Unique lipids of primate spermatozoa: desmosterol and docosahexaenoic acid[1]

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Abstract Spermatozoa represent a tissue readily accessible for study after various exogenous perturbations. To characterize the lipid composition of monkey sperm and to establish a baseline from which dietary or pharmaceutical influences may then be evaluated, we collected semen samples from five rhesus monkeys by electroejaculation and analyzed the sperm for sterols, fatty acid composition, and the molecular species of the ethanolamine glycerophospholipids. Two sterols were identified: cholesterol, 41%, and desmosterol, 59% of total sterols. Desmosterol was found only in the free form. Cholesterol existed in three different forms: free, 60%; esterified, 20%; and sulfated, 20%. Docosahexaenoic acid (22:6, DHA) was almost the only n-3 fatty acid in sperm phospholipids, 24% of the total fatty acids. DHA was present mainly in phosphatidylcholine and phosphatidylethanolamine. Oleic and palmitic acids were the predominant monounsaturated and saturated fatty acids. The ethanolamine glycerophospholipids were separated into three subclasses: diacyl 49%, alkenylacyl 43%, and alkylacyl 8%. Thirteen molecular species were identified and quantified. The *sn*-1 position of these molecular species contained exclusively 16:0, 18:0, or 18:1. The *sn*-2 position contained n-3, n-6, and n-9, as well as saturated fatty acids. The molecular species having n-3 fatty acids in the *sn*-2 position contributed 43, 73, and 100% of the total in the diacyl, alkenylacyl, and alkylacyl subclasses, respectively. The presence of the unusual sterol, desmosterol, a cholesterol precursor not found in measurable quantities in any other tissue suggests an important functional and structural role for desmosterol in spermatozoa. The other unique lipids, cholesterol sulfate and the n-3 docosahexaenoic acid, may also have a significant role in the function of spermatozoa.—**Lin, D. S., W. E. Connor, D. P. Wolf, M. Neuringer, and D. L. Hachey.** Unique lipids of primate spermatozoa: desmosterol and docosahexaenoic acid. *J. Lipid Res.* 1993. **34:** 491-499.

Supplementary key words sperm • monkey • fatty acids • sterols • molecular species

Spermatozoa are rich in lipids with phospholipids accounting for approximately 65-75% of the total (2). Sterols are the second major component. Phospholipids and cholesterol are important components of the lipid bilayer of cell membranes. Conceivably, the quantity and composition of these lipids in sperm could have a significant effect upon physico-chemical properties, and in turn, upon their functional characteristics.

Sperm contain unusually high concentrations of docosahexaenoic acid (22:6, DHA) (3, 4). The only other tissues that have a high content of this biologically important n-3 polyunsaturated fatty acid are the testes and the neural tissues (brain and retina). In the latter case DHA was found to be essential for development and function of these organs (5, 6).

Numerous reports have documented that lipids play an important role in the viability, maturity, fertility, and function of the sperm cells (7-14). In spite of their physiological and biochemical importance, relatively little is known concerning the lipid composition of primate sperm. Furthermore, most available studies were conducted in the 1970s before the development of modern analytical techniques. In the present investigation, we have determined the sterol content, the fatty acid composition of different lipid and phospholipid classes, and the phospholipid molecular species of rhesus monkey sperm, and attempted to deduce functional roles for these various lipids.

METHODS

Five healthy 7- to 12-year-old male rhesus monkeys (9-13 kg) were individually caged in a temperature con-

Abbreviations: DHA, docosahexaenoic acid; GLC, gas-liquid chromatography; MS, mass spectrometry; TMS, trimethylsilyl; BSTFA, N,O-bis-trimethylsilyltrifluoroacetamide; TLC, thin-layer chromatography.

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trolled (22°C), light-regulated (12 hr:12 hr, light:dark) room. Their diet consisted of Purina Monkey Chow (Ralston Purina Co., Richmond, IN) supplemented with fresh fruit three times weekly and water ad libitum. The study protocol was approved by the Animal Care and Use Committee of the Oregon Regional Primate Research Center.

Monkey semen was collected by a penile electroejaculation technique (15). Samples were twice washed in 3.0 ml Talp-HEPES medium containing 0.3% bovine serum albumin by sedimentation and resuspension. Washed sperm motility and density were determined microscopically. Sperm displayed normal motility (>90%) and sperm counts (total cells/ejaculate) for the five monkeys ranged from 271 to 820 × 10^6 cells. The cellular content was found to be 99% sperm cells. These monkeys had a record of inducing successful pregnancies in breeder females.

Sterol analysis

The lipids of the sperm pellet were extracted by the method of Folch, Lees, and Sloane Stanley (16). Sperm sterols were purified by digitonin precipitation, and identified by retention times on gas-liquid chromatography (GLC) and by chemical ionization mass spectrometry (MS). GLC-MS analysis of sperm sterols was carried out as follows.

