Selective changes of docosahexaenoic acid-containing phospholipid molecular species in monkey testis during puberty

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Abstract **Puberty has a profound effect upon the biochemical composition of the testis. We previously demonstrated that puberty was accompanied by great increases in the content of docosahexaenoic acid (DHA; 22:6 n-3) and dihomogamma-linoleic acid (20:3 n-6) and decreases in arachidonic acid (AA; 20:4 n-6) in the phospholipids of testis. In this report, we analyze the composition of the phospholipid molecular species of the ethanolamine and choline glycerophospholipids in the testis of prepubertal (2 years old) and young adult (7–8 years old) monkeys, There was an increase in the DHA species and a decrease in arachidonic species. Interestingly, with few exceptions, among the three molecules with DHA or AA at the sn-2 position, only 16:0-22:6 and 18:0-20:4 changed selectively in opposite directions for both ethanolamine and choline glycerophospholipids. In contrast, there was no such selectivity seen in molecular species containing dihomogamma-linoleic acid or linoleic acid at the sn-2 position. All three dihomogamma-linoleic acid species increased and all three linoleic acid species decreased during puberty. In summary, at puberty, i.e., the onset of spermatogenesis, there are selective changes in the phospholipid molecular species, particularly those containing DHA and AA. These changes suggest a specific functional role of DHA-containing molecular species in the lipid bilayer membranes of sperm cells. A possible link between the composition of DHA-phospholipid molecular species and cellular function is discussed.**—Lin, D. S., M. Neuringer, and W. E. Connor. **Selective changes of docosahexaenoic acid-containing phospholipid molecular species in monkey testis during puberty. *J. Lipid Res.* 2004.** 45: **529–535.**

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Phospholipids are an integral component of the lipid bilayers of cell membranes. These lipid bilayers are a mixture of phospholipid molecular species. Each molecular species is defined by the chemical nature of the polar head group, the type of linkage to glycerol, and the acyl groups at both the sn-1 and sn-2 positions. In vital organs, such as the brain, retina, and sperm, many different phospholipid molecular species were detected (1–3). The various molecular species have different metabolic and physical properties. The molecular species composition of membrane phospholipids is a major determinant of their biophysical properties and therefore influences lipid-protein interactions, including the activity of membrane-bound enzymes, receptors, and ion channels (4–7).

At puberty, the testes undergo profound physiological, biochemical, and structural changes, culminating in the onset of spermatogenesis. Because lipids play an important role in testicular structure and function, we previously analyzed the sterols and fatty acids of the testis of five groups of monkeys ranging in age from 2 days to 30 years old (8). We found several dramatic changes in testicular sterol and fatty acid composition during puberty. The desmosterol-cholesterol ratio increased by more than 30-fold, from 0.004 before puberty to 0.147 in adulthood. There were also significant changes in four important essential fatty acids in total lipids and in phospholipids: increases in docosahexaenoic acid (DHA; 22:6 n-3) and dihomogamma-linolenic acid (20:3 n-6) and decreases in arachidonic acid (AA; 20:4 n-6) and linoleic acid (18:2 n-6).

In the present study, to gain further insights into the changes in membrane phospholipids during puberty, we examined the composition of the phospholipid molecular species of ethanolamine and choline glycerophospholipids, including their diacyl, alkenylacyl, and alkylacyl subclasses, in the testis of prepubertal (juvenile, 2 years old) and young adult (7–8 years old) monkeys.

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METHODS

Testis samples were obtained through the Tissue Distribution Program of the Oregon National Primate Research Center from three juvenile, prepubertal male rhesus monkeys, all 2 years of age, and three young adults, 7–8 years of age. The samples were immediately frozen on dry ice and maintained at −80°C until extracted for lipid analysis.

