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Fatty acid composition of glycerophospholipids in seven tissues of cod (*Gadus morhua*), determined by combined high-performance liquid chromatography and gas chromatography

ØYVIND LIE* and GEORG LAMBERTSEN

Institute of Nutrition, Directorate of Fisheries, P.O. Box 1900, Bergen (Norway)

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ABSTRACT

A method for the separation from fish tissues of the four main glycerophospholipid classes, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine, using adsorption high-performance liquid chromatography with ultraviolet detection and consecutive gas chromatographic analysis, based on one injection for their fatty acid compositions, is described. Fatty acid 19:0 was used as an internal standard for the calculation of the relative concentrations of the phospholipids. The patterns of fatty acid distribution within each of the phospholipids from seven cod organs had some general similarities. Phosphatidylcholine had the highest levels of 16:0 and 18:2 $n-6$, and the ratio of 20:5 to 22:6 varied between 0.5 and 0.9. Phosphatidylethanolamine had the highest total polyunsaturated fatty acids, ($n-3$) polyunsaturated fatty acids, and 22:6 $n-3$, and the ratio of 20:5 to 22:6 varied between 0.2 and 0.5. Phosphatidylinositol showed the highest level of 18:0 and 20:4 $n-6$ and had the lowest ratio of ($n-3$) to ($n-6$). Phosphatidylserine had the highest ratio of ($n-3$) to ($n-6$) and the lowest ratio of 20:5 to 22:6. A generally low level (less than 1.5%) of the long-chain monoene, 22:1, was found in the phospholipids in all tissues.

INTRODUCTION

Polyunsaturated fatty acids (PUFA) in organs and tissues of marine fish are dominated by the $n-3$ series. A defined requirement for 18:3 $n-3$ has been proposed [1,2] for some fish species, whereas marine fish probably require long-chain $n-3$ PUFA and possibly also $n-6$ PUFA [3,4].

The phospholipids occupy a key role as a bridge between the dietary pool and the metabolic requirement for fatty acid precursors. The major phospholipids of marine animals are dominated by 20:5 $n-3$ and 22:6 $n-3$, resulting in a high ratio of ($n-3$) to ($n-6$). The exception is phosphatidylinositol (PI), which is unusual in being rich in 20:4 $n-6$ [3,5–7]. Relatively few studies of the lipids in the organs of marine fish have looked at the fatty acid compositions of individual phospholipids, and the role of $n-3$ and $n-6$ long-chain PUFA in marine phos-

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pholipids is not yet fully understood. Phospholipid classes are usually separated by thin-layer chromatography (TLC), but several high-performance liquid chromatographic (HPLC) methods have been proposed that offer advantages over TLC methods.

Our aim was to separate the four main phospholipid classes, phosphatidylcholine (PC), phosphatidylethanolamine (PE), PI, and phosphatidylserine (PS), in sufficient amounts after one injection for consecutive gas chromatographic (GC) analysis for their fatty acid compositions. This paper describes the results of the separation and the fatty acid composition study of four phospholipid classes from seven different organs and tissues in cod.

EXPERIMENTAL

Samples

Cod, hatched and reared at the Austevoll aquaculture station (Norway) were fed a commercial dry feed (T. Skretting, Stavanger, Norway) in a sheltered 25-m^3 tank supplied with running seawater at 8°C and 35‰ salinity.

Pooled samples from three fish (0.7–1.0 kg) of white and dark muscle, liver, roe, gill, heart and spleen were collected. The organs were immediately homogenized and total lipid was extracted with chloroform–methanol (3:1, v/v, Merck, Darmstadt, Germany), suitable for fish tissues. The lipid extracts were stored at −20°C until further analysis.

Analytical methods

HPLC was performed with a Constametric II solvent-delivery system and a variable-wavelength spectrophotometer (LDC Spectromonitor III). A Rheodyne (7125) sample-injection valve with a 20-μl loop was used (1–2 mg of lipids), and stepwise elution was achieved with a Rheodyne six-position rotary valve. The column (25 cm × 0.46 cm I.D.) was packed with silica gel (LiChrosorb 5 μm, Merck) by a Maximator (MSE 72) pump. All solvents of HPLC grade were from Rathburn Chemicals (Walkerburn, U.K.). The flow-rate was maintained at 1 ml/min, and the elution was monitored at 205 nm.