The sterol-O-trimethylsilyl (TMS) derivatives were prepared by addition of 250 μl of N,O-bis-trimethylsilyltrifluoroacetamide (BSTFA) to 500 μg of the sterol sample. The samples were heated at 80°C for 20 min. The TMS derivatives were analyzed by electron ionization and by methane chemical ionization mass spectrometry using a Hewlett-Packard HP 5988 GC/MS (Palo Alto, CA) equipped with an HP-5890 gas chromatograph. The samples were gas chromatographed on a 30 m × 0.32 mm DB-5 capillary column (J&W Scientific, Rancho Cordoba, CA) using helium as the carrier gas at 2.0 ml/min. A split injector was used with a split ratio of 30:1. The column temperature was programmed from 200 to 320°C at 10°C/min. Electron ionization spectra were obtained using an ion source temperature of 250°C and a 70 eV ionization potential. Methane chemical ionization spectra were obtained at an ion source temperature of 250°C using an ion source pressure of 1.1 torr CH_4. Spectra were recorded from *m/z* 100 to *m/z* 600 at 1.0 sec per scan.

For analysis of free and esterified sterols in sperm, aliquots of the lipid extracts were plated on silica gel G thin-layer chromatography (TLC) plates after [4-^{14}C]cholesterol and cholesteryl [^{14}C]oleate (New England Nuclear Corp., Boston, MA) were added as internal standards. The plates were developed in hexane-chloroform-ether-acetic acid 80:10:10:1. The free sterol band containing both cholesterol and desmosterol was removed and extracted with ether. Sterol esters were saponified with alcoholic KOH and the sterols were extracted with hexane. The sterol ester fatty acids were recovered by acidifying the aqueous phase and re-extracting with hexane for fatty acid analysis by the procedures described later. Sterol content was determined by GLC (Perkin-Elmer Model 8500, Norwalk, CT) on a 30 M SE-30 capillary column. The temperatures of column, detector, and injection post were 260°, 300°, and 300°C, respectively. Helium was used as the carrier gas. Cholestane was used as internal standard (17).

For the analysis of sterol sulfates, another aliquot of liquid extract was applied on silica gel H TLC plates. Cholesterol [4-^{14}C]sulfate was added as internal standard. The TLC plates were developed in chloroform-methanol-acetic acid 80:20:2 (18). Sterol sulfates were extracted from the TLC gel by chloroform and solvolyzed to liberate free sterols (19). The quantity of sterols was determined by GLC as described in the previous paragraph.

Analysis of fatty acids

The fatty acids of the lipid classes (phospholipids, free fatty acids, triglycerides, and sterol esters) of sperm lipids were determined. The four lipid classes were separated by TLC as described above. The individual phospholipids were separated by another TLC system (20), using pre-coated silica gel K6 plate (Whatman, Clifton, NJ) and a solvent system of chloroform-methanol-petroleum ether (bp 35-60°C)-acetic acid-boric acid 40:20:30:10:1.8 (v:v:v:v:w).

The fatty acids in each lipid class or phospholipid class were transmethylated with boron trifluoride-methanol (21) and their methyl esters were analyzed by GLC (22) on an instrument equipped with a hydrogen flame ionization detector (Perkin-Elmer Model Sigma 3B, Norwalk, CT) and a 30-m SP-2330 fused silica capillary column (Supelco, Bellefonte, PA). The temperatures of the column, detector, and injection port were 195°, 250°, and 250°C, respectively. Helium was used as the carrier gas. The split ratio was 1:170. The retention time and area of each peak were measured by an HP-3390 integrator, and a computer (HP85, Hewlett Packard, Palo Alto, CA) identified and quantified each individual fatty acid. A mixture of fatty acid standards was run daily.

Analysis of phospholipid molecular species

The molecular species of sperm ethanolamine glycerophospholipids were determined by established techniques (23). As noted above, sperm phospholipid classes were separated by TLC (20). Ethanolamine glycerophospholipids were extracted from gel scrapings with two washes of 5 ml of chloroform-methanol 1:1 (v/v), followed by one wash with 5 ml chloroform-methanol-water 65:45:12 (by volume) and one more with 5 ml of chloroform-methanol 1:1 (v/v) (24). The molecular species of ethanolamine glycerophospholipids were analyzed by the method of Blank et al. (25). Briefly, ethanolamine glycerophospholipids were hydrolyzed with phospholipase C for 4 h at room temperature (26). Diradylglycerols were extracted

from the hydrolysate by the Bligh and Dyer method (27) and benzoate derivatives were prepared by reaction with benzoic anhydride and 4-dimethyl aminopyridine for 1 h at room temperature (28). The reaction was stopped by addition of concentrated ammonium hydroxide, and the resulting diradylglycerobenzoates were extracted with hexane.

The diradylglycerobenzoates were separated into the alkenylacyl, alkylacyl, and diacyl subclasses by TLC on silica gel G with benzene–hexane–ethyl ether 50:45:4. Bands were scraped into a 1:1 ethanol and water mixture and the diradylglycerobenzoates were extracted with hexane. The samples were then filtered (Millex-HV 0.45 μm filter unit, Millipore Corp, Bedford, MA 01730), dried under nitrogen, and redissolved in acetonitrile–isopropanol 70:30 (v/v) for HPLC injection.

The separation of molecular species was accomplished with a Perkin-Elmer Model 410 LC BioPump system fitted with a μBondapak C18 pre-column insert and a 3.9 mm × 30 cm analytical column packed with Nova-pak C18 (Water 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 alkylacyl-glycerobenzoates were separated by isocratic elution with acetonitrile–isopropanol (v:v) in the ratios of 70:30, 65:35, and 63:37, respectively. Column flow rate was 1 ml/min.

