Long-term fatty acid modification of endothelial cells: implications for arachidonic acid distribution in phospholipid classes

Renée C. R. M. Vossen,[1,*] Marion A. H. Feijge,[*] Johan W. M. Heemskerk,[*,†] Maria C. E. van Dam-Mieras,[**] Gerard Hornstra,[†] and Robert F. A. Zwaal[*]

Departments of Biochemistry[*] and Human Biology,[†] University of Limburg, Maastricht, The Netherlands, and Department of Natural Sciences,[**] Open University of the Netherlands, Heerlen, The Netherlands

Abstract Human umbilical vein endothelial cells were cultured in various fatty acid-modified media until equilibrium conditions were reached (7–8 days). The effects on the fatty acid composition of phospholipid classes and on the metabolism of arachidonic acid (20:4(n-6)) were studied. The results showed that in every phospholipid class large changes in fatty acid composition, including 20:4(n-6) content, were induced by long-term modification with unsaturated as well as saturated fatty acids. However, the mean levels of saturated and unsaturated fatty acids per phospholipid class remained relatively constant, except for cells modified with oleic acid, which showed an increase in monounsaturated fatty acids at the expense of both saturated and polyunsaturated fatty acids. The rate of incorporation of radiolabeled 20:4(n-6) in endothelial lipids was not influenced by long-term fatty acid modification. Cells modified with 20:4(n-6) (having a high 20:4(n-6) content) tended to "store" excess 20:4(n-6) as the elongated product 22:4(n-6) mainly in phosphatidylserine and ethanolamine phospholipids. On the other hand, endothelial cells modified with 20:5(n-3) (having a low 20:4(n-6) content) differed typically from other fatty acid-modified cells by a relatively high level and high incorporation rate of 20:4(n-6) in phosphatidylinositol, with a low extent of elongation. These results indicate extensive homeostatic control of membrane unsaturation in each phospholipid class and economical control of 20:4(n-6) content in all modified endothelial cells, irrespective of a considerable variation of 20:4(n-6) levels in cellular lipids. Moreover, the observed maintenance of a critical level of 20:4(n-6) in phosphatidylinositol, when 20:4(n-6) supply was strongly decreased, may be important for maintaining proper signal transduction upon endothelial cell stimulation.—**Vossen, R. C. R. M., M. A. H. Feijge, J. W. M. Heemskerk, M. C. E. van Dam-Mieras, G. Hornstra, and R. F. A. Zwaal.** Long-term fatty acid modification of endothelial cells: implications for arachidonic acid distribution in phospholipid classes. *J. Lipid Res.* 1993. **34:** 409–420.

Supplementary key words phospholipid fatty acid composition • membrane unsaturation • elongation • radiolabeled arachidonic acid incorporation • eicosapentaenoic acid • phosphatidylinositol

Endothelial cells are actively involved in the maintenance of vascular tone and in providing selective permeability and an antithrombotic surface (1). The endothelial membrane phospholipids and their fatty acyl moieties play a role in these processes by controlling the physicochemical properties of the membrane (2, 3) and by providing precursors for bioactive mediators such as eicosanoids and platelet activating factor upon cell stimulation (4, 5). However, derailment of normal endothelial function may contribute to the pathogenesis of atherosclerosis (6). In this respect, the type of dietary lipid, which influences cellular fatty acid composition, may play a role (2). Insight into the relationship between endothelial fatty acid composition and the functional responses of these cells may contribute to understanding a relation between the type of dietary lipids and the incidence of cardiovascular disease.

Membrane phospholipids of endothelial cells contain relatively large amounts of arachidonic acid (20:4(n-6), which can be mobilized from the phospholipids by phospholipases and subsequently converted into bioactive eicosanoids upon cell stimulation (2, 7). Despite the established central role of 20:4(n-6) in cell activation, endothelial cells acquire it mainly from exogenous sources (4). Changes in membrane 20:4(n-6) content of endothelial cells can be induced by dietary modification. This may influence cellular responsiveness. Previous studies have shown that considerable changes in fatty acid composition can be induced in endothelial cells in vitro (3, 4, 8). Indeed, a relation between the extent of

