Comp. Biochem. Physiol. Vol. 115B, No. 2, pp. 255–266, 1996

ISSN 0305-0491/96/$15.00
PII S0305-0491(96)00111-3

In Vitro Metabolism of ^{14}C-Polyunsaturated Fatty Acids in Midgut Gland and Ovary Cells from *Penaeus kerathurus* Forskål at the Beginning of Sexual Maturation

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ABSTRACT. The incorporation and metabolism via the desaturase/elongase pathway of [1-^{14}C]18:2(n-6) linoleic acid (LA), [1-^{14}C]18:3(n-3) linolenic acid (LNA), [1-^{14}C]20:4(n-6) arachidonic acid (AA) and [1-^{14}C]20:5(n-3) eicosapentaenoic acid (EPA) were studied in midgut gland and ovary cell suspensions from wild-caught adult females of *Penaeus kerathurus* Forskål at the beginning of sexual maturation. The incorporation and recovery of radioactivity in total lipids of midgut gland cells was greater for [1-^{14}C]LA and [1-^{14}C]LNA than for [1-^{14}C]AA or [1-^{14}C]EPA, indicating a preferential retention of C_{18}-polyunsaturated fatty acids (PUFAs) in this organ. The recovery of radioactivity from all PUFA decreased during the time course. The incorporation of [1-^{14}C]PUFAs into total polar lipids in midgut gland cells increased during the time course (from 33.4% to 65.2%) with a concomitant decrease into total neutral lipids. These changes were due to significantly increased incorporation into phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI), with significantly decreased incorporation into triacylglycerol (TAG). [1-^{14}C]AA and [1-^{14}C]EPA were preferentially incorporated into PI, and [1-^{14}C]EPA was more important in phospholipid synthesis in midgut gland cells than [1-^{14}C]-labeled LA, LNA or AA. The incorporation and recovery of radioactivity in total lipids from ovary cells was significantly lower than in midgut gland cells but significantly increased during the time course with all the [1-^{14}C]PUFAs. The distribution of radioactivity from [1-^{14}C]AA and [1-^{14}C]EPA in ovary cells showed preferential retention into polar lipid classes compared with [1-^{14}C]LA or [1-^{14}C]LNA, and the general pattern was of net synthesis of PC at the expense of PE, free fatty acid and TAG. The results indicated that midgut gland and ovary cells have only a limited ability to convert C_{18}-PUFA to C_{20}- and C_{22}-highly unsaturated fatty acid. The recovery of radioactivity in 22:6(n-3) was ~10-fold greater with [1-^{14}C]EPA than with [1-^{14}C]LNA as precursor. Substantial amounts of radioactivity were recovered in 24:5(n-6), 24:5(n-3) and 24:6(n-3), particularly in cells incubated with [1-^{14}C]EPA, indicating that the conversion of EPA to DHA in both organs may occur by a pathway using Δ^6-desaturase activity rather than by a Δ^4-desaturation. COMP BIOCHEM PHYSIOL 115B;2:255–266, 1996.

KEY WORDS. Polyunsaturated fatty acid, metabolism, incorporation, lipid class, desaturation, elongation, midgut, ovary, sexual-maturation, *Penaeus kerathurus*

INTRODUCTION

The understanding of nutrition–reproduction interactions and the determination of nutrient requirements for successful maturation and spawning are necessary to enable the production of larval crustacean for grow-out operations. Lipids play several important roles in the biochemistry, metabolism and reproduction of decapod crustaceans and are an important component of the energetic and structural reserve materials that accumulate in the developing oocytes during oogenesis (1–3,5,7,8,14–16,21,26,29,33–36,42,50,52,56,57). In decapods, the midgut gland is the major lipid storage and metabolic center modifying dietary lipids (5,7,16,61), although during maturation the ovary becomes an additional center for lipid metabolism, including lipogenesis (16,46). Regarding ovarian lipids, it is not clear what proportion of the lipid in the eggs is derived directly from ingested food or previously stored reserves and what proportion is derived from *de novo* synthesized lipids (7,16,46). Thus, a controversy existed about the capacity of the midgut gland and ovary to synthesize fatty acids and particularly polyunsaturated fatty acids (PUFAs). Clarke (7), Shenker *et al.* (46) and Moreno *et al.* (31,32), using

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Abbreviations used—AA, all-*cis*-5,8,11,14-eicosatetraenoic acid (arachidonic acid, 20:4(n-6)); BHT, butylated hydroxytoulene; BSA, bovine serum albumin; C, free cholesterol; DHA, all-*cis*-4,7,10,13,16,19-docosahexaenoic acid (22:6(n-3)); DMEM, Dulbecco's modified Eagle's medium; EPA, all-*cis*-5,8,11,14,17-eicosapentaenoic acid (20:5(n-3)); FFA, free fatty acid; HBSS, Hank's balance salt solution; HPTLC, high-performance thin-layer chromatography; LA, all-*cis*-9,12-octadecadienoic acid (linoleic acid, 18:2(n-6)); LNA, all-*cis*-octadecatrienoic acid (linolenic acid, 18:3(n-3)); HUFA, highly unsaturated fatty acids ($\geq C_{20}$ and with ≥ 3 double bonds); PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PUFA, polyunsaturated fatty acid; TAG, triacylglycerol; TLC, thin-layer chromatography. Note: PC and PE represent diradyl (diacyl + alkenilacyl + alkylacyl) glycerophosphocoline and glycerophosphoethanolamine, respectively, whereas PA, PI and PS represent diacyl derivatives.

Received 12 January 1996; accepted 17 April 1996.

tritiated water, ^{14}C-acetate or ^{14}C-labeled palmitic, stearic or oleic acids as precursors, demonstrated that the calanoid copepod *Paracalanus parvus*, the polar shrimp *Chorismus antarcticus* and *Penaeus semisulcatus* are not capable of synthesizing PUFA and have to obtain them from their diet. In contrast, Whitney (64), Farkas and Nevenzel (12) and Farkas *et al.* (11) using ^{14}C-acetate detected *in vivo* and *in vitro* synthesis of PUFA in crustacean hepatopancreas and gill. Furthermore, although there has been abundant information showing the importance of PUFA for maturation (16), data on the metabolism of these particular fatty acids are few (4,22,51,55) and not related to reproduction.

In this context, and to achieve a better understanding of maturation, it is essential to determine the capacity of each crustacean species to desaturate and elongate C_{18}(n-3) and (n-6) fatty acids to essential C_{20} and C_{22} metabolites, as well as the contribution of the developing ovary (16). The present study investigated the incorporation and metabolism via the desaturase/elongase pathway of [1-^{14}C] linoleic acid (LA), [1-^{14}C] linolenic acid (LNA), [1-^{14}C] arachidonic acid (AA) and [1-^{14}C] eicosapentaenoic acid (EPA) in midgut gland and ovary cell suspensions from wild caught adult female of *Penaeus kerathurus* Forskål at the beginning of sexual maturation. A parallel aim of the present study was to determine the presence and levels of specific C_{24} highly unsaturated fatty acid (HUFA) that are intermediates in the putative pathway for the conversion of EPA to all-*cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA) without Δ^4-desaturase activity (62,63) to establish if this pathway was also operative in crustaceans.

MATERIALS AND METHODS

Experimental Animals

Wild adult females of *P. kerathurus* Forskål, caught in the Gulf of Cádiz (southwest Spain), near the mouth of the Guadalquivir river during the spring of 1994, were obtained from a commercial trawler. Females were transported alive to the laboratory and classified into different developmental stages according to ovary size and color (43). Only those females at maturation stage I that had mated were chosen for study. Average weight of these females was 12.9 ± 2.9 g, carapace length 30.3 ± 2.1 mm, midgut gland weight 0.8 ± 0.1 g and ovary weight 0.3 ± 0.1 g.

Total Lipid Extraction and Quantification

Lipids were extracted from preweighed ovaries and midgut glands by homogenization in chloroform/methanol (2:1, v/v), containing 0.01% butylated hydroxytoluene (BHT) as antioxidant, according to Folch *et al.* (13). Solvent was evaporated under a stream of nitrogen and lipid extracts desiccated overnight *in vacuo* before their mass was determined gravimetrically. Lipid extracts were redissolved in chloroform/methanol (2:1, v/v + BHT) at a concentration of 10 μg/μL and stored under an atmosphere of nitrogen at −20°C until analysis.