Testicular lipids were extracted by the methods of Folch, Leed, and Sloane Stanley (9). Individual glycerophospholipids were separated by TLC (10). Phospholipid molecular species were analyzed by the methods reported previously (1). The phospholipid classes were separated by a TLC system. Choline and ethanolamine glycerophospholipids were extracted from the gel scrapings with two washes of 5 ml of chloroform-methanol (1:1, v/v) followed by one wash with 5 ml of chloroform-methanol-water (65:45:12, v/v/v) and one more with 5 ml of chloroform-methanol (1:1, v/v). The molecular species of choline and ethanolamine glycerophospholipids were analyzed based on the method described by Blank et al. (11). Briefly, ethanolamine glycerophospholipids were hydrolyzed with phospholipase C for 4 h at room temperature. Diradylglycerols were extracted from the hydrolysate by the method of Bligh and Dyer (12), and benzoate derivatives were prepared by reaction with benzoic anhydride and 4-dimethylaminopyridine for 1 h at room temperature. The reaction was stopped with concentrated ammonium hydroxide, and the resulting diradylglycerobenzoates were extracted with hexane. Diradylglycerobenzoates were separated into the diacyl, alkenylacyl, and alkylacyl subclasses by TLC on silica gel G with benzene-hexane-ethyl ether (50:45:4, v/v/v). Bands were scraped into a 1:1 ethanol-water mixture, and the diraglycerobenzoates were extracted with hexane. The samples were then filtered (Millex-HV 0.43 μm filter unit; Millipore Corp., Bedford, MA), dried under nitrogen, and redissolved in acetonitrile-isopropanol (70:30, v/v) for HPLC injection.

Separation of the molecular species was accomplished by HPLC performed at ambient temperature with a Perkin-Elmer model 41:LC BioPump system fitted with a μBondapak C18 precolumn insert and a 3.9 mm × 30 cm analytical column packed with Novapak C18 (Waters Associates, Milford, MA). Peaks were monitored at 230 nm with a Perkin-Elmer LC-235 diode array detector and quantitated on a Perkin-Elmer LcI-100 integrator. Molecular species within the diacyl-, alkenylacyl-, and alkylacylglycerobenzoates were separated by isocratic elution with acetonitrile-isopropanol in ratios of 70:30, 63:35, and 63:37 (v/v), respectively. The column flow rate was 1 ml/min.

Identification of molecular species was accomplished by comparison with retention times in samples of bovine brain ethanolamine glycerophospholipid, as established by Blank et al. (11), and by gas chromatographic analysis of the collected peaks. The elution profile was similar to that obtained by Blank et al. (11). As a control for possible losses attributable to oxidation, an aliquot of bovine brain ethanolamine glycerophospholipid was included on every TLC plate and was worked up in parallel with experimental samples. Molecular species analysis of the three subclasses of the control bovine sample gave consistent results similar to those reported. Ethanolamine glycerophospholipid from bovine brain, phospholipase C from *Bacillus cereus*, benzoic anhydride, and 4-dimethylaminopyridine were purchased from Sigma (St. Louis, MO). Chloroform, acetonitrile, 2-propanol, methanol, hexane, and benzene were HPLC grade from Burdick and Jackson (Muskegon, MI), and anhydrous ethyl ether was from Mallinckrodt (Paris, KY).

The relative distribution of the diacyl, alkenylacyl and alkylacyl subclasses within each phospholipid was determined by comparison of chromatogram areas. Diradylglycerobenzoates from each phospholipid group were separated into the three subclasses on the same TLC plate and recovered by exhaustive extraction as described above. Aliquots of extract were then injected into the HPLC, and total peak areas from each chromatogram were calculated. The relative percentage of each of the three subclasses was then calculated by dividing the total peak area for the respective subclass by the sum of the total peak areas for all three subclasses.

Statistical analyses were performed by Student's *t*-test for group means (13).

RESULTS

During puberty, there were great changes in certain phospholipid molecular species. The major molecular species of juvenile and young adult monkey testis are listed in **Table 1** for each of the three subclasses of ethanolamine glycerophospholipid: diacyl, alkenylacyl, and alkylacyl. A total of 23 molecular species with nine different fatty acids at the sn-2 position are listed for each subclass. In particular, there were selective changes of the species with either DHA or AA at the sn-2 position. For example, in diacyl ethanolamine glycerophospholipid, 16:0-22:6 increased 2.5-fold during puberty and became the most abundant molecular species, but there were no changes in 18:0-22:6 or 18:1-22:6. In species with AA, 18:0-20:4 decreased by 36%,18:1-20:4 had a 46% decrease, but 16:0-20:4 increased by 64% (**Fig. 1**). The same pattern of selective changes in 16:0-22:6 and 18:0-20:4 was also observed in the alkenylacyl and alkylacyl subclasses (Table 1). In contrast, changes were uniform and nonselective in other species defined by the acyl group at the sn-2 position. For example, molecular species with 20:3 n-6 all increased and those with18:2 n-6 all decreased in the diacyl subclass (Fig. 1). Thus, the selective change of individual molecular species seemed a notable feature of molecular species with DHA and AA at the sn-2 position.

