The Influence of Dietary Fatty Acids and Environmental Temperature on the Fatty Acid Composition of Teleost Fish[1]

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Abstract

Marine and fresh water fish were depleted of tissue unsaturated fatty acids to various degrees and subsequently presented with linoleic and linolenic acids at different dietary levels, at different temperatures, with and without other dietary fat.

Examination of the tissue fatty acids demonstrated that marine and fresh water fish do not differ between themselves or from other classes of animals in the following basic mechanisms of deposition and interconversions of dietary fatty acids:

1) The fish are readily depleted of tissue polyunsaturated fatty acids.
2) Dietary linoleic and linolenic acids are deposited, the former to a greater degree than the latter.
3) At high levels of linoleic or linolenic acids in the diet there is no significant degree of their conversion to the longer chain more highly unsaturated acids typical of marine oils.
4) At low levels of linoleic or linolenic acids in the tissues there is a significant, but slight, conversion to the longer chain acids at low environmental temperatures.
5) The increase in the level of linoleic acid in tissue lipids which accompanies increases in the dietary levels, quickly tapers off above dietary levels of 5%.
6) Temparature differences between 13 and 23C had little or no influence on the deposition or interconversion of polyunsaturated acids.
7) Dietary cottonseed oil, which contains cyclopropene fatty acids, produces an increase in tissue stearic acid in the fundulus.

Introduction

IN PREVIOUS REPORTS from this laboratory on the origin of long chain polyunsaturated fatty acids of marine organisms, it was demonstrated that marine fish do not synthesize these acids *de novo;* rather, they either ingest them preformed or extend linoleic and linolenic acids (1,2,3,4). This communication reports further studies on the conditions conducive to the endogenous conversion of preformed 18 carbon acids to the 20 and 22 carbon acids and on other parameters of deposition and interconversions of dietary fatty acids by fish.

In these studies, both fresh water and marine fish were depleted of unsaturated fat and, subsequently, were presented diets containing linoleic [6] or linolenic [6] acids, or cottonseed, linseed, or safflower oils at different temperatures and at differing levels of dietary linoleic and linolenic acids. The degrees of return of the polyunsaturated acids under the different conditions were noted.

Experimental

Tests on the ability of dietary linoleic and linolenic acids, alone or in natural fats, to replace the C_{20} and C_{22} polyunsaturated acids depleted from marine fish by fat free rations. Small mullet, *Mugil cephalis,* and a limited number of fundulus, *Fundulus grandis,* obtained from Galveston Bay, Feb. 28, 1959, were placed on a low fat diet (Table I) in aquaria of circulating aerated sea water at room temperature. After 11 weeks on the low fat diet the fish were placed on test diets as follows:

15 mullet and 2 fundulus on 5% linoleic acid diet for 7 weeks.
15 mullet and 2 fundulus on 5% linolenic acid diet for 7 weeks.
10 mullet and 1 fundulus on 30% cottonseed oil diet for 25 and 18 weeks respectively.
10 mullet and 1 fundulus on 30% linseed oil diet for 25 and 18 weeks respectively.

There was a reduction in the number of mullet during the feeding period. Most of this resulted from some of the fish, especially those on the linseed oil diet, flipping out of the aquaria. At the end of the test there were 9 mullet in the fat free group, 7 in the cottonseed oil group, and 4 in the linseed oil

TABLE I
Basal Diet[a]

Major constituents	%
Corn starch	68.0
Albumin	25.0
Salt mixture No. 4	5.0
Glycine	1.7
Choline hydrochloride	0.25
Inositol	0.125
Water-soluble vitamins (mg/kg)	
Thiamin	6.0
Calcium pantothenate	25.0
Pyroxidine hydrochloride	8.0
Niacin	70.0
Folic acid	2.5
Para-amino benzoic acid	3.0
Lederle's extract for vitamin B_{12}	6.0
Oil-soluble vitamins (mg/kg)	
Mixed tocopherols	1.0
Menadione	1.5
Carotene	33.0
Vitamin D_3	0.08

[a] The major constituents and the water-soluble vitamins were mixed thoroughly with an equal weight of water. The fat-soluble vitamins were mixed with an equal volume of Tween 20 and emulsified with a small quantity of water. This was added to the major constituents and the whole was agitated thoroughly in a Waring Blendor. The resultant suspension was then cooked in a double boiler until solidified, cooled and stored in a refrigerator until used. Any fatty acid or fat added to the diet was substituted for corn starch.

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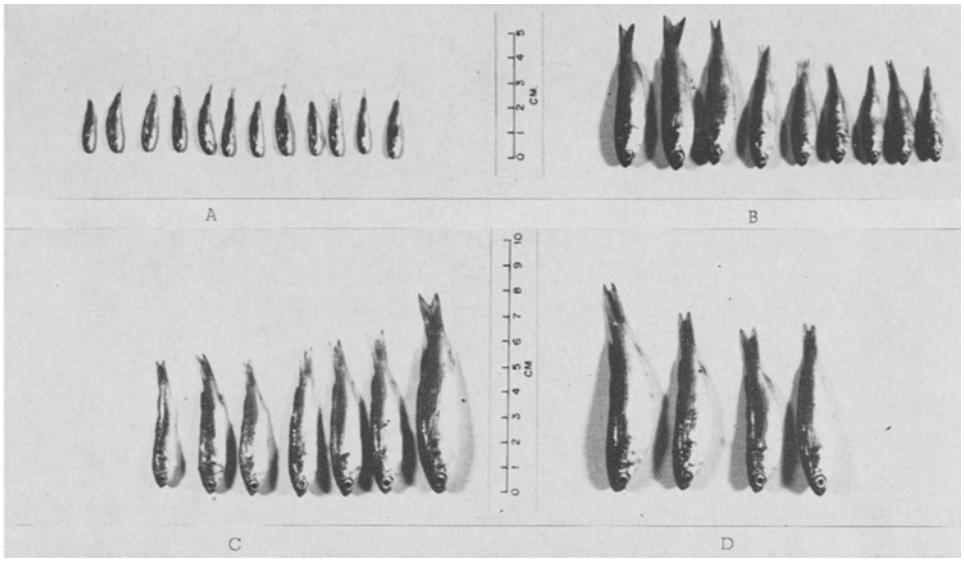