The initial solvent mixture of hexane–2-propanol–acetonitrile–water (364:486:94:56, v/v, solvent A) was run at room temperature for 27 min and eluted separately PE and PI, whereas neutral lipids and cardiolipin eluted with the front. PS and PC were separated by elution with hexane–2-propanol–water (394:526:80, v/v, solvent B) for 33 min. The four fractions were collected manually into vials. Reequilibration of the column was achieved by running solvent A until a stable baseline was attained (*ca.* 15 min), and the life of the column was increased by running hexane (0.2 ml/min) during the night. Columns not in use were stored in hexane.

The identity and purity of the isolated phospholipids were verified by TLC (Kieselgel 60 Merck) using standard phospholipids in a solvent system of ethyl

acetate–*n*-propanol–chloroform–methanol–0.25% aqueous KCL (15:25:25:10:4.5, v/v). No cross-contamination of the phospholipid classes was seen by visualization with a phosphate-detecting spray [8]. The phospholipid standards, PC, PE, PI and PS, were purchased from Sigma (St. Louis, MO, U.S.A.) and lysophosphatidylcholine was bought from Serdary Research Lab. (London, Canada).

The phospholipid fractions were evaporated and saponified, and 19:0 was added as internal standard. The fatty acids were esterified in 12% BF_3 in methanol. The methyl esters were separated in a Carlo Erba 2900 gas chromatograph ("on column" injection) connected to a Shimadzu C-R3A integrator equipped with a 50 m × 0.32 mm I.D. CP-Sil 88 (Chrompack, Middelburg, The Netherlands) fused-silica capillary column. The fatty acid composition was calculated using an integrator, and identification was ascertained by reference to a standard mixture of methyl esters (Nu-Chek, Elysian, MN, U.S.A.).

RESULTS

Standard phospholipids or tissue lipid extracts (1–2 mg) were separated by HPLC in a two-step elution procedure. The purity of the collected fractions was tested by TLC. The amount injected (1–2 mg) produced sufficient quantities of the least abundant phospholipids, PS and PI, for further determination of their fatty acid composition. Fatty acid 19:0 was added as an internal standard, and was used to calculate the relative levels of the different phospholipids from the sum of fatty acids in each fraction.

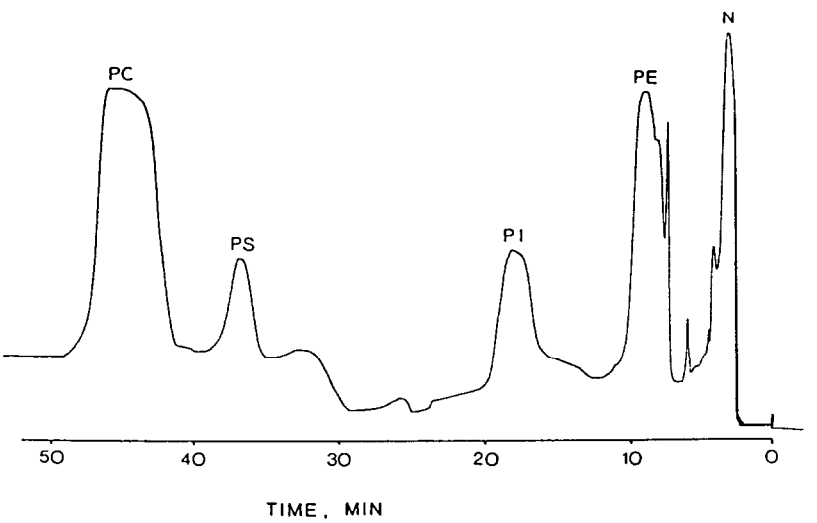

Fig. 1. HPLC separation of glycerophospholipids from lipid of cod white muscle, neutral lipid (N), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidylcholine (PC).