Identification of molecular species was accomplished by comparison with retention times in control samples of bovine brain ethanolamine glycerophospholipids, as established by Blank et al. (25) and by gas chromatographic analysis (22) of the collected peaks. The elution profiles of bovine brain ethanolamine glycerophospholipids were similar to those obtained by Blank et al. (25).

RESULTS

Although monkey sperm contained cholesterol as expected, there was actually a predominance of another sterol, desmosterol (**Fig. 1**). Of the total sterol content of monkey sperm, 359 μg per 10^9 cells (**Table 1**), 59% was desmosterol and 41% was cholesterol. Sperm cholesterol occurred in three different forms: free, esterified, and sulfated. Free cholesterol accounted for 60% of the total cholesterol. Cholesteryl ester and sulfate contributed 20% each. Desmosterol was present in the free form only. Sperm desmosterol was identified by GLC–MS which will be described subsequently. Other precursor sterols were not present in these analyses.

The fatty acid composition of sperm lipids and individual lipid classes (phospholipids, free fatty acid, triglycerides, and cholesteryl esters) is depicted in **Table 2.** In sperm lipids, saturated fatty acids comprised 48.7% of total fatty acids, with palmitate (16:0) and stearate (18:0)

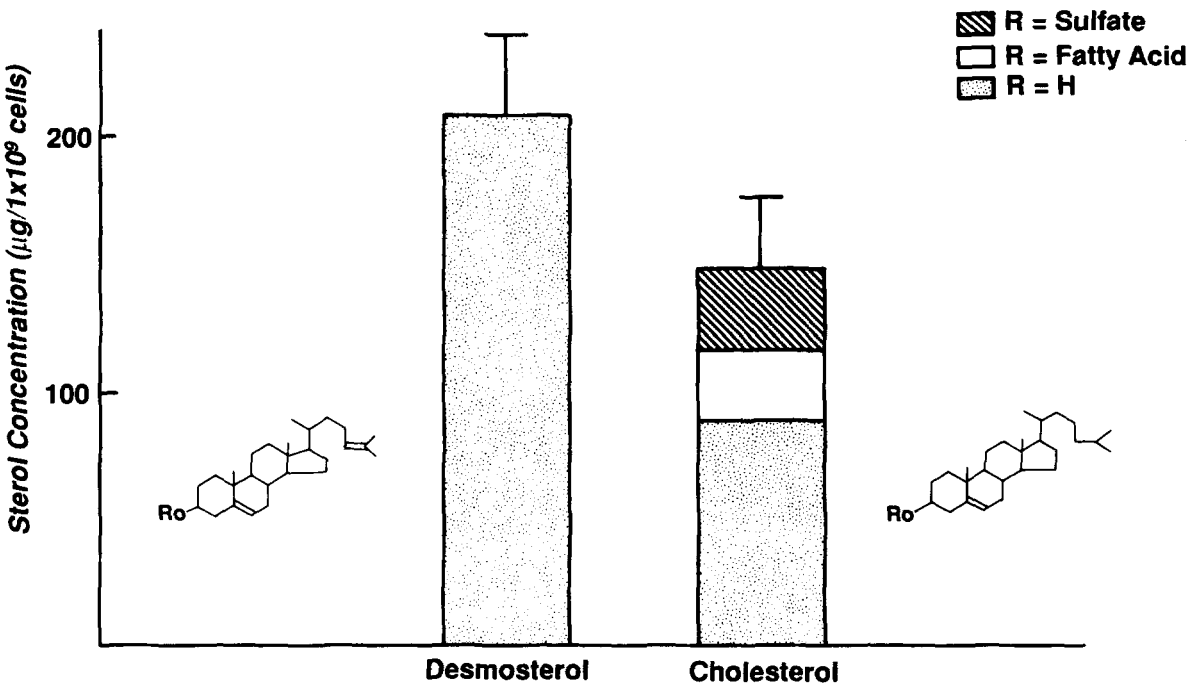

Fig. 1. The relative compositions of desmosterol and cholesterol in rhesus monkey spermatozoa (n = 5).

TABLE 1. Sterol composition of rhesus monkey sperm

Sterol	Content	Percent of Total Sterols
	μg/1 × 10⁹ cells	*%*
Cholesterol Free (60%) Ester (20%) Sulfate (20%)	149 ± 29.3	41.4 ± 5.8
Desmosterol Free (100%)	210 ± 31.0	58.6 ± 5.8
Total	359 ± 43.1	100

Values given as mean ± SD, n = 5.

predominating. Polyunsaturated fatty acids contributed 33.9% of the total fatty acids with the n-3 fatty acids (19.8%) higher than n-6 fatty acids (14.2%). The n-6 to n-3 ratio was 0.72. More than 95% of the n-3 fatty acids was DHA. Arachidonic acid (20:4) was the most abundant n-6 fatty acid (44% of total n-6 fatty acids). Linoleic acid (18:2) and 20:3 n-6 also contributed substantially (33 and 19% of total n-6 fatty acid, respectively).