Lipid Class Separation and Quantification

Lipid classes were separated by high-performance thin-layer chromatography (HPTLC) using a single-dimension double development method described previously (58). The classes were quantified by charring followed by calibrated densitometry using a Shimadzu CS-9001PC dual-wavelength flying spot scanner (40).

Fatty Acid Analysis

Fatty acid methyl esters from total lipids were prepared by acid-catalyzed transmethylation for 16 h at 50°C (overnight), using nonadecanoic acid (19:0) as internal standard (6) and purified by thin-layer chromatography (TLC) as described by Tocher and Harvie (58). The fatty acid methyl esters were analyzed in a Hewlett-Packard 5890 A Series II gas chromatograph equipped with a chemically bonded (PEG) Omegawax 320 fused-silica wall coated capillary column (30 m × 0.32 mm ID, Supelco Inc., Bellefonte, PA, USA), using an on-column injection system and Flame Ionization Detector (FID). Hydrogen was used as the carrier gas with an oven thermal gradient from 180 to 230°C. Individual fatty acid methyl esters were identified by reference to the retention time of authentic commercial standards (Supelco Inc.) and a well-characterized fish oil and quantified using Hewlett Packard 3365 Chemstation software in a personal computer linked to the gas chromatograph.

Preparation of Isolated Midgut Gland and Ovary Cell Suspensions

Two entire midgut glands and eight ovaries were dissected out, placed into ice-cold Hank's balance salt solution (HBSS) (Ca^{2+} and Mg^{2+} free and supplemented with 1.75% NaCl) and finely chopped for every incorporation time course experiment, and one entire midgut gland and four ovaries were used for the metabolism of ^{14}C-labeled PUFA experiments. Mixed cell suspensions were produced from each tissue by mechanical sieving of the chopped tissue through sterile nylon gauzes of 250-μm mesh. The suspension was centrifuged at 300 **g** for 10 min at 4°C and the cell pellet resuspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 4 mM glutamine, antibiotics (50 IU/mL penicillin and 50 μg/mL streptomycin and 25 μg/mL fungizone) and 0.35% NaCl. Cells were resuspended in 22 mL and 15 mL of medium for incorporation time course and metabolic experiments, respectively. The number of animals used in each incorporation time course and metabolic experiment were eight and four, respectively.

Incubation of Midgut Gland and Ovary Cells with ^{14}C-Labeled PUFA

For the incorporation time course of [1-^{14}C]PUFA experiments into lipid classes, 5 mL of either midgut gland or ovary cell suspension had 1.5 μCi of either [1-^{14}C]LA, [1-^{14}C]LNA, [1-^{14}C]AA or [1-^{14}C]EPA added, carrier free in 15 μl ethanol (final concentration, 6 μM PUFA and 0.3% ethanol). For metabolic experiments, 3 mL of either midgut gland or ovary cell suspension had 1.3 μCi of either [1-^{14}C]LA, [1-^{14}C]LNA, [1-^{14}C]AA or [1-^{14}C]EPA added, carrier free in 13 μL ethanol (final concentration, 8 μM PUFA and 0.4% ethanol). Incubations were performed in a shaking water bath at 18°C. For the time course incorporation experiments, samples were taken at 1, 2, 4, 6 and 8 h of incubation. For metabolic experiments, samples were taken after 24-h incubations.

Lipid Extraction

Samples of suspension (1 and 3 mL in incorporation and metabolic experiments, respectively) had 5 mL ice-cold HBSS containing 1% fatty acid free bovine serum albumin (BSA) added. After mixing, the samples were centrifuged at 500 **g** for 2 min and the supernatant aspirated. The cells were washed once more with HBSS as above and total lipid extracted essentially according to Folch *et al.* (13) as described in Tocher *et al.* (60). Lipid content of each suspension was determined in 1-mL sample extracted in parallel, with the total lipid content determined gravimetrically.

Incorporation of Radioactivity Into Total Lipids and Lipid Classes

One fifth of the total lipid extracts were addded to 4 mL of Beckman Ready Safe™ scintillation liquid in Mini poly Q-vials to determine the radioactivity recovered in total lipids. The remainder of the total lipid extracts were applied in 1-cm streaks to HPTLC plates. Lipid classes were separated using the single-dimension double development method mentioned above (58). Lipid classes were visualized by brief exposure to iodine vapor, marked and the iodine removed under vacuum. Individual classes were scraped into scintillation vials, 4 mL of Beckman Ready Safe™ scintillation liquid added and radioactivity determined using a Beckman LS 5000 CE liquid scintillation analyzer. Results were corrected for counting efficiency and quenching of ^{14}C under these conditions.

Incorporation of Radioactivity Into PUFA

Fatty acid methyl esters from total lipid from 3 mL midgut gland and ovary cell suspensions were prepared by acid-catalyzed transmethylation as described above. Methyl esters were separated by argentation-TLC as described previously (39). The labeled bands were scraped into scintillation vials and their radioactivity determined as above. Identification of labeled bands was confirmed by using authentic unlabeled standards run on parallel plates, with visualization by charring as above. The identity of some bands was confirmed by gas chromatography (GC)-mass spectrometric analysis of picolinyl derivatives (17).

Protein Content

Samples of 1 mL of each cell suspension were taken for protein determination according to Lowry *et al.* (28).

Materials

[1-^{14}C]PUFA (all 50–53 mCi · mmol^{-1} and 99% pure) were obtained from NEN Dupont, Investigación Técnica Industrial S.A. (ITISA), Madrid, Spain. BHT, fatty acid free BSA, silver nitrate, potassium chloride, DMEM, HBSS, glutamine and antibiotics were obtained from Sigma Chemical Co., Ltd. Alcobendas, Madrid, Spain. TLC (20 × 20 cm × 0.25 mm) and HPTLC (10 × 10 cm × 0.15 mm) glass plates, precoated with silica-gel 60 (without fluorescent indicator), were purchased from Merck (Darmstadt, Germany). All solvents were HPLC grade and were obtained from Fluka, Alcobendas, Madrid, Spain.

Statistical Analysis

Results are presented as means ± SD of triplicate experiments. The data were checked for homogeneity of the variances by Bartlett test and, where necessary, the data were arc-sin transformed before further statistical analysis. Differences between values were analyzed by one-way ANOVA followed (where appropriate) by Tukey's multiple comparison test (65).

RESULTS

Gross Composition, Lipid Class Composition and Total Lipid Fatty Acid Composition in Midgut Gland and Ovary

The gross composition and lipid class composition in midgut gland and ovary of *P. kerathurus* at maturation stage I are presented in Table 1. The midgut gland was significantly richer in dry matter content, total lipid and total carbohydrate, whereas the ovary was significantly richer in total protein content. The proportions of total polar lipids and individual polar classes were significantly greater in ovary than in midgut gland. The opposite was the case with to total neutral lipids and individual neutral classes, with the exception of cholesterol that was relatively more abundant in ovary.

The fatty acids in total lipids from midgut gland were

TABLE 1. Gross composition (dry weight percentage) and lipid class composition (total lipid percentage) in midgut gland and ovary from *Penaeus kerathurus* females at stage of maturation I

	Midgut gland	Ovary
Dry weight	30.6 ± 1.2	20.4 ± 0.4*
Protein	63.5 ± 0.6	70.3 ± 1.7*
Lipid	23.1 ± 0.2	9.6 ± 1.1*
Carbohydrate	5.3 ± 0.4	3.7 ± 0.2*
Others	8.0 ± 0.4	16.4 ± 1.2*
Total polar lipids	20.8 ± 0.3	58.8 ± 2.8*
Phosphatidylcholine	9.5 ± 0.1	21.7 ± 0.4*
Phosphatidylethanolamine	5.8 ± 0.2	21.7 ± 2.1*
Phosphatidylserine	1.4 ± 0.1	3.9 ± 0.2*
Phosphatidylinositol	1.3 ± 0.1	3.5 ± 0.4*
Phosphatidic acid/Cardiolipin	1.9 ± 0.1	5.7 ± 0.5*
Glycosylglycerides	0.3 ± 0.1	nd
Sphingomyelin	0.4 ± 0.1	2.1 ± 0.2*
Total neutral lipids	72.9 ± 0.7	33.1 ± 1.0*
Cholesterol	10.8 ± 0.9	24.6 ± 1.0*
Free fatty acids	2.8 ± 0.1	1.9 ± 0.3*
Triacylglycerol	50.0 ± 1.0	6.5 ± 1.8*
Sterol esters	9.2 ± 1.4	nd

Data are means ± SD ($n = 3$). SD = 0.0 implies an SD of <0.05. *Significantly different ($p < 0.05$).

