Phospholipid molecular species in human umbilical artery and vein endothelial cells

Hitoshi Takamura,* Hisanori Kasai,† Hitoshi Arita,† and Makato Kito[1,*]

Research Institute for Food Science,* Kyoto University, Uji, Kyoto 611, Japan, and Shionogi Research Laboratory,† Shionogi & Co., Ltd., Fukushima-ku, Osaka 533, Japan

Abstract Molecular species of several phospholipid classes and subclasses were quantitatively determined in human umbilical artery and vein endothelial cells. Both types of endothelial cells were similar in phospholipid class composition, whereas they were markedly different in phospholipid subclass and molecular species composition. The amounts of two ether subclasses in phosphatidylcholine and phosphatidylethanolamine were higher in artery endothelial cells than those in vein endothelial cells. The relative content of alkylacyl subclass in phosphatidylcholine, a precursor of platelet-activating factor, was about three times higher in artery endothelial cells than in vein endothelial cells. In artery endothelial cells, arachidonic acid was in highest amounts in alkenylacyl phosphatidylethanolamine, followed by diacyl phosphatidylcholine, diacyl phosphatidylethanolamine, and phosphatidylinositol. In the vein endothelial cells, arachidonic acid was highest in phosphatidylinositol, followed by diacyl phosphatidylethanolamine, diacyl phosphatidylcholine, and alkenylacyl phosphatidylethanolamine. Artery endothelial cells had higher amounts of molecular species containing arachidonic acid than vein endothelial cells in all phospholipid classes and subclasses. These differences are thought to reflect the functional differences of artery and vein endothelial cells.—**Takamura, H., H. Kasai, H. Arita, and M. Kito.** Phospholipid molecular species in human artery and vein endothelial cells. *J. Lipid Res.* 1990. **31:** 709–717.

Supplementary key words arachidonic acid • ether phospholipid • phosphatidylcholine • phosphatidylethanolamine • phosphatidylinositol • phosphatidylserine • cardiolipin

Biological membranes are composed of several phospholipid classes and subclasses consisting of various molecular species, which control membrane fluidity and function. In animal tissues and cells, membrane phospholipids contain considerable arachidonic acid (20:4), which is liberated upon stimulation and transformed via the cyclooxygenase and lipoxygenase pathways to various eicosanoids, e.g., prostaglandins, thromboxanes, and leukotrienes.

We have developed a sensitive analytical method for phospholipid molecular species to study the structure and function of membrane phospholipids (1, 2) and have reported the distribution and changes upon stimulation of phospholipid molecular species in human platelets, and emphasized the importance of 20:4 (3, 4). Endothelial cells also produce eicosanoids including prostaglandin I_2, which inhibits platelet aggregation and prevents arterial constriction (5–7). In addition, endothelial cells produce platelet-activating factor (PAF), a potent platelet agonist with a wide range of biological activities (8, 9).

Lipid analysis and metabolism in human endothelial cells have been reported using cells from umbilical veins (10–15). Blank et al. (12) reported the molecular species composition of diacyl PC and diacyl and alkenylacyl PE in human umbilical vein endothelial cells. However, alkylacyl PC, which is an important phospholipid as a precursor of PAF, and other phospholipid classes have not been analyzed on the molecular species level. In addition, it is known that artery endothelial cells have stronger biological activities such as angiotensin I-converting enzyme and prostaglandin I_2 formation than vein endothelial cells from human pulmonary capillary and umbilical cords (16). Hence, phospholipids of artery endothelial cells should be analyzed as well as those of vein endothelial cells in order to understand the function of the endothelial cell membrane.

We describe here the distribution of molecular species in various phospholipid classes and subclasses in human umbilical artery and vein endothelial cells.

Abbreviations: TLC, thin-layer chromatography; PBS, phosphate-buffered saline; BHT, butylated hydroxytoluene; DNB-DG, dinitrobenzoyldiradylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; CL, cardiolipin; PAF, platelet-activating factor. The fatty acids or radyl (acyl, alkenyl, and alkyl) groups are identified by the number of carbon atoms and double bonds. Thus, 20:4 represents arachidonic acid which contains 20 carbon atoms and 4 double bonds. The molecular species of phospholipids are identified by the two radyl groups combined by a slash, such as 18:0/20:4.

[1]To whom correspondence should be addressed.

MATERIALS AND METHODS

Materials

Collagenase was purchased from Worthington Biochemical Corp. (Freehold, NJ); endothelial mitogen was from Biomedical Technologies Inc.; human serum, Medium 199, amphotericin B solution were from Flow Laboratories Inc. (Scotland); and heparin was from Sigma Chemical Co. (St. Louis, MO). Rabbit anti-human Factor VIII-related antigen antiserum and fluorescein-conjugated goat anti-rabbit IgG were purchased from Immunon (Michigan) and ICN (Israel), respectively. Phospholipase C (from *Bacillus cereus*, grade I) was purchased from Boehringer-Mannheim GmbH (Mannheim, F.R.G.). PI-specific phospholipase C (from *Bacillus thuringiensis*) was from Funakoshi Pharmaceutical Corp. Ltd. (Tokyo, Japan). 3,5-Dinitrobenzoyl chloride and diethyl ether (acid value and peroxide value determination grade) were from Dojindo Laboratories (Kumamoto, Japan). Pyridine (silylation grade) was from Pierce Chemical Co. (Rockford, IL). Acetonitrile, 2-propanol, and hexane used for HPLC were of HPLC grade. All other chemicals were of reagent grade.