FIG. 1. Comparison of mullet on fat free, cottonseed oil and linseed oil diets. A. Mullet after 11 wk on fat free depletion diet. B. Mullet after 36 wk on fat free diet. C. Mullet after 25 wk on cottonseed oil diet following 11 wk depletion. D. Mullet after 25 wk on linseed oil diet after 11 wk depletion.

group (Fig. 1).

The composition of the linoleic and linolenic acids used in this study is given in Table II.

The fish carcasses were cut into small pieces and macerated in a Waring Blendor at top speed for 5 min with 10 volumes of chloroform. The chloroform was decanted into a large centrifuge bottle, and the residue was extracted in the blendor with half the volume of chloroform. The chloroform solution was centrifuged, the clear chloroform phase was dried with anhydrous sodium sulfate, and the dried solution was then reduced to a small volume.

In some instances, the chloroform extracts were slurried with silica gel to remove phospholipids. In these cases, subsequent fatty acid analyses were made on the triglycerides only, or on both the triglycerides and the mixed phospholipids, as indicated in the tables.

The lipids were saponified, and the non-saponifiable fractions were removed by pentane extraction. The soaps were decomposed by acidification and were extracted with pentane. The acids were converted to methyl esters by diazomethane.

Gas-liquid chromatographic analyses were made with a 6 ft, ¼ in. column packed with 20% ethyleneglycol succinate polyester on firebrick at 230C, and a flow rate of 60 ml/min of argon using a β-ray ionization detector.[7]

Results

The fatty acid composition of the mullet is given in Table III, and that of the fundulus in Table IV. As has been shown repeatedly in this series of reports on fish (1,2,3,4), polyunsaturated fatty acids are depleted from fish fat by a fat free diet. More important, under the conditions of this study, the ingestion of linoleic or linolenic acids in the form of either isolated acids or as cottonseed or linseed oils did *not* result in the return of the more highly polyunsaturated acids in either of the two species of fish. Reference to the previous reports of this series (1,2,3,4) reveals that this finding is consistent under the conditions used. There were some exceptions in which it appeared that there were slight increases in levels of acids of 4, 5, and 6 double bonds upon refeeding linoleic or linolenic acids, but in only one case did the new levels approach levels found in the native fish oil, that being the 20:5 acid found in mullet fed 30% linseed oil. It is especially significant that the most typical marine polyunsaturated acid, 22:6 did not return. Therefore, neither linolenic nor linoleic is the precursor of that acid in marine fish.

TABLE II

Composition of Dietary Linoleic and Linolenic Acids

Linoleic acid		Linolenic acid	
	%		%
14:0	Trace	14:0	Trace
16:0	0.9	16:0	0.4
16:1	1.9	16:1	0.3
18:1	3.6	18:1	1.4
18:2	88.2	18:2	5.7
18:3	2.5	18:3	85.5
20:1	2.9	20:1	2.5
		22:2	4.2

[7] Research Specialties, Series 600, Gas Chromatograph.

It is interesting that after cottonseed oil ingestion by the fundulus and possibly by the mullet, there was an increase in stearic acid levels. This has been reported for swine (5), hens (6,7), and rats (8). A high level of 18:4 after 30% linseed oil ingestion is also noteworthy.

The above observation suggested that some special conditions, such as environmental temperature or the levels of the linoleic and linolenic acid in the diet, as well as the levels and nature of the fatty acids fed with them, may be required for a high level of conversion, if, in fact, these conversions do take place to any marked degree in marine fish.

The influence of low levels of linoleic and linolenic acids in the diet, and of two environmental temperatures, on the conversion of these acids to longer chain, more highly unsaturated acids. Young mullet were placed on the basal diet on Jan. 11, 1960, until March 8, 1960 (8 weeks). They were then divided into four groups:

1) Basal diet at 13C.
2) Basal diet at 23C.
3) 20% fat diet (19.5% 1:1 ethyl myristate-ethyl laurate, 0.455% linolenic acid, 0.041% linoleic acid, and 0.004% oleic acid) at 13C.
4) 20% fat diet (same as in 3).

The fish were kept on the basal diet for 4 weeks and on the test diets for 6 weeks. The low temperature