TABLE 2. Fatty acid composition (weight percentage) from total lipids of the midgut gland and ovary of *Penaeus kerathurus* females at stage of maturation I

Fatty acid	Midgut	Ovary
14:0	2.3 ± 0.9	4.4 ± 0.1*
15:0	0.9 ± 0.3	0.9 ± 0.0
16:0	12.4 ± 0.3	11.1 ± 0.7*
16:1n-7	7.6 ± 0.8	4.9 ± 0.5*
16:2	2.9 ± 0.6	2.3 ± 0.1
16:3	1.6 ± 0.4	1.2 ± 0.1
18:0	6.6 ± 0.7	7.2 ± 0.1
18:1n-9	5.5 ± 0.2	8.5 ± 0.8*
18:1n-7	5.7 ± 0.5	3.9 ± 0.1*
18:2n-6	1.1 ± 0.2	0.4 ± 0.0*
18:3n-3	0.5 ± 0.1	0.3 ± 0.1
18:4n-3	0.5 ± 0.0	0.4 ± 0.1
20:0	0.3 ± 0.1	0.4 ± 0.1
20:1n-9	2.9 ± 0.2	1.8 ± 0.2*
20:4n-6	2.1 ± 0.3	5.6 ± 1.2*
20:3n-3	0.3 ± 0.0	0.2 ± 0.0
20:4n-3	0.9 ± 0.1	0.2 ± 0.0*
20:5n-3	11.7 ± 1.7	13.6 ± 1.0
22:1n-11	1.6 ± 0.2	0.2 ± 0.0*
22:5n-6	0.5 ± 0.0	0.5 ± 0.1
22:5n-3	2.2 ± 0.1	1.4 ± 0.1*
22:6n-3	7.5 ± 0.9	8.8 ± 0.3
Total saturated	23.7 ± 0.7	25.9 ± 0.5*
Total monounsaturated	26.8 ± 1.3	20.0 ± 1.1*
Total polyunsaturated	36.8 ± 0.8	39.3 ± 1.6
Unknown	12.7 ± 0.6	14.8 ± 0.9*
Total (n-9)	9.1 ± 0.2	10.6 ± 0.7*
Total (n-7)	16.1 ± 1.4	9.3 ± 0.5*
Total (n-6)	9.6 ± 0.8	10.9 ± 1.7
Total (n-3)	27.2 ± 0.8	28.4 ± 0.2
Total (n-6)HUFA	3.6 ± 0.3	6.9 ± 1.4*
Total (n-3)HUFA	23.6 ± 1.0	24.7 ± 0.5

Data are means ± SD ($n = 3$). SD = 0 implies an SD of <0.05. *Significantly different ($p < 0.05$). HUFA, C_{20} and C_{22} fatty acids with at least three double bonds. Totals include some minor components not shown.

significantly poorer in saturated but richer in monounsaturated fatty acids than in ovary, but no significant differences were found between total polyunsaturated where the proportions of their major components EPA and DHA in both organs were not different. It is noteworthy that the proportion of AA was significantly higher in ovary (Table 2).

Time Course Incorporation of [1-^{14}C]PUFA Into Lipid Classes

The incorporation of radioactivity from [1-^{14}C]LA and [1-^{14}C]LNA into total lipids and individual lipid classes in midgut gland cell suspensions during the incubation period are presented in Table 3. The absolute incorporation of [1-^{14}C]LA as well as the recovery in total lipids from midgut gland cells decreased significantly from 2 h of incubation onward, whereas the absolute incorporation and recovery of [1-^{14}C]LNA into total lipids significantly decreased during the whole time course. The radioactivity from [1-^{14}C]LA was initially distributed primarily in neutral lipids, with almost 57% of the total recovered in triacylglycerol (TAG). However, the percentage of radioactivity of [1-^{14}C]LA increased significantly in polar lipids during the time course, mainly in phosphatidylcholine (PC) and phosphatidylethanolamine (PE), with a concomitant significant decrease in neutral lipids. A similar trend was shown in the incorporation of [1-^{14}C]LNa into lipid classes.

The incorporation of radioactivity from [1-^{14}C]AA and [1-^{14}C]EPA into total lipids and individual lipid classes in midgut gland cell suspensions during the incubation period are presented Table 4. The absolute incorporation of both [1-^{14}C]AA or [1-^{14}C]EPA as well as the recovery of radioactivity in total lipids from midgut gland cells were lower than those for [1-^{14}C]LA or [1-^{14}C]LNA and showed a downward trend during the time course. As with [1-^{14}C]LA and [1-^{14}C]LNA, the radioactivity from either [1-^{14}C]AA or [1-^{14}C]EPA was initially distributed primarily in neutral lipids, with over 55% and 45% of the total recovered in TAG, respectively. Similarly, the percentage of radioactivity from both [1-^{14}C]AA and [1-^{14}C]EPA increased in polar lipids during the time course. The increase in relative incorporation into polar lipids was observed in all classes but was most significant in PC, PE and particularly phosphatidylinositol (PI), whereas the concomitant decrease in relative incorporation in neutral lipids was exclusively due to a decrease in TAG.

The incorporation of radioactivity from [1-^{14}C]LA, [1-

TABLE 3. Incorporation of radioactivity from [1-^{14}C]18:2(n-6) and [1-^{14}C]18:3(n-3) in total lipids as nmol of ^{14}C-PUFA/mg of protein and the recovery into total lipids and different lipid classes in midgut gland cells of *Penaeus kerathurus* during the incubation period