Among the four lipid classes, phospholipids contained the most polyunsaturated fatty acids (44%). N-3 fatty acids were 25% and n-6 fatty acids were 19% of total fatty acids. The n-6 to n-3 ratio was 0.76. Most of the n-3 fatty acid was DHA (95% of total n-3 fatty acids). Arachidonic, linoleic acids, and homogamma linolenic (20:3 n-6) were the major n-6 fatty acids. In the other three lipid classes, polyunsaturated fatty acids were in relatively low concentration (11-14% of total fatty acids), predominated by the n-6 family (79-85% of total polyunsaturated fatty acids). The n-6 to n-3 ratio varied between 3.46 and 6.79.

The fatty acid compositions of eight different phospholipids in monkey sperm are presented in **Table 3.** Phosphatidylethanolamine and phosphatidylcholine had the highest polyunsaturated fatty acid content at 49% and 35%, respectively. The ratio of n-6 fatty acids to n-3 fatty acids was 0.58 for phosphatidylcholine and 0.57 for phosphatidylethanolamine. Over 90% of the n-3 fatty acids was DHA. The predominant saturated fatty acid was palmitic acid (16:0) (58-72% of total saturated fatty acids). Phosphatidic acid and cardiolipin had the second most polyunsaturated fatty acids (22 and 35% of total fatty acid, respectively). Unlike phosphatidylethanolamine and phosphatidylcholine, n-6 fatty acids were the most predominant polyunsaturated fatty acids in phosphatidic acid and cardiolipin. The n-6/n-3 ratio was 6.47 for phosphatidic acid and 20.75 for cardiolipin. Linoleic acid (18:2) and homogamma linolenic acid (20:3) were the major n-6 fatty acids of these two phospholipids. Phosphatidylinositol, phosphatidylserine, and phosphatidylglycerol all had low polyunsaturated fatty acid content (11-18% of total fatty acids). The majority of their polyunsaturated fatty acids were in the n-6 family. Sphingomeylin contained the lowest amount of polyunsaturated fatty acids (6.9% of total fatty acids). Monounsaturated fatty acids were the predominant fatty acids in this phospholipid (52% of total fatty acids).

As DHA is the predominant polyunsaturated fatty acid in sperm, and as the ethanolamine glycerophospholipids

TABLE 2. *Fatty acid composition of total lipids and lipid classes of rhesus monkey sperm*

		Lipid Classes			
Fatty Acids	Total Lipids	Phospholipids	Free Fatty Acids	Triglycerides	Cholesterol esters
		% of total fatty acids			
16:0	32.0 ± 2.3	28.2 ± 6.4	30.4 ± 4.8	25.0 ± 5.0	28.6 ± 9.5
18:0	12.4 ± 0.8	8.6 ± 1.1	24.2 ± 3.6	14.2 ± 1.5	17.6 ± 2.7
Total saturated	48.7 ± 3.2	38.4 ± 6.4	60.9 ± 7.2	46.0 ± 5.1	54.4 ± 11.5
18:1	12.0 ± 1.3	10.9 ± 3.9	18.5 ± 2.1	31.7 ± 7.2	20.1 ± 8.1
Total monounsaturated	15.2 ± 2.3	15.5 ± 0.2	23.4 ± 1.5	38.8 ± 7.8	31.4 ± 9.3
18:2 n-6	4.7 ± 0.3	5.4 ± 0.8	5.8 ± 1.1	5.2 ± 0.6	3.6 ± 1.2
20:3 n-6	2.7 ± 0.4	3.7 ± 0.1	0.9 ± 0.5	0.3 ± 0.2	*tr*
20:4 n-6	6.2 ± 1.1	9.1 ± 0.9	3.2 ± 2.0	0.7 ± 0.4	1.2 ± 1.0
22:5 n-6	tr	tr	tr	0.8 ± 0.5	1.6 ± 1.2
Total n-6	14.2 ± 1.9	19.0 ± 1.6	11.1 ± 3.3	10.2 ± 5.7	9.5 ± 4.9
20:5 n-3	tr	0.4 ± 0.3	tr	0.8 ± 1.3	
22:5 n-3	0.6 ± 0.4	0.5 ± 0.2		tr	
22:6 n-3	18.9 ± 3.0	23.9 ± 3.7	1.9 ± 1.4	1.4 ± 0.9	1.1 ± 1.0
Total n-3	19.8 ± 3.1	25.1 ± 5.2	2.5 ± 1.4	2.8 ± 1.4	1.4 ± 1.5
Total polyunsaturated	33.9 ± 4.3	44.1 ± 6.6	13.6 ± 4.3	13.0 ± 6.7	10.9 ± 4.8
n-6/n-3	0.72	0.76	4.44	3.46	6.79

Values given as mean ± SD; n = 5; tr, trace.