TABLE III
Mullet Fatty Acids after Depletion and Refeeding

Fatty acids	Linoleic and Linolenic Acid Diets (caught 2-28-59)[h] Low fat diet to 5-15-59 (11 wk) Test diets to 7-24-59 (7 wk)				Cottonseed Oil and Linseed Oil Diets (caught 2-28-59)[h] Low fat diet to 7-24-59 (23 wk) Test diets to 1-18-60 (25 wk)			
	When caught	Low fat[e]	5% L.[a] acid diet[e]	5% Ln.[b] acid diet[e]	When caught	Low fat[f]	30% CSO[c] diet[g]	30% LSO[d] diet[g]
	% of carcass fatty acids				% of carcass fatty acids			
12:0	1.3	0.8	0.1	0.4	0.1	0.1	0.1	0.1
14:0	6.2	12.8	4.4	3.7	0.1	4.4	1.3	0.8
15:0	5.3	4.8	1.5	1.2	trace	0.4	0.1	0.2
16:0	24.0	26.2	27.6	28.3	13.3	34.0	21.4	10.6
16:1	15.8	24.2	15.2	13.1	0.1	16.4	3.9	4.7
17:0	6.7	7.8	1.9	2.4	0.0	0.7	0.3	0.2
18:0	4.1	1.1	1.9	2.0	0.1	1.3	4.2	3.5
18:1	12.3	11.2	11.5	11.1	16.2	18.5	21.4	16.4
18:2	2.4	6.5	30.0	5.8	10.6	12.9	43.5	12.0
18:3	1.8	0.5	0.3	24.6	0.5	2.2	1.0	14.4
18:4	1.3	1.8	2.6	3.1	2.7	4.6	1.1	24.4
20:1	0.0	0.0	2.3	0.0	1.2	0.0	0.0	0.0
20:2	0.0	0.0	0.0	0.0	0.0	0.9	0.2	0.0
20:3	trace	0.6	0.7	trace	1.9	0.8	0.3	2.9
20:4	0.9	1.7	trace	1.5	9.4	0.3	0.1	4.1
20:5	5.2	trace	trace	0.7	5.8	0.8	0.4	5.5
22:2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:3	0.0	0.0	0.0	0.0	10.4	0.2	0.1	0.2
22:4	trace	trace	trace	trace	0.3	0.4	0.1	trace
22:5	3.6	trace	0.0	trace	2.1	0.4	0.2	trace
22:6	9.1	0.0	trace	trace	25.5	0.7	0.3	trace
24:4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24:5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Linoleic. [b] Linolenic. [c] Cottonseed oil. [d] Linseed oil. [e] 5 fish. [f] 12 fish. [g] 10 fish. [h] The mullet used for the natural oil diet study were older and larger than those used for the linoleic and linolenic acid diet study although also caught on Feb. 28, 1959.

was deleterious. At 13C, only two of the five fish on the basal diet and only three of six fish on the fat diets survived.

The fatty acid composition of the mixed fatty acids of the entire carcasses of the fish is given in Table V.

There is no apparent significant change in the fatty acids of the fish held at 13C that could be related to diet except a possible reduction in the 20:4 [8] and 22:4 acids on the fatty diet. The group fed fat at 23C did appear to have a shift from 16:1 and 16:2 acids to 12:0 and 14:0 acids. There was also a possible decrease in the 18:1 and 18:2 acids which may have been utilized rather than inter-converted and, perversely, an increase in 20:4.

TABLE IV
Fundulus Fatty Acids after Depletion and Refeeding

Fatty acids	Linoleic and Linolenic Acid Diets (caught 2-28-59) Low fat diet to 5-15-59 (11 wk) Test diets to 7-3-5-9 (7 wk)				Cottonseed and Linseed Oil Diets (caught 2-28-59) Low fat diet to 5-15-59 (11 wk) Test diets to 12-1-59 (19 wk)	
	When caught	Low fat	5% L.[a] acid diet	5% Ln.[b] acid diet	30% CSO[c] diet	30% LSO[d] diet
	% of carcass fatty acids				% of carcass fatty acids	
12:0	2.1	2.5	3.2	3.3	0.1	0.1
14:0	4.3	9.1	6.6	6.0	1.0	0.8
15:0	2.8	4.6	3.9	3.0	0.3	0.3
16:0	9.7	16.7	14.1	18.8	19.1	7.8
16:1	16.6	16.1	12.9	11.0	3.5	4.9
17:0	0.0	1.5	1.7	2.6	0.0	0.0
18:0	4.6	0.6	0.8	2.6	9.3	3.1
18:1	13.2	25.9	25.5	19.4	21.1	39.5
18:2	6.0	10.3	16.5	18.2	28.7	11.7
18:3	2.7	1.5	1.2	10.0	0.6	18.3
18:4	1.1	0.6	0.6	2.0	1.4	3.1
20:1	0.0	0.0	1.0	0.0	6.3	1.2
20:2	0.0	0.0	0.0	0.0	0.0	0.0
20:3	0.0	1.0	0.8	0.1	0.8	0.0
20:4	8.5	1.9	1.7	0.8	3.4	2.6
20:5	9.2	2.2	2.1	0.2	0.7	2.5
22:2	0.0	0.0	0.0	0.0	0.0	0.0
22:3	0.5	0.0	0.0	0.0	0.6	0.1
22:4	1.0	1.0	1.6	0.2	1.1	0.3
22:5	6.6	1.6	1.9	0.5	0.2	1.2
22:6	11.1	2.9	3.9	1.3	0.8	2.4
24:4	0.0	0.0	0.0	0.0	0.5	0.0
24:5	0.0	0.0	0.0	0.0	0.5	0.0

[a] Linoleic acid.
[b] Linolenic acid.
[c] Cottonseed oil.
[d] Linseed oil.

[8] The figure before the colon represents the number of carbon atoms; the figure after the colon, the number of double bonds.

The influence of temperature during depletion from body fat of high levels of linoleic and linolenic acids. A group of mullet were fed the 30% linseed oil for 4 weeks and were then placed on a diet containing 20% 1:1 ethyl myristate-ethyl laurate at 13C and 23C for 4 weeks (Table VI).

This is the first study in this series which has demonstrated the conversion of linoleic and linolenic to more highly unsaturated fatty acids to any significant degree. At 13C very little of the lauric and myristic acids of the diet were deposited, and there was a significant degree of conversion of the 16:0, 18:0, 18:1, 18:2, and 18:3 acids to 20 and 22 carbon unsaturated acids. At the higher temperature the lauric and myristic acids were deposited, similar to the results in Experiment B, Table V, and there was little change in the 18, 20, and 22 carbon unsaturated acids.