Preparation of primary cultures

Endothelial cells from arteries and veins of human umbilical cords were prepared by a modification of the method of Jaffe et al. (17). Umbilical cords obtained after normal vaginal deliveries were used. The umbilical vessel was first rinsed thoroughly with phosphate-buffered saline (PBS), filled with 0.2% collagenase in PBS, and clamped at both ends with a two-way stopcock. The umbilical cord was set in a petri dish containing moist, sterile gauze and incubated for 10–15 min at 37°C. The cord was gently rubbed together and the cell suspension was drained into 10 ml of Medium 199, centrifuged, and the cells were seeded onto plastic dishes coated with 0.2% gelatin. Endothelial cells were cultured in Medium 199 containing 20% inactivated human serum, penicillin (50 IU/ml), streptomycin (50 μg/ml), amphotericin B (2.5 μg/ml), endothelial mitogen (100 μg/ml), and heparin (90 μg/ml). The dishes were incubated at 37°C under 5% CO_2. After serial subculture, endothelial cells harvested in the sixth

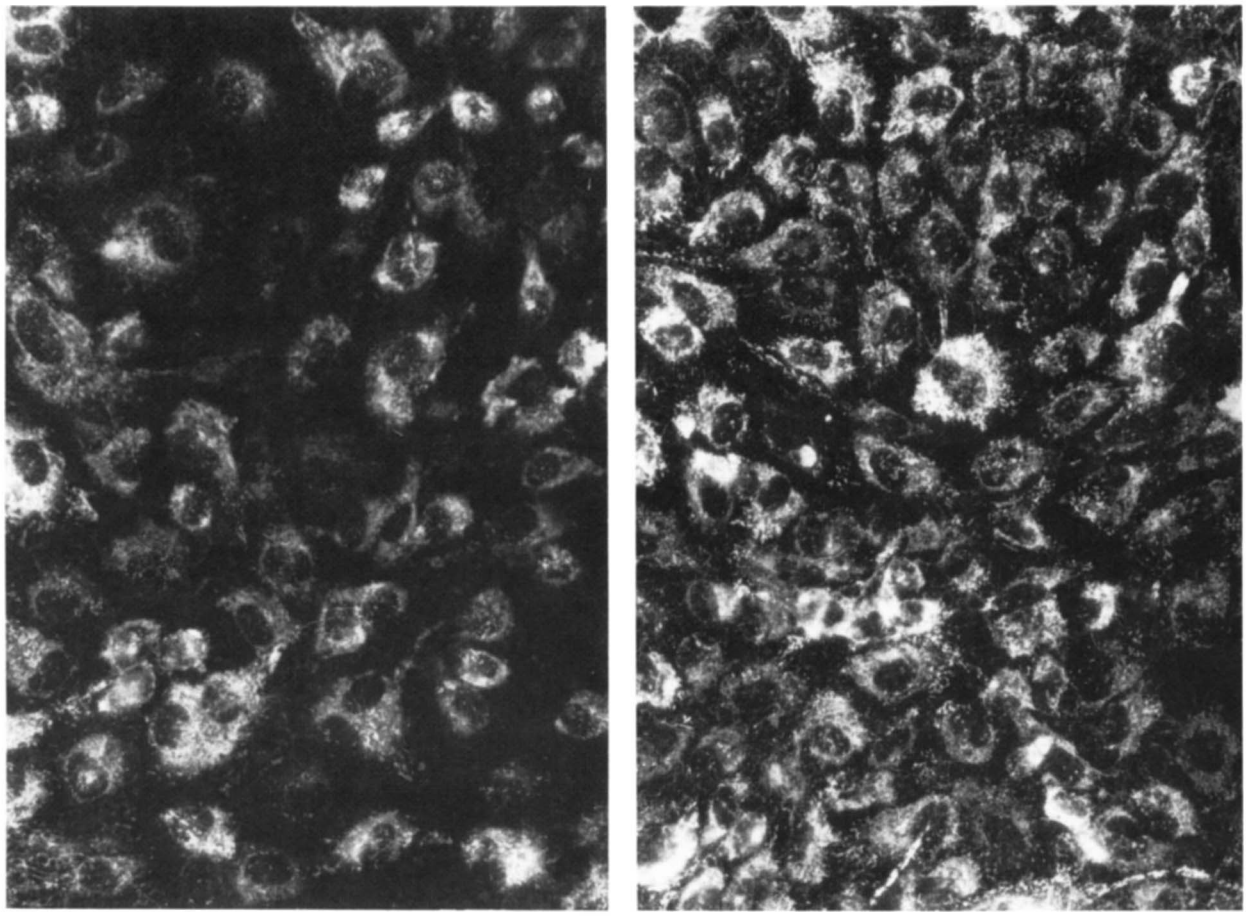

Fig. 1. Immunofluorescent localization of Factor VIII-related antigen in endothelial cells. The cells in the left panel were from umbilical artery, and those in the right were from umbilical vein. Both cultures were in the third passage.

TABLE 1. Phospholipid class composition of human umbilical artery and vein endothelial cells

Phospholipid Class	Cells	
	Artery	Vein
	mol %	
Phosphatidylcholine	49.0 ± 0.1	50.5 ± 0.1
Phosphatidylethanolamine	28.1 ± 0.3	25.5 ± 0.4
Phosphatidylserine	9.0 ± 0.2	9.1 ± 0.2
Phosphatidylinositol	6.0 ± 0.3	5.9 ± 0.4
Cardiolipin	2.0 ± 0.1	2.3 ± 0.2
Sphingomyelin	5.9 ± 0.1	6.7 ± 0.1

Values (mol%) were calculated from phosphorus determination and are presented as means ± SD (n = 4).

passage were used in the experiments. For subculture, cells were harvested with trypsin-EDTA solution (0.05% trypsin, 53 mM EDTA).

