Oestradiol-induced changes in the composition of phospholipid classes of quail oviduct: specific replacement of arachidonic acid by docosahexaenoic acid in alkenylacyl-glycerophosphoethanolamine

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The phospholipid composition and the molecular species of the major subclasses of ethanolamine and choline glycerophospholipids were determined during the natural or oestradiol-induced development of the quail oviduct. The phospholipid concentration increased significantly during oviduct development, and the proportion of ethanolamine glycerophospholipids (EPL) remained constant while that of choline glycerophospholipids increased. The immature oviduct contained the majority of its endogenous arachidonic acid mass (71 %) in EPL, mainly in alkenylacyl-glycerophosphoethanolamine (alkenylacyl-GPE) (49 % of the total). Oestrogen treatment induced the depletion of

20:4,*n*–6 specifically from this pool, which indicates the biological importance of 20:4,*n*–6 molecular species in alkenylacyl-GPE as substrates for the oviduct phospholipases activated by oestradiol, and suggests that this EPL subclass is involved in the oestrogen-induced cell proliferation. Another striking result was the marked increase in 22:6,*n*–3 EPL molecular species following the oestradiol treatment and more particularly the strict substitution of 20:4,*n*–6 by 22:6,*n*–3 in alkenylacyl-GPE. We speculate that alkenylacyl-GPE molecular species containing 22:6,*n*–3 may participate in the arrest of oestrogen-induced proliferation.

INTRODUCTION

Growth and differentiation of the oviduct and uterus are under steroid hormone control, and changes in lipid metabolism have been implicated. Oestradiol stimulates phosphatidylinositol (PI) turnover [1,2] and phospholipase A₂ (PLA₂) activity [3–5], and increases both the release of arachidonate from phospholipids and the production of eicosanoids [6,7]. These lipid compounds, which include prostaglandins, leukotrienes and also platelet-activating factor (PAF), play many physiological roles in reproduction. They are considered to be important mediators of implantation and decidualization (see [8] for a review) and have also been implicated together with thromboxane [9], non-esterified fatty acids [10], and phosphatidic acid (PA) and lysophosphatidic acids [11,12] in the proliferation of several cell types. PLA₂ itself is known to be involved in the mitogenic signal triggered by epidermal growth factor [13].

We have recently shown that the fatty acid composition of oviduct phospholipids changed significantly during sexual maturation or after oestradiol stimulation of immature quail [14]. Most notable was the progressive decrease in the proportions of arachidonic acid (20:4,*n*–6) and adrenic acid (22:4,*n*–6) and the accumulation of linoleic acid (18:2,*n*–6) and docosahexaenoic acid (22:6,*n*–3). A time-related reduction in the content of 20:4,*n*–6 after oestradiol injection has also been reported in the mouse and rat uterus [15,16], and this reduction is relevant to the stimulation of PLA₂ activity and the increased synthesis and release of 20:4,*n*–6 oxygenated derivatives. Despite these studies, little is known about the distribution of 20:4,*n*–6 and other polyunsaturated fatty acids in diacyl and

ether phospholipids of the oviduct and uterus, and about the occurrence of a phospholipid class primarily responsible for the 20:4,*n*–6 release after oestrogen stimulation.

In the present study we investigated the contents and the fatty acyl chain compositions of alkenylacyl-, alkylacyl- and diacyl-*sn*-glycerophosphocholine (GPC) and -*sn*-glycerophosphoethanolamine (GPE) in the oviduct of immature, oestradiol-treated and laying quails. We show that in the immature quail oviduct the majority of its endogenous 20:4,*n*–6 mass resides in the alkenylacyl-GPE molecular species and that the oestrogen treatment induced a specific depletion of 20:4,*n*–6 from this pool and its exclusive replacement at the *sn*-2 position by 22:6,*n*–3.

MATERIALS AND METHODS

Materials

Phospholipase C from *Bacillus cereus* was purchased from Boehringer Mannheim (Meylan, France). L- α -Diheptadecanoyl phosphatidylethanolamine, L- α -diheptadecanoyl phosphatidylcholine, standard fatty acid methyl esters (FAME) and phospholipids, benzoic anhydride and 4-dimethylaminopyridine were obtained from Sigma (St. Louis, MO, U.S.A.). The solvents used were of h.p.l.c. grade from Fluka (Basel, Switzerland).