The effect of a low dietary level of linolenic acid with a high dietary level of palmitic acid on the inter-

TABLE V
Fatty Acid Composition of Carcasses of Mullet Fed Low Level Linoleic and Linolenic Acid Diets at Two Temperature Levels [a]

Fatty acids	13C		23C	
	Basal diet 4 wk	20% Fat diet[b] 4 wk[a]	Basal diet 6 wk[a]	20% Fat diet[b] 6 wk
	% of carcass fatty acids			
12:0	0.5	0.6	0.6	6.0
14:0	3.7	4.8	5.5	15.5
15:0	0.5	0.9	0.9	0.9
16:0	27.1	26.5	29.9	23.4
16:1	14.5	17.0	15.9	11.4
17:0	2.6	3.1	5.0	2.5
18:0	7.2	5.9	6.5	5.9
18:1	16.9	15.7	14.9	12.6
18:2	11.6	11.8	12.3	8.7
18:3	2.0	1.7	1.4	2.1
18:4 & 20:1	0.8	0.4	0.5	0.7
20:2	2.0	3.0	3.4	2.7
20:4	2.0	1.0	0.6	1.3
20:5	1.8	1.6	0.4	1.3
22:2	0.0	0.0	0.0	0.7
22:4	1.5	0.7	0.0	0.9
22:5	0.6	0.9	2.2	0.0
22:6	4.7	4.4	0.0	3.4

[a] All fish were held at room temperature (about 23C) on the basal diet for 2 months before the test temperatures and diets.
[b] 19.5% 1:1 ethyl myristate-ethyl laurate
0.455% linolenic acid
0.040% linoleic acid
0.005% oleic acid.

TABLE VI

Fatty Acid Composition of Mullet Fed Linseed Oil then Depleted of Body Linoleic and Linolenic Acid by a Myristate-Laurate Diet [a]

Fatty acids	30% Linseed oil diet	4 wk M-L diet [b, c] 13C	4 wk M-L diet [b, c] 23C
	% of carcass fatty acids		
12:0	0.5	1.2	5.5
14:0	1.4	2.5	11 9
15:0	1.0	0.4	0.1
16:0	14.1	10.9	15.9
16:1	2.4	2.1	6.8
17:0	0.0	0.0	0.9
18:0	10.2	6.5	4.3
18:1	22.2	18.0	16.4
18:2	16.3	13.9	11.7
18:3	25.1	19.1	20.3
18:4 & 20:1	0.9	1.7	1.5
20:4	1.7	3.5	0.6
20:5	1.0	2.6	0.3
22:3	0.4	1.5	0.4
22:4	0.3	5.2	0.6
22:5	0.6	3.7	0.6
22:6	2.0	7.2	2.2

[a] Diet contained 20% of 1:1 ethyl myristate-ethyl laurate.
[b] Low fat diet Jan. 11, 1960–March 15: 30% linseed oil March 15–April 11.
[c] Low fat diet Jan. 11, 1960–March 15: 30% linseed oil March 15–April 11: Saturated fat depletion diet April 11–May 10.

conversion of the linolenic acid. The positive effect of depletion at a low temperature on the conversion of depot linoleic and linolenic fatty acids to more highly unsaturated acids suggested that the conversion may take place only under conditions in which a reduced supply of unsaturated acids is available. In order to test this hypothesis, a group of 10–15 cm mullet, after being on the basal diet for 2 months, was placed on a diet containing 19% ethyl palmitate, but only 0.9% linolenic acid, 0.08% linoleic acid, and 0.02% oleic acid for 2, 4, 6, and 8 weeks at room temperature. The fatty acid compositions of the groups of fish are given in Table VII.

There is a very definite response to the dietary palmitate: however, the very highly unsaturated acids, especially the 22:6, also appear to have increased in the presence of linolenic acid as 0.9% of the diet. Thus, at low levels, approximately 1% linolenic acid in the diet is converted to the polyunsaturated acids

TABLE VII

Fatty Acid Composition of Mullet Fed a Diet Containing Low Levels of Linoleic and Linolenic Acids with a High Level of Saturated Acids [a] for Varying Periods at Room Temperature

Fatty acid	As caught	Basal diet		Fat diet			
		4 wk	12 wk	2 wk	4 wk	6 wk	8 wk
	% of carcass fatty acids						
12:0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
14:0	4.3	4.7	4.4	1.6	0.9	0.9	1.5
15:0	4.3	8.0	1.3	1.8	1.3	0.8	1.0
16:0	17.4	23.0	28.3	36.9	53.9	43.2	41.1
16:1	18.1	16.1	13.7	14.4	2.4	4.4	7.6
17:0	0.0	0.0	3.3	0.0	0.0	0.0	0.0
18:0	10.4	12.6	7.9	5.7	8.5	10.3	9.0
18:1	11.0	15.0	15.8	9.3	8.8	12.1	12.2
18:2	2.4	5.5	13.7	6.8	2.6	2.3	5.6
18:3	2.2	2.6	2.4	1.7	2.2	2.1	5.1
18:4 & 20:1	1.1	1.5	0.6	2.3	1.2	0.8	1.1
20:2	1.7	2.3	2.8	1.1	0.7	0.0	0.0
20:4	4.3	2.3	1.5	3.2	4.4	4.0	3.2
20:5	10.6	1.2	1.0	5.2	4.6	3.4	2.7
22:2	0.0	0.0	0.0	0.0	0.0	0.0	0.7
22:4	0.9	1.7	0.5	4.4	1.1	2.5	1.7
22:5	4.0	1.7	0.7	1.1	2.3	3.6	1.7
22:6	7.3	1.8	1.8	4.3	5.3	9.5	5.9

[a] 19% ethyl palmitate, 0.9% linolenic acid, 0.08% linoleic acid, 0.02% oleic acid.

at 23C, at least to some degree, in contrast to the non-conversion when fed at the 5% level.