Fig. 1 shows the results of HPLC separation of a total lipid extract from white muscle of cod: PE, PI, PS and PC were well separated. PE and PC plasmalogens eluted with PE and PC, respectively. The analyses aimed to establish the detailed fatty acid composition and did not include plasmalogens which would complicate the procedure. Cardiolipin eluted with the neutral lipids.

PC and PE made up 54–69 and 20–28% of the sum of phospholipids, respectively, and PI and PS accounted for 6–11 and 4–12%, respectively (Table I). These figures may be slight underestimates, owing to the presence of plasmalogens mainly in PE. In gills and spleen the proportion of PS was 11–12%, which is double that in the other organs.

In PC, saturated fatty acids accounted for 27–35% of total fatty acids, being highest in gills and lowest in the dark muscle (Table II). A characteristic high content of 16:0, 23–29%, was seen in PC in all organs, more than double the average content in all other phospholipid fractions. The monoenes accounted for 14–34% of the fatty acids, being lowest in white and dark muscle. The major monoene was 18:1 $n-9$, exceeding 20% in the gills, whereas only trace amounts of 22:1 isomers were present. The polyenes dominated all samples, with the exception of the gills, which contained only 26%. The sum of the two major long-chain $n-3$ fatty acids, 22:6 and 20:5, exceeded 40% in both white and dark muscle PC. Noteworthy high levels, 6–10%, of 18:2 $n-6$ were found, resulting in a ratio of $(n-3)$ to $(n-6)$ of between 2.5 and 6.0.

In PE (Table III), the monoenes, primarily 18:1 and 20:1, accounted for 12–30% of total fatty acids, while the saturates ranged from 12 to 18%. Highest levels were found in liver PE, and lowest levels in white muscle for both groups. Even more than PC, PE was characterized by high levels of polyenes, exceeding 50 mol% in all organs, being lowest in the gills. The major PUFA was 22:6 $n-3$, and 18:2 $n-6$ was also prominent. Together with 20:4 $n-6$, this gave a ratio of $(n-3)$ to $(n-6)$ of between 6 and 10. Low GC recoveries of known fatty acids, particularly in the gills, may be explained by the presence of plasmalogens in PE. In the microsomal fraction isolated from the gills of cod, Bell *et al.* [6] found

TABLE I

RELATIVE LEVELS OF THE DIFFERENT PHOSPHOLIPIDS IN DIFFERENT ORGANS IN COD

Phospholipid	Relative concentration (%)						
	White muscle	Dark muscle	Liver	Roe	Gill	Heart	Spleen
PC	69	65	54	62	61	62	58
PE	20	24	28	24	21	24	24
PI	7	7	11	8	7	6	7
PS	4	4	7	5	11	8	12

TABLE II

FATTY ACID COMPOSITION OF PHOSPHATIDYLCHOLINE FROM DIFFERENT ORGANS IN COD

Fatty acid	Composition (%)						
	White muscle	Dark muscle	Liver	Roe	Gill	Heart	Spleen
14:0	1.2	1.2	1.7	1.9	2.5	2.1	2.5
16:0	25.5	24.8	23.0	27.9	29.3	24.4	26.6
16:1[a]	2.0	1.9	2.7	7.2	5.3	2.4	3.2
18:0	1.2	1.3	3.4	1.4	3.6	3.3	2.2
18:1 $n-9$	9.4	10.3	11.9	8.8	21.4	16.3	15.9
18:1 $n-7$	1.7	2.3	3.0	1.9	3.9	3.0	3.2
18:2 $n-6$	9.8	6.4	7.5	6.5	6.1	8.4	6.5
18:3 $n-3$	0.5	0.7	0.7	0.5	0.5	1.1	0.7
18:4 $n-3$	0.9	0.7	0.9	0.6	0.4	0.7	0.9
20:1[a]	0.9	1.6	3.1	1.3	3.0	3.2	2.5
20:4 $n-6$	1.1	1.4	1.1	1.2	1.3	1.1	1.9
20:4 $n-3$	0.6	0.5	0.6	0.4	0.4	0.5	0.5
20:5 $n-3$	14.6	17.4	14.5	17.3	6.9	12.6	13.3
22:1[a]	–	0.1	0.3	–	0.2	0.5	0.7
22:5 $n-3$	1.1	1.0	0.7	0.9	0.7	0.8	0.9
22:6 $n-3$	27.3	26.2	21.2	18.9	9.7	16.4	14.7
24:1	–	–	–	–	–	–	0.5
$\sum$[b]	97.8	97.8	96.3	96.7	95.2	96.8	96.8
$\sum$S[b]	27.9	27.3	28.1	31.2	35.4	29.8	28.1
$\sum$M[b]	14.0	16.2	21.0	19.2	33.8	25.4	26.0
$\sum$P[b]	55.9	54.3	47.2	46.3	26.0	41.6	39.4
$(n-3/(n-6)$	4.1	6.0	4.5	5.0	2.5	3.4	3.7