The major molecular species in choline glycerophospholipid of juvenile and young adult monkey testis are listed in **Table 2**. The diacyl subclass showed the same basic pattern of changes in DHA- and AA-containing molecular species as seen in diacyl ethanolamine glycerophospholipid. Specifically, 16:0-22:6 increased 2-fold, whereas there was little change in the other two DHA species, 18:0-22:6 and 18:1-22:6. At the same time, 18:0-20:4 decreased by 42%, whereas there were no changes in 16:0-20:4 and 18:1-20:4. Again, as in the ethanolamine glycerophospholipids, all three 20:3 n-6 species increased and all three 18:2 n-6 species decreased. The changes in two other subclasses are much less consistent, perhaps because of their small quantities.

No changes occurred during puberty in the proportion of the three subclasses of testicular choline and ethanolamine glycerophospholipids (**Table 3**). The distribution of the diacyl, alkenylacyl, and alkylacyl subclasses for ethanolamine phosphoglycerides of the testis of prepubertal monkeys was 54.0, 43.3, and 2.7%, respectively. For the choline phosphoglycerides, the distribution was 92.2, 4.2, and 3.6%, respectively.

TABLE 1. The major molecular species in ethanolamine glycerophospholipids of the testes of juvenile and young adult monkeys

Molecular Species	Diacyl		Alkenylacyl		Alkylacyl	
	Juvenile (n = 3)	Young Adult (n = 3)	Juvenile (n = 3)	Young Adult (n = 3)	Juvenile (n = 3)	Young Adult (n = 3)
n-3						
16:0-22:6	8.8 ± 1.4	31.4 ± 2.4[a,b]	18.7 ± 8.6	33.6 ± 3.4[c]	14.2 ± 1.1	65.9 ± 3.4[b]
18:0-22:6	6.4 ± 0.9	5.7 ± 0.8	10.9 ± 2.7	10.0 ± 2.2	9.6 ± 1.1	2.5 ± 0.0[b]
18:1-22:6	2.8 ± 0.7	3.0 ± 0.3	1.9 ± 1.0	6.7 ± 1.1[d]	11.0 ± 1.6	2.6 ± 1.1[d]
n-6						
16:0-20:4	8.3 ± 1.2	13.6 ± 0.5[d]	14.2 ± 2.9	9.6 ± 0.1	8.0 ± 1.5	2.4 ± 0.6[d]
18:0-20:4	29.4 ± 1.0	18.7 ± 0.4[b]	28.8 ± 0.7	10.4 ± 1.2[b]	11.2 ± 2.2	0.7 ± 1.2[d]
18:1-20:4	6.7 ± 1.6	3.0 ± 0.4[e]	6.0 ± 1.2	4.7 ± 1.0	8.1 ± 2.5	2.0 ± 0.2[c]
16:0-18:2	2.6 ± 0.7	0.9 ± 0.1[c]	0.7 ± 0.3	2.1 ± 0.1[d]	1.7 ± 0.5	2.4 ± 0.5
18:0-18:2	3.8 ± 0.3	1.1 ± 0.0[b]	1.1 ± 0.2	0.5 ± 0.1[e]	2.6 ± 1.2	0.5 ± 0.1[c]
18:1-18:2	3.0 ± 0.4	0.3 ± 0.0[b]	0.4 ± 0.1	0.2 ± 0.0	0.4 ± 0.0	0.0 ± 0.0[b]
16:0-20:3	1.1 ± 0.3	6.4 ± 0.7[b]	0.5 ± 0.2	1.2 ± 0.1[e]	1.1 ± 0.3	1.4 ± 0.3
18:0-20:3	2.3 ± 0.2	6.2 ± 0.2[b]	0.5 ± 0.1	0.5 ± 0.1	1.2 ± 0.6	0.5 ± 0.1
18:1-20:3	0.3 ± 0.0	1.0 ± 0.1[b]	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
16:0-22:4	1.7 ± 0.5	0.9 ± 0.1	3.0 ± 1.0	5.9 ± 0.4[e]	6.8 ± 2.2	6.7 ± 1.5
18:0-22:4	3.3 ± 0.3	1.2 ± 0.0[b]	2.1 ± 0.4	1.5 ± 0.2	4.9 ± 2.3	1.6 ± 0.4
18:1-22:4	0.2 ± 0.0	0.3 ± 0.0	1.0 ± 0.2	0.5 ± 0.1[c]	0.9 ± 0.1	0.1 ± 0.0[b]
n-9						
16:0-18:1	3.1 ± 0.3	1.7 ± 0.1[d]	1.6 ± 0.3	2.4 ± 0.3	3.7 ± 1.7	2.4 ± 0.6
18:0-18:1	6.1 ± 0.7	1.5 ± 0.1[b]	1.9 ± 1.2	1.7 ± 0.5	0.9 ± 0.8	0.3 ± 0.2
18:1-18:1	2.6 ± 1.0	0.3 ± 0.3[c]	1.4 ± 0.6	2.9 ± 0.6[c]	0.8 ± 1.3	2.4 ± 0.3
16:0-20:1	0.6 ± 0.1	0.0 ± 0.0[b]	0.3 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	0.0 ± 0.0
18:0-20:1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
18:1-20:1	1.3 ± 1.1	0.3 ± 0.3	1.4 ± 0.8	1.5 ± 1.0	4.2 ± 3.7	0.7 ± 0.6
Saturated						
16:0-16:0	0.9 ± 0.9	0.5 ± 0.5	0.4 ± 0.7	0.9 ± 1.5	0.6 ± 1.0	3.1 ± 1.4
16:0-18:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.9	0.0 ± 0.0	0.3 ± 0.5