	Time of incubation (h)				
	1	2	4	6	8
Incorporation of [1-^{14}C]18:2(n-6)					
Incorporation into total lipids	15.1 ± 0.3^{a}	16.0 ± 0.8^{a}	12.4 ± 0.2^{b}	8.7 ± 0.2^{c}	5.8 ± 0.3^{d}
Recovery in total lipids (%)	35.8 ± 1.3^{a}	37.9 ± 2.7^{a}	29.5 ± 1.6^{b}	20.6 ± 1.3^{c}	13.8 ± 0.9^{d}
Total polar lipids	36.2 ± 0.5^{a}	41.4 ± 0.6^{b}	44.4 ± 1.1^{c}	43.8 ± 0.7^{c}	47.5 ± 1.0^{d}
Phosphatidylcholine	28.6 ± 0.1^{a}	31.6 ± 0.3^{b}	34.2 ± 0.8^{c}	32.2 ± 0.5^{b}	35.2 ± 0.8^{c}
Phosphatidylethanolamine	4.4 ± 0.1^{a}	4.4 ± 0.0^{a}	5.3 ± 0.0^{b}	5.3 ± 0.1^{b}	5.5 ± 0.1^{b}
Phosphatidylserine	0.7 ± 0.0^{a}	2.5 ± 0.1^{b}	1.3 ± 0.1^{c}	1.4 ± 0.1^{cd}	1.6 ± 0.0^{d}
Phosphatidylinositol	1.4 ± 0.0^{a}	1.2 ± 0.0^{b}	1.9 ± 0.1^{c}	2.0 ± 0.0^{c}	1.9 ± 0.1^{c}
Phosphatidic acid	1.0 ± 0.0^{a}	0.9 ± 0.0^{a}	1.1 ± 0.1^{a}	1.0 ± 0.0^{a}	1.5 ± 0.2^{b}
Sphingomyelin	0.1 ± 0.0^{a}	0.8 ± 0.0^{b}	0.6 ± 0.0^{b}	1.9 ± 0.2^{c}	1.8 ± 0.3^{c}
Total neural lipids	63.6 ± 0.5^{a}	58.4 ± 0.6^{b}	55.4 ± 1.1^{c}	55.9 ± 0.7^{c}	52.4 ± 1.0^{d}
Free fatty acid	7.0 ± 0.2^{a}	4.2 ± 0.1^{b}	3.7 ± 0.1^{b}	2.9 ± 0.2^{c}	3.7 ± 0.3^{b}
Triacylglycerol/steryl ester	56.6 ± 0.4^{a}	54.2 ± 0.4^{b}	51.7 ± 1.0^{c}	53.0 ± 0.4^{bc}	48.7 ± 0.7^{d}
Incorporation of [1-^{14}C]18:3(n-3)					
Incorporation into total lipids	19.6 ± 0.5^{a}	14.9 ± 0.2^{b}	11.4 ± 0.2^{c}	8.1 ± 0.4^{d}	3.2 ± 0.3^{e}
Recovery in total lipids (%)	45.0 ± 2.5^{a}	34.2 ± 2.8^{b}	26.2 ± 1.4^{c}	18.7 ± 0.9^{d}	7.3 ± 1.1^{e}
Total polar lipids	33.7 ± 0.8^{a}	38.4 ± 0.6^{b}	40.1 ± 0.7^{bc}	42.6 ± 0.9^{c}	59.9 ± 2.3^{d}
Phosphatidylcholine	25.9 ± 0.5^{a}	28.4 ± 0.3^{ab}	29.8 ± 0.5^{bc}	32.4 ± 0.5^{c}	37.8 ± 2.1^{d}
Phosphatidylethanolamine	5.2 ± 0.1^{a}	5.1 ± 0.1^{a}	5.7 ± 0.3^{ab}	6.0 ± 0.1^{b}	9.6 ± 0.4^{c}
Phosphatidylserine	0.4 ± 0.0^{a}	1.4 ± 0.1^{b}	1.3 ± 0.2^{b}	0.9 ± 0.1^{b}	3.9 ± 1.4^{c}
Phosphatidylinositol	0.9 ± 0.2^{a}	1.1 ± 0.2^{a}	1.3 ± 0.3^{ab}	1.2 ± 0.1^{ab}	1.7 ± 0.1^{b}
Phosphatidic acid	1.1 ± 0.3^{a}	1.3 ± 0.3^{a}	1.2 ± 0.1^{a}	1.3 ± 0.3^{a}	2.4 ± 0.5^{b}
Sphingomyelin	0.2 ± 0.0^{a}	1.1 ± 0.3^{b}	0.8 ± 0.3^{ab}	0.8 ± 0.2^{ab}	4.5 ± 0.4^{c}
Total neural lipids	66.2 ± 0.8^{a}	61.5 ± 0.6^{b}	59.9 ± 0.7^{bc}	57.2 ± 0.9^{c}	40.0 ± 2.3^{d}
Free fatty acid	6.0 ± 0.3^{a}	4.6 ± 0.2^{b}	3.2 ± 0.2^{cd}	2.6 ± 0.3^{c}	3.5 ± 0.4^{d}
Triacylglycerol/steryl ester	60.2 ± 0.6^{a}	56.9 ± 0.4^{b}	56.7 ± 0.5^{b}	54.6 ± 0.5^{b}	36.5 ± 1.9^{c}

The recovery in total lipids is expressed as percentage of total radioactivity added and the recoveries in lipid classes are percentages of the total radioactivity recovered. Data are means ± SD (n = 3). SD = 0.0 implies an SD <0.05. Values within a given row not bearing the same superscript letter are significantly different at $p < 0.05$. If no superscript appears, values are not different.

^{14}C]LNA, [1-^{14}C]AA and [1-^{14}C]EPA into total lipids and lipid clases in ovary cell suspensions are presented in Tables 5 and 6. The absolute incorporation of all [1-^{14}C]PUFAs as well as the recovery in total lipids from ovary cells was lower than that experienced in midgut gland cells, but in contrast, showed an upward trend during the time course. The radioactivity from [1-^{14}C]LA and [1-^{14}C]LNA was distributed approximately in similar proportions between polar and neutral lipids with almost 40% of the total recovered in PC. PE and free fatty acid (FFA) showed a downward trend from 10% to 6% and 14% to 3%, respectively, and TAG showed an upward trend from 30% to 44%. In contrast, the radioactivity from [1-^{14}C]AA and [1-^{14}C]EPA was distributed primarily in polar lipids. The percentage of radioactivity from [1-^{14}C]AA recovered in PC during the time course was constant at over 50%, PE showed and downward trend from 17% to 12%, PI was about 5%, FFA decreased significantly from 14% to 5% and TAG increased significantly from 7% to 15%. Finally, the radioactivity from [1-^{14}C]EPA was initially distributed primarily in polar lipids, with PC showing an upward trend from 57% to 70%, PE remained constant at about 14%, PI was also about 5%, FFA showed a downward trend from 13% to 5%, whereas TAG remained constant at about 1%.

Metabolism of [1-^{14}C]PUFA in Midgut Gland and Ovary Cell Suspensions via Desaturation Elongation

Most of the radioactivity from [1-^{14}C]PUFAs in midgut gland and ovary cell suspensions was recovered unmetabolized (Table 7). The percentage of [1-^{14}C]LA unmetabolized in midgut gland cells was significantly higher than in ovary cells. The total percentages of [1-^{14}C]LA desaturated were relatively small (3.1% and 4.3% for midgut gland and ovary, respectively) but significantly higher in ovary cells. AA was synthesized, from [1-^{14}C]LA, more actively in ovary than in midgut gland cells. Whereas the percentages of direct elongation products of [1-^{14}C]LA, 20:2(n-6) and 22:2(n-6) (4.5% and 9.2% for midgut gland and ovary cells, respectively) were also significantly more abundant in ovary cell suspensions. The radioactivity from [1-^{14}C]LNA recovered unmetabolized in midgut gland and ovary cell suspensions

TABLE 4. Incorporation of radioactivity from [1-^{14}C]20:4(n-6) and [1-^{14}C]20:5(n-3) in total lipids as nmol of ^{14}C-PUFA/mg of protein and the recovery into total lipids and different lipid classes in midgut gland cells of *Penaeus kerathurus* during the incubation period