Animals and treatments

Female Japanese quails (*Coturnix coturnix japonica*; 17–50 days old) were maintained in groups at a constant temperature (23 °C) on a daily cycle of light (14 h) and darkness (10 h). Chicken powder chow (UAR 115, Villemoisson, France) and water were

Abbreviations used: CPL, choline glycerophospholipids; DMA, dimethylacetals; EPL, ethanolamine glycerophospholipids; FAME, fatty acid methyl esters; GPC, *sn*-glycero-3-phosphocholine; GPE, *sn*-glycero-3-phosphoethanolamine; OB, oestradiol benzoate; PA, phosphatidic acid; PAF, platelet-activating factor; PI, phosphatidylinositol; PLA₂, phospholipase A₂; PS, phosphatidylserine; RRT, relative retention time; RT, retention time; Sph, sphingomyelin.

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provided *ad libitum*. Under these conditions, the sexual maturation of the quail started after 21 days and reached the laying state at 45 days. Hormonal treatment was given to immature quails (17 days old) only. Oestradiol benzoate (OB; 0.5 mg/kg dissolved in olive oil) was injected intramuscularly in a total volume of 0.1 ml once a day for three consecutive days. Controls received the vehicle alone. Treated and control animals were killed 24 h after the last injection. The oviduct was excised and the magnum was dissected, weighed and used immediately or stored in liquid nitrogen until lipid analysis. For this study, three groups of animals were considered: immature quails (controls), OB-treated animals and laying birds (45–50 days old).

Tissue extraction

The magnum (70–250 mg of pooled or individual tissues) was homogenized using a glass–glass tissue homogenizer (Kontes P22; Polylabo P. Block, Strasbourg, France) in 6 ml of chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene and internal standards (10–30 µg of L- α -diheptadecanoyl phosphatidylcholine and L- α -diheptadecanoyl phosphatidylethanolamine). The homogenate was kept at 18 °C for 30 min and then centrifuged at 800 g for 10 min at 20 °C. The resulting pellet was dissolved into 6 ml of chloroform/methanol (2:1, v/v) and centrifuged. The two supernatants were combined and the resulting lipid extract was washed with 3 ml of 0.88% (w/v) KCl. The chloroform layer was removed, dried under vacuum and the residue redissolved in chloroform. Control assays (without tissue) were carried out for each series of experiments.

Separation of phospholipid classes

Phospholipids were purified from total lipid extracts using Sep-Pac (Waters, Milford, MA, U.S.A.) silica chromatography [17]. The individual phospholipid classes were then separated by t.l.c. on silica gel 60 plates (20 cm × 20 cm, 0.25 mm thick; Merck, Darmstadt, Germany) using chloroform/methanol/propan-2-ol/0.25% (w/v) KCl/triethylamine (90:30:75:18:54, by vol.) as the developing solvent. Bands were detected under u.v. light after spraying with 0.05% 2',7'-dichlorofluorescein in methanol or were delimited according to the mobility of authentic standards spotted on the plate. The phospholipid bands corresponding to ethanolamine glycerophospholipids (EPL), PI, PA, phosphatidylserine (PS), choline glycerophospholipids (CPL) and sphingomyelin (Sph) were scraped off and the fatty acids were directly converted to methyl esters using 10% (v/v) BF₃ in methanol [14]. FAME were then analysed and quantified by g.l.c. after addition of pentadecanoic acid methyl ester (5–20 µg) as an internal standard in each sample.

Separation of phospholipid subclasses

CPL and EPL were separated by t.l.c. as described above, the gel was extracted twice using 4 ml of chloroform/methanol/water (5:5:1, by vol.) and phospholipids were converted to benzoate derivatives following phospholipase C hydrolysis [18,19]. Aliquots of the diradylglycerobenzoate samples were then analysed by h.p.l.c. according to the procedure described by Patton et al. [20]. Briefly, the diradylglycerobenzoates were dissolved into 100 µl of hexane and injected on to a 250 mm × 4.6 mm Lichrospher Si 100 (5 µm spherical particle size) column and eluted

with cyclohexane/hexane/methyl-*tert*-butyl ether/acetic acid (375:125:8.5:0.1, by vol.) at a flow rate of 0.8 ml/min. Peaks of alkenylacyl- [retention time (RT) 6.0 min], alkylacyl- (RT 7.6 min) and diacyl-benzoylglycerol (RT 13.2 min) were detected by absorbance at 230 nm and their area measured using a Shimadzu CR5A integrator. The proportion of each subclass was then computed assuming the same response factor.