Abbreviations: EDTA, ethylenediamineyetraacetic acid; HEPES, n-2-hydroxy-ethylpiperazine-n-2-ethanesulfonic acid; SM, sphingomyelin; CP, choline phospholipid; PS, phosphatidylserine; PI, phosphatidylinositol; EP, ethanolamine phospholipid; 16:0, palmitic acid; 18:0, stearic acid; 18:1(n-9), oleic acid; 18:2(n-6), linoleic acid; 20:4(n-6), arachidonic acid; 20:5(n-3), eicosapentaenoic acid; 22:4(n-6), docosatetraenoic acid; 22:5(n-3), docosapentaenoic acid; 22:6(n-3), docosahexaenoic acid.

[1]To whom correspondence should be addressed.

modification of the 20:4(n-6) content of total phospholipids and eicosanoid release upon stimulation has been found.

Several studies (5, 9-17) have described the rapid incorporation of radiolabeled 20:4(n-6) in endothelial cell phospholipids after relatively short incubation periods (5 min to 24 h). In addition, other studies have shown that the time scale to reach equilibrium conditions for the distribution of radiolabeled 20:4(n-6) over the various phospholipid classes and for the elongation and desaturation processes was much longer (18-20). However, the effect of long-term modification with different fatty acids in vitro on endothelial fatty acid composition and 20:4(n-6) metabolism was not investigated. Insight in the extent of fatty acid modification induced by various fatty acids and the influence of these modifications on the 20:4(n-6) content of the individual phospholipid classes is of importance, considering the specific roles of different phospholipid classes upon cell stimulation (2, 21).

In this study human endothelial cells were modified with saturated, monounsaturated, and polyunsaturated fatty acids, by long-term culture in various fatty acid-modified media until equilibrium conditions were reached (7-8 days). The fatty acid compositions of each phospholipid class were analyzed. Furthermore, the influence of long-term fatty acid modification on the incorporation, redistribution, and modification of [1-^{14}C]arachidonic acid into endothelial cell phospholipid classes was studied after both short incubation (2 h) and prolonged culture (3 days) with radiolabeled 20:4(n-6). The results indicate considerable alterations of 20:4(n-6) levels in every phospholipid class of long-term fatty acid-modified endothelial cells. This may account for the observed variation in eicosanoid production of these cells (8). Nevertheless, in every phospholipid class extensive homeostatic control of membrane unsaturation and economical control of the 20:4(n-6) content was found. As was shown in 20:5(n-3)-modified cells with a low 20:4(n-6) content, a critical 20:4(n-6) level appeared to be maintained in phosphatidylinositol. This may be important for maintaining proper signal transduction upon endothelial cell stimulation and it may explain why the observed functional cellular reactivity remains virtually unaltered (8) despite the gross changes in fatty acid patterns.

EXPERIMENTAL PROCEDURES

Materials

Culture media M199 and RPMI 1640 (with L-glutamine and 20 mM HEPES) and 2.5% (w/v) trypsin solution were obtained from Flow Lab (Bioggio, Switzerland). Tissue culture dishes (60 cm², F3003) were from Falcon (Etten Leur, The Netherlands). EDTA, HEPES, and L-glutamine were obtained from Serva (Heidelberg, Germany). Free fatty acids and their sodium salts were obtained from Sigma (St. Louis, MO). All organic solvents were purchased from Merck (Darmstadt, Germany) and contained butylated hydroxy toluene (50 mg/l, Sigma) as antioxidant. [1-^{14}C]arachidonic acid (54.4 mCi/mmol) was obtained from Amersham (Houten, The Netherlands). All chemicals used were of the highest grade of purity available.

Several buffers were used: phosphate-buffered saline contained 0.137 M NaCl, 2.6 mM KCl, 8.1 mM Na_2HPO_4, 1.15 mM KH_2PO_4 (pH 7.4). Trypsin solution contained 0.125% (w/v) trypsin in phosphate-buffered saline containing EDTA (0.33 mM). The standard culture medium consisted of 20% human serum in M199/RPMI1640 (1:1, v/v) supplemented with L-glutamine (2 mM), gentamicin (50 mg/ml), $NaHCO_3$ (11.9 mM), and endothelial cell growth supplement (100 μg protein/ml). Endothelial cell growth supplement was prepared from bovine brain as described by Maciag et al. (22).