[a] Sum of isomers.

[b] $\sum$ = total fatty acids; $\sum$S = total saturated; $\sum$M = total monoenes; $\sum$P = total polyenes.

dimethyl acetals only in the PE fraction, implying that approximately one third of PE was present as plasmalogens [6].

In PI the saturated fatty acids accounted for 30–40% of the total fatty acids, as in PC, but in contrast to the high content of 16:0 in PC, 18:0 was the major saturated fatty acid in PI (15–32%) (Table IV). The monoenes represented 13–19% of the fatty acids, except for the liver PI which contained 30%. The fatty acid composition of PI was characterized primarily by high levels of arachidonic acid (4–19%). In liver, gills and heart, 20:4 $n-6$ was the major polyene, and consequently PI had the lowest ratio of $(n-3)$ to $(n-6)$ of all the phospholipids, except for white and dark muscle. Another prominent PUFA in PI was 20:5 $n-3$, and this was also the major polyene in spleen. Wide differences were found be-

TABLE III

FATTY ACID COMPOSITION OF PHOSPHATIDYLETHANOLAMINE FROM DIFFERENT ORGANS IN COD

Fatty acid	Composition (%)						
	White muscle	Dark muscle	Liver	Roe	Gill	Heart	Spleen
14:0	–	–	1.1	0.5	0.6	0.4	0.4
16:0	8.3	7.2	10.2	15.6	8.0	5.4	7.4
16:1[a]	0.2	0.3	2.2	1.7	0.9	0.5	0.3
18:0	4.1	5.5	6.7	2.0	8.8	5.9	5.4
18:1 $n-9$	9.3	9.1	12.8	7.7	11.1	8.2	9.7
18:1 $n-7$	3.5	3.4	5.8	3.5	5.3	4.8	4.6
18:2 $n-6$	4.5	4.8	6.0	3.9	3.6	6.3	4.8
18:3 $n-3$	0.5	0.5	0.6	0.2	–	1.3	0.3
18:4 $n-3$	–	–	0.4	–	–	0.3	–
20:1[a]	3.2	4.4	7.8	2.0	5.3	4.6	5.0
20:4 $n-6$	1.4	1.3	1.3	1.8	2.3	2.6	3.5
20:4 $n-3$	0.6	0.7	0.6	0.3	0.4	1.3	0.5
20:5 $n-3$	13.6	9.4	14.0	13.2	7.9	12.5	14.4
22:1[a]	–	–	1.4	0.1	–	0.4	0.6
22:5 $n-3$	1.6	1.7	0.8	1.3	1.5	1.8	2.3
22:6 $n-3$	44.7	47.4	25.5	38.5	27.9	35.7	31.8
24:1	–	–	–	0.3	–	0.1	–
$\sum$[b]	95.5	95.7	97.2	92.6	83.6	92.1	91.0
$\sum$S[b]	12.4	13.7	18.0	18.1	17.4	11.7	13.2
$\sum$M[b]	12.7	17.2	30.0	15.3	22.6	18.6	20.2
$\sum$P[b]	66.9	65.8	49.2	59.2	43.6	61.8	57.6
$(n-3)/(n-6)$	10.3	9.8	5.7	9.4	6.4	5.9	5.9

[a] Sum of isomers.

