

Effect of maternal diet on the distribution of phospholipids and their fatty acid composition in *Xenopus laevis* embryos

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We determined the total phospholipid content, the percentage distribution of different phospholipid classes and their fatty acid composition in 6-day-old embryos obtained from *Xenopus laevis* females fed on two different diets. A first group of females was fed on beef liver, and a second one was nourished with commercial fish food very rich in ω -3 fatty acids. The embryos showed different patterns of phospholipids that had dissimilar fatty acid compositions. Phosphatidylinositol content was particularly affected. Due to the functional roles of this phospholipid as part of the transmembrane signaling machinery, it is possible to hypothesize that maternal diet might influence cell metabolism in amphibian embryos. (J. Nutr. Biochem. 10:44–48, 1999) © Elsevier Science Inc. 1999. All rights reserved.

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Introduction

The important role played by phospholipids in the membrane architecture and in the transmembrane signaling cascade is now well established. We know that content, distribution, and fatty acid composition of plasma membrane phospholipids can affect both the organization and the functional behavior of the cells,^{1,2} and that these parameters can be influenced by different factors, including diet, temperature, cell stage, development, and aging.³

The different phospholipid fatty acids can generate distinct prostanoids when phospholipase A₂ is activated.⁴ On the other hand, it is well known that activation of membrane receptors causes breakdown of specific membrane phospholipids to produce second messengers, such as diacylglycerol (DAG), which is obtained through phospholipase C action. However, this molecule can act differently (e.g., modulating protein kinase C activity) depending on

the fatty acid present in the 2 position.^{5–7} Therefore, manipulation of phospholipid content and of the fatty acid composition of phospholipids may result in modifications of cell metabolism. Supporting this theory are studies performed in mammals during development that demonstrate the importance of essential fatty acids in the mother's diet.^{8–11}

In this article, we present data on the effects exerted by maternal diet on the composition of different lipid classes in 6-day-old amphibian embryos. For this purpose a commercial fish food that was very rich in ω -3 fatty acids was compared with a diet based on beef liver, in which these fatty acids are far lower.

Methods and materials

Xenopus laevis, maintained in aquaria (Tecniplast, Varese, Italy), were fed *ad libitum* twice a week for 1 month with beef liver (from butchery) or commercial fish food (Veronesi Verona spa, Acquanegra Cremonese, Cremona) before *in vitro* fertilization.

In vitro fertilization was performed as previously described.¹² Briefly, gonadotropin injected females (five in each treatment) were induced to lay eggs that were immediately inseminated with sperm suspension (obtained by mincing the testes in 1 to 2 mL of

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Table 1 Fish food and beef liver composition (mg/g dry wt)

Type	Proteins	Cholesterol	Triglycerides	Glycolipids	Phospholipids
Fish food	234.3	4.50	28.4	0.909	8.35
Beef liver	194.8	3.81	6.87	0.643	65.5

cold De Boers Tris (DBT) solution). After 1 minute, FETAX solution was added to the eggs. The composition of the saline solution (FETAX) was (in mg/L): NaCl, 625; NaHCO₃, 96; KCl, 30; CaCl₂, 15; CaSO₄ · 2H₂O, 60; and MgSO₄, 70. DBT composition was (in mmol/L): NaCl, 119; KCl, 2.5; CaCl₂, 1.8; and Tris/HCl, 15 (pH 7.5).

After a few minutes, eggs successfully fertilized were oriented with the dark side (animal pole) up. A first screening, performed approximately 2 hours postfertilization (PF), allowed us to discard the 'bad' and unfertilized eggs. This screening was followed by a second one (6 hours PF) when all the irregularly segmented embryos were eliminated.

Embryos from different females were kept separate, maintained in a thermostatic chamber at 23 ± 0.5°C, collected by large bore pipettes at day 6 of development, and stored at -20°C.