Endothelial cell culture and modification of the fatty acid composition

Human umbilical vein endothelial cells were isolated from umbilical cord veins and cultured in standard culture medium containing 20% human serum (pool of at least 200 healthy donors) as described previously (8). For modification of the endothelial cell fatty acid composition, the cells were cultured in various fatty acid-supplemented media from passage 2 to 4 (7 or 8 days). The fatty acid-supplemented media (200 μM final concentration) were prepared by addition of a particular fatty acid sodium salt to warm human serum (37°C) prior to addition of culture medium as described previously (8). In each experiment, unmodified endothelial cells were always compared to a set of four differently fatty acid-modified cells, obtained from the same umbilical cord.

Lipid analysis

Endothelial cell monolayers were washed twice with cold phosphate-buffered saline (4°C). The cells were trypsinized and washed twice with cold phosphate-buffered saline containing 1.35 mM EDTA (4°C), counted with a Coulter counter, and frozen (−20°C) until use (within 2 weeks). Preliminary experiments showed no appreciable differences between total fatty acid compositions of trypsinized and scraped endothelial cells.

For lipid analysis, the cells were thawed rapidly and the lipids were extracted according to Bligh and Dyer (23). Aliquots were taken to quantify phospholipid content by measuring phosphorus content according to Böttcher, van Gent, and Pries (24) and to quantify cholesterol content using reagents provided by a kit (free cholesterol 310328, Boehringer, Mannheim, Germany). The lipid extract was

subjected to thin-layer chromatography (TLC) and gas–liquid chromatography (GLC) as described below.

Total phospholipids were separated from neutral lipids by TLC on silica gel 60 plates (Merck) as described by Rand, Hennissen, and Hornstra (25). Phospholipid classes were separated by TLC as described by Touchstone, Chen, and Beaver (26). Lipid bands co-migrating with known standards were visualized by spraying with 0.5% (w/v) 8-anilino-1-naphtalene sulfonic acid in methanol and UV detection. Appropriate bands were scraped and phospholipids were extracted with methanol–chloroform–water 90:10:2 (v/v). Phosphorus content was quantified and fatty acids were methylated, extracted, and analyzed by GLC as described by Rand et al. (25) with some modifications: a Perkin-Elmer autosystem capillary gaschromatograph with a CP-sil 5 CB column (Chrompack, Middelburg, The Netherlands) was used with 19:0 as internal standard. Data were collected and analyzed using a Turbochrome II workstation (Perkin-Elmer, Gouda, The Netherlands).

Plasmalogen EP was separated from diacyl EP by TLC. The TLC plate was subjected to HCl fumes for 15 min to hydrolyze the aldehyde moiety of plasmalogen EP, prior to development in chloroform–methanol–water 65:25:4 (v/v). Thereby diacyl EP was separated from the lysoEP formed. Thus, only the fatty acids of the *sn*-2 position of plasmalogen EP were analyzed. Appropriate bands were scraped and analyzed as described above.

Labeling of endothelial cells with [1-^{14}C]arachidonate and lipid analysis of radiolabeled cells

Endothelial cells were cultured from passage 2 to 4 in fatty acid-modified media. Subconfluent modified endothelial cells (passage 4) were cultured for 3 days in the same media supplemented with 0.625 μCi sodium [1-^{14}C]arachidonate (2.3 μM). In a parallel experiment, confluent modified endothelial cells (passage 4) were incubated for 2 h with 0.625 μCi sodium [1-^{14}C]arachidonate (2.3 μM) in RPMI 1640 containing human serum albumin (5 g/l). Then, the cells were washed twice with phosphate-buffered saline, incubated with saline for 5 min, and trypsinized. Lipids were extracted as described above and aliquots were taken to quantify incorporated radioactivity. Phospholipid classes were separated by TLC as described above, radioactive spots were scraped off, and radioactivity was measured.

For analysis of elongation products, fatty acids of total phospholipids were methylated (25) and separated by high performance liquid chromatography (HPLC) using a C_{18} reversed phase column (100RP, 5 μm, Merck), eluted with methanol–acetonitrile–water 85.5:9.5:5 (v/v). Radioactive peaks were analyzed by a radioisotope detector (Beckmann 171, Palo Alto, CA).