	Time of incubation (h)				
	1	2	4	6	8
Incorporation of [1-^{14}C]20:4(n-6)					
Incorporation into total lipids	7.2 ± 0.3^{a}	6.3 ± 0.2^{b}	4.4 ± 0.1^{c}	2.9 ± 0.0^{d}	0.6 ± 0.0^{e}
Recovery in total lipids (%)	18.3 ± 0.8^{a}	15.9 ± 1.2^{b}	11.2 ± 0.9^{c}	7.4 ± 0.9^{d}	1.5 ± 0.4^{e}
Total polar lipids	33.4 ± 0.7^{a}	40.5 ± 2.9^{b}	45.4 ± 1.5^{bc}	49.1 ± 1.0^{c}	57.8 ± 3.6^{d}
Phosphatidylcholine	19.6 ± 0.6^{a}	22.2 ± 1.1^{a}	29.3 ± 0.6^{b}	30.6 ± 1.1^{b}	30.9 ± 2.4^{b}
Phosphatidylethanolamine	7.6 ± 0.1^{a}	7.7 ± 0.5^{a}	8.1 ± 0.3^{a}	8.4 ± 0.1^{a}	11.2 ± 1.9^{b}
Phosphatidylserine	1.2 ± 0.0^{a}	3.9 ± 0.5^{b}	1.9 ± 0.4^{a}	2.0 ± 0.1^{a}	3.4 ± 0.7^{b}
Phosphatidylinositol	3.7 ± 0.1^{a}	4.6 ± 0.3^{b}	4.4 ± 0.1^{b}	5.5 ± 0.1^{c}	6.3 ± 0.4^{d}
Phosphatidic acid	1.1 ± 0.0^{a}	1.5 ± 0.4^{a}	1.1 ± 0.0^{a}	1.6 ± 0.5^{a}	3.1 ± 0.3^{b}
Sphingomyelin	0.2 ± 0.0^{a}	0.6 ± 0.2^{ab}	0.6 ± 0.2^{ab}	1.0 ± 0.4^{b}	2.9 ± 0.1^{c}
Total neural lipids	66.4 ± 0.7^{a}	59.3 ± 2.9^{b}	54.6 ± 1.5^{bc}	50.6 ± 1.0^{c}	42.0 ± 3.6^{d}
Free fatty acid	11.1 ± 0.6	10.5 ± 0.8	12.4 ± 1.8	10.8 ± 0.9	9.0 ± 2.4
Triacylglycerol/steryl ester	55.3 ± 0.3^{a}	48.8 ± 3.1^{a}	42.2 ± 1.6^{b}	39.8 ± 0.9^{b}	33.0 ± 4.1^{c}
Incorporation of [1-^{14}C]20:5(n-3)					
Incorporation into total lipids	13.1 ± 0.3^{a}	9.6 ± 0.5^{b}	7.5 ± 0.4^{c}	5.9 ± 0.6^{d}	1.7 ± 0.1^{e}
Recovery in total lipids (%)	30.2 ± 1.2^{a}	22.0 ± 1.2^{b}	17.3 ± 0.8^{c}	13.5 ± 1.7^{d}	4.0 ± 0.7^{e}
Total polar lipids	45.0 ± 1.1^{a}	48.1 ± 1.2^{ab}	52.6 ± 1.0^{b}	49.0 ± 2.7^{ab}	65.2 ± 2.2^{c}
Phosphatidylcholine	30.8 ± 0.3^{a}	31.9 ± 1.5^{a}	36.8 ± 1.0^{b}	34.7 ± 2.8^{ab}	39.0 ± 1.3^{b}
Phosphatidylethanolamine	6.9 ± 0.2^{ab}	7.2 ± 0.1^{b}	6.8 ± 0.4^{ab}	5.9 ± 0.4^{a}	7.7 ± 0.6^{b}
Phosphatidylserine	1.1 ± 0.0^{a}	2.8 ± 0.8^{ab}	1.8 ± 0.6^{a}	1.8 ± 0.1^{a}	4.1 ± 1.2^{b}
Phosphatidylinositol	4.4 ± 0.1^{ab}	4.2 ± 0.1^{a}	4.1 ± 0.1^{a}	4.6 ± 0.2^{ab}	4.8 ± 0.4^{b}
Phosphatidic acid	1.1 ± 0.1	1.8 ± 0.6	1.3 ± 0.3	1.3 ± 0.5	2.4 ± 0.9
Sphingomyelin	0.7 ± 0.1^{a}	0.2 ± 0.1^{a}	1.8 ± 0.7^{a}	0.7 ± 0.3^{a}	7.2 ± 2.7^{b}
Total neural lipids	55.1 ± 1.1^{a}	51.8 ± 1.2^{ab}	47.2 ± 1.0^{b}	50.8 ± 2.7^{ab}	34.7 ± 2.2^{c}
Free fatty acid	9.5 ± 1.0^{a}	13.9 ± 0.9^{b}	12.3 ± 0.7^{b}	13.2 ± 1.1^{b}	4.9 ± 0.3^{c}
Triacylglycerol/steryl ester	45.6 ± 1.3^{a}	37.9 ± 1.0^{b}	34.9 ± 0.8^{bc}	37.6 ± 3.1^{b}	29.8 ± 2.4^{c}

The recovery in total lipids is expressed as percentage of total radioactivity added and the recoveries in lipid classes are percentages of the total radioactivity recovered. Data are means ± SD (n = 3). SD = 0.0 implies an SD <0.05. Values within a given row not bearing the same superscript letter are significantly different at $p < 0.05$. If no superscript appears, values are not different.

were not significantly different and accounted for about 90% of the total radioactivity. The total percentages of [1-^{14}C]LNA desaturated were slightly higher than for [1-^{14}C]LA but also relatively small, 6.5% and 7.3% in midgut gland and ovary, respectively. The percentages of synthesized EPA (1.8% and 1.7%) and DHA (0.6% and 0.4%), from [1-^{14}C]LNA, were not significantly different between the cell suspensions of both organs. As with [1-^{14}C]LA and [1-^{14}C]LNA, ~90% of the radioactivity from [1-^{14}C]AA was recovered unmetabolized but midgut gland cells presented higher desaturase activity than ovary cells (8% versus 3.3%), whereas the elongase activity was the same (4.7–4.9%). In contrast, the radioactivity from [1-^{14}C]EPA recovered unmetabolized was below 90% and significantly different for midgut gland and ovary cells (82.3% versus 87.4%). Approximately 10% of the radioactivity from [1-^{14}C]EPA was recovered as desaturated products in midgut gland cells with 5.5% as 22:6(n-3) and 4.4% as 24:6(n-3). In contrast, only ~5% of the radioactivity from [1-^{14}C]EPA was recovered as desaturated products in ovary cell suspensions, with 3.4% as 22:6(n-3) and 1.5% as 24:6(n-3). The radioactivity recovered as products of the elongation of [1-^{14}C]EPA [22:5(n-3) and 24:5(n-3)] was the same in the cell suspensions of both organs (7.7–7.8%).

Discussion

The present experiment was designed to determine to what extent essential (n-6) and (n-3) HUFA such as AA, EPA and DHA were synthesized in midgut gland and ovary cells from adult *P. kerathurus* females at the beginning of sexual maturation. Studies using [^{14}C]-acetate or [^{14}C]-palmitic acid have demonstrated the absence of *de novo* synthesis of LA, LNA, EPA and DHA in penaeids (4,23–25,32). However, feeding trials and radiotracer studies have shown the bioconversion of LNA to other (n-3) PUFAs, particularly (n-3) HUFAs, by crustaceans (9,24,31,41,51,55). In this context, and regarding the large amount of PUFAs such as EPA and DHA accumulated in ovary and egg, determination of the contribution of desaturation and elongation of precursors of these HUFAs in midgut gland and ovary at different stages of maturation was an important objective in penaeid species of commercial interest such as *P. kerathurus*.

The biometric data of the animals used for this experi-

TABLE 5. Incorporation of radioactivity from [1-^{14}C]18:2(n-6) and [1-^{14}C]18:3(n-3) in total lipids as nmol of ^{14}C-PUFA/mg of protein and the recovery into total lipids and different lipid classes in ovary cells of *Penaeus kerathurus* during the incubation period

