Table 1. Each pooled liver of control and starved fish was used for the extraction of lipids.

Table 1. *Biological measurements and the characteristics*

Experiment*1	Days*2 starved	Fish no. examined	Body length cm*3	Body weight g*3	Liver wt/Body wt %*3
I	0	12	7.4	7.6	6.1
	1	12	7.2	6.6	6.3
	3	12	7.2	6.2	5.8
	5	12	7.4	7.0	7.4
	7	12	7.2	6.5	5.1
	9	12	7.4	6.4	7.1
II	0	5	13.7	54.5	6.6
	1	4	14.4	52.2	7.8
	3	4	14.2	51.3	9.5
	5	4	13.0	39.2	6.9
III	0	12	11.9	35.8	7.0
	17	5	12.8	31.2	2.7

*1 Fish were collected at Kamiiso near Hakodate, Dec. '72 for experiments I and II, and June '70 for experiment III; temperature of the sea surface *in situ* were 9.0°C for the former and 20.8°C for the latter.

*2 Zero shows the non-starved fish as control. *3 Mean value

Experimental Methods

Total lipids in the liver were extracted by the method of Bligh and Dyer[13]. Non-polar lipids were separated from polar lipids in a column packed with a silicic acid (Mallinckrodt Co.,)-Celite (2:1, w/w) activated at 110°C for 5 hrs. Non-polar lipids were eluted by passing chloroform through the column. The iodine and acid values of non-polar lipids were determined by the usual way. Triglycerides in non-polar lipids of experiment II were separated in a column packed with a silicic acid activated at 110°C for 5 hrs, using 10% diethyl ether-hexane after 4% diethyl ether-hexane as the solvent for development. It was ascertained that the triglyceride fraction had fewer contaminating components by analysis with thin-layer chromatography (TLC). The technique used a thin layer of 0.25 mm silicic acid (Wako gel B-5) activated at 110°C for 60 min; a developing solvent: hexane, diethyl ether, acetic acid (90:10:1, v/v); and a reagent: 15% phosphomolybdic acid in ethanol. Triglyceride contents of non-polar lipids for experiments I and II were determined with a densitometer (Yamato-Asuka Ozumor 82) by calculating the spot areas of the copying-paper transcribed from the chromatogram after TLC. The developing solvent was benzene, and other analytical conditions were the same as previously described.

The fatty acid compositions of non-polar lipids and triglycerides were determined with a Yanagimoto gas chromatograph (model G8 or GCG-5DH) equipped with a dual hydrogen flame detector. The column was 1.5 m×3 mm i.d., U-shaped of stainless steel, and packed with 10% diethyleneglycol-succinate on Chromosorb W AW, 80/100 mesh (Gaschro Kogyo Co.,). The column and detector temperature were 190°C and 240°C, and flow rates of nitrogen, hydrogen and air were 0.7 kg/cm^2, 15 ml/min and 600 ml/min, respectively. The fatty acid methyl esters were prepared by boron trifluoride-methanol[14] and were identified

of the lipids in the liver of puffer during starvation.

Total lipid %*4	Non-polar lipid %*4	Iodine value	Acid value	Triglyceride*5 %*4
35.8	35.1	146.5	8.2	24.2
41.2	40.3	126.8	14.3	28.4
38.4	37.9	137.0	13.0	26.1
46.5	46.2	132.8	9.3	31.1
36.8	35.8	131.8	13.2	24.1
41.6	40.9	130.5	8.0	28.9
39.0	38.2	137.6	8.1	27.5(27.1)
41.7	40.8	120.6	9.3	26.8(26.8)
46.4	45.9	133.5	7.3	31.5(31.3)
39.9	39.3	136.0	9.8	25.8(25.9)
23.0	22.7	189.9	—	—
14.3	7.1	188.4	—	—

*4 % to wet weight of liver tissue

*5 Determined by TLC-densitometer analysis, and the values in parentheses: determined by chromatography of a silicic acid column.

by comparison with commercial standard reagents (14:0, 16:0, 18:0, 16:1, 18:1, 18:2 and 18:3 acid). The identification of other fatty acids was accomplished by a log-plot of retention times against the number of carbons in the chain, and the comparison of equivalent chain length values with those in the literature. The percentage of each fatty acid was calculated by measuring the area of the peaks.