TABLE 3. Fatty acid composition of individual phospholipids of rhesus monkey sperm

Fatty Acids	Sphingomyelin	PC	PI	PS	PE	PG	PA	Cardiolipins
				% of total fatty acids				
16:0	19.8 ± 2.9	30.8 ± 1.4	29.8 ± 7.2	23.2 ± 8.1	22.7 ± 2.7	52.5 ± 7.0	17.3 ± 3.6	15.8 ± 3.8
18:0	10.2 ± 2.4	6.4 ± 1.2	20.0 ± 4.1	19.3 ± 3.7	12.8 ± 4.2	8.4 ± 7.7	6.9 ± 3.6	6.2 ± 2.5
Total saturated	36.5 ± 4.7	42.2 ± 3.3	58.1 ± 7.6	55.1 ± 9.0	39.4 ± 6.3	67.7 ± 4.7	35.7 ± 4.5	29.8 ± 7.0
18:1	6.6 ± 1.0	12.5 ± 0.5	8.9 ± 3.5	7.1 ± 2.8	3.6 ± 1.8	6.6 ± 2.1	9.5 ± 4.9	11.3 ± 2.3
Total monounsaturated	51.9 ± 5.1	17.8 ± 2.9	19.9 ± 6.2	25.7 ± 5.9	9.8 ± 2.3	17.9 ± 5.2	37.4 ± 7.2	31.2 ± 4.3
18:2 n-6	3.3 ± 1.5	3.1 ± 0.6	4.1 ± 1.5	3.2 ± 1.0	1.5 ± 0.6	2.6 ± 0.9	7.2 ± 5.7	21.5 ± 1.0
20:3 n-6	1.1 ± 0.6	2.8 ± 0.2	2.1 ± 0.9	1.5 ± 1.2	3.0 ± 1.2	4.7 ± 2.5	7.8 ± 3.1	9.0 ± 1.2
20:4 n-6	0.5 ± 0.4	4.1 ± 1.2	1.2 ± 0.9	3.6 ± 1.4	9.8 ± 1.7	tr	tr	1.6 ± 0.2
22:5 n-6		1.4 ± 0.3	4.1 ± 2.7	4.0 ± 3.2	1.9 ± 0.6	tr	tr	tr
Total n-6	5.6 ± 1.4	13.1 ± 3.0	13.8 ± 5.4	13.8 ± 6.5	17.7 ± 1.8	9.5 ± 2.6	19.4 ± 4.9	33.2 ± 2.9
20:5 n-3		0.5 ± 0.3			tr		1.0 ± 1.2	tr
22:5 n-3					0.5 ± 0.6			
22:6 n-3	0.9 ± 1.1	21.6 ± 3.7	3.6 ± 2.8	0.6 ± 0.6	30.4 ± 7.2	0.9 ± 1.2	1.8 ± 1.6	1.1 ± 0.7
Total n-3	1.4 ± 1.8	22.4 ± 4.1	4.3 ± 2.8	1.2 ± 0.5	31.1 ± 7.3	1.0 ± 1.1	3.0 ± 2.2	1.6 ± 0.9
Total polyunsaturated	6.9 ± 3.1	35.4 ± 6.5	18.1 ± 5.4	14.9 ± 6.9	48.8 ± 8.9	10.5 ± 2.9	22.4 ± 7.5	34.8 ± 3.5
n-6/n-3	4.00	0.58	3.21	11.50	0.57	9.50	6.47	20.75

Values given as mean ± SD; n = 5; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PA, phosphatidic acid; tr, trace.

of sperm are richest in this fatty acid, we chose this phospholipid class for molecular species analysis (**Table 4**). Molecular species were determined for each of the three subclasses of this phospholipids (diacyl, alkenylacyl, and alkylacyl). In the diacyl subclass, a total of ten different molecular species were identified and quantified. While the *sn*-1 position always contained only 16:0, 18:0, or 18:1, the fatty acids in the *sn*-2 position differed markedly. There were three n-3 species (species that contained n-3 fatty acid in the *sn*-2 position), five n-6 species, one n-9 species, and one saturated series containing saturated fatty acid in both *sn*-1 and *sn*-2 positions (16:0-16:0). The n-3 species contributed 43% of the total molecular species and the n-6 species amounted to 53%. The n-9 and saturated species were only about 2% each. Of the n-3 species, 16:0-22:6 predominated (62% of total n-3 species). The ratio of 18:1-22:6/16:0-22:6/18:0-22:6 was 1/4.2/1.5. Interestingly, for the n-6 species, species with 18:0 at the *sn*-1 position were prevalent. For example, the ratio of 18:1-20:4/16:0-20:4/18:0-20:4 was 1/1.6/2.6. In alkenylacyl subclasses, a total of eleven different molecular species were identified. The n-3, n-6, and n-9 species were 73, 12, and 15%, respectively. Again 16:0-22:6 was the predominant n-3 species (80% of total n-3 species). Most of the n-9 molecular species were located in this subclass. In the alkylacyl subclass, only n-3 species were found, and 16:0-22:6 contributed 79% of the total.

As the HPLC detector response is proportional to the molar concentration of the glycerobenzoate derivatives of the different molecular species (25), the relative concentrations of the three subclasses (diacyl, alkenylacyl and alkyacyl) could be calculated by summing the total area of all peaks (**Table 5**). The relative distributions of diacyl, alkenylacyl, and alkylacyl subclasses in sperm ethanolamine glycerophospholipids were 49, 43, and 8%, respectively.