The influence of medium and high-level dietary linoleic acid on the polyunsaturated fatty acids of marine lipids. The purpose of this study was to determine the maximum levels to which the polyunsaturated acids in marine teleost fish may be raised by the inclusion of linoleic acid in their diet.

Two species of marine fish, *Fundulus dispar* and *Cyprinodon variegatus,* were maintained on the basal fat free diet, the basal diet plus 5% safflower oil containing 75% linoleic acid, and the basal diet plus 20% safflower oil. The fatty diets thus contained 3.75% and 15.0% linoleic acid respectively.

The experiments were conducted as follows: Three groups of small *Cyprinodon variegatus* and three groups of *Fundulus dispar* were placed in different 45 gal aquaria containing filtered natural sea water which had a salinity of 34–36 parts/thousand. The aquaria were held at constant temperature and equipped with a continuous filter. The water was recycled through glass wool and charcoal at a rate of 50–100 gal/hr. The tanks were also provided with an air supply of approximately 1 liter/min. Once daily, the fish were fed a sufficient amount of finely divided food. At the end of one month the fish were killed and the carcass fat was analyzed.

The polyunsaturated acids were determined by the official ultraviolet spectrophotometric method of the AOCS (9).[9] This method has not been recognized for the determination of polyunsaturated fatty acids of fish oils because of evidence that there were ethylene interrupted double bonds present. However, it has been demonstrated (10) that the position of the double bonds in fish oil fatty acids, like other natural polyunsaturated fatty acids, are all methylene interrupted. Thus the AOCS method should also be valid for fish oil. Fatty acids were assumed to constitute 66.7% of the phospholipids and 95.6% of the triglycerides.

In spite of the fact that the isomerization technique has been replaced by gas-liquid chromatography, the conclusions reached by its use in the present case are valid.

It may be seen in Table VIII that the dienoic acid did not exceed 30% of either triglycerides or phospholipids, nor did tetraenoic acid exceed 10% of the phospholipids. Also, the increase of linoleic acid in the diet from 3.75–15% did not result in a very great increase in linoleic acid or any acid derived from linoleic acid.

TABLE VIII

Composition of Triglyceride and Phospholipid Polyunsaturated Fatty Acids of Fish Ingesting Diets Containing 3.75% and 15.0% Linoleic Acids as Safflower Oil

	Fundulus			Cyprinodon		
	Diets [a]			Diets [a]		
	1	2	3	1	2	3
	%	%	%	%	%	%
Triglyceride F. A.						
Dienoic	15.5	25.0	25.7	21.6	29.2	22.6
Trienoic	4.9	2.5	4.1	3.5	4.4	3.3
Tetraenoic	4.8	1.0	2.3	3.0	3.1	1.8
Pentaenoic	1.6	0.6	1.2	1.8	1.3	0.9
Phospholipid F. A.						
Dienoic	.....	21.4	22.4	.....	19.2	22.3
Trienoic	.....	3.3	4.6	.....	3.2	3.5
Tetraenoic	.....	4.6	7.8	.....	5.6	6.7
Pentaenoic	.....	3.1	4.7	.....	3.6	3.6
Hexaenoic	.....	2.8	3.2	.....	2.1	2.3

[a] Diet 1: Basal low fat diet.
Diet 2: 5% Safflower oil (3.75% linoleic acid).
Diet 3: 20% Safflower oil (15% linoleic acid).

[9] This study was made before gas-liquid chromatographic equipment was obtained in the laboratory.

TABLE IX
Growth of Goldfish on Low Fat Diet for 104 Days[a]

Sample No.	Description	No. of fish	Age of fish	Avg length at end of experiment	Original wt	Avg wt at end of experiment	Avg wt gain	
			days	cm	g	g	g	%
1	Initial sample	5	92	2.4	0.4	0.4	....	....
2	Original low fat	6	196	3.0	0.4	1.6	1.2	....
3	Low fat 13C	6	316	3.3	2.0	2.3	0.2	11.6
4	Linoleic 13C	6	316	3.7	2.4	2.7	0.3	11.6
5	Linolenic 13C	6	316	4.1	2.4	3.0	0.6	29.1
6	Low fat 19C	6	316	3.4	2.1	2.3	0.2	10.5
7	Linoleic 19C	6	316	3.6	2.3	2.5	0.3	18.4
8	Linolenic 19C	6	316	3.6	1.8	2.3	0.4	23.0
9	Low fat 25C	6	316	3.4	2.3	2.5	0.2	6.4
10	Linoleic 25C	6	316	3.5	2.1	2.4	0.3	14.0
11	Linolenic 25C	6	316	3.4	2.1	2.5	0.4	19.2

[a] Fish raised in hatchery for 92 days before being placed on diet.