Results

As shown in Table 1, the proportion of liver to body weight in experiments I and II fluctuated repeatedly during the early period of starvation. But, those measures in the prolonged starved fish of experiment III decreased clearly as compared with the control. The livers of the starved fish were small and red as contrasted with the creamy colour of the livers of the controls. The contents of total lipids, non-polar lipids and triglycerides, and iodine and acid values of non-polar lipids, of the liver of the starved and control fish of experiments I, II and III are listed in Table 1. In the second experiment, the contents of triglycerides eluted from the column (Fig. 1) agreed with the results of the TLC-densitometer (Fig. 2). For convenience, triglyceride contents for first experiment were determined by the method of TLC-densitometer.

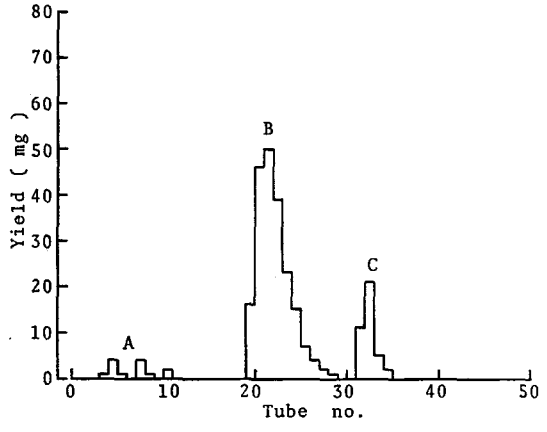

Fig. 1. A typical chromatogram of the non-polar lipids in the liver of puffer.
Column: 1.8 (i.d.)×28.5 cm, 30 g of a silicic acid
Eluent: Fr. 1–15, 4% diethyl ether-hexane (300 ml); Fr. 16–30, 10% diethyl ether-hexane (300 ml); Fr. 31–45, ether (300 ml)
Sample: 310 mg
A: Hydrocarbon and steryl ester B: Triglyceride
C: Fatty acid, sterol and partial glyceride

The puffer characteristically had a high lipid content in the liver (23.0% of wet weight basis) as compared with other tissues (flesh, 0.6% and viscera, 2.5%). It was found that the total lipid content of the liver fluctuated repeatedly during the early period of starvation. The fluctuations corresponded with the proportions of liver to body weight. It is probable that the variations of the liver-body weight

proportion were influenced by the variations in the lipids. The variation of the total lipids was due to the changes in the triglyceride contents, which were the major components of non-polar lipids.

For experiment II, triglyceride contents of the starved fish (starved 5 days) decreased slightly as compared with those of the control. In experiment III, both total lipids and non-polar lipids were decreased significantly, compared with the control. In addition, it was also found that the amount of total lipids and non-polar lipids in experiment III decreased from 0.6% to 0.4% for total lipids and from 47.5% to 40.0% for non-polar lipids in the flesh, and from 2.5% to 2.0% for total lipids and 76.9% to 66.7% for non-polar lipids in the viscera.

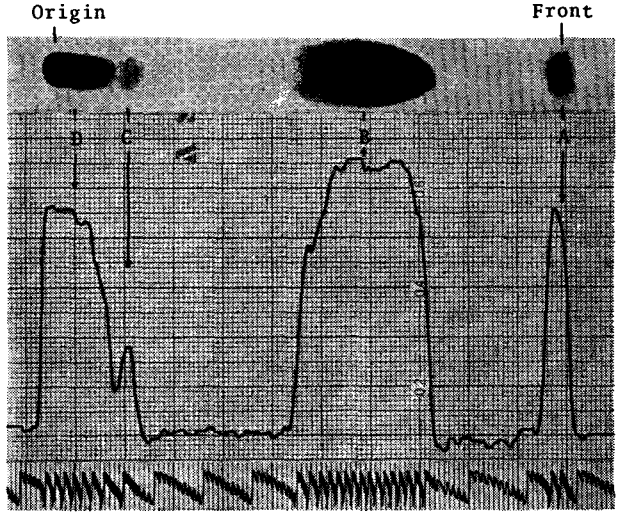

Fig. 2. A typical pattern by TLC-densitometer analysis for non-polar lipids in the liver of puffer (experiments I and II).
A: Hydrocarbon and steryl ester B: Triglyceride C: Sterol
D: Fatty acid and partial glyceride

As shown in Table 1, iodine values of non-polar lipids in the liver during starvation for experiments I and II fluctuated. A decreased iodine value for the starved fish (one day) in experiments I and II was due to a relative increase of saturated acids in non-polar lipids. In the prolonged starvation of experiment III, little difference was observed in iodine values for the liver as compared with the control. However, it was observed that those for the flesh and viscera of the starved fish decreased from 196.0 to 167.9, and from 199.4 to 175.4, respectively, compared with the control. Acid values of non-polar lipids for experiments I and II also fluctuated repeatedly during starvation.