The identification of desmosterol was confirmed by

TABLE 4. Major molecular species of ethanolamine glycerophospholipids of rhesus monkey sperm

Molecular species	Composition		
	Diacyl	Alkenylacyl	Alkylacyl
		mol %	
n-3			
18:1-22:6	6.4 ± 1.4	7.7 ± 3.0	16.9 ± 10.3
16:0-22:6	26.9 ± 4.0	58.9 ± 11.6	78.6 ± 13.6
18:0-22:6	9.8 ± 0.7	6.8 ± 3.7	4.5 ± 7.7
Total	43.1 ± 4.3	73.4 ± 12.8	100 ± 0
n-6			
18:1-20:4	7.0 ± 2.1	3.0 ± 1.0	tr
16:0-20:4	11.5 ± 1.1		
18:0-20:4	15.2 ± 2.1	0.7 ± 1.2	tr
16:0-20:3	5.9 ± 1.9	5.4 ± 3.0	tr
18:0-20:3	10.1 ± 2.7	2.6 ± 1.4	tr
Total	52.7 ± 5.4	11.7 ± 4.2	
n-9			
18:1-18:1	1.5 ± 1.3	1.6 ± 2.3	
17:0-18:1	tr	6.5 ± 2.4	
18:0-18:1	tr	3.8 ± 6.6	
18:1-20:1	tr	3.0 ± 4.0	
Total	1.5 ± 1.3	15.0 ± 8.3	
Saturated			
16:0-16:0	2.3 ± 2.5		

Values given as mean ± SD; n = 3; tr, trace.

TABLE 5. Relative distribution of the subclasses of ethanolamine glycerophospholipids of rhesus monkey sperm

Subclass	Distribution
	percent of total
Diacyl	49.1 ± 5.6
Alkenylacyl	42.9 ± 4.0
Alkylacyl	7.6 ± 5.0

Values given as mean ± SD; n = 3.

chromatographic retention time and by electron ionization and methane chemical ionization mass spectra (29). Desmosterol elutes at 12.21 min, slightly later than cholesterol at 11.91 min, as shown in **Fig. 2.** The relative areas under the total ion chromatogram peaks were 42% for cholesterol and 58% for desmosterol. The electron ionization mass spectrum (not shown) was identical to the published spectrum of the desmosterol-O-TMS derivative (29). A weak molecular ion of 2% abundance was present at *m/z* 456 in the E1 spectrum. The methane chemical ionization spectrum (**Fig. 3**) provided additional confirmation of desmosterol identity. The spectrum had a weak protonated molecular ion [M + H] at *m/z* 457, together with a slightly more abundant hydride abstraction ion [M-H] at *m/z* 455. Other structurally useful ions were present at *m/z* 441 [M-H-CH_3], *m/z* 395 [M-H-$(CH_3)_2Si$], and *m/z* 367 [M + H-$(CH_3)_3$ SiOH]. These ions (both E1 and C1) were 2 amu lower than corresponding ions observed in the spectrum of the cholesterol-O-TMS derivative, which indicated the presence of an additional double bond in the sterol.

DISCUSSION

Phospholipids and sterols are the two predominant lipid components of monkey sperm representing more than 85% of the total lipids (2). In the present study, we analyzed the detailed composition of these two lipid components in the sperm of the nonhuman primate, the rhesus monkey. Our data demonstrated for the first time the presence of desmosterol as the predominant sterol, confirmed by mass spectroscopy. We have also detected rather high concentrations of desmosterol in the testis of adult rhesus monkeys, 13% of total sterols, (W. E. Connor, D. S. Lin, and M. D. Neuringer, unpublished data). This sterol has previously been identified tentatively in the sperm of only two other species, the hamster and boar (30-32). While no quantitative analyses of desmosterol were made in hamster sperm, desmosterol accounted for only 14% of total sterols in boar sperm. In contrast, monkey sperm contained desmosterol as the predominant sterol. A low level of Δ^{24}-reductase and/or the presence of an inhibitor of this enzyme in the monkey gamete during spermatogenesis are two likely mechanisms by which desmosterol might accumulate.

Large quantities of desmosterol in primate spermatozoa and testes present a unique biological finding. From the pioneer observations of Stokes, Fish, and Hickey (33), desmosterol was discovered initially as a transitory sterol in the chick brain. Its presence has also been observed by us in the brain of 14-day-old chick embryos but not in the brain of the 21-day-old chicks (34). A small amount of desmosterol was found in the brain of rat pups, but the amount decreased with age (35). Desmosterol is a usual constituent of human milk, in which it represents about 8-12% of the total milk sterols (36). No special biological function has been attributed to desmosterol in human milk. While desmosterol is well known as a transitory trace product in the synthesis of cholesterol, the biological significance of desmosterol in spermatozoa, presumably derived from desmosterol in spermatogonia, is unknown. In view of its significant quantitative presence, it could be important in the function of spermatozoa.