Data shown are mol% (mean ± SD).
[a] Young adult monkeys versus juvenile monkeys.
[b] $P < 0.001$.
[c] $P < 0.05$.
[d] $P < 0.005$.
[e] $P < 0.01$.

DISCUSSION

The most interesting observation of this study was the selective increase in one of the DHA-containing phospholipid molecular species (16:0-22:6) in the testis during puberty. At puberty, spermatogenesis is accompanied by a tremendous proliferation of spermatogonia. The changes in the phospholipid molecular species composition of the testis may reflect the phospholipid molecular species of newly generated sperm cells. Indeed, we previously showed that 16:0-22:6 is a major molecular species in sperm. **Figure 2** shows the similarity between the sperm cells and the testes of young adult monkeys in the composition of DHA-containing phospholipid molecular species (Fig. 2). The predominance of the 16:0-22:6 molecular species suggests that it serves a specific functional role in

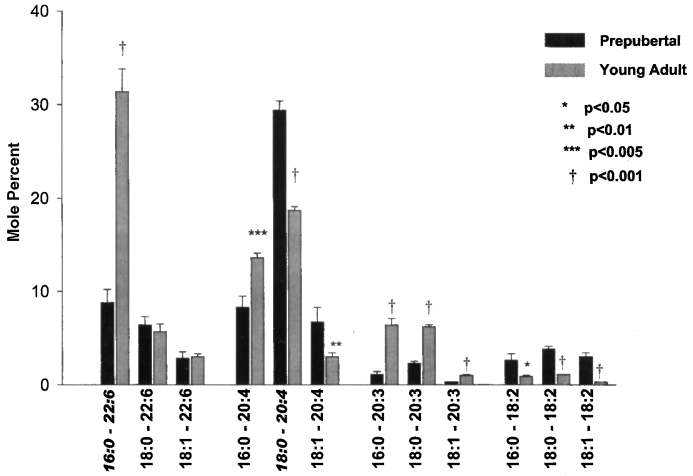

Fig. 1. The effects of puberty on the concentrations of the major molecular species of diacyl ethanolamine glycerophospholipids in monkey testes (mean ± SD).

TABLE 2. The major molecular species in choline glycerophospholipids of the testes of juvenile and young adult monkeys