The composition or contents of the major fatty acids of non-polar lipids or triglycerides for experiments I, II and III are given in Tables 2 and 3. For experiment I, the percentages of saturated and polyenoic acids of non-polar lipids showed a tendency to decrease with a trend for monoenoic acids to increase during

Table 2. *Changes in the compositions and contents of major fatty acids of non-polar lipids in the liver of puffer during starvation (experiment I).*

Fatty acid	Days starved											
	0	1	3	5	7	9	0	1	3	5	7	9
	%						Mg*1					
14:0	5.3	5.3	4.9	4.5	5.3	4.6	17	20	18	19	18	18
16:0	27.6	26.8	27.6	26.5	27.1	25.7	90	102	99	117	92	99
18:0	5.9	5.2	5.5	5.2	4.7	4.9	19	20	20	23	16	19
16:1	23.7	22.4	21.3	22.8	24.4	21.2	78	85	76	105	83	82
18:1	20.8	24.4	24.7	24.0	22.8	29.4	68	92	88	106	78	113
20:1	1.9	1.7	1.7	1.6	1.8	1.1	6	6	6	7	6	4
20:5	4.9	4.3	4.7	4.8	3.8	3.2	16	16	17	21	13	12
22:5	2.6	3.2	3.2	2.3	2.7	3.0	9	12	11	10	9	12
22:6	2.6	2.2	3.1	1.2	1.9	2.3	9	8	11	5	7	9
Sat.*2	39.6	38.2	38.6	37.4	38.3	36.2	130	145	138	165	130	140
Mono.*3	47.4	49.6	48.4	50.9	50.0	52.8	155	188	173	224	170	203
Poly.*4	13.0	12.2	13.0	11.7	11.7	11.0	43	46	46	52	40	42

*1 Values in the liver tissues of 1000 mg.
*2 To include the minor components of 15:0 and 17:0 acids.
*3 To include the minor components of 14:1, 15:1, 17:1 and 19:1 acids.
*4 To include the minor components of 18:2, 18:3, 20:4 and 21:5 acids.

starvation. The fatty acid contents of non-polar lipids or triglycerides in 1000 mg liver tissue fluctuated in the starved fish of experiment I. And in experiment II the non-polar lipids and triglycerides behaved in a similar manner. However, during the 17 day period of starvation in experiment III, the amount of each fatty acid, and those of saturated, monoenoic and polyenoic acids of the starved fish decreased strikingly, compared with the control (Table 3). As shown in Table 4, for experiment III the polyenoic acids of the prolonged starved fish decreased, compared with the control, and did so at the same rate as the saturated and monoenoic acids. In the starved fish of experiments I and II, polyenoic acids decreased on 5 or 7 days for non-polar lipids, and after 3 days for triglycerides. Saturated or monoenoic acids of non-polar lipids and triglycerides tended to decrease slightly prior to polyenoic acids in the early period of starvation.

Discussion

It was found that liver weight and the amount of non-polar lipids in the liver decreased markedly during prolonged starvation. This showed that the energy expended during starvation was provided by the lipid reserves, which was similar to other observations in that the lipids in the tissues of fish were reduced during starvation[5-9]. In this study, particularly during the early period of starvation, the liver lipids fluctuated due to variations in triglycerides. The same was also true for the observations on the lipids of starved eels[7], rainbow trout[8], and rats[15]. Increased liver lipids, influenced by starvation, might be due to the

Table 3. *Changes in the contents of major fatty acids of non-polar lipids or triglycerides in the liver of puffer during starvation (experiments II and III).*