[b] $\sum$ = total fatty acids; $\sum$S = total saturated; $\sum$M = total monoenes; $\sum$P = total polyenes.

tween organs. Liver, heart and spleen had approximately equal levels of 20:4 $n-6$ and 20:5 $n-3$, and low levels of 22:6 $n-3$. In roe, however, 22:6 $n-3$ was the major polyene, and in the gills 20:5 $n-3$ and 22:6 $n-3$ were about equal in proportion, but half the level of 20:4 $n-6$. In both white and dark muscle 22:6 $n-3$ was the major PUFA, with relatively low levels of 20:4 $n-6$.

The fatty acid composition of PS varied less than the other phospholipids between organs (Table V). The saturated fatty acids accounted for 25–35%. The monoenes varied between 20 and 28%, mainly 18:1 isomers, but quite high levels of 20:1 isomers were found in this phospholipid. The polyenoic fatty acids (34–48%) were again dominated by 22:6 $n-3$, with contents about ten times higher

TABLE IV

FATTY ACID COMPOSITION OF PHOSPHATIDYLINOSITOL FROM DIFFERENT ORGANS IN COD

Fatty acid	Composition (%)						
	White muscle	Dark muscle	Liver	Roe	Gill	Heart	Spleen
14:0	0.4	0.9	2.1	0.7	1.3	0.8	0.8
16:0	6.3	6.8	10.8	12.4	7.9	6.8	6.2
16:1[a]	1.0	1.1	2.7	1.4	1.6	0.6	1.5
18:0	25.8	32.2	17.8	14.8	31.9	29.1	24.2
18:1 $n-9$	9.0	7.5	15.7	10.9	9.1	9.1	10.6
18:1 $n-7$	3.2	2.1	3.7	3.2	2.9	2.8	3.0
18:2 $n-6$	0.8	1.4	3.3	2.2	2.6	3.7	2.4
18:3 $n-3$	–	–	0.4	–	–	0.5	0.1
18:4 $n-3$	–	–	0.4	–	–	0.4	–
20:1[a]	1.5	1.6	5.9	1.9	3.0	2.7	3.0
20:4 $n-6$	3.5	5.9	11.9	13.1	17.2	18.5	17.8
20:4 $n-3$	–	–	0.6	0.3	0.1	0.2	–
20:5 $n-3$	7.7	10.0	10.9	16.3	7.9	14.3	18.1
22:1[a]	–	0.3	1.5	0.4	0.6	0.3	0.7
22:5 $n-3$	0.8	0.9	1.0	0.6	0.7	0.8	0.8
22:6 $n-3$	37.6	28.0	6.5	19.1	8.8	7.7	7.5
$\sum$[b]	97.8	98.6	95.2	97.3	95.6	98.3	96.7
$\sum$S[b]	32.5	39.9	30.7	27.9	41.1	36.7	31-2
$\sum$M[b]	14.7	12.5	29.5	17.8	17.2	15.5	18.8
$\sum$P[b]	50.6	46.2	35.0	51.6	37.3	46.1	46.7
$(n-3)/(n-6)$	10.7	5.3	1.3	2.4	0.9	1.1	1.3

[a] Sum of isomers.

[b] $\sum$ = total fatty acids; $\sum$S = total saturated; $\sum$M = total monoenes; $\sum$P = total polyenes.

than that of 20:5 $n-3$ in all organs. The $n-6$ fatty acids were generally low, giving the highest ratio (9–24) of $(n-3)$ to $(n-6)$ among the four phospholipids.

DISCUSSION

The HPLC method used to separate the glycerophospholipids was developed by combining and modifying two UV-transparent solvent systems proposed for the separation of phospholipids: the acetonitrile–methanol–water system first described by Jungalwala *et al.* [9], and the hexane–2-propanol–water system developed by Geurts van Kessel *et al.* [10].