TABLE X
Growth of Goldfish on Low Fat Diet for 261 Days[a]

Sample No.	Description	No. of fish	Age of fish	Avg length at end of experiment	Original wt	Avg wt at end of experiment	Avg wt gain	
			days	cm	g	g	g	%
1	Initial sample	5	92	2.5	0.4	0.4	....	....
2	Original low fat	6	196	3.0	0.4	1.6	1.2	....
3	Low fat 13C	5	429	3.8	2.8	3.0	0.2	7.9
4	Linoleic 13C	5	429	3.2	2.4	2.8	0.4	14.5
5	Linolenic 13C	5	429	3.9	3.6	4.0	0.4	12.0
6	Low fat 19C	5	429	3.8	3.1	3.3	0.2	6.5
7	Linoleic 19C	5	429	3.6	3.3	3.5	0.2	7.1
8	Linolenic 19C	5	429	4.0	2.5	2.9	0.4	16.4
9	Low fat 25C	5	429	3.2	2.3	2.5	0.2	9.1
10	Linoleic 25C	5	429	3.7	2.7	3.0	0.3	11.3
11	Linolenic 25C	5	429	3.1	2.5	3.0	0.5	21.5

[a] Fish raised in hatchery for 92 days before being placed on diet.

These phenomena are similar to those found in the laying hen (11,12) and in the pig (13), and they thus hold for three classes of animals: fish, birds, and mammals.

The influence of temperature and dietary linoleic and linolenic acids on the fatty acid composition of fresh water fish. In one experiment, *Carrassius auratus* goldfish, hatched on April 10, 1960, and reared in a hatchery on a stock diet, were placed on the basal low fat diet on July 11, 1960, at 92 days of age, and maintained on that diet until Oct. 21, 1960 (104 days) to deplete them of their unsaturated fatty acids. At the end of the depletion period, the fish were maintained on test diets at 13C, 19C, and 25C for 4 months.

In a second experiment, 92 day old goldfish were kept on the basal low fat diet for 267 days. One group was continued on the low fat diet at the three temperatures for 200 days. Other groups were given the test fats at the test temperatures for 76 days.

Three diets were used: the basal low fat diet; 5% linoleic acid diet; and 5% linolenic acid diet.

The growth data of these fish are given in Tables IX and X.

It is immediately apparent that both linoleic and linolenic acid had a growth stimulating effect with that of linolenic acid being the greater. It is not conclusive whether the latter is peculiar to the linolenic acid alone, as it contained 5.6% linoleic (Table I). Linolenic acid had an effect in addition to the linoleic acid, or the two are synergistic. In general, the lower the temperature the greater the growth. In spite of the greater growth, there was a higher mortality at 13C than at the higher temperature. The fish were less active and consumed about half as much food.

The fatty acid composition of the goldfish, fat, as modified by diet and environmental temperature, and as determined by gas-liquid chromatography (Beckman, 1GC-2A Gas Chromatograph), is given in Tables XI, XII, XIII, and XIV. The effects of the various diets and temperatures may be summarized as follows:

I. Depletion period on low fat diet:
  A. Triglyceride fatty acids
    1) Depletion effects
      a) Increase in 18:1 and 20:3
      b) Decrease in 18:0, 18:2, and 20:4
    2) Temperature effects—none
  B. Phospholipid fatty acids
    1) Depletion effects
      a) Increase in 20:1, 20:3, 22:2, 22:3
      b) Decrease in 18:0, 18.3, 22.4, 22:5, 22:6
    2) Temperature effects—questionable

II. Linoleic acid diet after depletion:
  A. Triglyceride fatty acids
    1) Linoleic acid effects
      a) Increase in 18:2, 18:3 (γ linoleic), and 20:4
    2) Temperature effects—none

TABLE XI
Changes in Goldfish Triglyceride Fatty Acids after 120 Days on Test Diets (First Experiment)

Fatty acid	Stock diet (92 days)	Depletion diet (104 days)	Low fat diet (120 days)			Linoleic (120 days)			Linolenic (120 days)		
			13C	19C	25C	13C	19C	25C	13C	19C	25C
	%	%	%	%	%	%	%	%	%	%	%
18:0	8.4	4.6	3.6	3.3	3.7	6.9	2.2	5.0	3.5	4.3	2.4
18:1	38.9	42.0	44.1	46.0	47.2	32.9	44.5	43.5	45.3	42.0	46.7
18:2	7.5	5.4	3.6	3.5	4.0	9.8	10.3	9.4	3.4	3.8	2.3
18:3	0.6	0.2	trace	0.1	0.1	0.2	0.4	1.0	3.4	4.4	2.0
18:4 & 20:1	1.2	1.7	2.3	2.3	2.3	3.1	3.6	2.3	2.4	2.3	1.9
20:2	1.0	1.1	1.8	1.5	1.7	2.0	1.7	1.5	1.3	0.6	1.5
20:3	1.4	2.4	2.4	2.5	2.9	0.3	2.2	2.5	2.2	1.9	2.2
20:4	0.8	0.4	0.3	0.6	0.4	1.7	0.8	0.8	0.5	0.4	0.2
20:5	0.3	0.0	0.7	0.4	0.0	trace	0.1	0.0	0.3	0.3	0.2
22:1	trace	0.0	0.0	trace	0.0	trace	0.0	0.0	0.2	0.2	0.1
22:2	0.4	0.4	0.9	0.6	0.6	1.4	0.4	0.4	0.5	0.5	0.5
22:3	0.3	0.2	0.2	0.2	0.3	0.5	0.2	0.0	0.3	0.3	0.1
22:4	0.5	0.0	0.0	trace	0.3	0.3	0.2	0.5	trace	0.5	0.0
22:5	0.6	0.0	0.0	trace	0.3	0.0	trace	0.0	0.9	0.3	0.1
22:6	0.2	0.0	0.0	trace	0.0	0.0	trace	0.0	0.7	0.5	0.2

TABLE XII

Changes in Goldfish Triglyceride Fatty Acids after 76 Days on Test Diets (Second Experiment)