Molecular Species	Diacyl		Alkenylacyl		Alkylacyl	
	Juvenile (n = 3)	Young Adult (n = 3)	Juvenile (n = 3)	Young Adult (n = 3)	Juvenile (n = 3)	Young Adult (n = 3)
n-3						
16:0-22:6	5.4 ± 1.1	15.0 ± 1.9[a,b]	35.1 ± 7.5	32.9 ± 11.7	8.8 ± 1.6	18.3 ± 5.5[c]
18:0-22:6	0.8 ± 0.1	1.6 ± 0.1[d]	0.7 ± 0.2	1.4 ± 0.3[c]	1.2 ± 0.3	1.6 ± 0.7
18:1-22:6	0.6 ± 0.2	0.8 ± 0.1	0.5 ± 0.3	6.1 ± 7.3	5.4 ± 2.3	1.9 ± 1.0
n-6						
16:0-20:4	14.4 ± 3.4	15.0 ± 1.1	10.5 ± 1.9	11.3 ± 2.4	15.0 ± 1.5	7.9 ± 1.7[e]
18:0-20:4	8.5 ± 0.0	4.9 ± 0.4[d]	10.9 ± 5.1	5.2 ± 1.6	7.5 ± 0.7	20.7 ± 1.3[d]
18:1-20:4	4.0 ± 1.4	2.3 ± 0.3	3.9 ± 1.5	3.0 ± 0.9	12.7 ± 5.7	2.7 ± 0.1[c]
16:0-18:2	17.1 ± 1.2	5.8 ± 0.6[d]	5.0 ± 1.4	1.5 ± 0.3[c]	7.1 ± 1.9	1.5 ± 0.4[e]
18:0-18:2	4.7 ± 0.8	2.0 ± 0.0[b]	1.2 ± 0.4	0.8 ± 0.1	2.4 ± 0.2	1.4 ± 0.2[b]
18:1-18:2	3.1 ± 0.4	0.6 ± 0.0[d]	2.4 ± 0.8	0.5 ± 0.1[c]	4.5 ± 1.1	0.6 ± 0.2[b]
16:0-20:3	3.7 ± 0.3	22.3 ± 2.1[d]	1.1 ± 0.3	5.9 ± 1.3[b]	1.6 ± 0.4	5.8 ± 1.5[e]
18:0-20:3	1.1 ± 0.2	5.3 ± 0.1[d]	0.3 ± 0.1	2.2 ± 0.2[d]	0.5 ± 0.0	3.7 ± 0.5[d]
18:1-20:3	0.6 ± 0.1	0.9 ± 0.1[c]	0.4 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.4
16:0-22:4	0.6 ± 0.0	0.5 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.1 ± 0.0[c]
18:0-22:4	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
18:1-22:4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0[d]
n-9						
16:0-18:1	18.0 ± 3.0	11.8 ± 0.2[c]	4.5 ± 1.3	4.9 ± 0.5	9.1 ± 0.6	8.1 ± 1.1
18:0-18:1	3.4 ± 1.3	1.0 ± 0.3[c]	0.8 ± 1.0	0.9 ± 0.1	0.6 ± 0.6	0.0 ± 0.0
18:1-18:1	1.2 ± 1.1	1.3 ± 0.5	0.8 ± 1.5	3.0 ± 0.4	2.3 ± 1.1	1.7 ± 1.5
16:0-20:1	0.5 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.0[c]	0.1 ± 0.1	0.0 ± 0.0
18:0-20:1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 1.1	0.0 ± 0.0
18:1-20:1	0.3 ± 0.5	0.3 ± 0.6	4.7 ± 0.1	4.9 ± 1.9	1.7 ± 1.9	0.5 ± 0.8
Saturated						
16:0-16:0	6.8 ± 0.5	5.5 ± 1.1	4.9 ± 1.3	9.0 ± 1.0[c]	6.1 ± 1.2	16.7 ± 2.7[b]
16:0-18:0	2.2 ± 0.6	0.2 ± 0.3[e]	3.3 ± 3.1	1.3 ± 1.1	3.6 ± 1.7	1.9 ± 0.5

Data shown are mol% (mean ± SD).
[a] Young adult monkeys versus juvenile monkeys.
[b] $P < 0.005$.
[c] $P < 0.05$.
[d] $P < 0.001$.
[e] $P < 0.01$.

sperm cells. Previously, we found that 99% of the DHA in sperm is concentrated in the sperm tail, whereas the sperm head has a very low DHA concentration (14). This finding suggested that the highly polyunsaturated DHA may contribute to the membrane properties necessary for sperm motility.

Two other tissues, the brain and retina, are also rich in DHA. Increasing evidence has suggested that a high level of DHA is necessary for optimal photoreceptor function and for visual and neural development (15–19). However, although the ethanolamine glycerophospholipids of these tissues have a DHA content similar to that of sperm (3, 20), their phospholipid molecular species composition is distinctly different (Fig. 2). Most notably, the predominant molecular species in both the retina and the brain is 18:0-22:6, rather than 16:0-22:6 as in mature testis and sperm. Thus, specific DHA-containing molecular species may be required for the characteristic functions of particular cell membranes.