All fatty acid data are expressed as nmol fatty acid or dpm radiolabel per amount of cells. We have previously shown that cell number and phospholipid content are highly correlated, and not influenced by fatty acid modification of the cells (8). As cellular phospholipid content can be quantified more accurately than cell number in small amounts of cells (by measuring phosphorus content (24)), this was measured as the determinant of cell number. All data are expressed per 8.3×10^6 or 8.3×10^3 cells, which is equivalent to 1 μmol or 1 nmol cellular phospholipid, respectively.

RESULTS

Fatty acid composition of phospholipid classes of unmodified human endothelial cells

The distribution of the different phospholipid classes in the endothelial cell membranes is shown in **Table 1** and the fatty acid compositions of these classes are summarized in **Fig. 1.** The results show that particular fatty acids are esterified into various phospholipid classes to different extents. For example, in SM 40.6% of total fatty acids is 16:0 (Fig. 1A), 9.1% is 24:0, and 21.1% is 24:1(n-9) (**Table 2**). 16:0 is also incorporated to a high extent into CP (33.8% of total CP fatty acids). CP actually shows the highest 16:0 mass incorporation (Fig. 1B). As CP comprises 53.4% of total phospholipids, its fatty acid composition determines to a considerable extent that of the total phospholipids (Fig. 1B). It is also evident from Fig. 1A that the relative amount of 18:0 is high in PS (35.1%) and PI (34.0%), although Fig. 1B shows that EP contains the highest 18:0 mass. Also, PI is very rich in 20:4(n-6) (30.7%), while EP contains the highest 20:4(n-6) mass content (Fig. 1B). EP, which comprises 27.0% of total

TABLE 1. Distribution of phospholipid classes in endothelial cell membranes

PL Classes	% of Total PL
SM	6.7 (1.5)
CP	53.4 (1.6)
PS	6.8 (0.6)
PI	6.1 (0.5)
EP	27.0 (2.8)
Plasmalogen EP	10.4 (1.8)
Diacyl EP[a]	16.6 (2.0)

Human umbilical vein endothelial cells were cultured to passage 4 in standard medium containing 20% human serum. Lipids were extracted, phospholipid classes were separated, and phosphorus content was analyzed as described in Experimental Procedures. The distribution of phospholipid classes is expressed as percentage of total phospholipids. Values represent means (SEM), n = 6. Abbreviations: PL, phospholipid; SM, sphingomyelin; CP, choline phospholipid; PS, phosphatidylserine; PI, phosphatidylinositol; EP, ethanolamine phospholipid.

[a]Diacyl EP including alkyl-acyl EP (< 0.5% of total PL).