Unlike desmosterol, which was found only in the free form only, sperm cholesterol existed in free, esterified, and sulfated forms. Cholesterol sulfate has also been detected in boar and human sperm (31, 37). It has been postulated

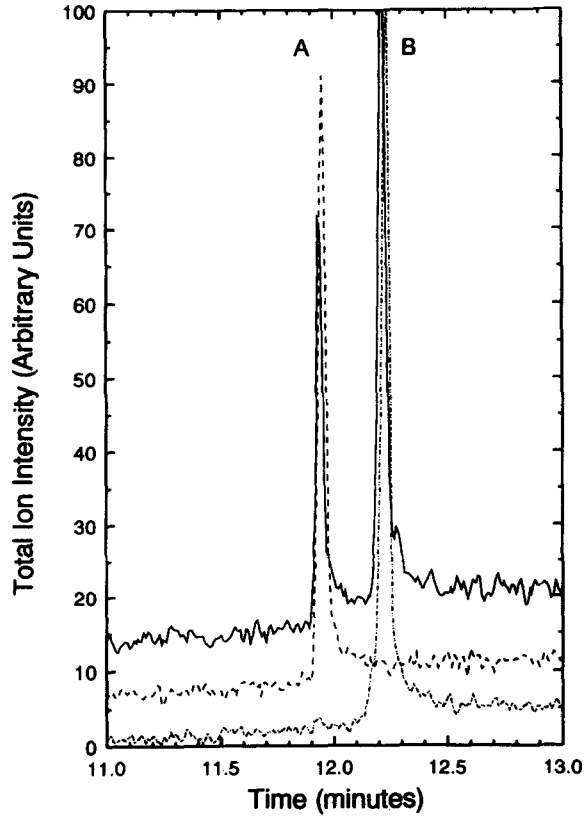

Fig. 2. Methane chemical ionization total ion chromatograms of the TMS derivatives of rhesus monkey spermatozoa sterols (—), a cholesterol standard (----), and a desmosterol standard (-··-··). Peak A was identified as cholesterol (11.91 min) and peak B was identified as desmosterol (12.21 min.).

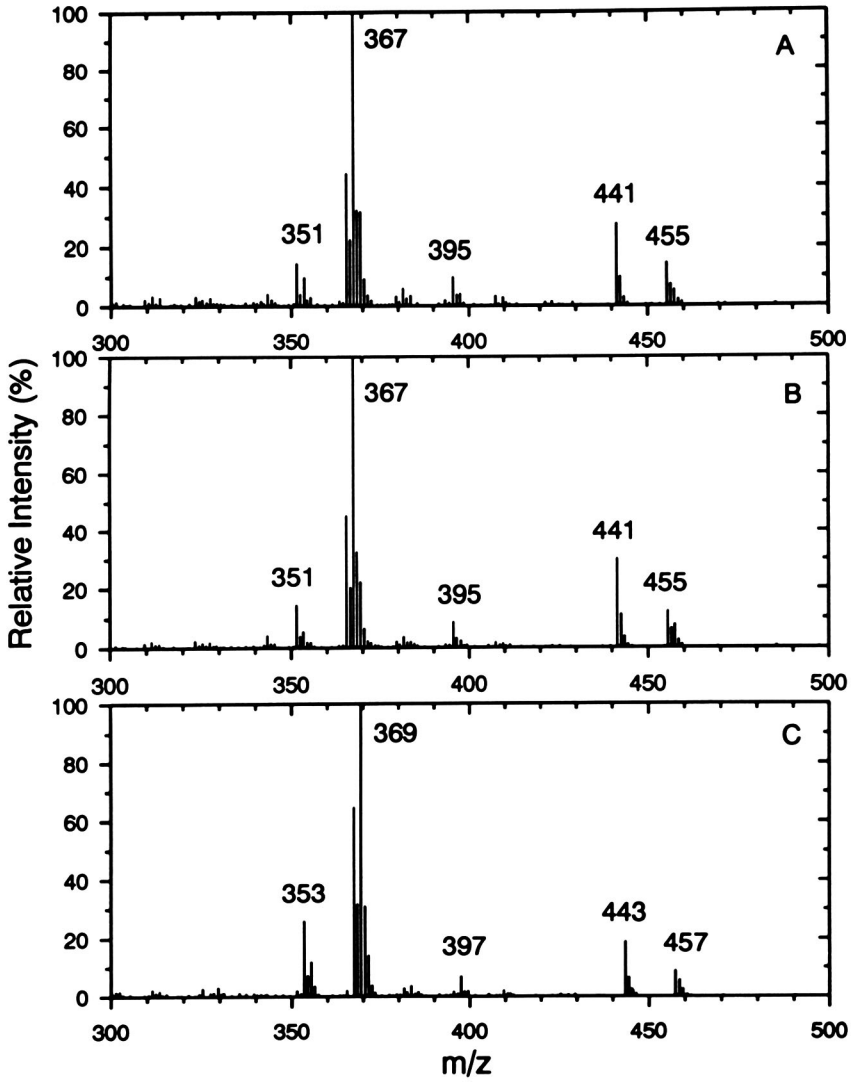

Fig. 3. The methane chemical ionization mass spectra of the TMS derivatives of the rhesus monkey sterol at 12.21 min (A), a desmosterol standard (B), and a cholesterol standard (C).

that cholesterol sulfate acts as membrane stabilizer and enzyme inhibitor during sperm maturation in the epididymis and thus may play an important role in sperm capacitation and fertilization (9, 11). In contrast to our results, desmosterol sulfate comprised 95% of the sterol sulfate fraction of the epididymal spermatozoa in hamsters (30). Roberts (11) reported that the testis is the major source of cholesterol sulfate in humans, whereas the epididymis is the source of desmosterol sulfate in the hamster.