An analogous situation may be seen in the work of Farkas et al. (21), who studied the variation of DHA-containing phospholipid molecular species in the brains of several types of fish living at different environmental temperatures. Despite similarity in the overall amount of brain DHA, their distribution in phospholipid molecular species varied according to body temperature. Particularly in diacyl ethanolamine glycerophospholipid, there was a significant increase of 18:1-22:6 and a decrease in the ratio of 18:0-22:6 to 16:0-22:6 with decreasing body temperature. These authors propose that the compositional changes are associated with an increasing membrane disorder, reduced acyl chain packing, and altered membrane curvature, all properties that help to maintain the efficiency of lipid-protein interactions at lower temperatures. Similarly, such different molecular species distributions of DHA may provide the optimal membrane characteristics for the distinct functions of different tissues within the same organism.

TABLE 3. Distribution of the subclasses of testicular ethanolamine and choline glycerophospholipids of prepubertal and young adult monkeys

Subclass	Ethanolamine Glycerophospholipids		Choline Glycerophospholipids	
	Prepubertal (n = 3)	Young Adult (n = 3)	Prepubertal (n = 3)	Young Adult (n = 3)
Diacyl	54.0 ± 4.4	60.7 ± 5.7	92.2 ± 3.4	93.1 ± 0.9
Alkenylacyl	43.3 ± 5.0	33.1 ± 6.6	4.2 ± 3.2	3.1 ± 0.6
Alkylacyl	2.7 ± 1.1	6.3 ± 1.7	3.6 ± 0.8	3.8 ± 0.3

Data shown are percentage of total.

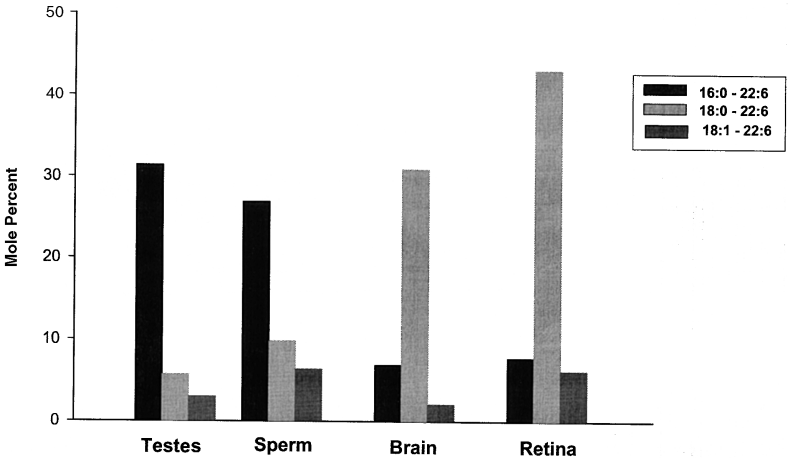

Fig. 2. Composition of docosahexaenoic acid (DHA) molecular species in diacyl ethanolamine glycerophospholipids of the tissues with high DHA concentration. [The brain, retina, and sperm data are from refs. (1–3), respectively, and the data for the testes are from the present study.]

In the late pregnancy rat, Burdge, Hunt, and Postle (22) found specific increases in hepatic 16:0-22:6 in both phosphatidylethanolamine (PE) and phosphatidylcholine (PC). These authors suggested that the supply of PUFA to the developing fetal rat is the result of specific adaptation to maternal hepatic phospholipid biosynthesis rather than of passive transfer from the maternal diet.

The change of phospholipid molecular species composition in cell membranes is the result of synthesis and turnover. The major pathways for hepatic PE and PC synthesis are the CDP-ethanolamine and CDP-choline pathways (23–26). Holub (27) reported that CDP-ethanolamine:1,2 diacyl-sn-glycerol ethanolaminephosphotransferase and CDP-choline:1,2 diacyl-sn-glycerol cholinephosphotransferase can exert significant selectivities. This author suggested that such selectivity may be important in maintaining the characteristic composition of fatty acid chains in membrane phospholipids. Phospholipid molecular species with different turnover rates were observed in rat rod outer segment membranes and in rabbit erythrocytes (28, 29). Studying the relative degradation of different molecular species of choline glycerophospholipids in thrombin-stimulated human platelets, Mahadevappa and Holub (30) reported that the different compartmentalization of PC molecular species according to their metabolic origin can influence their susceptibility to hydrolysis. Samborski, Ridgway, and Vance (31) reported that only newly made PE is methylated to PC. Unlike PC and phosphatidylinositol, PE is not significantly deacylated-reacylated in rat hepatocytes. Obviously, the special composition of phospholipid molecular species of different cells is the result of a series of complex reactions. The precise mechanisms responsible for changes in the phospholipid molecular species composition of testicular membranes during sexual maturation are not known.