Characterization of individual molecular species

Diradylglycerobenzoates were separated on silica gel 60 plates using toluene/hexane/diethyl ether (50:45:4, by vol.). The alkenylacyl-, alkylacyl- and diacyl-glycerobenzoates were visualized using a 2',7'-dichlorofluorescein spray. The corresponding bands were scraped, extracted three times using 2 ml of hexane/diethyl ether (1:1, v/v), and the solvent was evaporated under N₂. The distribution of molecular species of 1,2-diacyl-GPC, 1,2-diacyl-GPE and 1-*O*-alk-1'-enyl-2-acyl-GPE subclasses was analysed by reversed-phase h.p.l.c. [18]. Separation of molecular species within each subclass was achieved with a Kontron 420 h.p.l.c. system fitted with a C₁₈ precolumn and a 250 mm × 4.6 mm analytical column. Both columns were packed with 5 µm particle size Ultrabase-ODS (SFCC-Shandon, Eragny, France). Molecular species were quantified at 230 nm using a Kontron 432 u.v./visible detector coupled to a CR5A integrator. The 1,2-diacyl- and 1-*O*-alk-1'-enyl-2-acyl-glycerobenzoates were separated isocratically with acetonitrile/propan-2-ol (70:30, v/v) as solvent and a flow rate of 1.0 ml/min. Authentic standard molecular species were prepared as described above from standard phospholipids and analysed by h.p.l.c. and g.l.c. for identification of the major individual molecular species. Relative retention times (RRTs) were determined as described by Patton et al. [21] and a graphical relationship using log RRT was constructed for prediction of the elution sequence of molecular species [18,21,22].

Individual molecular species collected from h.p.l.c. were also identified as FAME and dimethylacetals (DMA) by g.l.c. after transmethylation with 10% BF₃/methanol and addition of a known amount of pentadecanoic acid methyl ester as an internal standard.

Gas chromatography

FAME and DMA were analysed with a Girdel 3000 (Suresnes, France) gas chromatograph coupled with a CR5A integrator. The capillary column was either a CPWax 52 CB (0.32 mm × 50 m; Chrompack, Middelburg, The Netherlands) or a SP2380 (0.32 mm × 30 m; Supelco, Bellefonte, PA, U.S.A.). The temperature was programmed from 145 °C to 225 °C at 1.2 °C/min; helium was used as the carrier gas (20 cm/s). FAME and DMA were identified by comparison with the RRTs of known standards. The percentage and concentration of each fatty acid were computed using the internal standard (pentadecanoic acid) and correction for recovery was determined by means of the heptadecanoic acid internal standard [14].

Statistical analysis

Data are expressed as means ± S.E.M. Statistical significance ($P < 0.05$) was tested using Mann–Whitney or Kruskal–Wallis non-parametric rank tests followed by the multiple comparison procedure described by Conover [23]. When compositions of molecular species were reported, data were first transformed according to Aitchinson [24] and standard methods were then applied.

RESULTS

The natural sexual maturation of the female quail is characterized by significant proliferation and differentiation of oviduct cells, resulting in a marked increase in magnum weight from 8.2 ± 0.4 mg (mean $\pm$ S.E.M.; 7 pools of 10 animals) for 21-day-old immature quails to 3652 ± 298 mg ($n = 7$) for laying quails. The magnum growth is clearly related to the circulating level of oestradiol [25,26], and treatment of immature quails for three

Table 1 Composition of CPL and EPL subclasses

Results are expressed as (a) μ mol/g of tissue and (b) mol% of total CPL or EPL subclasses, and are means $\pm$ S.E.M. of n assays. *, Significant difference ($P < 0.05$) compared with the immature group.