	Time of incubation (h)				
	1	2	4	6	8
Incorporation of [1-^{14}C]18:2(n-6)					
Incorporation into total lipids	1.6 ± 0.1^{a}	2.7 ± 0.1^{b}	3.9 ± 0.2^{c}	3.4 ± 0.2^{bc}	3.5 ± 0.7^{bc}
Recovery in total lipids (%)	5.7 ± 0.6^{a}	9.5 ± 0.7^{b}	13.9 ± 0.9^{c}	11.9 ± 0.8^{c}	12.4 ± 1.0^{c}
Total polar lipids	53.0 ± 3.1	55.5 ± 2.3	54.5 ± 1.4	51.8 ± 1.1	52.6 ± 1.7
Phosphatidylcholine	37.6 ± 1.2^{a}	41.3 ± 1.5^{ab}	42.5 ± 1.4^{b}	39.6 ± 1.3^{ab}	42.7 ± 2.9^{b}
Phosphatidylethanolamine	10.1 ± 0.9^{a}	8.8 ± 0.7^{a}	7.0 ± 0.6^{b}	6.4 ± 0.4^{b}	6.1 ± 0.3^{b}
Phosphatidylserine	1.6 ± 0.4^{a}	0.7 ± 0.1^{b}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}	0.6 ± 0.2^{b}
Phosphatidylinositol	2.2 ± 0.3	2.1 ± 0.2	2.1 ± 0.4	2.1 ± 0.4	2.0 ± 0.3
Phosphatidic acid	1.3 ± 0.1	1.5 ± 0.3	0.9 ± 0.1	1.4 ± 0.6	0.8 ± 0.1
Sphingomyelin	0.2 ± 0.0^{a}	1.1 ± 0.7^{ab}	1.6 ± 0.4^{b}	1.9 ± 0.4^{b}	0.4 ± 0.1^{a}
Total neural lipids	46.8 ± 3.1	44.3 ± 2.3	45.4 ± 1.4	48.0 ± 1.1	47.2 ± 1.7
Free fatty acid	13.9 ± 1.6^{a}	10.4 ± 1.4^{b}	7.6 ± 0.8^{bc}	6.0 ± 0.5^{c}	4.9 ± 0.6^{c}
Triacylglycerol/steryl ester	32.9 ± 3.6^{a}	33.9 ± 2.5^{a}	37.8 ± 1.6^{ab}	42.0 ± 1.2^{b}	42.3 ± 0.7^{b}
Incorporation of [1-^{14}C]18:3(n-3)					
Incorporation in total lipids	2.1 ± 0.1^{a}	3.0 ± 0.1^{b}	4.2 ± 0.2^{c}	4.6 ± 0.1^{d}	3.1 ± 0.1^{b}
Recovery into total lipids (%)	7.1 ± 0.5^{a}	10.4 ± 0.7^{b}	14.3 ± 0.9^{c}	15.7 ± 0.7^{c}	10.5 ± 0.4^{b}
Total polar lipids	58.1 ± 1.0^{a}	57.5 ± 1.2^{ab}	57.2 ± 2.7^{ab}	54.9 ± 1.2^{ab}	53.1 ± 2.1^{b}
Phosphatidylcholine	42.9 ± 1.3	42.4 ± 1.4	44.9 ± 3.3	43.0 ± 1.5	40.4 ± 1.9
Phosphatidylethanolamine	10.2 ± 1.7^{a}	9.9 ± 0.3^{a}	8.7 ± 0.7^{ab}	7.9 ± 0.9^{ab}	7.1 ± 0.8^{b}
Phosphatidylserine	1.3 ± 0.4^{a}	0.7 ± 0.1^{a}	0.5 ± 0.1^{a}	0.7 ± 0.2^{a}	2.5 ± 0.7^{b}
Phosphatidylinositol	1.9 ± 0.2	1.9 ± 0.2	2.2 ± 0.2	2.0 ± 0.0	1.9 ± 0.1
Phosphatidic acid	1.2 ± 0.1^{a}	1.4 ± 0.2^{a}	0.7 ± 0.1^{bc}	0.9 ± 0.0^{b}	0.6 ± 0.0^{c}
Sphingomyelin	0.6 ± 0.0^{ab}	1.2 ± 0.7^{b}	0.2 ± 0.0^{a}	0.4 ± 0.0^{ab}	0.6 ± 0.2^{ab}
Total neural lipids	41.7 ± 1.0^{a}	42.4 ± 1.2^{ab}	42.8 ± 2.7^{ab}	45.0 ± 1.2^{ab}	46.7 ± 2.1^{b}
Free fatty acid	11.5 ± 0.5^{a}	9.1 ± 1.2^{ab}	6.6 ± 0.1^{b}	6.6 ± 1.7^{b}	3.0 ± 0.1^{c}
Triacylglycerol/steryl ester	30.2 ± 0.7^{a}	33.3 ± 0.7^{b}	36.2 ± 0.9^{c}	38.4 ± 1.3^{c}	43.7 ± 1.5^{d}

The recovery in total lipids is expressed as percentage of total radioactivity added and the recoveries in lipid classes are percentages of the total radioactivity recovered. Data are means ± SD (n = 3). SD = 0.0 implies an SD <0.05. Values within a given row not bearing the same superscript letter are significantly different at $p < 0.05$. If no superscript appears, values are not different.

ment were similar to those found for the same species and the same stage of maturation (36). At this stage, the ovary presents a higher proportion of polar and structural lipids (primarily PC, PE and C) and a much lower proportion of reserve neutral lipids. In contrast, the midgut gland shows a much higher proportion of neutral lipids (primarily TAG). This has also been shown for other crustacean species (14,15,21,30,34,36). At this stage of maturation, the total lipid fatty acid profile was very similar in both organs.

The incorporation and recovery of radioactivity in total lipids of midgut gland was generally greater for [1-^{14}C]LA and [1-^{14}C]LNA than for [1-^{14}C]AA and [1-^{14}C]EPA, perhaps indicating a preferential retention of C_{18} PUFA in this organ. The midgut gland appears to be an organ with a high metabolic activity because the incorporation of radioactivity was maximal only 1 h after and then declined throughout the time course. Furthermore, a similar pattern was shown for the incorporation of the four [1-^{14}C]PUFAs studied in this organ during the time course. The proportion of radioactivity from all [1-^{14}C]PUFAs incorporated into midgut gland cells increased in total polar lipids during the time course (from ~33.4% to 65.2%) with a concomitant decrease in total neutral lipids (form 66.4% to 34.7%). The increase in polar lipids was primarily due to significant increases in the PC, PE and PI. The labeled TAG, the major neutral lipid class, significantly decreased. [1-^{14}C]AA and [1-^{14}C]EPA were preferentially incorporated into PI.

The pattern of incorporation of [1-^{14}C]EPA into lipid classes of midgut gland cells from *P. kerathurus* was different to that from midgut gland of *P. esculentus* where in the neutral lipids, most of the radioactivity was recovered as FFA instead of TAG, and phosphatidic acid was the second highest labeled fraction instead of PE (10). The proportions of the labeled fatty acids in the polar and neutral lipid fractions in midgut gland cells differed from the composition data. The range of the polar lipid/neutral lipid ratios of the labeled [1-^{14}C]PUFAs fractions was 0.5–1.9 during the time course, whereas the normal polar lipid/neutral lipid ratio for midgut gland was only 0.3 (excluding cholesterol). This fact is indicative of the selective incorporation of [1-^{14}C]PUFAs into polar lipids, primarily PC and PE, and may reflect to the function of the midgut gland in the synthesis of phospholipids, expelled into the hemolymph and transported to various tissues including the ovary (5,10), because

TABLE 6. Incorporation of radioactivity from [1-^{14}C]20:4(n-6) and [1-^{14}C]20:5(n-3) in total lipids as nmol of ^{14}C-PUFA/mg of protein and the recovery into total lipids and different lipid classes in ovary cells of *Penaeus kerathurus* during the incubation period