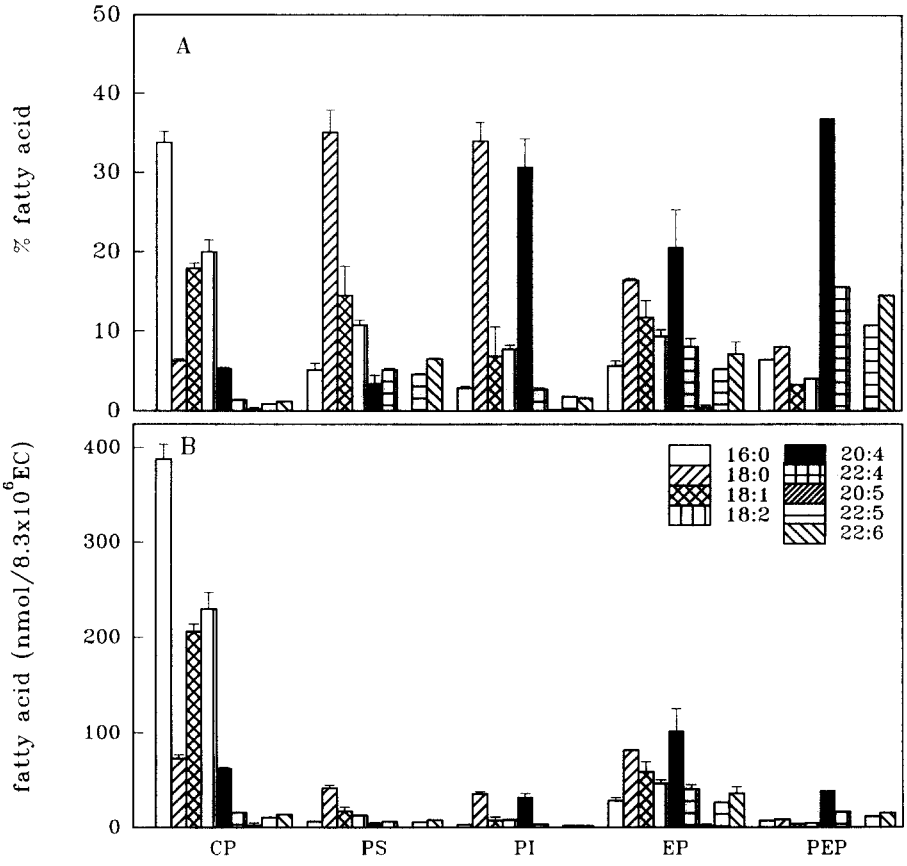

Fig. 1. Fatty acid composition of phospholipid classes in unmodified human endothelial cells. Endothelial cells were cultured to passage 4 in standard culture medium and fatty acid compositions of phospholipid classes were analyzed. Only the major fatty acids are depicted here. A: The amounts of fatty acid in a phospholipid class are expressed as percentage of total fatty acids in that class. B: Shows the fatty acid mass content in various phospholipid classes, expressed as nmol fatty acid per 8.3 × 10^6 cells (equivalent to 1 μmol cellular phospholipid). These values are calculated from the data given in Table 1 and Fig. 1A. The mean values ± SEM of three independent experiments are represented. Abbreviations: EC, endothelial cells; PEP, plasmalogen EP (values represent only the % fatty acyl composition at the *sn*-2 position).

phospholipids, has a relatively high content of 18:0, 18:1(n-9), and 20:4(n-6) (Fig. 1A) and a relatively large mass content of 18:0, 20:4(n-6), 22:4(n-6), 22:5(n-3), and 22:6(n-3) (Fig. 1B). The polyunsaturated fatty acid composition of plasmalogen EP (*sn*-2 position) shows no gross deviation from that of diacyl EP, except for 18:1(n-9) and 18:2(n-6), which are present at both the *sn*-1 and *sn*-2 positions (**Table 3**). Only 18:1(n-9) is uniformly distributed over all phospholipid classes, except for SM and plasmalogen EP.

Changes in fatty acid composition of phospholipid classes in fatty acid-modified endothelial cells

In order to study the effect of fatty acid modification on the distribution of fatty acids in the different phospholipid classes, endothelial cells were cultured from passage 2 to 4 in media supplemented with 200 μM of a particular fatty acid. Preliminary studies showed that higher concentrations of supplemented fatty acids inhibit cell growth. As the medium also contains 20% human serum (containing ca. 2 mM total fatty acids), the concentration of the supplemented fatty acids is at most doubled, assuming that this fatty acid represents 10% or more of the total fatty acids in serum. However, serum fatty acids are in part esterified which may limit their availability for incorporation into cellular lipids.

The fatty acid compositions of the phospholipid classes of unmodified cells and fatty acid-modified cells are given in Table 2. As plasmalogen EP constitutes about 39% of total EP, the fatty acid modifications in the subclasses plasmalogen EP and diacyl EP (including alkyl-acyl EP) were analyzed as well (Table 3). No appreciable differences in lipid phosphorus, phospholipid class distributions, and cholesterol contents between fatty acid modified cells and control cells were found. Regarding the relevance of 20:4(n-6) for cellular activity, the fatty acid

TABLE 2. Fatty acid compositions of the phospholipid classes of differently modified endothelial cells