Subclass		Immature ($n = 5$)	OB-treated ($n = 7$)	Laying ($n = 7$)
Alkenylacyl-GPC	(a)	0.08 ± 0.01	0.06 ± 0.01	0.11 ± 0.01
	(b)	1.40 ± 0.12	$0.81 \pm 0.07^*$	$0.92 \pm 0.05^*$
Alkylacyl-GPC	(a)	0.37 ± 0.02	0.30 ± 0.02	$0.16 \pm 0.01^*$
	(b)	6.71 ± 0.47	$3.96 \pm 0.29^*$	$1.42 \pm 0.17^*$
Diacyl-GPC	(a)	5.04 ± 0.41	$7.17 \pm 0.71^*$	$11.36 \pm 0.89^*$
	(b)	91.87 ± 0.43	$95.19 \pm 0.52^*$	$97.66 \pm 1.47^*$
Alkenylacyl-GPE	(a)	2.23 ± 0.08	1.99 ± 0.12	$3.21 \pm 0.20^*$
	(b)	57.19 ± 1.73	$43.19 \pm 1.49^*$	$43.70 \pm 1.48^*$
Alkylacyl-GPE	(a)	0.14 ± 0.01	$0.31 \pm 0.02^*$	$0.39 \pm 0.01^*$
	(b)	3.59 ± 0.31	$6.59 \pm 0.67^*$	$5.48 \pm 0.60^*$
Diacyl-GPE	(a)	1.53 ± 0.10	$2.31 \pm 0.11^*$	$3.70 \pm 0.18^*$
	(b)	39.22 ± 1.89	$50.17 \pm 1.39^*$	$50.81 \pm 1.28^*$

consecutive days with 0.5 mg/kg OB per day increased the magnum weight to 374 ± 55 mg ($n = 8$).

Phospholipid composition

The natural development of the quail oviduct is accompanied by a significant increase in the tissue concentration of total phospholipids (from 12.6 ± 0.6 μ mol/g of tissue in immature quails to 21.6 ± 1.5 μ mol/g in laying birds). The immature undifferentiated oviduct phospholipids largely comprised CPL and EPL, which made up 44.3 ± 1.3 and $33.7 \pm 1.5\%$ of total phospholipids respectively. During the oviduct development, the proportion of CPL increased ($P < 0.05$) and represented $53.2 \pm 1.6\%$ of total phospholipids in laying birds, whereas the proportion of EPL remained constant. Sph, PS and PA+PI represented 4.8 ± 0.5 , 6.3 ± 0.6 and $8.7 \pm 0.5\%$ of total phospholipids in immature tissue respectively. These proportions were significantly reduced in fully developed oviduct (1.9 ± 0.1 , 4.0 ± 0.2 and 3.2 ± 0.2 respectively).

In OB-treated quails, the concentrations of total phospholipids and CPL (15.0 ± 0.8 and 7.5 ± 0.8 μ mol/g of tissue respectively) were significantly increased, but less than in the laying group. The proportion of PA+PI was also reduced whereas those of EPL, Sph and PS were unaffected.

EPL and CPL subclasses

The composition of CPL and EPL subclasses are listed in Table 1. In the oviduct of immature animals, CPL comprised mainly diacyl (91.9%) and alkylacyl (6.7%) subclasses. Interestingly, during natural and OB-induced development of the oviduct, the concentration and proportion of alkylacyl-GPC decreased whereas those of 1,2-diacyl-GPC increased.

EPL from immature oviducts comprised mainly alkenylacyl (57.2%) and diacyl (39.2%) subclasses. The OB treatment induced a significant increase in concentrations and proportions of alkylacyl- and diacyl-GPE, and significantly reduced that of alkenylacyl-GPE. Similar changes in EPL subclasses occurred throughout natural oviduct development.

Changes in CPL and EPL molecular species during sexual maturation and following OB treatment

Representative chromatograms of the reverse-phase h.p.l.c. separation of diacyl-GPC and alk-1-enylacyl-GPE individual molecular species are shown in Figure 1. The fatty acid composition of molecular species of diacyl-GPC and -GPE in immature, OB-treated and laying quail oviducts are listed in Table 2. The major molecular species in diacyl-GPC of immature oviducts were 16:0/18:1 (39.4 mol%), 16:0/18:2, $n=6$ (16.1 mol%) and 18:0/18:2, $n=6$ (13.7 mol%). In OB-treated animals, the only significant variation in the major molecular species was a small increase in 16:0/18:2, $n=6$ (+18%). Fully maturation of the oviduct (laying) resulted in a large increase of the 16:0/18:2, $n=6$ molecular species (+170%) and a reciprocal significant decrease of the 18:0/18:2 (−73%), 18:0/20:4, $n=6$ (−70%), 18:0/18:1 (−56%) and 16:0/18:1 (−20%) molecular species.