The method described for the isolation of phospholipids and the subsequent

TABLE V

FATTY ACID COMPOSITION OF PHOSPHATIDYLISERINE FROM DIFFERENT ORGANS IN COD

Fatty acid	Composition (%)						
	White muscle	Dark muscle	Liver	Roe	Gill	Heart	Spleen
14:0	1.2	1.4	2.9	1.0	1.2	0.3	0.8
16:0	10.4	8.9	14.9	13.0	9.0	6.9	9.9
16:1[a]	1.4	2.2	3.7	1.3	2.0	0.5	0.8
18:0	20.0	19.6	18.3	13.2	22.1	17.4	16.0
18:1 $n-9$	10.6	12.4	10.4	6.8	8.8	5.3	5.0
18:1 $n-7$	2.5	2.8	3.5	3.3	3.7	3.6	3.3
18:2 $n-6$	2.1	1.4	2.0	1.4	2.1	1.5	1.5
18:3 $n-3$	–	–	–	–	0.1	–	–
18:4 $n-3$	–	–	–	–	–	–	–
20:1[a]	4.9	8.2	6.9	6.3	8.1	8.9	10.8
20:4 $n-6$	0.8	0.2	0.7	1.4	1.4	1.3	1.6
20:4 $n-3$	–	–	–	–	–	0.8	–
20:5 $n-3$	3.6	2.2	2.8	3.4	2.7	4.2	3.2
22:1[a]	0.6	1.2	0.6	0.6	0.6	1.5	1.2
22:5 $n-3$	1.0	1.0	0.3	1.3	0.8	1.7	1.4
22:6 $n-3$	34.9	34.8	27.8	39.6	26.1	38.1	39.6
24:1	0.5	–	–	0.9	0.4	1.2	0.6
$\sum$[b]	94.5	96.3	94.8	93.5	89.1	93.2	95.7
$\sum$S[b]	31.6	29.9	36.1	27.2	32.3	24.6	26.7
$\sum$M[b]	20.5	26.8	25.1	19.2	23.6	21.0	27.7
$\sum$P[b]	42.4	39.6	33.6	47.1	33.2	47.6	47.3
$(n-3)/(n-6)$	13.6	23.8	11.4	15.8	8.5	16.0	14.3

[a] Sum of isomers.

[b] $\sum$ = total fatty acids; $\sum$S = total saturated; $\sum$M = total monoenes; $\sum$P = total polyenes.

determination of the fatty acid composition has several advantages. The easy preparation of samples eliminates time-consuming pre-cleaning or pre-separation steps from the total lipid extract. Triglycerides (and other non-polar lipids) elute with the solvent front and do not interfere with the phospholipid separation, even for the lipid extract derived from the liver consisting mainly of triglycerides. There is no need for derivatization procedures to increase the detector sensitivity in the HPLC step, owing to the content of highly unsaturated fatty acids in the phospholipids. On the other hand, this fact excludes a direct quantitative determination. The use of 19:0 as an internal standard in the subsequent GC analysis gives an indirect quantification of the relative amounts of phospholipids, based on the sum of fatty acids.

The complete analysis through phospholipid separation and fatty acid determination is carried out in a closed system, as opposed to the open TLC methods, thereby preventing unwanted oxidation. This is of particular value in studies on the highly unsaturated fish lipids. From only one injection the method allows the collection of PC, PE, PI and PS in sufficient amounts and purity for further fatty acids analysis, owing to the high sensitivity of capillary GC.

The columns used for adsorption HPLC on silica gel, as in this method, have a somewhat limited lifetime when water-containing solvent systems are used. Such columns are easily refilled, however, and give reproducible results. Small modifications in the composition of solvents A and B may be necessary when columns are exchanged, since no two columns have identical adsorption characteristics.