PL Class	Fatty Acid	Fatty Acid Supplemented to Culture Medium							
		None	16:0	18:0	18:1	18:2	20:4	20:5	22:6
SM	16:0	40.6	47.0	36.9	31.6	36.1	46.2	50.2	45.5
	18:0	4.8	7.5	16.4	3.6	2.7	4.6	5.9	5.3
	18:1(n-9)	0.3	0.1	0.7	1.2	0.7	2.0	0.6	0.3
	18:2(n-6)	1.1	1.6	1.4	0.6	3.1	1.1	1.1	2.0
	20:4(n-6)	0.7	1.0	2.6	1.1	0.7	1.4	1.6	1.3
	22:6(n-3)	0.0	0.0	0.0	0.2	0.1	0.3	0.0	0.7
	22:0	4.1	6.0	12.0	3.2	2.9	4.7	5.7	4.3
	24:0	9.1	10.3	9.5	4.2	4.0	7.2	8.7	8.9
	24:1(n-9)	21.1	9.7	8.6	40.5	10.1	15.4	15.3	17.1
	24:2(n-6)	9.3	4.2	2.9	5.7	33.2	3.7	3.5	8.7
CP	16:0	33.8	41.1	23.7	24.1	26.4	35.1	34.7	34.2
	18:0	6.3	6.0	17.1	3.9	4.0	4.3	5.6	6.4
	18:1(n-9)	17.9	13.4	17.4	44.9	8.9	15.0	16.1	14.9
	18:2(n-6)	20.0	14.8	17.1	10.8	43.1	11.9	14.9	18.8
	20:2(n-6)	1.4	0.8	0.7	0.7	5.3	0.6	0.5	0.8
	20:3(n-6)	1.6	2.3	2.3	1.0	1.5	1.7	0.7	1.4
	20:4(n-6)	5.4	9.5	10.1	3.0	2.5	14.9	2.0	4.7
	20:5(n-3)	0.2	0.5	0.5	0.1	0.3	0.1	8.3	0.9
	22:4(n-6)	1.4	1.2	1.1	1.2	1.2	7.9	2.9	0.4
	22:5(n-3)	0.9	1.2	1.0	0.7	0.3	0.2	5.9	0.7
	22:6(n-3)	1.2	1.6	1.7	0.9	0.6	0.3	0.2	8.8
PS	16:0	5.2	7.4	2.3	6.1	7.8	8.7	5.0	7.8
	18:0	35.1	34.5	40.5	29.1	32.2	32.1	35.6	37.1
	18:1(n-9)	14.5	11.2	15.3	31.3	8.9	12.7	13.0	13.1
	18:2(n-6)	10.8	8.5	10.5	5.5	23.9	5.8	8.3	11.5
	20:2(n-6)	0.9	4.1	0.6	0.8	4.2	0.5	0.4	0.8
	20:3(n-6)	3.8	5.1	4.2	2.1	2.8	1.7	1.7	3.1
	20:4(n-6)	3.5	6.0	4.9	1.6	1.8	7.4	1.0	1.9
	20:5(n-3)	0.0	0.3	0.0	0.0	0.3	0.0	3.0	0.2
	22:4(n-6)	5.2	6.7	4.5	4.3	5.3	22.7	1.5	0.8
	22:5(n-3)	4.6	5.2	4.5	3.1	2.9	0.0	22.0	0.0
	22:6(n-3)	6.6	8.1	6.8	4.1	3.1	1.0	1.2	16.7
PI	16:0	2.9	7.3	1.7	1.8	2.2	2.9	1.9	2.3
	18:0	34.0	32.7	39.6	29.7	33.0	32.2	35.4	35.0
	18:1(n-9)	6.9	4.0	3.3	19.5	4.8	5.8	5.5	5.2
	18:2(n-6)	7.8	4.1	4.6	4.9	21.7	3.3	5.0	7.5
	20:2(n-6)	0.5	0.4	0.4	0.4	2.1	0.2	0.3	0.5
	20:3(n-6)	2.9	2.7	1.7	2.2	2.6	0.9	2.0	2.4
	20:4(n-6)	30.7	36.0	35.5	26.4	21.6	40.7	22.9	32.2
	20:5(n-3)	0.1	0.1	0.2	0.1	0.1	0.0	13.9	0.4
	22:4(n-6)	2.7	2.5	2.5	2.7	3.0	6.2	0.3	1.1
	22:5(n-3)	1.8	1.9	1.1	1.2	0.4	0.0	8.5	0.0
	22:6(n-3)	1.6	1.9	1.9	1.2	1.0	0.2	0.1	5.9
EP	16:0	5.7	8.3	2.7	5.5	6.3	4.7	4.0	4.7
	18:0	16.5	13.3	21.3	14.2	16.2	19.0	19.0	18.7
	18:1(n-9)	11.8	6.7	6.5	33.4	9.2	8.7	10.0	8.2
	18:2(n-6)	9.4	5.7	5.9	5.5	22.4	4.4	7.1	6.4
	20:2(n-6)	1.0	0.1	0.3	0.7	6.0	0.5	0.4	0.5
	20:3(n-6)	2.0	2.1	1.9	1.3	1.8	1.2	1.1	1.3
	20:4(n-6)	20.6	26.0	27.0	11.2	10.6	22.8	4.9	12.3
	20:5(n-3)	0.4	0.5	0.8	0.2	0.4	0.0	16.5	0.9
	22:4(n-6)	8.1	9.5	8.2	7.7	9.3	27.1	3.1	1.5
	22:5(n-3)	5.3	6.8	4.0	4.4	1.5	0.0	24.0	0.0
	22:6(n-3)	7.2	7.6	8.2	6.3	5.8	1.2	1.2	33.9
Total PL	16:0	21.1	25.3	9.4	16.5	18.7	22.3	23.5	22.0
	18:0	13.3	12.7	23.4	10.2	11.3	12.7	14.9	14.1
	18:1(n-9)	17.5	10.7	16.9	37.0	7.0	11.5	12.2	12.5
	18:2(n-6)	14.9	11.3	13.9	8.6	33.3	8.8	11.4	12.5
	20:2(n-6)	1.3	0.7	0.4	0.8	5.2	0.6	0.4	0.6
	20:3(n-6)	1.8	2.4	0.1	1.1	1.7	1.3	0.9	1.4
	20:4(n-6)	11.4	16.0	16.8	6.7	5.8	18.8	3.9	8.3
	20:5(n-3)	0.3	0.4	0.4	0.1	0.3	0.1	10.4	1.0
	22:4(n-6)	3.8	4.1	3.2	3.4	3.8	13.8	0.7	1.0
	22:5(n-3)	2.9	3.1	3.0	2.2	1.6	0.5	11.3	1.1
	22:6(n-3)	3.5	4.4	3.8	2.7	2.1	0.5	0.5	15.3