Immunocytochemical characterization

Cultured cells were rinsed in PBS, fixed with acetone at 4°C for 5 min, and air-dried. After washing with PBS, fixed cells were incubated for 3 h with anti-serum Factor VIII-related antigen at room temperature, and then incubated with fluorescein-conjugated goat anti-rabbit IgG for 1 h at room temperature. After washing and mounting, they were examined with a Nikon FX-S RFL fluorescence microscope.

Extraction of lipids from endothelial cells

Endothelial cell lipids were extracted according to the previous method for platelets (3, 4). To 3 ml of cell suspension (1×10^8/ml), 6 ml of chloroform–methanol 1:2 (v/v) containing 0.01% BHT and 0.1 mM tocopherol, and 3 ml of 2 M KCl–0.1 M EDTA were added with vigorous shaking for 10 min. Then, 6 ml of chloroform was added with shaking for an additional 10 min. After centrifugation, the organic layer was removed and the remaining lipids in the aqueous layer were extracted twice with 8 ml of chloroform. The combined organic fractions were dried by evaporation. The extracted lipids were solubilized with 10 ml of chloroform and washed twice with 30 ml of water.

Separation of phospholipid classes

We divided the cell lipid extract into four portions and repeated four separate analyses. The lipids were separated into phospholipid classes by two-step single-dimensional TLC (18). Samples were concentrated by evaporation and applied to TLC plates (Art. 11845, Merck, Darmstadt, F.R.G.). The plates were first developed with chloroform–acetone–methanol–water–acetic acid 100:100:50:10:4 (v/v), dried in vacuo for more than 30 min, and redeveloped in the same direction with chloroform–methanol–acetic acid–water 180:150:30:10 (v/v). The appropriate area of each phospholipid fraction was scraped off. Phosphorus of individual phospholipid classes was determined by the method of Bartlett (19) and Keenan, Schmidt, and Tanaka (20). For further analysis, separated phospholipid classes were extracted with chloroform- methanol 2:1 (v/v). Then the extracts were washed with water and filtered through a disk filter (Chromatodisc 13N, 0.45 μm, Kurabo Industrial Ltd., Osaka, Japan) to eliminate silica powder. All solvents included 0.01% BHT.

Preparation of the DNB-DG derivatives

Approximately 0.25 mg of PC, 0.1 mg of PE, and 0.05 mg of PS and CL were dried under N_2 flow and dispersed by sonication in 1.25 ml of 10 mM Tris-HCl buffer (pH 7.5) containing 30 mM H_3BO_4. Then 200 units of phospholipase C (from *Bacillus cereus*) and 2 ml of water-saturated diethyl ether were added. After shaking vigorously at 30°C for 3 h, the diradylglycerols were extracted twice with the water-saturated ether. The ether extracts were dried under N_2 flow and in vacuo. This incubation was sufficient for the complete hydrolysis of these phospholipid classes. PI was hydrolyzed by PI-specific phospholipase C. Approximately 0.05 mg of PI was dried and dispersed in the same buffer. Then 0.5 unit of PI-specific phospholipase C (from *Bacillus thuringiensis*) was added. The reaction was performed as described above.

Approximately 25 mg of 3,5-dinitrobenzoyl chloride and 0.5 ml of dry pyridine were added to the diradylglycerols. The mixture was heated in a sealed vial at 60°C for

TABLE 2. Subclass composition of PC and PE in human umbilical artery and vein endothelial cells

Subclass	Phospholipid			
	PC		PE	
	Artery	Vein	Artery	Vein
	mol %			
Diacyl	90.7 ± 0.0	97.3 ± 0.5	51.3 ± 0.1	65.1 ± 3.1
Alkenylacyl	1.9 ± 0.1	0.2 ± 0.0	47.7 ± 0.1	34.2 ± 3.1
Alkylacyl	7.4 ± 0.1	2.5 ± 0.4	1.0 ± 0.1	0.7 ± 0.0

Values (mol%) were calculated from total relative peak areas with respect to the internal standard in each subclass (4) and are presented as means ± SD (n = 4).

TABLE 3. Relative retention times of the molecular species of the DNB-DG derivatives

Molecular Species	Subclass		
	Diacyl	Alkenylacyl	Alkylacyl
	relative retention time		
18:2/22:6	1.20	1.45	-
18:2/22:5	1.40	1.69	-
16:1/20:4	1.46 (1.83)	-	-
18:2/20:4	1.46 (1.96)	1.75	-
18:1/20:5	1.56	1.86	2.27
16:0/20:5	1.63 (2.03)	1.95	2.43 (3.07)
18:1/22:6	1.63 (2.26)	2.00	2.43 (3.45)
16:0/22.6	1.68	2.09	2.59
16:1/18:2	1.73 (2.03)	-	-
18:2/18:2	1.73 (2.17)	-	-
18:1/22.5	1.99	2.35	2.88
16:0/22.5	2.04 (2.55)	2.46 (3.16)	3.01
18:1/20:4	2.04 (2.46)	2.46 (3.03)	3.07
16:0/20:4	2.12	2.56	3.20
18:0/20:5	2.30	2.77	-
18:1/16:1	2.41 (2.50)	-	-
18:1/18:2	2.41 (2.68)	2.86	3.62
18:0/22:6	2.50 (3.15)	3.00 (3.84)	3.74
16:0/16:1	2.50 (2.47)	-	-
16:0/18:2	2.50 (2.65)	3.00 (3.30)	3.87
18:1/20:3	2.50 (2.94)	3.00 (3.53)	3.99
16:0/20:3	2.59 (2.90)	3.09 (3.53)	4.13 (4.45)
16:0/14:0	2.59 (2.43)	-	-
18:1/22:4	2.59 (3.19)	3.09 (3.89)	4.13 (4.89)
16:0/22:4	2.70	3.30	4.30
18:0/22.5	2.91	3.55	4.48
18:0/20:4	3.04	3.70	4.67
18:1/18:1	3.42 (3.39)	4.18	5.21
20:1/18:2	3.42 (3.56)	-	-
16:0/18:1	3.60	4.45 (4.15)	5.45
18:0/18:2	3.67	4.45 (4.54)	5.60
16:0/16:0	3.78 (3.39)	4.64 (4.16)	5.88
18:0/20:3	3.78 (4.02)	4.64 (4.93)	-
18:0/22:4	3.92	4.81	-
17:0/18:1	4.26	-	-
18:0/18:1	5.27	6.54	-
18:0/16:0	5.55 (4.75)	6.88	8.68
18:0/20:2	5.55 (4.95)	-	-