In diacyl-GPE, the major molecular species of immature oviducts were 18:0/20:4, $n=6$ (39.4 mol%), 16:0/18:1 (20.9 mol%) and 16:0/18:2, $n=6$ (11.0 mol%). The OB treatment decreased the proportions of 16:0/18:2, $n=6$ (−32%) and 16:0/22:6, $n=3$ (−63%) species and increased that of 18:0/22:6, $n=3$ (+152%). In laying birds, the proportion of 18:0/20:4, $n=6$ was significantly reduced (−66%) whereas those of 18:1/22:6, $n=3$ (+560%) and 18:0/22:6, $n=3$ (+278%) species were markedly increased.

Figure 1 H.p.l.c. separation of (a) diacyl-GPC and (b) alkenylacyl-GPE molecular species from quail oviduct

Refer to Tables 2 and 3 for peak identification. *, Peak corresponding to 17:0/17:0 which was the internal standard.

Table 2 Composition of diacyl-GPC and diacyl-GPE molecular species of immature, OB-treated and laying quail oviducts

Peak numbers correspond to those shown in Figure 1(a). Results are expressed as mol% of total subclasses and are means $\pm$ S.E.M. of three independent assays. *, Significant difference ($P < 0.05$) compared with the immature group; tr, trace amount.

Peak no.	Molecular species	Immature		OB-treated		Laying	
		GPC	GPE	GPC	GPE	GPC	GPE
1	16:1/22:6, <i>n</i> -3	0.31 $\pm$ 0.04	1.14 $\pm$ 0.13	0.26 $\pm$ 0.02	1.49 $\pm$ 0.18	0.05 $\pm$ 0.01*	0.90 $\pm$ 0.08
2	18:2, <i>n</i> -6/20:4, <i>n</i> -6	0.10 $\pm$ 0.01	0.18 $\pm$ 0.03	0.15 $\pm$ 0.01	0.47 $\pm$ 0.06*	0.03 $\pm$ 0.01	0.63 $\pm$ 0.07*
3	18:1/22:6, <i>n</i> -3	0.61 $\pm$ 0.04	1.93 $\pm$ 0.19	1.25 $\pm$ 0.10*	2.03 $\pm$ 0.22	1.79 $\pm$ 0.19*	12.73 $\pm$ 1.28*
4	18:2, <i>n</i> -6/18:2, <i>n</i> -6	0.34 $\pm$ 0.04	tr	0.32 $\pm$ 0.02	tr	0.58 $\pm$ 0.07	tr
	16:0/22:6, <i>n</i> -3	tr	3.33 $\pm$ 0.29	tr	1.25 $\pm$ 0.09*	tr	tr
5	18:1/20:4, <i>n</i> -6	1.23 $\pm$ 0.10	2.37 $\pm$ 0.21	1.41 $\pm$ 0.13	3.62 $\pm$ 0.41	1.04 $\pm$ 0.12	2.49 $\pm$ 0.20
6	16:0/20:4, <i>n</i> -6	6.39 $\pm$ 0.67	4.98 $\pm$ 0.51	6.22 $\pm$ 0.49	6.01 $\pm$ 0.25	6.78 $\pm$ 0.41	4.29 $\pm$ 0.56
7	16:0/22:5, <i>n</i> -6	tr	0.18 $\pm$ 0.01	0.19 $\pm$ 0.03	0.41 $\pm$ 0.06	tr	tr
8	18:1/18:2, <i>n</i> -6	3.28 $\pm$ 0.24	tr	4.37 $\pm$ 0.32	tr	2.91 $\pm$ 0.22	tr
	18:0/22:6, <i>n</i> -3	tr	3.17 $\pm$ 0.04	tr	8.00 $\pm$ 0.68*	tr	11.98 $\pm$ 1.42*
9	16:0/18:2, <i>n</i> -6	16.05 $\pm$ 0.82	11.05 $\pm$ 0.88	18.93 $\pm$ 0.61*	7.50 $\pm$ 0.68*	43.24 $\pm$ 1.48*	13.02 $\pm$ 1.12
10	16:0/22:4, <i>n</i> -6	1.32 $\pm$ 0.21	0.98 $\pm$ 0.29	1.26 $\pm$ 0.09	0.93 $\pm$ 0.07	0.80 $\pm$ 0.07*	0.12 $\pm$ 0.04*
11	18:0/20:4, <i>n</i> -6	5.70 $\pm$ 0.49	39.41 $\pm$ 1.50	4.69 $\pm$ 0.35	36.41 $\pm$ 1.92	1.73 $\pm$ 0.09*	13.21 $\pm$ 1.37*
12	18:0/22:5, <i>n</i> -6	0.83 $\pm$ 0.14	0.57 $\pm$ 0.07	0.68 $\pm$ 0.08	0.58 $\pm$ 0.11	0.89 $\pm$ 0.05	0.12 $\pm$ 0.01*
13	18:1/18:1	2.60 $\pm$ 0.31	2.05 $\pm$ 0.20	3.03 $\pm$ 0.33	0.90 $\pm$ 0.07*	1.91 $\pm$ 0.10*	4.99 $\pm$ 0.41*
14	16:0/18:1	39.43 $\pm$ 1.58	20.87 $\pm$ 1.39	39.05 $\pm$ 1.80	19.38 $\pm$ 1.54	31.77 $\pm$ 1.09*	26.08 $\pm$ 1.81*
15	18:0/18:2, <i>n</i> -6	13.70 $\pm$ 1.12	tr	10.52 $\pm$ 0.58	0.13 $\pm$ 0.03	3.74 $\pm$ 0.09*	tr
16	18:0/18:1	6.12 $\pm$ 0.44	7.79 $\pm$ 0.51	6.49 $\pm$ 0.41	9.07 $\pm$ 1.02	2.71 $\pm$ 0.18*	9.38 $\pm$ 0.92