Endothelial cells were cultured from passage 2 to 4 in media supplemented with various fatty acid sodium salts (200 μM) as indicated. Fatty acid compositions of phospholipid classes were determined as described in Experimental Procedures. Only the major fatty acids are shown. Data are expressed as molar percentage of total fatty acids in a phospholipid class. Standard errors did not exceed 12% of mean value, n = 3.

TABLE 3. Fatty acid compositions of the ethanolamine phospholipid subclass of differently modified endothelial cells

EP Subclass	Fatty Acid	Fatty Acid Supplemented to Culture Medium						
		None	18:0	18:1	18:2	20:4	20:5	22:6
Diacyl EP	16:0	6.7	2.5	5.5	7.7	7.8	7.0	6.4
	18:0	22.0	37.9	18.3	24.1	28.3	32.0	29.1
	18:1(n-9)	14.0	10.0	43.7	8.4	11.1	15.5	11.2
	18:2(n-6)	11.0	8.2	7.4	26.6	5.8	11.1	9.0
	20:2(n-6)	0.8	0.4	0.6	7.6	0.6	0.0	0.6
	20:3(n-6)	2.0	2.1	0.6	2.2	1.3	0.0	1.5
	20:4(n-6)	18.8	19.7	7.1	6.7	20.6	4.7	9.6
	20:5(n-3)	0.3	0.6	0.1	0.0	0.0	13.5	1.3
	22:4(n-6)	6.8	5.1	4.7	6.6	20.2	0.0	1.2
	22:5(n-3)	4.7	3.5	1.4	2.4	0.0	13.0	1.0
	22:6(n-3)	6.5	6.2	3.8	3.4	0.0	0.0	24.6
Plasmalogen EP	16:0	6.5	1.3	5.0	2.5	2.9	2.9	1.9
	18:0	8.1	2.6	3.3	2.5	5.7	3.9	3.6
	18:1(n-9)	3.3	1.5	9.2	1.6	3.3	3.2	2.7
	18:2(n-6)	4.1	1.8	2.7	11.7	1.7	2.7	2.1
	20:2(n-6)	0.0	0.0	0.1	1.7	0.0	0.1	0.0
	20:3(n-6)	0.1	0.8	1.4	0.5	0.4	0.5	0.3
	20:4(n-6)	36.9	46.2	27.2	27.8	35.7	3.2	18.8
	20:5(n-3)	0.0	0.0	0.0	0.0	0.0	29.8	2.8
	22:4(n-6)	15.6	17.0	20.5	25.2	44.8	3.6	4.3
	22:5(n-3)	10.8	11.0	11.4	10.1	1.5	43.5	2.4
	22:6(n-3)	14.6	15.4	17.1	15.5	2.1	1.9	57.6