The DNB-DG derivatives were separated into molecular species by using acetonitrile–2-propanol 80:20 (v/v) as the eluting solvent. The overlapped peaks were then re-chromatographed by using methanol–2-propanol 95:5 (v/v). The values with or without parentheses were calculated from the chromatogram with methanol–2-propanol 95:5 or acetonitrile–2-propanol 80:20, respectively, with respect to the 12:0/12:0 (diacyl) molecular species regarded as 1.00 (1, 2, 4).

10 min, and the reaction was stopped by adding 2 ml of methanol–water 80:20 (v/v) and 2 ml of water. Then, the reaction mixture was applied to a SepPak C_{18} cartridge (Waters Associates, Milford, MA), and eluted with 25 ml of methanol–water 80:20 to clean up the sample. The product was recovered by eluting with 30 ml of methanol. After evaporation, the residue was dissolved in hexane and washed with water. The diethyl ether and methanol contained 0.01% BHT.

Separation of the derivatives into subclasses

The DNB-DG derivatives thus prepared from PC and PE were separated into diacyl, alkenylacyl, and alkylacyl subclasses by single-step TLC (4). Fluorescence plates (Art. 11798, Merck) were developed with hexane–diethyl ether 70:30 (v/v) containing 0.01% BHT. After visualization under UV light (254 nm), an equal amount (approximately 5–10 nmol) of the 12:0/12:0 (diacyl) DNB-DG derivative was applied to each spot as an internal standard to determine relative peak areas and relative retention times of molecular species peaks (4). Then, the band of each subclass was scraped off and extracted twice with water-saturated diethyl ether (containing 0.01% BHT). The extract was washed with water, dried under N_2 flow, and dissolved in acetonitrile.

Separation and quantification of the molecular species

Separation and quantification of the molecular species were accomplished by reverse-phase HPLC (4) using a Hitachi Model 655A-12 liquid chromatograph with detection at 254 nm using a Hitachi Model L-4200 UV-VIS detector. Data were processed using a Hitachi Model 833A data processor. An Ultrasphere ODS column (5 μm, 4.6 × 250 mm, Altex Scientific, Berkeley, CA) was used for separation at 25°C. The eluting solvent was acetonitrile–2-propanol 80:20 (v/v) for each subclass, with a flow rate of 1.0 ml/min. Peaks containing more than one molecular species were collected from HPLC and re-chromatographed using methanol–2-propanol 95:5 (v/v) as the eluting solvent (2). Identification of the molecular species peaks was carried out as described previously (4).

RESULTS

Characterization of the endothelial cells

The endothelial nature of the cell cultures was documented by the presence of Factor VIII-related antigen in all cells (**Fig. 1**) and characteristic morphology by electron microscopy. Cells on the subculture grew in an organized manner which took place on a "cobblestone" morphology. Any contamination of the other types of cells, i.e., smooth muscle cells or fibroblast, was not detected via immunocytochemical procedures as shown in Fig. 1.

Phospholipid class and subclass composition

Phospholipid compositions of artery and vein endothelial cells were determined (**Table 1**). There was little difference between the two kinds of cells. PC was the predominant phospholipid class (49.0–50.5%), followed by PE (25.5–28.1%), PS (9.0–9.1%), sphingomyelin (5.9–6.7%), and PI (5.9–6.0%).

The subclass compositions of PC and PE are shown in **Table 2**. The values were calculated from the relative peak areas of the molecular species in each subclass by referring to the internal standards (4). In both cells, PC

TABLE 4. Molecular species composition of PC subclasses in human umbilical artery and vein endothelial cells