During puberty, there were significant increases of all molecular species with dihomogamma-linolenic acid (20:3 n-6) at the sn-2 position. This fatty acid is the precursor of 1-series prostaglandins (32). It has been suggested that prostaglandins may play a role in the maturation process of spermatozoa (33–35). Conversely, the concentration of AA, the precursor of the 2-series prostaglandins, decreased during puberty (8). In most species, the 20:3 n-6 content of tissue phospholipids is far lower than the content of AA, and prostaglandins of the 2-series predominate. However, human and monkey seminal vesicles contain high levels of the 1-series prostaglandins, especially 19-hydroxy-prostaglandin E_1 (PGE_1), derived from 20:3 n-6 (36, 37). In rhesus monkey semen, 19-hydroxy-PGE_1 is present at five times the level of 19-hydroxy-PGE_2. Thus, the specific increase in 20:3 n-6 at puberty appears to reflect the importance of 1-series prostaglandins in mature testicular function.

We also analyzed the fatty acid composition of two phospholipid classes [ethanolamine (PE) and choline (PC) glycerophospholipids]. During puberty, DHA increased from 12.3% to 29.9%; dihomogamma-linoleic acid increased from 1.2% to 4.8% in PE, whereas AA decreased from 29.5% to 16.0% and linoleic acid decreased from 3.6% to 1.3% (our unpublished observations). In PC, DHA increased from 2.6% to 11.8%, and dihomogamma-linoleic acid increased from 2.1% to 12.9%. Linoleic acid was reduced from 14.6% to 4.2%. AA level in PC did not change significantly (from 12.9% to 11.8%). Comparison of these changes of fatty acid composition in phospholipids with the changes of phospholipid molecular species suggested some interesting divergences. For example, in PC, there was an increase of dihomogamma-linoleic acid during puberty. Similarly, the three dihomogamma-linoleic acid species were also increased. However, when DHA in PC increased during puberty, only 16:0-22:6 species increased; the other two DHA species did not change. On the other hand, although the AA of PC did not change during puberty, there was a significant decline of 18:0-20:4 species. The other two arachidonic species did not change. This comparison demonstrated clearly that the subtle changes of phospholipid molecular species composition in response to either physiological or pathological changes cannot be detected by only fatty acid analysis of phospholipids.

The three subclasses of tissue glycerophospholipids are defined by the type of linkage at position 1 attached to the glycerol moiety. Alkyl ethers include alkenylacyl (plasmalogen), in which the ether-linked alkyl chain contains a

double bond between C_1 and C_2, and the alkylacyl subclass, in which the alkyl group is without a double bond. Alkenyl and alkyl ethers are present in particularly high concentrations in cancer cells (38). It was suggested that the alkyl ether bond is more stable than the ester bond found in the diacyl phospholipids (38). Therefore, the presence of alkyl ether derivatives may provide additional stability to cellular membrane structure. In the testis, we found that in ethanolamine glycerophospholipid, the diacyl, alkenylacyl, and alkylacyl subclasses constituted 91, 5, and 4%, respectively. In choline glycerophospholipid, the composition was 54, 43, and 3%, respectively. These proportions did not change with puberty, and they also are similar to those found in the testes of several other species (39). Furthermore, in our previous analysis of the ethanolamine glycerophospholipid of monkey brain and retina, we found subclass compositions that were also similar to that of the testis (1, 2). Thus, it seems that phospholipid subclass composition remains relatively stable despite substantial differences in phospholipid molecular species composition.

In conclusion, the composition of DHA phospholipid molecular species as well as the concentration of DHA are specific for different tissues and developmental stages. The DHA phospholipid molecular species may be tailored to optimize different cell functions. Several intriguing questions remain to be investigated. Why do different cells require different phospholipid molecular species composition? How is this achieved? Are these compositions changeable in disease states or by nutritional, pharmaceutical, and genetic manipulation?

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