Fatty acid	Days starved									
	0	1	3	5	0	1	3	5	0	17
	Non-polar lipid				Triglyceride				Non-polar lipid	
	Mg*1									
14:0	11	12	19	8	12	9	13	7	12	9
16:0	118	124	148	134	88	76	104	61	37	20
18:0	16	21	22	17	14	14	15	13	12	5
16:1	51	50	61	59	36	35	40	44	28	16
18:1	140	130	140	135	82	80	88	93	34	15
20:1	11	14	16	7	8	9	14	4	7	2
20:4	—	—	—	—	—	—	—	—	7	3
20:5	9	8	9	7	6	8	10	6	27	10
22:5	6	6	6	5	4	5	5	6	8	6
22:6	7	11	8	10	7	10	4	9	14	7
Sat.*2	147	160	192	160	118	105	137	85	72	39
Mono.*3	203	197	221	196	130	130	148	146	78	37
Poly.*4	28	32	32	27	23	33	28	28	68	30

*1 Values in the liver tissues of 1000 mg.
*2 To include the minor components of 15:0, 17:0, 19:0 and 20:0 acids.
*3 To include the minor components of 14:1, 17:1, 19:1 and 22:1 acids.
*4 To include the minor components of 18:2, 18:3, 18:4, 20:2 and 21:5 acids.

Table 4. *The variations in the contents**1 *of saturated, monoenoic and polyenoic acids of non-polar lipids or triglycerides in the liver of puffer during starvation (experiments I, II and III).*

Days starved	Experiment I			Experiment II						Experiment III		
	Non-polar lipid			Non-polar lipid			Triglyceride			Non-polar lipid		
	Sat.	Mono.	Poly.	Sat.	Mono.	Poly.	Sat.	Mono.	Poly.	Sat.	Mono.	Poly.
0	100	100	100	100	100	100	100	100	100	100	100	100
1	111	121	107	109	97	114	89	100	143			
3	95	92	100	120	112	100	130	114	85			
5	120	129	113	83	89	84	62	62	100			
7	79	76	77									
9	107	119	105									
17										55	47	46

*1 Values calculated from Tables 2 and 3 where if the left column (L) is 130 and the right column (R) is 145, then calculated L=100 and R=111.

transport of the stored lipids from the peripheral tissues to the liver and/or to an increase of the lipids synthesized endogenously. Yamamoto[16] studied the morphology of liver cell in the starved goldfish, *Carassius auratus*, and suggested

that lipoprotein containing triglycerides were transproted as a component of mobilized lipids *via* blood to the liver. However, it was also observed that triglyceride synthesis in the liver of the fish subjected to prolonged starvation decreased[17].

The oxidation of fatty acids in fish as an energy reserve was reviewed by Mead and Kayama[18]. However, they gave little information on the selective utilization of fatty acids by fish. In this study, it was found that the fish starved for a long period of time reduce polyenoic acids at the same rate as saturated and monoenoic acids. However, in the early period of starvation, monoenoic or saturated acids were reduced slightly prior to polyenoic acids.

In addition, the authors examined the non-polar lipids of the flesh of sardine, *Engraulis japonica*, 90 min after capture (30 min transport and 60 min in an aquarium) and found a relatively high iodine value. The amount of saturated and monoenoic acids were reduced 22% and 8%, respectively, compared with the control; while polyenoic acids were unchanged (unpublished data). It is, thus, inferred that polyenoic acids were utilized by fish in the early stages of starvation, after about 3 days to about a week without feeding. Kaneko *et al.*[11] reported that the proportion of polyenoic acids in the muscle of rainbow trout increased during starvation, presumably indicating utilization of the saturated acids instead. The authors[12] have also observed that polyenoic acids in the flesh of lower contents during the season of harvest for sardine, *Sardinops melanosticta*, were at a high level, suggesting that more saturated acids were preferentially utilized. These observations show that in utilization of fatty acids as a source of energy by fish, polyenoic acids have a peculiar biological role; those are utilized during a stress condition such as prolonged starvation.

The authors have determined the fatty acid composition of non-polar lipids in the liver of a number of deep-sea fish and found higher concentrations of monoenoic acids than in epipelagic fish[2–4]. It is probable that one reason for this phenomenon might be a result of shifts in the fatty acid metabolism of deep-sea fish as they are affected by chronic starvation caused by low and erratic food suplies that are characteristic of deep-sea areas. In this study, during first stages of starvation monoenoic acids had a tendence to increase while saturated and polyenoic acids tended to decrease. This was also true in the liver of a starved rat[19] which also had an increase in monoenoic acids. A detailed investigation of the changes of the fatty acids of fish under a chronic starving condition is necessary to resolve the question.