Molecular Species	Subclass: Diacyl		Subclass: Alkylacyl	
	Artery	Vein	Artery	Vein
	mol %			
18:2/22:6	T[a]	T	-	-
18:2/22:5	T	T	-	-
16:1/20:4	T	T	-	-
18:2/20:4	1.2 ± 0.0	T	-	-
18:1/20:5	T	T	T	1.2 ± 0.3
16:0/20:5	T	T	T	1.0 ± 0.1
18:1/22:6	T	T	1.2 ± 0.0	1.9 ± 0.3
16:0/22:6	2.1 ± 0.0	2.4 ± 0.2	1.3 ± 0.0	3.1 ± 0.5
18:2/18:2	2.4 ± 0.0	2.5 ± 0.1	-	-
18:1/22:5	1.7 ± 0.0	2.4 ± 0.1	1.3 ± 0.0	2.2 ± 0.3
16:0/22:5		1.1 ± 0.1	2.6 ± 0.1	4.3 ± 0.4
18:1/20:4	3.1 ± 0.0	1.1 ± 0.1	11.3 ± 0.1	6.7 ± 0.3
16:0/20:4	4.3 ± 0.0	2.1 ± 0.1	11.5 ± 0.1	8.1 ± 0.3
18:0/20:5	T	T	-	-
18:1/16:1	T	1.0 ± 0.1	-	-
16:0/16:1	2.6 ± 0.0	2.8 ± 0.2	-	-
18:1/18:2	4.9 ± 0.0	7.5 ± 0.5	2.3 ± 0.0	2.2 ± 0.1
16:0/18:2	12.9 ± 0.0	12.9 ± 0.1	6.1 ± 0.0	6.6 ± 0.1
18:0/22:6	T	1.0 ± 0.1	T	1.2 ± 0.1
18:1/20:3	1.7 ± 0.0	2.3 ± 0.3	3.3 ± 0.1	2.4 ± 0.2
16:0/20:3				
16:0/14:0	1.5 ± 0.0	1.0 ± 0.1	-	-
18:1/22:4	2.4 ± 0.0	2.4 ± 0.1	2.9 ± 0.1	3.8 ± 0.1
16:0/22:4				
18:0/22:5	T	T	1.3 ± 0.0	1.0 ± 0.1
18:0/20:4	4.5 ± 0.0	2.3 ± 0.1	5.5 ± 0.0	2.5 ± 0.1
18:1/18:1	3.4 ± 0.0	5.4 ± 0.3	1.3 ± 0.0	
16:0/18:1	18.3 ± 0.1	19.0 ± 0.4	11.4 ± 0.1	25.1 ± 1.3
18:0/18:2	4.9 ± 0.0	4.8 ± 0.2	3.2 ± 0.1	
16:0/16:0	16.1 ± 0.0	10.8 ± 0.9	18.6 ± 0.3	17.4 ± 1.2
17:0/18:1	T	T	-	-
18:0/18:1	3.2 ± 0.0	3.1 ± 0.9	-	-
18:0/16:0	3.0 ± 0.0	2.1 ± 0.2	5.1 ± 0.0	5.1 ± 0.5
Unidentified	1.5	5.1	8.0	6.7
20:4[b]	13.4 ± 0.0	6.6 ± 0.2	28.3 ± 0.3	17.3 ± 0.4
Polyene[c]	24.0 ± 0.1	21.6 ± 0.7	43.9 ± 0.3	39.3 ± 1.9

Values are expressed as mol% of each subclass and presented as means ± SD (n = 4).
[a]T, trace amounts (less than 1%).
[b]20:4, summation of the molecular species containing 20:4.
[c]Polyene, summation of the molecular species containing 20:3, 20:4, 20:5, 22:4, 22:5, and 22:6.

consisted mostly of the diacyl subclass. The relative content of the alkylacyl subclass, which is a precursor of PAF, was about three times higher in artery endothelial cells (7.4%) than that in vein endothelial cells (2.5%). The major subclasses of PE were diacyl and alkenylacyl types. PE contained about equal amounts of diacyl and alkenylacyl subclasses in artery endothelial cells, whereas the amount of alkenylacyl subclass was about half that of the diacyl subclass in vein endothelial cells.

Relative retention times of molecular species of the DNB-DG derivatives

Relative retention times of molecular species in diacyl, alkenylacyl, and alkylacyl subclasses of the DNB-DG derivatives are shown in **Table 3**. The derivatives were separated into molecular species by using acetonitrile–2-propanol 80:20 (v/v) as the eluting solvent. The overlapped peaks were re-chromatographed by using methanol–2-propanol 95:5 (v/v). We could not detect 20:4/20:4 and 22:6/20:4 molecular species, though 20:4/20:4 PC and PE in V79-UF cells have been reported from this laboratory (21).

Molecular species composition of PC subclasses

The molecular species compositions of diacyl and alkylacyl PC in artery and vein endothelial cells are shown in **Table 4**. The major molecular species of the diacyl subclass were 16:0/18:1, 16:0/16:0, and 16:0/18:2 in

TABLE 5. Molecular species composition of PE subclasses in human umbilical artery and vein endothelial cells

Molecular Species	Subclass			
	Diacyl		Alkenylacyl	
	Artery	Vein	Artery	Vein
	mol %			
18:2/22:6	T[a]	T	T	T
18:2/22:5	T	T	T	T
18:2/20:4	T	T	T	T
18:1/20:5	T	T	T	T
16:0/20:5	T	T	T	1.5 ± 0.0
18:1/22:6	T	1.9 ± 0.3	1.4 ± 0.0	2.9 ± 0.1
16:0/22:6	1.5 ± 0.1	3.5 ± 0.5	6.1 ± 0.0	9.5 ± 0.6
18:2/18:2	T	1.2 ± 0.2	-	-
18:1/22:5	1.0 ± 0.1	2.2 ± 0.3	1.2 ± 0.0	2.6 ± 0.1
16:0/22:5	T	2.0 ± 0.2	7.1 ± 0.0	10.4 ± 0.3
18:1/20:4	2.7 ± 0.0	2.1 ± 0.3	7.1 ± 0.0	4.5 ± 0.1
16:0/20:4	4.5 ± 0.1	2.6 ± 0.1	22.3 ± 0.1	16.7 ± 0.3
18:0/20:5	1.6 ± 0.4	1.2 ± 0.1	1.4 ± 0.0	1.7 ± 0.0
18:1/18:2	3.9 ± 0.3	4.6 ± 0.1	T	T
16:0/18:2		2.6 ± 0.4	T	T
18:0/22:6	3.7 ± 0.1	4.5 ± 0.9	3.3 ± 0.1	5.1 ± 0.4
18:1/20:3	1.0 ± 0.2	T	T	T
16:0/20:3	1.1 ± 0.1	T	1.7 ± 0.0	1.0 ± 0.0
18:1/22:4	2.2 ± 0.1	3.6 ± 0.1	1.9 ± 0.0	3.0 ± 0.2
16:0/22:4	2.3 ± 0.1	2.2 ± 0.2	8.5 ± 0.0	7.0 ± 0.2
18:0/22:5	5.1 ± 0.2	6.2 ± 1.0	4.7 ± 0.0	5.5 ± 0.2
18:0/20:4	26.0 ± 0.1	16.0 ± 1.7	17.0 ± 0.1	9.5 ± 0.2
18:1/18:1	2.6 ± 0.1	6.4 ± 0.2	T	T
16:0/18:1	4.1 ± 0.6	4.0 ± 1.1	1.3 ± 0.1	1.8 ± 0.4
18:0/18:2	8.2 ± 0.3	7.4 ± 0.5	T	T
16:0/16:0	T	T	T	T
18:0/20:3	3.4 ± 0.1	2.0 ± 0.1	T	T
18:0/22:4	6.7 ± 0.0	5.8 ± 0.2	4.6 ± 0.0	3.1 ± 0.1
20:0/20:4	-	-	T	T
20:1/18:1	T	T	-	-
18:0/18:1	7.6 ± 0.5	8.0 ± 0.9	T	T
18:0/16:0	T	T	T	T
18:0/20:2	T	T	-	-
Unidentified	6.0	4.3	3.6	7.7
20:4[b]	33.7 ± 0.0	21.2 ± 1.8	47.4 ± 0.2	31.6 ± 0.4
Polyene[c]	65.4 ± 1.2	59.5 ± 4.4	91.5 ± 0.1	86.7 ± 1.9