Table 3 Composition of alkenylacyl-GPE molecular species of immature, OB-treated and laying quail oviducts

Peak numbers correspond to those shown in Figure 1(b). Results are expressed as mol% of total subclasses and are means $\pm$ S.E.M. of three independent assays. *, Significant difference ($P < 0.05$) compared with the immature group.

Peak no.	Molecular species	Immature	OB-treated	Laying
1	18:1/22:6, <i>n</i> -3	0.26 $\pm$ 0.01	1.03 $\pm$ 0.07*	0.53 $\pm$ 0.02*
2	16:0/22:6, <i>n</i> -3	2.06 $\pm$ 0.12	4.26 $\pm$ 0.49*	2.12 $\pm$ 0.32
3	18:1/22:5, <i>n</i> -3	tr	0.18 $\pm$ 0.01	0.33 $\pm$ 0.02
4	18:1/20:4, <i>n</i> -6	3.87 $\pm$ 0.23	3.12 $\pm$ 0.28	2.02 $\pm$ 0.17*
5	16:0/20:4, <i>n</i> -6	14.31 $\pm$ 1.12	9.50 $\pm$ 0.85*	4.56 $\pm$ 0.43*
6	16:0/22:5, <i>n</i> -6	1.26 $\pm$ 0.10	1.78 $\pm$ 0.32	1.23 $\pm$ 0.23
7	18:0/22:6, <i>n</i> -3	4.65 $\pm$ 0.39	12.24 $\pm$ 1.17*	14.99 $\pm$ 1.45*
8	18:1/18:2, <i>n</i> -6	1.26 $\pm$ 0.09	0.32 $\pm$ 0.07*	0.67 $\pm$ 0.06*
9	16:0/18:2, <i>n</i> -6	5.00 $\pm$ 0.41	3.31 $\pm$ 0.37*	3.39 $\pm$ 0.42
10	16:0/22:4, <i>n</i> -6	3.75 $\pm$ 0.43	3.11 $\pm$ 0.42	4.89 $\pm$ 0.44
11	18:0/20:4, <i>n</i> -6	40.36 $\pm$ 1.59	35.23 $\pm$ 1.01*	27.94 $\pm$ 1.78*
12	18:0/22:5, <i>n</i> -3	1.44 $\pm$ 0.43	3.77 $\pm$ 0.49*	3.05 $\pm$ 0.44
13	18:0/22:4, <i>n</i> -6	17.01 $\pm$ 0.52	14.46 $\pm$ 0.12*	15.67 $\pm$ 0.23*
14	16:0/18:1	1.56 $\pm$ 0.16	3.58 $\pm$ 0.53*	4.47 $\pm$ 0.39*
15	18:0/18:1	3.21 $\pm$ 0.22	2.85 $\pm$ 0.24	11.42 $\pm$ 1.25*