Samples (five in each treatment, constituted by 100 embryos from a single female, 600 mg) were analyzed after manual discard of excess water, extracted in 4 mL of three different chloroform-methanol mixtures (1:2, 2:1, 1:1 v/v), and partitioned in two steps with 1/5 volume of water and chloroform/methanol/water 3/48/47 (v/v/v) to obtain an organic and an aqueous phase.¹³

The organic phase was dried under vacuum, resuspended in 3 to 4 mL chloroform, and applied to a silicic acid column eluted with the following sequence of solvents: 100 mL chloroform, 20 mL ethyl acetate, 80 mL acetone-methanol 9/1 (v/v), and 100 mL methanol.¹⁴ Total cholesterol and triglyceride content in the chloroform fraction was determined according to Pearson et al.¹⁵ and by a commercial kit (Triglycerid GPO-PAP, Boehringer, Mannheim), respectively. Total phospholipids as phospholipid-phosphorus were quantified in the methanol fraction according to Bartlett¹⁶; the phospholipid pattern was determined by high performance thin layer chromatography (HPTLC) on silica gel plates as previously described.¹³ Neutral glycolipids and sulfate, eluted in the acetone-methanol fraction, were separated by HPTLC developed in chloroform/methanol/water 110/40/6 (v/v/v) as solvent system, and detected by the diphenylamine reagent.¹⁷ Quantitative analysis was performed by scanning densitometry of the plates at 660 nm.¹⁸

Individual phospholipids were separated as previously described¹³ using five samples for each treatment, and their fatty acid composition was analyzed, as methyl esters, by gas liquid chromatography (GLC) equipped with flame ionization detector (FID) (DANI, model 86.10 Monza, Italy) and capillary column 32 m with an inner diameter of 0.32 mm (AT Silar, Alltech).¹⁹ Temperature was maintained at 80°C for 2 minutes, and then increased to 180°C at 8°C/min. Helium flow rate was 0.7 mL/min. Injector and detector temperatures were 250°C and 275°C, respectively. Every day an external standard, composed of a mixture of a known amount of standard fatty acid methyl esters was used to standardize the equipment and to quantify each fatty acid. The results were expressed as weight percentage.

The defatted residue was assayed for the content of proteins²⁰ and DNA.²¹ Fish food and beef liver were extracted and analyzed by the same methods used for *Xenopus* embryos lipid analysis.

The data were analyzed using unpaired, two tailed *t*-test (*N* = 5 for each treatment).

Table 2 Phospholipid percentage distribution in fish food and beef liver

	PE	PI	PS	PC	SM	LPC
Fish food	3.13	5.82	2.91	58.5	12.1	17.6
Beef liver	37.5	4.28	7.22	42.8	8.27	0

PE—phosphatidylethanolamine. PI—phosphatidylinositol. PS—phosphatidylserine. PC—phosphatidylcholine. SM—sphingomyelin. LPC—lysophosphatidylcholine.

Results

Protein and lipid contents of beef liver and fish food diets are reported in *Table 1*. The two diets differed both in protein and lipid content. The fish food contained a higher amount of proteins, cholesterol, and triglycerides. In contrast, beef liver was richer in phospholipids. Percentage distribution of phospholipids, reported in *Table 2*, indicated a higher content of phosphatidylethanolamine (PE) in beef liver and the presence of lysophosphatidylcholine (LPC) in fish food.

The fatty acid composition of triglycerides and phospholipids from the two diets are reported in *Table 3*. Both lipid classes from fish food were richer in ω-3 fatty acids (about 30% vs. 4% of beef liver), mainly represented by C18:4, C20:4, C20:5, and C22:6.

Results of the chemical analysis of 6-day-old embryos are reported in *Table 4*. Higher amount (*P* < 0.05) of proteins, triglycerides, and phospholipids (as lipid phosphorus) were present in the embryos obtained from the fish-food fed mothers. In contrast, no differences were present in cholesterol and glycolipid contents.

Significant differences (*P* < 0.01) were observed in the phospholipid distribution (*Table 5*). The fish food embryos had higher percentages of phosphatidylinositol (PI) and sphingomyelin (SM), and a lower percentage of phosphatidylserine (PS) compared with embryos of the beef liver fed mothers.

Fatty acid weight percentages of the different phospholipid classes are reported in *Table 6*. Embryos from mothers fed fish food had a higher weight percentage of C20:3 + C20:4 ω-6 and a lower weight percentage of C18:0 fatty acids than embryos from mothers fed beef liver. However, weight percentage of total ω-3 fatty acids in the phospholipid fractions from embryos of the two groups were similar.