Esterified cholesterol accounted for 20% of the total cholesterol in rhesus monkey sperm as opposed to approximately 30% in rat epididymal sperm (38). Quinn and White (39) observed a progressive increase in cholesteryl ester content in ram sperm collected from caputal, caudal epididymis and in semen, suggesting that esterification occurs during sperm transit.

The n-3 fatty acid DHA (22:6) is highly concentrated in retina and brain and is essential for the development and function of these neural tissues (5, 6). Sperm is the only other tissue in the body known to contain a high concentration of this n-3 polyunsaturated fatty acid (3, 4). However, the analysis of individual phospholipids and molecular species indicate that there are some differences in the lipid biochemistry of neurons and sperm. First, sperm DHA is present mainly in phosphatidylethanolamine and phosphatidylcholine, whereas brain and retinal DHA is concentrated in phosphatidylethanolamine and phosphatidylserine. Second, differences were found between the fatty acid composition of phosphatidylethanolamine of sperm, cerebral cortex, and retina (**Table 6**). The most striking difference between sperm and two neural tissues occurs in the concentrations of the two major satu-

TABLE 6. Comparison of the fatty acids of phosphatidylethanolamine in sperm, brain and retina of the rhesus monkey

Fatty Acids	Sperm	Brain[a]	Retina[a]
16:0	22.7 ± 2.7	6.3 ± 2.3	3.1 ± 0.2
18:0	12.8 ± 4.2	31.4 ± 2.4	23.8 ± 6.0
Total saturated	39.4 ± 6.3	39.2 ± 1.6	30.6 ± 4.9
18:1	3.6 ± 1.8	7.1 ± 1.2	5.9 ± 1.1
Total monounsaturated	9.8 ± 2.3	10.3 ± 1.6	8.8 ± 1.1
20:4 n-6	9.8 ± 1.7	12.4 ± 0.9	16.0 ± 1.6
22:4 n-6	tr	9.4 ± 0.8	2.4 ± 0.4
22:5 n-6	1.9 ± 0.6	1.4 ± 0.3	0.7 ± 0.7
Total n-6	17.7 ± 1.8	24.9 ± 1.5	22.6 ± 2.3
22:6 n-3	30.4 ± 7.2	22.3 ± 0.3	36.4 ± 5.8
Total n-3	31.1 ± 7.3	22.5 ± 0.5	37.2 ± 4.7
Total polyunsaturated	48.8 ± 8.9	47.3 ± 1.0	59.8 ± 7.0
n-6/n-3	0.57	1.11	0.61

Values given as mean ± SD.
[a]From reference 5.

rated fatty acids (16:0 and 18:0). While palmitic acid was the predominant saturated fatty acid in sperm, stearic acid was the major saturated fatty acid in brain and retina. This difference was reflected in molecular species composition. Thus, the major n-3 molecular species in sperm was 16:0-22:6, whereas in brain 18:0-22:6 predominated (23). This tissue specificity of molecular species suggests that different molecular species may have important roles in cellular structural and function. Third, differences were found in the distribution of n-3 molecular species in subclasses. In the diacyl subclass, these species contributed about 43% of total molecular species in both sperm and cerebral cortex (23). However, while the contribution of the n-3 species remained about the same in the other two subclasses in the cerebral cortex (about 45%), it increased in sperm to 73% in the alkenylacyl subclass and 100% in the alkylacyl subclass. Fourth, there was dissimilarity of the relative concentrations of the three subclasses (diacyl, alkenylacyl, and alkylacyl) of ethanolamine glycerophospholipids between rhesus monkey sperm and cerebral cortex. Sperm have lower diacyl (50 vs. 68%) but higher alkenylacyl (43 vs. 30%) and alkylacyl subclasses (8 vs. 3%).

The presence of alkenylacyl subclass (plasmalogens) has been reported in bull, ram, rabbit, boar, and human sperm (40). The precise function of the membrane phospholipids with ether linkages has yet to be elucidated. However, it has been suggested that they may play a direct role in protecting animal cell membranes against oxidative stress (41) and perhaps also in fertility. Minhas and colleagues (42) have identified the presence of platelet activating factor-like activity in human spermatozoa and suggested that this factor may be related to the fertility potential of sperm. A metabolic pathway of ether-linked glycerophospholipids, the conversion of alkylacyl glycerophosphocholine to alkylacyl glycerophosphoethanolamine, was recently proposed by Daniel et al. (43).

The results of this study clearly define the unique lipid composition of primate spermatozoa. Because lipids play an important role in the viability, maturity, fertility, and function of the sperm cells (6-13), the question arises: can sperm lipid composition and, in turn, sperm function be altered by dietary or pharmacologic means? The lipid composition of rhesus monkey brain, retina, and other tissues has been shown to be sensitive to dietary changes (5, 22, 23). One case report suggests dramatic effects of dietary n-3 fatty acids on sperm motility and production. A healthy but elderly man who consumed only marine food, rich in n-3 fatty acids, and water for 100 days produced immobile sperm and eventually became azoospermic (44). The total amount of prostaglandins in his seminal fluid decreased significantly and only a small amount of the 3-series prostaglandins appeared. These interesting questions require further exploration.

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