The molecular species of alkenylacyl-GPE are listed in Table 3. In immature tissue, the most abundant molecular species were 18:0/20:4,*n*-6 (40.4 mol%), 18:0/22:4,*n*-6 (17.0 mol%) and 16:0/20:4,*n*-6 (14.3 mol%) respectively. OB treatment significantly decreased the proportions of 16:0/20:4,*n*-6 (-34%) and to a lesser extent 18:0/20:4,*n*-6 (-13%) and 18:0/22:4,*n*-6 (-15%). By contrast, the proportions of all the 22:6,*n*-3-containing species were strongly increased. Complete sexual maturation was characterized by a marked decrease of the major 20:4,*n*-6-containing species (18:0/20:4,*n*-6, -31%; 16:0/20:4,*n*-6, -68%) and by increased proportions of

Table 4 Changes in 20:4,*n*-6, 22:6,*n*-3, 18:1 and 18:2,*n*-6 content at the *sn*-2 position of major phospholipid subclasses during sexual maturation and following OB treatment

Results are expressed as mmol/mol of each subclass and were computed from results presented in Tables 2 and 3.

Fatty acid at <i>sn</i> -2 position	Phospholipid subclass	Immature	OB-treated	Laying
20:4, <i>n</i> -6	Diacyl-GPC	134	125	96
	Diacyl-GPE	469	465	206
	Alkenylacyl-GPE	585	478	345
22:6, <i>n</i> -3	Diacyl-GPC	9	15	33
	Diacyl-GPE	96	128	256
	Alkenylacyl-GPE	68	175	176
18:1	Diacyl-GPC	482	486	364
	Diacyl-GPE	304	294	405
	Alkenylacyl-GPE	48	64	159
18:2, <i>n</i> -6	Diacyl-GPC	338	341	505
	Diacyl-GPE	111	75	130
	Alkenylacyl-GPE	63	36	41

18:0/22:6,*n*-3 (+222%), 18:0/18:1 (+255%) and 16:0/18:1 (+186%).

The proportions of 20:4,*n*-6, 22:6,*n*-3, 18:1 and 18:2,*n*-6 at the *sn*-2 position of major CPL and EPL subclasses in immature, OB-treated and laying quail oviduct were computed from our results and are summarized in Table 4. The major effect of the OB treatment was the specific depletion of 20:4,*n*-6 from alkenylacyl-GPE (-107 mmol/mol) and its replacement by 22:6,*n*-3 (+107 mmol/mol). The complete development of the oviduct from immature to laying states resulted in stronger depletion of arachidonate than that observed after the OB treatment, and both diacyl- and alkenylacyl-GPE were depleted (-263 and -240 mmol/mol respectively). In alkenylacyl-GPE,

20:4,*n*-6 was replaced by 22:6,*n*-3 (+108 mmol/mol) and 18:1 (+111 mmol/mol). In diacyl-GPE, arachidonate was also replaced by 22:6,*n*-3 (+160 mmol/mol) and 18:1 (+101 mmol/mol). From immature to laying states, the main variation in diacyl-GPC was a depletion of 18:1 (-118 mmol/mol) and to a lesser extent of 20:4,*n*-6 (-38 mmol/mol) which were replaced by 18:2,*n*-6 (+167 mmol/mol).

DISCUSSION

Previous studies from our laboratory [25,26] have shown that growth and maturation of the quail oviduct is divided into three phases. The first phase (from 15 mg up to 300 mg magnum weight) consists of a very active proliferation of epithelial cells associated with a regular increase in the circulating level of oestradiol and a constant low level of progesterone. This phase was mimicked, in our study, by OB injection into immature quails [27]. Then cellular proliferation decreases and egg white proteins synthesis takes place in glandular cells. This second phase (up to 1.5 g magnum weight) is related to increasing levels of oestradiol and progesterone. Further growth involves only the accumulation of secretory products and this third phase is associated with a constant level of oestradiol and increasing values of plasma progesterone.

The phospholipid concentration increased significantly in the oviduct during natural or OB-induced development and proportions of the different phospholipid classes were altered. The molar percentage of CPL increased with differentiation whereas proportions of PI + PA, PS and Sph decreased. The proportions of EPL remained constant.

Analysis of CPL subclasses indicated that only the proportion of diacyl-GPC increased whereas that of alkylacyl-GPC markedly decreased during the sexual maturation (-79% from immature to laying states) or after the OB treatment (-41%). This is of particular interest because the cellular pool of alkyl-arachidonoyl-GPC is the precursor pool of PAF [28]. PAF of uterine origin [29,30] and PAF-binding sites in rabbit endometrium [31] have been described. More recently, regulation of PAF production in oophorectomized rat uterus by oestradiol has been suggested [16]. The decrease of the oviduct pool of alkylacyl-GPC during sexual maturation and after the OB treatment of immature animals suggests that large amounts of PAF may be synthesized throughout the oviduct development with only partial or no renewal of the precursor. The physiological role of endogenous PAF in the oviduct is still unknown, but as PAF is a potent vasoactive compound [32], it might affect the oviduct vasculature. It is noteworthy that alkylacyl-GPE varied in the opposite direction; its proportion increased by 83% after the OB treatment.