Fatty acid	Stock diet (92 days)	Depletion diet (267 days)	Low fat diet (76 days)			Linoleic (76 days)			Linolenic (76 days)		
			13C	19C	25C	13C	19C	25C	13C	19C	25C
	%	%	%	%	%	%	%	%	%	%	%
18:0	8.4	4.6	2.2	3.0	2.0	2.8	1.6	0.3	2.6	2.5	1.7
18:1	38.9	42.0	46.0	44.9	43.7	44.4	42.3	43.0	46.2	44.2	45.2
18:2	7.5	5.4	3.0	3.1	3.3	5.5	7.8	10.6	3.0	4.5	4.4
18:3	0.7	0.2	0.1	0.2	0.1	0.4	0.3	0.3	1.3	2.1	2.2
18:4 & 20:1	1.2	1.7	2.6	0.0	1.9	2.1	2.1	2.1	2.4	2.1	2.2
20:2	1.0	1.9	1.8	2.3	1.8	1.8	1.8	2.4	1.3	1.4	1.7
20:3	1.4	2.4	3.0	1.2	2.9	3.7	2.6	3.0	2.8	2.4	2.7
20:4	0.8	0.4	0.3	3.3	2.3	0.3	0.5	0.3	0.2	0.3	0.4
20:5	0.3	0.0	0.5	trace	0.1	0.0	0.0	trace	0.0	0.1	0.1
22:1	trace	0.0	0.0	0.4	0.0	0.0	0.1	trace	0.0	0.0	0.0
22:2	0.4	0.4	0.0	0.5	0.5	0.6	0.5	0.6	0.5	0.5	0.5
22:3	0.3	0.2	0.0	0.0	0.3	0.0	0.2	0.1	0.0	0.0	0.2
22:4	0.5	0.0	0.0	0.0	0.0	0.3	0.0	0.2	0.0	0.0	0.1
22:5	0.6	0.0	0.0	trace	0.0	0.0	0.2	trace	0.0	1.5	0.3
22:6	0.2	0.0	0.0	trace	0.0	0.0	0.0	0.0	0.0	0.0	trace

B. Phospholipid fatty acids
   1) Linoleic acid effects
      a) Increase in 18:2 and 20:4
      b) Reduction in 18:3 and 20:3
   2) Temperature effects
      a) Increase in 18:1, 18:2, and 18:3 at higher temperatures
      b) Questionable decrease in 20:1, 20:2, 20:4 and 20:5 higher temperatures.

III. Linolenic acid diet
   A. Triglyceride fatty acids
      1) Linolenic acid effects
         a) Increase in 18:3
      2) Temperature effects—none
   B. Phospholipid fatty acids
      1) Linolenic acid diet effects
         a) Increase in 18:3
         b) 20:4, 22:2, and 22:3 reduced toward control levels
      2) Temperature effects
         a) 18:3 highest at 13C

As usual dietary linoleic was deposited to a greater degree than linolenic.

Discussion

The data presented above make it clear that although the marine teleost is able to convert linoleic and linolenic acids to longer chain, more highly unsaturated acids, it does not do so to more than a slight degree if the two acids are offered to it at the 5%, or above, level. However, at the 1% level, there was appreciable conversion. Furthermore, if large amounts of linolenic and linoleic acids are deposited in tissues previously depleted of C_{20} and C_{22} acids, and the animals are again depleted, the tissue 18:2 and 18:3 acids will be converted to the longer chain, more highly unsaturated acids in significant amounts.

The increase in the level of stearic acid in fish (fundulus) on cottonseed oil ingestion adds another class of animals to the birds (6,7) and mammals (8,9) which have been demonstrated to thus respond to oils containing cyclopropene acids. The mechanism of this phenomenon is under study in this laboratory.

The lack of significant influence of low and high temperatures on the unsaturated fatty acid composition of both marine and fresh water fish was surprising. It was interesting, however, that dietary lauric and myristic acids are not deposited, as such, at 13C, but are deposited at 23C. It is possible that these saturated fatty acids are not absorbed at the lower temperature.

The fact that the level of tissue linoleic acid tends to approach a maximum of about 30% or slightly above as the level in the diet increases from 5–20% is consistent with other studies in this laboratory, as yet unpublished, that the excess polyunsaturated acids are preferentially utilized. The very high levels of deposition of linoleic acid found by Tove and Smith (15,16,17), 66% on 50% safflower oil diet, can thus be explained since at that level the linoleic acid in the diet may exceed the amount which can be utilized for energy, and therefore, would have to be stored. This would result in two plateaus of percentage of depot linoleic when plotted against increasing levels in the diet. Tove and Smith found that the level of linoleic acid deposition or depletion does change stepwise, and attributed this phenomenon to differences in the affinity of linoleic acid for the 2 and the 1,3

TABLE XIII

Changes in Goldfish Phospholipid Fatty Acids after 120 Days on Test Diets (First Experiment)