Discussion

Xenopus laevis is an animal model widely used in developmental studies because it is easy to handle and the *in vitro* fertilization allows for embryo collection at the same stage of development. The dietary management of this amphibian, however, is not well established. Different sources of foods, such as liver, heart, and commercial foods, have been used without taking into account the possible effects of maternal diet on embryonic lipid composition or general embryonic metabolism.

Table 3 Percentage fatty acid distribution of triglycerides and phospholipids from fish food and beef liver

	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3 ω6	C18:3 ω3	C20:0	C18:4 ω3	C20:4 + C20:3 ω6	C20:4 ω3	C20:5 ω3	C22:5	C22:6	Total ω	Others
TG FA																	
Fish food	5.29	18.57	6.85	4.47	19.74	13.66	2.03	2.62	0.00	4.61	0.54	8.04	5.70	0.89	5.79	27.65	1.20
Beef liver	4.86	28.00	2.98	10.31	26.19	22.62	0.00	0.00	1.93	0.00	3.12	0.00	0.00	0.00	0.00	0.00	0.00
PL FA																	
Fish food	4.76	18.75	6.47	5.04	20.48	14.28	2.78	2.69	0.00	4.53	0.00	8.44	5.08	1.31	5.39	27.45	0.00
Beef Liver	0.12	11.16	0.37	34.93	10.38	25.23	0.00	0.00	1.11	0.00	10.09	0.00	0.00	2.43	1.38	3.80	2.81

TG FA—triglyceride fatty acids. PL FA—phospholipid fatty acids.

Although embryonic development takes place independently of the mother's body, the embryos do not eat during the first 6 days of development. The metabolic requirements for growth are satisfied by the utilization of the carbohydrates, proteins, and lipids stored in the egg. Thus, the diet utilized by the mother before ovulation takes place could influence the chemical composition and, possibly, the development of the embryo.

In our study, we compared two different diets used in many laboratories: beef liver and commercial fish food. The latter is richer in ω-3 fatty acids. Data from the literature indicate the importance of ω-3 fatty acids during central nervous system development.^{8,22,23}

Results of the present study clearly indicate that maternal diet influences the lipid composition and protein content of the amphibian embryo. Interestingly, the main differences were found in phospholipids. For example, changes were observed in both the content of bioactive phospholipids, such as PI, PS, and SM, and for the pattern of important fatty acids such as C:18:0 and C20:4.

PI and PS are important in the control of cell proliferation due to their synergistic action in the modulation of calcium-phospholipid dependent protein kinase C (PKC) activity.²⁴ PI is the precursor of phosphatidylinositol 4,5 bisphosphate (PIP₂); its hydrolysis, catalyzed by phospholipase C, generates two second messengers, inositoltriphosphate (IP₃) and DAG, which directly activate PKC.^{5,25} PS is required for the full activation of PKC; moreover, it removes the possible inactivation of phospholipase C by phosphatidylcholine (PC).^{26,27} In this view the lower content of PS in the fish food embryos may counterbalance the increase of proliferation rate due to the possible PKC activation by PI. Furthermore, in embryos from fish-food fed mothers we observed an increase of SM, and it is known that cell proliferation may also be influenced by SM content through its hydrolysis products.^{28–30}

No difference was found in the total ω-3 fatty acid percentage in the embryos despite its very high concentration in the fish food diet. The reasons could be various: The ω-3 fatty acids, present in the mother diet, are delivered to eggs in an unknown percentage; moreover the essential fatty acids (EFA) and highly unsaturated fatty acids (HUFA) metabolism in the early developmental phase in *Xenopus* as well as in other species is far from being completely understood. Finally ω-3 can be metabolized to bioactive products or incorporated in special tissues (e.g., retina). Embryos from fish-food fed mothers had significantly greater percentages of C20:4 ω-6 in all phospholipid classes. This result might indicate that ω-3 fatty acids influence the incorporation of arachidonic acid in the phospholipid (PL) fractions, particularly in PI where its content is doubled.