In general, the compositions of PC, PE and PS confirm the results reported for the fatty acid composition of the flesh lipid fraction of wild caught cod [11]. The higher level of linoleic acid, 18:2 $n-6$, was the only exception, pointing to the influence of the diet. The composition of dark and white muscle differed little. The liver is the main lipid depot in cod, containing 50–60% of fat and accounting for 8–12% of the total weight of the fish [12]. Numerous analyses have been published on the fatty acid composition of cod liver lipid (and cod liver oil) [13] but, as far as we know, none on individual phospholipids.

Data for individual phospholipid fatty acid compositions of cod roes from ripe female cod immediately before spawning were given by Tocher and Sargent [5] and Bell [14]. They reported the relative proportions of saturated acids, monoenes and polyenes similar to the present results. Some differences were observed, probably owing to difference in roe ripeness, as our analyses were carried out on immature roe.

In land mammals $n-6$ PUFA and their eicosanoid metabolites are very important in osmoregulatory (renal) physiology [15–17]. The gills, which constitute the osmoregulatory tissue in teleost fish, have been extensively examined in cod by Bell *et al.* [6]. Our results were in general accordance with their results.

The haemopoietic sites in cod are primarily the spleen (and kidney) since fish bone does not contain marrow for haemopoiesis [18]. The spleen phospholipids had somewhat elevated levels of the long-chain monoenes 20 and 22, as we also found in erythrocytes from cod [7]. The heart phospholipids differed very little from the spleen phospholipids.

Lipids in the marine food chain are characterized by high ratios of $(n-3)$ to $(n-6)$ polyenes, and high contents of $n-3$ polyenes in the phospholipids are to be expected in wild caught cod. The cod in this experiment were fed a commercial dry feed, the lipid of which contained 7–10% 18:2 $n-6$ owing to the use of wheat as a feed ingredient. This was reflected in the fatty acid composition, as 18:2 $n-6$ accounted for 6–10 and 4–6% in PC and PE, respectively, less in PI and PS. The commercial dry feed also has high levels of 20:1 (10–15%) and 22:1 (15–20%) owing to the use of capelin meal and oil. Despite the high levels of 22:1 in the diet, only minor amounts of this fatty acid were found in the phospholipids in cod.

Dietary 22:1 therefore may be preferentially utilized as an energy source by cod. Similar observations have been reported from rainbow trout [19] and cod [12,20].

White and dark muscle PE contained nearly 70% polyenoic fatty acids, whereas in PE from roe, heart and spleen the polyenes accounted for 60% of total fatty acids. These values imply the existence of di-PUFA molecular species in PE, although the presence of plasmalogens may lead to overestimation of the relative level of PUFA. Di-PUFA molecular species of PC and PE have been reported in ripe roe of cod [14]. Tocher and Harvie [21] also found that the fatty acid composition of PE from fish retinas, especially from cod, implied the existence of di-PUFA and even di-22:6 $n-3$ molecular species. These may be of particular importance for the regulation of localized membrane structure and functionality in actively metabolizing tissues. Nevertheless, the presence of di-PUFA molecular species is contrary to earlier ideas on phospholipid structure, and their properties and role in biomembrane function remain to be elucidated [14].

The fatty acid composition of PI of organs and tissues in cod warrants special attention. Recently several authors [3,5–7, 22] have shown that PI from marine fish contains arachidonic acid, 20:4 $n-6$, as a major acid, where as phospholipids generally are dominated by $n-3$ PUFA. The characteristic composition of PI, with 18:0/20:4 $n-6$ as a major molecular species [14,23], corresponds well with that found in terrestrial mammals and suggests that the fatty acid composition in PI is strictly controlled.

As for mammalian PI, the high arachidonic acid level in fish PI points to the pivotal metabolic role of this minor phospholipid [24], as the source of arachidonic acid for eicosanoid synthesis in marine species. However, the presence also of substantial amounts of 20:5 $n-3$ in PI in cod tissue phospholipids suggests an eicosanoid production of the 3-series. Only in the gill PI did 20:4 $n-6$ dominate over 20:5 $n-3$. In liver, heart and spleen PI, 20:5 $n-3$ was found in higher levels than 22:6 $n-3$, which is unique for the PI fatty acid composition.

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