References

1) Sargent, J.R. (1976). The structure, metabolism and function of lipids in marine organisms. *In* Biochemical and biophysical perspectives in marine biology. Vol. 3, pp. 149–212, Academic Press, London.

2) Hayashi, K. and Yamada, M. (1975). The lipids of marine animals from various habitat depths-II. On the fatty acid compositions of the netural lipids in six species of Gadiformes. *Bull. Jap. Soc. Sci. Fish.* **41**, 1153–1160. (In Japanese with English abstract).

3) Hayashi, K. and Yamada, M. (1975). *Ditto*-III. On the characteristics of the component fatty acids of the neutral lipids in deep-sea fishes. *Ibid.* **41**, 1161–1175.

(In Japanese with English abstract).

4) Hayashi, K. and Yamada, M. (1975). *Ditto*-IV. On the fatty acid composition of the neutral lipids in nine species of flatfishes. *Bull. Fac. Fish. Hokkaido Univ.* **26**, 265–276.

5) Love, R.M. (1958). Studies on the North Sea cod-III. Effect of starvation. *J. Sci. Food Agric.* **9**, 617–620.

6) Ehrlich, K.F. (1973). Chemical changes during growth and starvation of herring larvae. *The Early Life Hist. Fish, Proc. Int. Symp.* 1973 (Pub. 1974), 301–323.

7) Inui, Y. and Ohshima, Y. (1966). Effect of starvation on metabolism and chemical composition of eels. *Bull. Jap. Soc. Sci. Fish.* **32**, 492–501.

8) Takashima, F., Hibiya, T., Watanabe, T., and Hara, T. (1971). Endocrinological studies on lipid metabolism in rainbow trout-I. Differences in lipid content of plasma, liver and visceral adipose tissues between sexually immature and mature females. *Ibid.* **37**, 307–311.

9) Shimma, Y., Ichimura, H., and Shibata, N. (1976). Effect of starvation on body weight, lipid contents, and plasma constitutents of maturing rainbow trout. *Ibid.* **42**, 83–89. (In Japanese with English abstract).

10) Bilinski, E. (1974). Biochemical aspects of fish swimming. *In* Biochemical and biophysical perspectives in marine biology. Vol. 1, pp. 239–288, Academic Press, London.

11) Kaneko, T., Takeuchi, M., Ishii, S., Higashi, H., and Kikuchi, T. (1966). Effect of dietary lipids on fish under cultivation-IV. Changes of fatty acid composition in flesh lipids of rainbow trout on non-feeding. *Bull. Jap. Soc. Sci. Fish.* **33**, 56–58. (In Japanese with English abstract).

12) Hayashi, K. and Takagi, T. (1977). Seasonal variation in lipids and fatty acids of sardine, *Sardinops melanosticta*. *Bull. Fac. Fish. Hokkaido Univ.* **28**, 83–94.

13) Bligh, E.G. and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, 911–917.

14) Morrison, W.R. and Smith, L.M. (1962). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J. Lipid Res.* **3**, 600–608.

15) Majima, Y. and Kurata, C. (1965). Translation of depot fat in starvation. *Eiyo to Shokuryo* **18**, 374–376. (In Japanese with English abstract).

16) Yamamoto, T. (1964). Some observations on the fine structure of the liver cells in the starved goldfish (*Carassius auratus*), with special reference to the morphology of fat mobilization during starvation to the liver. *Arch. Hist. Jap.* **24**, 335–345.

17) Phleger, C.F. (1971). Liver triglyceride synthesis failure in post-spawning salmon. *Lipids* **6**, 347–349.

18) Mead, J.F. and Kayama, M. (1967). Lipid metabolism in fish. *In* Fish oil; their chemistry, technology, stability, nutritional properties, and uses. pp. 289–299, The Avi Publishing Comp. Inc., Westport, Connecticut.

19) Imai, Y. and Sakagami, T. (1966). *Shishitsu no Seikagaku*. pp. 284, Asakura, Tokyo. (In Japanese).