Values are expressed as mol% of each subclass and presented as means ± SD (n = 4).
[a]T, trace amounts (less than 1%).
[b]20:4, summation of the molecular species containing 20:4.
[c]Polyene, summation of the molecular species containing 20:3, 20:4, 20:5, 22:4, 22:5, and 22:6.

both cell types. Molecular species containing 20:4 comprised 13.4% of the total molecular species of the diacyl subclass in artery endothelial cells and 6.6% of that in vein endothelial cells. In the alkylacyl subclass, the major molecular species were 16:0/18:1 and 16:0/16:0 in both cells, and 16:0/20:4 and 18:1/20:4 in the arterial cells. This subclass contained larger amounts of molecular species containing 20:4 than the diacyl subclass. The summations of the molecular species containing 20:4 were 28.3% and 17.3% in artery and vein endothelial cells, respectively. Molecular species analysis of the alkenylacyl subclass of PC was difficult due to very low levels.

Molecular species composition of PE subclasses

PE was composed of molecular species containing 20:4 and other polyenoic acids, as compared with PC (**Table 5**). In the diacyl subclass, the predominant molecular species was 18:0/20:4. The summations of the molecular species containing 20:4 were 33.7% and 21.2% in artery and vein endothelial cells, respectively. The alkenylacyl subclass contained 16:0/20:4 rather than 18:0/20:4, and comprised 47.4% and 31.6% of the molecular species containing 20:4 in artery and vein endothelial cells, respectively. Molecular species of the alkylacyl subclass was negligible.

Molecular species composition of PI, PS, and CL

PI consisted mostly of the molecular species containing 20:4, which amounted to 80.9% and 67.0% in artery and vein endothelial cells, respectively (**Table 6**). In both cells, 18:0/20:4 comprised more than half of the molecular species of PI. Molecular species containing fatty acids other than 20:4 were also present, although they were not found in human platelets (3).

In PS, the major molecular species was 18:0/18:1. Most of the PS molecular species contained 18:0. Although more than half of the PS molecular species contained polyenoic fatty acids, summations of the molecular species containing 20:4 were 11.7% and 7.3% in artery and vein endothelial cells, which were similar to their distribution in the diacyl subclass of PC.

The molecular species composition of CL was remarkably different from those of other phospholipid classes. Species 18:1/18:2 comprised nearly half of CL and the molecular species containing 18:2 accounted for approximately 80%, whereas the molecular species containing 20:4 comprised only 3–4%. CL molecular species containing 18:2 have been reported to be closely related to mitochondrial function in rat hearts (22).

Distribution of the molecular species containing 20:4 in phospholipid classes and subclasses

Table 7 summarizes the distribution of the molecular species containing 20:4 among individual phospholipid classes and subclasses. Arachidonic acid was highest in alkenylacyl PE, followed by diacyl PC, diacyl PE, and PI

TABLE 6. Molecular species composition of PI, PS, and CL in human artery and vein endothelial cells