In the immature oviduct, ethanolamine glycerophospholipids were predominantly composed of alkenylacyl (57%) and diacyl (39%) subclasses. During sexual development, the molar percentage of alkenylacyl-GPE decreased by 24% while that of diacyl-GPE increased by 29% (laying versus immature group). The same changes in proportions of EPL subclasses were induced by a 3 day OB treatment, which indicates that these alterations occur specifically during the proliferative phase of the tissue and are probably under oestrogen control.

In the immature quail, < 30% of the 20:4,*n*-6 content of the oviduct is in CPL and > 70% in EPL. As ascertained by g.l.c. and reversed-phase h.p.l.c., only 13% of diacyl-GPC molecular species contained 20:4,*n*-6 esterified at the *sn*-2 position, while the proportion reached 47% in diacyl-GPE molecular species and more than 58% in alkenylacyl-GPE molecular species. In

the quail oviduct, alkenylacyl-GPE represented the major phospholipid storage depot for 20:4,*n*-6. This distribution of 20:4,*n*-6 is quite different from that observed in the oophorectomized rat uterus [16] where arachidonate-CPL is predominant. However, similar high amounts of arachidonic acid in alkenylacyl-GPE have been described in platelets, endothelial cells, kidney cells, alveolar macrophages and the vascular smooth muscle [33-37]. During the sexual maturation or after oestrogen stimulation, one of the most notable changes was the decreased content of 20:4,*n*-6 in oviduct PL. Similar results have been recently reported in the mouse [15] and rat [16] uterus treated with oestrogen and must be connected with the activation of PLA₂ and the synthesis and release of prostaglandins occurring during oestrogen stimulation [3-7].

The most striking results of our studies were that, after the oestrogen treatment, arachidonic acid was (a) depleted almost exclusively from 16:0/20:4,*n*-6 and 18:0/20:4,*n*-6 alkenylacyl-GPE molecular species, and (b) almost exclusively replaced by 22:6,*n*-3 at the *sn*-2 position. During further development and differentiation of the oviduct, arachidonic acid was mainly depleted from diacyl-GPE and replaced by 22:6,*n*-3 and 18:1. The specific loss of 20:4,*n*-6 from alkenylacyl-GPE during the oestrogen stimulation suggests the occurrence of an oestrogen-dependent plasmalogen-selective PLA₂ in the oviduct, a hypothesis which is under investigation. Selective plasmalogen phospholipases have been described in canine myocardium and sheep platelets [38,39]. These results also suggest an important biological role for alkenylacyl-GPE in oestrogen-induced proliferation. It should be noted that high levels of ether phospholipids are found in neoplastic tissues and that their proportions increase with tumour growth [40].

Another striking result was the large increase in 22:6,*n*-3 EPL molecular species following the OB treatment, and more particularly the strict substitution of the 20:4,*n*-6 by 22:6,*n*-3 in alkenylacyl-GPE. The physiological functions of docosa-hexaenoic acid are still unclear. Highly unsaturated fatty acids have specific effects on the lipid bilayer structure and dynamics with potential influence on membrane enzyme activities [41], and there is evidence that *n*-3 fatty acids competitively inhibit the uterine *n*-6 essential fatty acid metabolism [42]. 22:6,*n*-3 may also be converted by lipoxygenases to form *n*-3 hydroxy derivatives which antagonize the *n*-6 derivative activity [43]. However, the biological relevance of such an effect is questionable as 22:6,*n*-3 is minimally mobilized from phospholipids under stimulation by agonists involving phospholipases in their mechanism of action [44,45]. More recently 22:6,*n*-3 was shown to inhibit the growth of human breast and prostate cancer cell lines [10,46]. The data presented here suggest that the induction of oestrogen target cell proliferation in the oviduct involves the participation of 20:4,*n*-6 from alkenylacyl-GPE, while 22:6,*n*-3 molecular species from the subclass might be associated with cell growth inhibition. Further investigations are needed to check this working hypothesis.

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