Fatty acid	Stock diet (92 days)	Depletion diet (104 days)	Low fat diet (120 days)			Linoleic 5% (120 days)			Linolenic 5% (120 days)		
			13C	19C	25C	13C	19C	25C	13C	19C	25C
	%	%	%	%	%	%	%	%	%	%	%
18:0	11.0	7.8	8.6	8.2	3.2	7.3	6.9	5.4	6.0	6.0	4.4
18:1	34.7	36.4	31.5	29.0	42.0	33.8	32.8	36.0	39.9	35.8	38.7
18:2	1.7	3.2	1.1	1.4	1.7	4.7	9.4	7.1	2.3	2.5	3.2
18:3	1.4	1.2	1.6	1.2	3.4	0.3	0.2	0.1	5.6	4.2	2.3
18:4 & 20:1	0.6	1.8	2.3	2.3	1.6	2.8	2.2	1.8	2.3	2.4	2.0
20:2	2.7	1.4	1.9	2.8	1.0	4.2	1.2	1.1	1.2	1.0	1.4
20:3	2.4	6.6	5.4	5.9	2.2	1.3	1.8	1.6	2.9	2.1	2.3
20:4	1.1	2.4	3.3	3.6	0.4	4.1	3.0	0.9	1.6	1.3	0.6
20:5	3.6	trace	0.2	0.4	0.1	0.4	0.2	0.2	0.4	0.6	0.3
22:1	0.0	0.0	0.1	0.1	0.1	0.4	0.5	0.1	0.1	0.3	0.0
22:2	trace	0.7	0.8	0.9	0.6	0.3	0.4	0.3	0.4	0.4	trace
22:3	trace	0.3	0.8	0.7	0.4	trace	0.1	trace	0.2	0.1	0.3
22:4	1.4	0.1	trace	0.1	trace	trace	0.2	trace	0.7	0.0	0.1
22:5	1.9	1.0	2.3	1.3	1.1	0.4	0.9	1.0	1.3	1.3	1.1
22:6	1.3	0.7	1.2	0.7	0.3	2.5	0.5	0.8	1.1	0.8	1.4

TABLE XIV
Changes in Goldfish Phospholipid Fatty Acids after 76 Days on Test Diets (Second Experiment)

Fatty acid	Stock diet (92 days)	Depletion diet (267 days)	Low fat diet (76 days)			Linoleic 5% (76 days)			Linolenic 5% (76 days)		
			13C	19C	25C	13C	19C	25C	13C	19C	25C
	%	%	%	%	%	%	%	%	%	%	%
18:0	11.0	7.7	4.9	10.5	3.8	8.2	5.8	6.3	6.9	5.5	8.9
18:1	34.7	36.4	37.7	27.7	35.7	30.3	35.3	32.7	35.2	34.4	31.5
18:2	1.7	3.2	2.3	3.0	2.9	3.0	6.0	6.5	2.3	3.3	1.7
18:3	1.4	1.2	0.3	0.0	0.0	0.3	0.1	0.5	1.4	2.1	1.4
18:4 & 20:4	0.6	1.8	2.4	1.9	2.3	2.2	2.1	2.2	0.7	2.4	2.2
20:2	2.7	1.4	2.1	1.1	2.1	4.0	1.4	4.4	1.6	3.0	1.1
20:3	2.4	6.6	5.9	12.7	7.0	3.9	3.5	3.3	3.8	2.8	5.2
20:4	1.1	2.4	1.2	2.7	3.7	2.2	4.8	4.7	1.2	0.8	4.9
20:5	3.6	trace	0.3	0.0	0.1	0.4	0.2	0.0	1.4	0.4	0.9
22:1	0.0	0.0	0.4	0.0	trace	0.0	0.0	trace	0.2	0.1	0.0
22:2	trace	0.7	1.1	1.3	1.0	0.6	0.5	0.6	0.8	0.8	0.6
22:3	trace	0.3	0.5	1.4	0.8	0.2	0.0	trace	1.0	trace	trace
22:4	1.4	0.1	0.6	0.3	trace	0.4	0.2	0.1	trace	trace	trace
22:5	1.9	1.0	0.4	3.0	1.1	1.7	1.2	0.8	6.1	2.9	2.0
22:6	1.3	0.7	0.5	1.9	0.9	0.9	1.0	0.8	3.2	2.4	6.1

positions in the triglyceride molecule.

It is thus apparent that fresh water and marine fish probably do not differ in any basic way in their mechanism for the deposition, synthesis and interconversions of fatty acids, nor do they differ from other animals.

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Separation of the Methyl Esters of Oleic, Linoleic, and Linolenic Acids by Column Chromatography Using Cation Exchange Resin Containing Silver Ion

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Abstract

By taking advantage of the tendency of silver ion to form complexes with unsaturated compounds, the methyl esters of oleic, linoleic, and linolenic acids have been cleanly separated in good yield by column chromatography. The silver ion was supported by means of a cation exchange resin; no silver was ever eluted from the column. Aqueous methanol, pure methanol, and ethanol saturated with butene-1 were employed as solvents.

Introduction

IT HAS BEEN known for many years that silver ion undergoes a reversible reaction with unsaturated compounds to form rather weak and unstable complexes. In 1938 Winstein and Lucas (1) studied these complexes in some detail, including the determination of equilibrium constants between silver ion and a number of olefins. Subsequent investigations of these compounds were performed by Lucas, et al. (2), and Traynham (3), while Andrews (4) and Keefer (4b) studied the more unstable complexes of silver ion and aromatic compounds. Recently Muhs and Weiss (5) determined the equilibrium constants of a great many silver-olefin complexes and discussed the factors affecting their stability.

With the increased use of chromatography, advantage has been taken of these silver complexes to effect the separation of various unsaturated compounds. Bednas and Russell (6), Bradford et al. (7), Tenny (8), and Smith and Ohlson (9), used silver nitrate solutions in gas chromatography to separate low molecular weight olefins. Muhs and Weiss (5), used a similar gas chromatographic system. But while these systems are excellent for the separation of low molecular weight materials, the elevated temperatures needed for gas chromatographic analysis of substances of higher molecular weight cause this method to be definitely limited; this is due to the non-volatility and thermal instability of many such substances, as well as possible thermal decomposition of the column itself (6).

Discussion

Since we in this laboratory are interested in the separation of various phospholipids according to the degree of unsaturation of their fatty acid substituents, column chromatography involving a cation exchange resin containing silver ion was investigated,