	Time of incubation (h)				
	1	2	4	6	8
Incorporation of [1-^{14}C]20:4(n-6)					
Incorporation into total lipids	1.8 ± 0.1^{a}	3.1 ± 0.3^{bc}	3.5 ± 0.1^{c}	3.3 ± 0.5^{c}	2.3 ± 0.4^{ab}
Recovery in total lipids (%)	6.8 ± 0.5^{a}	11.8 ± 1.1^{b}	13.1 ± 0.8^{b}	12.5 ± 0.9^{b}	8.8 ± 0.7^{a}
Total polar lipids	78.9 ± 1.6	83.3 ± 1.2	82.1 ± 5.1	77.3 ± 4.7	80.1 ± 1.1
Phosphatidylcholine	52.0 ± 1.8	55.7 ± 1.5	56.2 ± 5.9	51.8 ± 5.2	57.5 ± 0.9
Phosphatidylethanolamine	17.2 ± 0.6^{a}	18.5 ± 0.8^{a}	15.5 ± 1.8^{ab}	13.9 ± 0.9^{bc}	11.8 ± 1.2^{c}
Phosphatidylserine	2.8 ± 0.6	1.8 ± 0.2	2.2 ± 0.9	1.9 ± 0.6	2.6 ± 0.9
Phosphatidylinositol	4.7 ± 0.0^{a}	5.1 ± 0.2^{ab}	4.1 ± 0.6^{a}	5.9 ± 0.4^{b}	4.7 ± 0.4^{a}
Phosphatidic acid	1.9 ± 0.0^{a}	1.7 ± 0.1^{a}	2.3 ± 0.3^{ab}	3.2 ± 0.4^{b}	2.6 ± 0.6^{ab}
Sphingomyelin	0.3 ± 0.1^{a}	0.5 ± 0.1	1.8 ± 0.3^{b}	0.6 ± 0.4^{a}	0.9 ± 0.3^{a}
Total neural lipids	21.1 ± 1.6	16.6 ± 1.2	17.8 ± 5.1	22.5 ± 4.7	19.8 ± 1.1
Free fatty acid	14.2 ± 0.7^{a}	7.0 ± 0.4^{bc}	10.2 ± 2.8^{b}	4.2 ± 0.5^{c}	5.0 ± 0.3^{c}
Triacylglycerol/steryl ester	6.9 ± 0.1^{a}	9.6 ± 2.3^{a}	7.6 ± 0.8^{a}	18.3 ± 0.9^{b}	14.8 ± 0.9^{b}
Incorporation of [1-^{14}C]20:5(n-3)					
Incorporation into total lipids	2.2 ± 0.1^{a}	2.5 ± 0.2^{ab}	2.5 ± 0.1^{ab}	3.2 ± 0.4^{c}	2.9 ± 0.1^{bc}
Recovery in total lipids (%)	7.5 ± 0.4^{a}	8.5 ± 0.6^{ab}	8.6 ± 0.4^{ab}	11.0 ± 0.7^{c}	9.8 ± 0.8^{bc}
Total polar lipids	85.2 ± 4.9^{a}	87.2 ± 1.8^{ab}	90.6 ± 2.8^{ab}	94.2 ± 1.3^{b}	93.8 ± 1.4^{b}
Phosphatidylcholine	57.4 ± 5.5^{a}	58.2 ± 1.9^{a}	65.7 ± 3.1^{ab}	67.3 ± 1.9^{b}	70.3 ± 1.6^{b}
Phosphatidylethanolamine	14.2 ± 1.2	13.1 ± 3.6	15.6 ± 0.6	15.1 ± 1.0	14.9 ± 0.2
Phosphatidylserine	2.2 ± 0.2^{a}	7.0 ± 0.6^{b}	1.8 ± 0.5^{a}	5.2 ± 0.7^{c}	1.5 ± 0.1^{a}
Phosphatidylinositol	5.1 ± 0.4^{a}	6.5 ± 0.5^{b}	4.5 ± 0.3^{a}	4.3 ± 0.6^{a}	5.3 ± 0.5^{ab}
Phosphatidic acid	2.1 ± 0.3^{a}	1.8 ± 0.1^{ab}	1.9 ± 0.2^{ab}	1.5 ± 0.2^{bc}	1.2 ± 0.1^{c}
Sphingomyelin	4.2 ± 0.5^{a}	0.6 ± 0.1^{c}	1.1 ± 0.3^{b}	0.8 ± 0.2^{b}	0.6 ± 0.1^{b}
Total neural lipids	14.8 ± 4.9^{a}	12.6 ± 1.8^{ab}	9.2 ± 2.8^{ab}	5.7 ± 1.3^{b}	6.3 ± 1.4^{b}
Free fatty acid	13.1 ± 0.3^{a}	11.4 ± 3.8^{a}	7.9 ± 2.3^{ab}	4.4 ± 0.6^{b}	5.1 ± 0.8^{b}
Triacylglycerol/steryl ester	1.7 ± 0.3	1.2 ± 0.2	1.3 ± 0.1	1.3 ± 0.3	1.2 ± 0.3

The recovery in total lipids is expressed as percentage of total radioactivity added and the recoveries in lipid classes are percentages of the total radioactivity recovered. Data are means ± SD ($n = 3$). SD = 0.0 implies an SD <0.05. Values within a given row not bearing the same superscript letter are significantly different at $p < 0.05$. If no superscript appears, values are not different.

the role of phospholipids in lipid transport in shrimps is well documented (48,49,53,54). Similar results were also found by Teshima *et al.* (56) when feeding [1-^{14}C]LNA to *P. japonicus*. The recovery of radioactivity represent the net result of initial incorporation, and metabolism via β-oxidation resulting in the loss of radioactivity as $^{14}CO_2$. The abundance of label in FFA as well as the reduction of label recovered in TAG during the time course in the midgut gland cells suggested that labeled fatty acids were being mobilized either for phospholipid synthesis or for oxidation to supply energy. The rate of oxidation of the different [1-^{14}C]PUFAs was not measured in the present study but in midgut gland cells is presumably very high because the losses of radioactivity from the lipidic fraction during the 8-h time course ranged from 61.6% to 91.7% and possibly a great part of this label could be incorporated in CO_2 as the final product of [1-^{14}C]PUFA β-oxidation.

The distribution of label in the lipids of the digestive gland cells suggested that EPA was more important in phospholipid synthesis than LA, LNA or AA, because it was primarily and more uniformly distributed in polar lipid classes. This was also observed when using dietary ^{14}C labeled fatty acids, particularly ^{14}C-labeled EPA with *P. esculentus* (10). However, in an *in vivo* study by Kanazawa and Koshio (22), injecting [1-^{14}C]EPA in the spiny lobster *Palinurus japonicus*, the ratio of incorporation in polar lipid/neutral lipid was 10:1 1 week after the injection and PC was the most labeled fraction (74.7%).

The incorporation and recovery of radioactivity in total lipids from ovary cells was significantly lower and showed a different pattern of incorporation than in the midgut gland cells. There was a significant upward trend for the incorporation of all [1-^{14}C]PUFAs during the time course, with values quantitatively similar for all of them. The increased incorporation into total lipids during the time course varied from 21.7% for [1-^{14}C]AA to 54.3% for [1-^{14}C]LA. The distribution of radioactivity in lipid classes from [1-^{14}C]LA in ovary cells showed a polar lipid/neutral lipid ratio of ~1 and indicated primarily a net synthesis of PC and TAG (which increased by 11.9% and 22.2%, respectively) at the expense of PE, phosphatidylserine, PI and FFA (which decreased during the time course by 39.6%, 62.5%, 38.5% and 64.7%, respectively). The incorporation and distribution of [1-^{14}C]LNA in ovary cells showed a simi-

TABLE 7. Metabolism via the desaturase/elongase pathway of ^{14}C-polyunsaturated fatty acids in midgut gland and ovary cells of female *Penaeus kerathurus* at stage of maturation I

	^{14}C-Polyunsaturated fatty acid							
	^{14}C-18:2(n-6)		^{14}C-18:3(n-3)		^{14}C-20:4(n-6)		^{14}C-20:5(n-3)	
	Midgut	Ovary	Midgut	Ovary	Midgut	Ovary	Midgut	Ovary
18:2(n-6)	92.6 ± 1.4	86.6 ± 0.9*	—	—	—	—	—	—
18:3(n-6)	0.7 ± 0.0	0.5 ± 0.0*	—	—	—	—	—	—
18:3(n-3)	—	—	91.3 ± 0.9	90.6 ± 0.1	—	—	—	—
18:4(n-3)	—	—	2.8 ± 0.1	3.4 ± 0.1*	—	—	—	—
20:2(n-6)	4.0 ± 1.1	7.5 ± 0.1*	—	—	—	—	—	—
20:3(n-6)	0.2 ± 0.0	0.7 ± 0.0*	—	—	—	—	—	—
20:3(n-3)	—	—	1.1 ± 0.1	1.6 ± 0.1*	—	—	—	—
20:4(n-6)	0.9 ± 0.0	1.6 ± 0.2*	—	—	87.3 ± 0.7	91.7 ± 0.9*	—	—
20:4(n-3)	—	—	0.4 ± 0.0	0.5 ± 0.1	—	—	—	—
20:5(n-3)	—	—	1.8 ± 0.1	1.7 ± 0.1	—	—	82.3 ± 1.3	87.4 ± 1.2*
22:2(n-6)	0.5 ± 0.0	1.7 ± 0.3*	—	—	—	—	—	—
22:3(n-3)	—	—	1.1 ± 0.3	0.8 ± 0.0	—	—	—	—
22:4(n-6)	0.9 ± 0.0	0.4 ± 0.0*	—	—	4.7 ± 0.2	4.9 ± 0.9	—	—
22:5(n-6)	0.1 ± 0.0	0.4 ± 0.0*	—	—	4.9 ± 0.4	2.2 ± 0.0*	—	—
22:5(n-3)	—	—	0.4 ± 0.2	0.3 ± 0.0	—	—	5.9 ± 0.2	6.2 ± 0.3
22:6(n-3)	—	—	0.6 ± 0.2	0.4 ± 0.0	—	—	5.5 ± 0.4	3.4 ± 0.3*
24:4(n-6)	tr	tr	—	—	tr	tr	—	—
24:5(n-6)	0.4 ± 0.0	0.6 ± 0.0*	—	—	3.1 ± 0.2	1.1 ± 0.1*	—	—
24:5(n-3)	—	—	0.1 ± 0.0	0.2 ± 0.0*	—	—	1.9 ± 0.0	1.5 ± 0.1*
24:6(n-3)	—	—	0.4 ± 0.0	0.5 ± 0.0*	—	—	4.4 ± 0.9	1.5 ± 0.0*