Molecular Species	Phospholipid					
	PI		PS		CL	
	Artery	Vein	Artery	Vein	Artery	Vein
			mol %			
18:2/22:5	T[a]	T	-	T	-	-
18:2/20:4	T	T	-	T	1.9 ± 0.1	1.2 ± 0.2
18:1/22:6	T	T	T	T	-	-
16:0/22:6	T	T	T	T	-	-
16:1/18:2	-	-	-	-	3.3 ± 0.1	3.0 ± 0.6
18:2/18:2	-	T	T	T	26.8 ± 0.5	20.1 ± 4.3
18:1/22:5	6.4 ± 0.4	1.5 ± 0.2	T	T	-	-
16:0/22:5			T	T	-	-
18:1/20:4		8.0 ± 0.8	T	T	T	T
16:0/20:4	4.0 ± 0.2	3.0 ± 0.3	T	T	T	T
18:0/20:5	T	T	T	T	-	-
18:1/18:2	1.1 ± 0.0	3.5 ± 0.3	2.0 ± 0.1	2.6 ± 0.1	46.1 ± 0.4	48.0 ± 2.9
16:0/18:2	T	T			3.4 ± 0.0	3.0 ± 0.6
18:0/22:6	T	T	9.4 ± 0.1	14.5 ± 0.0	-	-
18:1/20:3	T	T	-	-	-	-
16:0/20:3	T	1.1 ± 0.1	-	-	-	-
18:1/22:4	T	1.2 ± 0.1	1.4 ± 0.0	T	-	-
16:0/22:4	T			T	-	-
18:0/22:5	2.3 ± 0.1	3.2 ± 0.3	9.6 ± 0.6	11.9 ± 1.0	-	-
18:0/20:4	70.1 ± 0.3	55.5 ± 2.8	10.1 ± 0.3	6.1 ± 0.4	1.2 ± 0.2	T
18:1/18:1	T	2.3 ± 0.5	1.7 ± 0.0	3.8 ± 0.6	3.9 ± 0.0	5.8 ± 0.4
20:1/18:2	-	-	-	-	1.1 ± 0.0	1.8 ± 0.1
16:0/18:1	T	T	4.3 ± 0.2	4.1 ± 0.4	5.6 ± 0.3	7.7 ± 3.4
18:0/18:2	3.3 ± 0.4	5.2 ± 1.2	13.9 ± 0.1	13.1 ± 1.2	1.1 ± 0.1	2.6 ± 1.1
18:0/20:3	2.9 ± 0.3	2.7 ± 0.3	10.0 ± 0.0	5.2 ± 0.1	-	-
18:0/22:4	2.6 ± 0.1	3.0 ± 0.3	11.5 ± 0.1	9.0 ± 0.7	-	-
18:0/18:1	1.3 ± 0.3	2.2 ± 0.5	17.8 ± 0.6	18.0 ± 1.4	1.6 ± 0.3	1.4 ± 0.8
18:0/16:0	T	T	T	1.6 ± 0.1	-	-
18:0/20:2	T	T	1.0 ± 0.0	1.0 ± 0.0	-	-
Unidentified	0.8	2.6	3.1	4.2	2.8	3.7
20:4[b]	80.9 ± 1.0	67.0 ± 3.4	11.7 ± 0.3	7.3 ± 0.5	4.3 ± 0.0	3.0 ± 0.2
Polyene[c]	91.5 ± 0.7	82.2 ± 3.1	55.0 ± 0.8	51.3 ± 1.3	4.3 ± 0.0	3.0 ± 0.2

Values are expressed as mol% of each phospholipid class and presented as means ± SD (n = 4).
[a]T, trace amounts (less than 1%).
[b]20:4, summation of the molecular species containing 20:4.
[c]Polyene, summation of the molecular species containing 20:3, 20:4, 20:5, 22:4, 22:5, and 22:6.

TABLE 7. Distribution of the molecular species containing 20:4 in phospholipid classes and subclasses of human umbilical artery and vein endothelial cells

Lipid Class	Subclass	Distribution of Subclass		Molecular Species Containing 20:4	
		Artery	Vein	Artery	Vein
		mol %		*mol%*	
PC	Diacyl	44.5	49.1	5.95	3.25
	Alkylacyl	3.6	1.3	1.02	0.22
PE	Diacyl	14.4	16.6	4.86	3.51
	Alkenylacyl	13.4	8.7	6.36	2.75
PS	Diacyl	9.0	9.1	1.05	0.66
PI	Diacyl	6.0	5.9	4.86	3.98
CL	Diacyl	2.0	2.3	0.08	0.07
Others[a]		7.1	7.0	-	-
Total		100.0	100.0	24.18	14.44

Data from Tables 1–5 are summarized, and the values were expressed as mol% of the total phospholipids.
[a]Others, alkenylacyl PC, alkylacyl PE, and sphingomyelin.

in artery endothelial cells. In vein endothelial cells, 20:4 was highest in PI, followed by diacyl PE, diacyl PC, and alkenylacyl PE. Artery endothelial cells had higher amounts of the phospholipid molecular species containing 20:4 than vein endothelial cells. The relative contents of the molecular species containing 20:4 in the total phospholipids were 24.18% and 14.44% in artery and vein endothelial cells, respectively.

DISCUSSION

Phospholipid class compositions were similar in artery and vein endothelial cells (Table 1). However, the subclass compositions were different among these cells (Table 2). The arterial cells contained higher amounts of two ether subclasses in both PC and PE than vein cells. In particular, the level of alkylacyl subclass in PC was three times higher in artery endothelial cells than that in vein endothelial cells. This suggests that artery endothelial cells are capable of producing higher amounts of PAF than vein endothelial cells. The subclass composition obtained in this study was not in agreement with those of Blank et al. (12) and Takayama et al. (15), in which ether subclasses of both PC and PE in vein endothelial cells were higher than our results and the alkenylacyl subclass was higher than the alkylacyl subclass in PC. This discrepancy may be due to the differences in culture conditions of endothelial cells or the analytical methods.

There was a great difference between artery and vein endothelial cells with regards to molecular species containing 20:4 (Tables 4–7). In all phospholipid classes and subclasses, artery endothelial cells had higher amounts of the molecular species containing 20:4 than vein endothelial cells. Especially in PC, both diacyl and alkylacyl subclasses contained about twofold higher levels of the molecular species containing 20:4 in artery endothelial cells than those in vein endothelial cells (Table 4). Hornstra et al. (23) recently reported the fatty acid composition of total phospholipids in human umbilical arteries and veins; artery endothelial cells contained a larger amount of 20:4 than vein endothelial cells. Our results support their findings. The higher content of molecular species containing 20:4 in artery endothelial cells may be related to the higher production of prostaglandin I_2 in artery endothelial cells than in vein endothelial cells, as reported in human pulmonary endothelial cells (16). However, the levels of phospholipid molecular species containing 20:4 were considerably low compared with the results in human platelets (3). This suggests that phospholipid hydrolysis in endothelial cells upon stimulation has to be kept to a minimum in order to maintain the homeostasis of vascular walls. In addition, endothelial cells are capable of producing prostaglandin I_2 not only from endogenous 20:4 but also from prostaglandin H_2 originating from 20:4-containing phospholipid molecular species in platelets (6, 24).

In this study, we found for the first time that artery endothelial cells are different from vein endothelial cells in the composition of alkylacyl PC and the phospholipid molecular species containing 20:4 which are the precursors of lipid mediators. These differences may be related to the functional differences between artery and vein endothelial cells.