In conclusion, the constituents of the maternal diet affect the composition of embryos and must be considered when development of nonmammalian species is studied. In particular, maternal diet influences phospholipid pattern of the embryo, which also must be considered when biochemical experiments are conducted with these animal species.

Table 4 Composition of 6-day-old embryos from mothers fed with fish food and beef liver

µg/embryo	DNA	Proteins	Triglycerides	Cholesterol	Glycolipids	PLPi
Fish food	6.14 ± 0.23	206.9 ± 15.4*	4.06 ± 0.49*	10.9 ± 0.55	0.54 ± 0.03	1.36 ± 0.07*
Beef liver	5.71 ± 1.47	159.1 ± 25.8	1.35 ± 0.22	9.73 ± 1.08	0.68 ± 0.13	1.03 ± 0.09

* $P < 0.05$; $N = 5$ fish food vs. beef liver.
PLPi—phospholipidic phosphorus.

Table 5 Phospholipid percentage distribution of 6-day-old embryos from mothers fed with fish food and beef liver

	PE	PI	PS	PC	SM	LPC
Fish Food	23.4 ± 1.33	9.51 ± 1.06*	5.52 ± 0.54*	51.1 ± 1.34	9.83 ± 1.12*	1.99 ± 0.42
Beef Liver	25.9 ± 1.53	1.11 ± 0.20	12.9 ± 1.11	54.5 ± 2.54	3.17 ± 0.50	3.58 ± 0.56

* $P < 0.01$; $N = 5$ fish food vs. beef liver.

PE—phosphatidylethanolamine. PI—phosphatidylinositol. PS—phosphatidylserine. PC—phosphatidylcholine. SM—sphingomyelin. LPC—lysophosphatidylcholine.

Table 6 Percentage fatty acid distribution of phospholipids of 6-day-old embryos from mothers nourished with fish food (FF) or beef liver (BL)

	SM		PC		PS		PI		PE	
	FF	BL	FF	BL	FF	BL	FF	BL	FF	BL
C14:0 + C14:1	2.58	10.4*	0.75	0.85	1.82	2.81	2.47	4.09*	0.48	1.08*
C16:0	31.4	34.0	30.4	36.5 [†]	16.7	17.9	10.4	15.6 [†]	11.6	14.8*
C16:1	3.63	2.27	4.02	6.23*	2.38	1.76	1.61	1.96	1.07	1.37
C18:0	13.7	28.9 [†]	3.25	3.82 [†]	20.1	30.6 [†]	23.8	33.8 [†]	13.8	16.0 [†]
C18:1	19.9	10.2 [†]	21.8	21.8	13.1	15.7	13.4	12.7	14.3	15.8
C18:2C	8.79	2.66*	14.1	10.1 [†]	6.45	5.26	2.54	3.03	6.92	6.11
C18:3 ω6	1.38	3.87	0.42	0.32	0.42	1.53	0.52	1.68	0.33	0.61
C18:3 ω3	1.74	2.62	0.32	1.28	0.92	1.23	0.44	1.19	0.30	0.99
C20:3 + C20:4 ω6	11.0	3.61*	16.8	11.4 [†]	15.6	11.4*	29.4	16.5 [†]	30.5	21.1 [†]
C20:5 ω3	ND	ND	0.95	1.72	1.36	2.38 [†]	0.51	ND [†]	1.61	5.50
C22:5 ω3	ND	ND	0.84	1.04	1.68	ND [†]	1.94	ND [†]	2.60	2.60
C22:6 ω3	3.43	1.50	4.71	4.14	11.2	9.38	6.31	9.37*	13.3	12.2
Total ω3	5.17	4.12	6.82	8.19	15.2	13.0	9.20	10.6	17.5	21.3
OTHERS	2.42	ND	1.68	0.81	8.22	ND	6.56	ND	3.50	1.82

* $P < 0.05$; [†] $P < 0.01$; $N = 5$ FF vs. BL.

SM—sphingomyelin. PC—phosphatidylcholine. PS—phosphatidylserine. PI—phosphatidylinositol. PE—phosphatidylethanolamine. ND—not detected.

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