Values represent the radioactivity found in each fatty acid fraction expressed as a percentage of total radioactivity recovered and are means ± SD (n = 3). SD = 0.0 implies an SD < 0.05. tr implies < 0.05%. *Significantly different ($p < 0.05$).

lar pattern to that of [1-^{14}C]LA, but there was no increase in the proportion of labeled PC but, in contrast, the increase of radioactivity recovered in TAG was higher (by 30.9%). Similar results were obtained when feeding *P. japonicus* with ^{14}C-labeled LNA but the ratio of labeled polar lipid/neutral lipid was higher for that species in that study (56). The distribution of radioactivity in ovary cells from [1-^{14}C]AA and [1-^{14}C]EPA showed polar lipid/neutral lipid ratios higher than those for C_{18}-labeled PUFAs, indicated that C_{20} PUFA are preferentially incorporated into polar lipid classes in ovary cells. In particular, EPA showed polar lipid/neutral lipid ratios ranging from 5.7 to 15 during the time course and very low incorporations into TAG. Similar results were obtained for the incorporation of injected dietary [1-^{14}C]EPA in whole body lipids of *P. japonicus*, *P. orientalis* and *Macrobrachium rosenbergii* (51). The general pattern suggested a net synthesis of PC at the expense of PE, FFA and TAG.

The decreased percentages of radioactivity in neutral lipid were due to decreased percentages in FFA and TAG. Therefore, the initial incorporation of [1-^{14}C]PUFAs in ovary cells was into TAG, with subsequent hydrolysis of fatty acids that were selectively reacylated into phospholipids, primarily PC. In any case, the incorporation of [1-^{14}C]PUFAs in ovary cells in comparison with midgut gland cells predominated over the ôxidation. In ovary cells, [1-^{14}C]PUFAs were retained selectively in membrane polar lipids, primarily PC, whereas neutral lipids, mainly TAG and FFA represented a reservoir of fatty acids for energy production via β-oxidation. This does not exclude a role for TAG as a supply of PUFA for ovary moieties during remodeling reactions of deacylation/reacylation or *de novo* phospholipid synthesis involved in membrane biosynthesis and turnover (45).

The specific incorporation of both [1-^{14}C]AA and [1-^{14}C]EPA into PI in ovary cells was noteworthy. The role of AA and EPA as precursors of eicosanoids in marine organisms is well known (44). Different roles such as the regulation of ion flux, temperature regulation and reproductive biology have been assigned to eicosanoids in invertebrates (47). Monohydroxy and trihydroxy derivatives of EPA have been found to act as hatching factors in barnacles (19,20). EPA may also act as a modulator in the formation of eicosanoids by competing with the enzyme systems converting AA to eicosanoids (44). In addition, and according to Middleditch *et al.* (29), there were no studies on endogenous prostaglandins in crustaceans, and their occurrence and functions in reproduction is an important area of investigation that will lead to a better understanding of the role of dietary (n-6) and (n-3) fatty acids and their interactions (16). In this respect and because prostaglandin synthesis is related to the AA and EPA content of membrane phospholipids, the constancy of these particular fatty acids in PI could be related to prostaglandin synthesis in ovary cells, although further research is required to elucidate this area.

As few studies have investigated the metabolism of

PUFAs, via the desaturation–elongation pathway, in crustaceans (4,25,31,33,5,55) and such studies in different organs during sexual maturation are lacking (16), we aimed to investigate the characteristics of PUFAs metabolism in midgut gland and ovary during maturation. Part of the results from that project are presented in the present paper, which contains the characteristics of PUFAs metabolism at the beginning of maturation in *P. kerathurus* females from the wild.

Teshima *et al.* (55) demonstrated that fatty acid metabolism varied during development, where larval *P. japonicus* had a greater ability to convert dietary [1-^{14}C]LNA than older animals. Therefore, variations may occur in PUFA metabolism in midgut gland and particularly in ovary throughout sexual maturation. Some studies have shown that maturation of the ovary involves an increase in lipid synthesis and some fatty acids, primarily saturated and monoenes, are synthesized *de novo* by the maturing female (7,46). However, there is a lack of information about PUFA metabolism in sexual maturation. The results obtained in the present study showed a pattern of bioconversion of ^{14}C-labeled PUFA similar to that shown by other marine crustaceans (4,24,31,55) and marine or piscivorous freshwater fish (18,24,27,37–39,55,59). Most of the ^{14}C-labeled precursor (~80–90%) remained unmetabolized, with values ranging from 3.1 to 9.9% as desaturation products and 2.2 to 7.8% as elongation products. [1-^{14}C]LA and [1-^{14}C]LNA were more actively bioconverted in ovary cell suspensions, whereas [1-^{14}C]AA and [1-^{14}C]EPA were more actively metabolized, via desaturation/elongation, in midgut gland cells. Focusing our attention on (n-3) HUFA (EPA and DHA), considered essential fatty acids in most marine organisms (44), EPA was 10-fold more effective as a precursor of DHA than LNA, either in midgut gland or ovary cells. These results are also similar to those found for the marine fish species mentioned above. It is, however, noteworthy, that Teshima *et al.* (51) did not find any bioconversion of injected or dietary [1-^{14}C]EPA to 22:5(n-3) or 22:6(n-3) in *P. japonicus*, *P. orientalis* and M.*rosenbergii*. Therefore, the low percentages of [1-^{14}C]PUFAs desaturated, in both midgut gland and ovary cells of *P. kerathurus*, support the lack of sufficient Δ^5-desaturase activity as the mechanism underpinning low production of DHA from LNA. As has been also demonstrated in marine fish species (19,27,37,59), the presence of significant amounts of label in both 24:5(n-3) and 24:6(n-3) suggests that the pathway for the production of DHA via a Δ^6 desaturation, as proposed by Voss *et al.* (62,63), could also be operating in midgut gland and ovary cells from *P. kerathurus*.

Enzyme-substrate interactions in fatty acid metabolism are more dependent on weaker "hydrophobic" interactions such as Van der Waals and dispersion forces than on strong ionic and hydrogen bond interactions. This implies a low substrate specificity, with 16:1(n-7), 18:1(n-9), 18:2(n-6), 18:3(n-3) and possibly 24:4(n-6) and 24:5(n-3) all competing for a single enzyme, Δ^6 fatty acid desaturase (45,62,63). This emphasizes the complex interactions and multitude of potential rate controls that can occur in the conversions of dietary unsaturated fatty acids that determine final PUFA/HUFA compositions of phospholipids that could be synthesized either in the midgut gland or in the ovary as part of the anabolic mechanisms occuring during the sexual maturation.

In conclusion, the pattern of PUFA metabolism in midgut gland and ovary cells from *P. kerathurus* is consistent with that previously obtained with marine fish, with relatively low Δ^5-desaturase activity, and up to 10-fold more DHA produced from EPA compared with LNA as substrate. These results strongly support the need of dietary supply of (n-6) and (n-3) HUFA at the beginning of maturation. It remains to be determined if in more advanced stages of maturation, PUFA metabolism is more active compared with non-mature females.

This work was supported by the Comisión Interministerial de Ciencia y Tecnologia, Spanish Ministry of Education and Sciences, CICYT project AGF93-0173. I also thank Dr. Douglas R. Tocher, NERC Unit of Aquatic Biochemistry, University of Stirling, Stirling, Scotland (UK), for critical reading of the manuscript.

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