The authors would like to thank Ms. Kazumi Nakano for her technical assistance. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

Manuscript received 18 September 1989 and in revised form 12 December 1989.

REFERENCES

1. Kito, M., H. Takamura, H. Narita, and R. Urade. 1985. A sensitive method for quantitative analysis of phospholipid molecular species by high-performance liquid chromatography. *J. Biochem.* **98:** 327–331.
2. Takamura, H., H. Narita, R. Urade, and M. Kito. 1986. Quantitative analysis of polyenoic phospholipid molecular species by high performance liquid chromatography. *Lipids.* **21:** 356–361.
3. Takamura, H., H. Narita, H. J. Park, K. Tanaka, T. Matsuura, and M. Kito. 1987. Differential hydrolysis of phospholipid molecular species during activation of human platelets with thrombin and collagen. *J. Biol. Chem.* **262:** 2262–2269.
4. Takamura, H., K. Tanaka, T. Matsuura, and M. Kito. 1989. Ether phospholipid molecular species in human platelets. *J. Biochem.* **105:** 168–172.
5. Marcus, A. J., B. B. Weksler, and E. A. Jaffe. 1978. Enzymatic conversion of prostaglandin endoperoxide H_2 and arachidonic acid to prostacyclin by cultured human endothelial cells. *J. Biol. Chem.* **253:** 7138–7141.
6. Moncada, S. 1982. Prostaglandin and arterial wall biology. *Arteriosclerosis.* **2:** 193–207.
7. Hopkins, N. K., T. D. Oglesby, G. L. Bundy, and R. R. Gorman. 1984. Biosynthesis and metabolism of 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid by human umbilical vein endothelial cells. *J. Biol. Chem.* **259:** 14048–14053.
8. Camussi, G., M. Aglietta, F. Malavasi, C. Tetta, W. Piacibello, F. Sanavio, and F. Bussolino. 1983. The release of platelet-activating factor from human endothelial cells in culture. *J. Immunol.* **131:** 2397–2403.
9. Prescott, S. M., G. A. Zimmerman, and T. M. McIntyre. 1984. Human endothelial cells in culture produce platelet-activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) when stimulated with thrombin. *Proc. Natl. Acad. Sci. USA.* **81:** 3534–3538.
10. Spector, A. A., J. C. Hoak, G. L. Fry, G. M. Denning, L. L. Stoll, and J. B. Smith 1980. Effect of fatty acid modification on prostacyclin production by cultured human endothelial cells. *J. Clin. Invest.* **65:** 1003–1012.
11. Spector, A. A., T. L. Kaduce, J. C. Hoak, and R. L. Czervionke. 1983. Arachidonic acid availability and prostacyclin production by cultured human endothelial cells. *Arteriosclerosis.* **3:** 323–331.
12. Blank, M. L., A. A. Spector, T. L. Kaduce, and F. Snyder. 1986. Composition and incorporation of [^{3}H]arachidonic acid into molecular species of phospholipid classes by cultured human endothelial cells. *Biochim. Biophys. Acta.* **877:** 211–215.
13. Rosenthal, M. D. 1987. Apparent specificity of the thrombin-stimulated deacylation endothelial glycerolipid for polyunsaturated fatty acids with a delta-5 desaturation. *Biochim. Biophys. Acta.* **917:** 279–289,
14. Takayama, H., M. A. Gimbrone, Jr., and A. I. Schafer. 1987. Preferential incorporation of eicosanoid precursor fatty acids into human umbilical vein endothelial cell phospholipids. *Biochim. Biophys Acta.* **922:** 314–322.
15. Takayama, H., M. H. Kroll, M. A. Gimbrone, Jr., and A. Schafe. 1989. Turnover of eicosanoid precursor fatty acids among phospholipid classes and subclasses of cultured human umbilical vein endothelial cells. *Biochem. J.* **258:** 427–434.
16. Johnson, A. R. 1980. Human pulmonary endothelial cells in culture. Activities of cells from arteries and cells from veins. *J. Clin. Invest.* **65:** 841–850.
17. Jaffe, E. A., R. L. Nachman, C. G. Becker, and C. R. Minick. 1973. Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. *J. Clin. Invest.* **52:** 2745–2756.
18. Ishinaga, M., H. Takamura, H. Narita, and M. Kito. 1985. Changes of linoleic, arachidonic and eicosapentaenoic acids in rat platelet, aorta and plasma lipids after changing from a sardine oil diet to a corn oil diet. *Argic. Biol. Chem.* **49:** 2741–2746.
19. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
20. Keenan, R. W., G. Schmidt, and T. Tanaka. 1968. Quantitative determination of phosphatidal ethanolamine and other phosphatidates in various tissues of the rat. *Anal. Biochem.* **23:** 555–566.
21. Hayashi, Y., R. Urade, and M. Kito. 1987. Distribution of phospholipid molecular species containing arachidonic acid and cholesterol in V79-UF cells. *Biochim. Biophys. Acta.* **918:** 267–273.
22. Yamaoka, S., R. Urade, and M. Kito. 1990. Cardiolipin molecular species in rat heart mitochondria are sensitive to essential fatty acid-deficient dietary fats and oils. *J. Nutr.* In press.
23. Hornstra, G., A. C. van Houwelingen, M. Simonis, and J. M. Gerrard. 1989. Fatty acid composition of umbilical arteries and veins: possible implications for the fetal EFA-status. *Lipids.* **24:** 511–517.
24. Chesterman, C. N., R. Owe-Young, J. Macpherson, and S. A. Krilis. 1986. Substrate for endothelial prostacyclin production in the presence of platelets exposed to collagen is derived from the platelets rather than the endothelium. *Blood.* **67:** 1744–1750.