The EP subclass compositions of plasmalogen EP and diacyl EP were analyzed. Diacyl EP also contains alkyl-acyl EP (< 0.5% of total PL). Only the major fatty acids are shown. Data are expressed as molar percentage of total fatty acids in an EP subclass. It should be noted that the values of plasmalogen EP represent only the fatty acid composition at the *sn*-2 position, in contrast to the values of diacyl EP which are derived from the *sn*-1 plus *sn*-2 positions.

contents of different phospholipid classes in 20:4-modified endothelial cells are also shown in **Fig. 2.** Comparison of Figs. 1 and 2 show that supplementation of 20:4(n-6) to the culture medium results in considerable changes in fatty acid composition of every phospholipid class. Not only was the content of 20:4(n-6) increased in all phospholipid classes (except for SM, see Table 2), but also its elongation product 22:4(n-6). PS and EP especially were enriched with 22:4(n-6) (Fig. 2A)). In plasmalogen EP, 22:4(n-6) content was somewhat higher than that of 20:4(n-6), while absolute mass of 20:4(n-6) was highest in CP.

Some examples of preferential incorporation of supplemented fatty acids in phospholipid classes of modified cells are as follows (Tables 2 and 3): 22 carbon polyunsaturated fatty acids (especially elongation products in 20:4- and 20:5-modified endothelial cells) were preferentially incorporated into PS and EP. Plasmalogen EP was enriched in 22:4(n-6), 22:5(n-3), and 22:6(n-3) in all cells modified with polyunsaturated fatty acids. In plasmalogen EP of saturated fatty acid-modified cells, an increased content of 20:4(n-6) was found compared to that of diacyl EP. The fatty acids 20:4(n-6) and 18:0 were highly incorporated into PI of 18:0-modified cells, and 20:4(n-6) and 16:0 were enriched in PI of 16:0-modified cells. SM was enriched in the elongation products 24:1(n-9) and 24:2(n-6) in 18:1- and 18:2-modified cells, respectively.

Comparison of Figs. 1 and 2 also show that an increase of 20:4(n-6) and 22:4(n-6) is accompanied by a decrease of the fatty acids 18:2(n-6), 22:5(n-3), and 22:6(n-3). This compensation mechanism was also seen in every phospholipid class. Only SM, which has an extremely low 20:4(n-6) content, was somewhat divergent and showed a remarkable increase of 16:0 (from 40.6% to 46.2%) with smaller decreases of 18:0 and 18:1(n-9) in 20:4-modified cells (Fig. 2A). It is evident from the data in Tables 2 and 3 that, for cells modified with polyunsaturated fatty acids, increases in these fatty acids in each phospholipid class were balanced by decreases in other polyunsaturated fatty acids. For cells modified with saturated fatty acids, increases in saturated fatty acids were balanced by increases in 20:4(n-6) content in every phospholipid class and plasmalogen EP (except SM, see Table 2).

In general, the net effect of all fatty acid changes was a fairly constant distribution pattern of saturated, monounsaturated, and polyunsaturated fatty acids in each phospholipid class, as is shown in **Fig. 3.** PI and EP have a relatively high polyunsaturated fatty acid content, whereas SM has a fairly high saturated fatty acid content with somewhat divergent fatty acid group distributions.

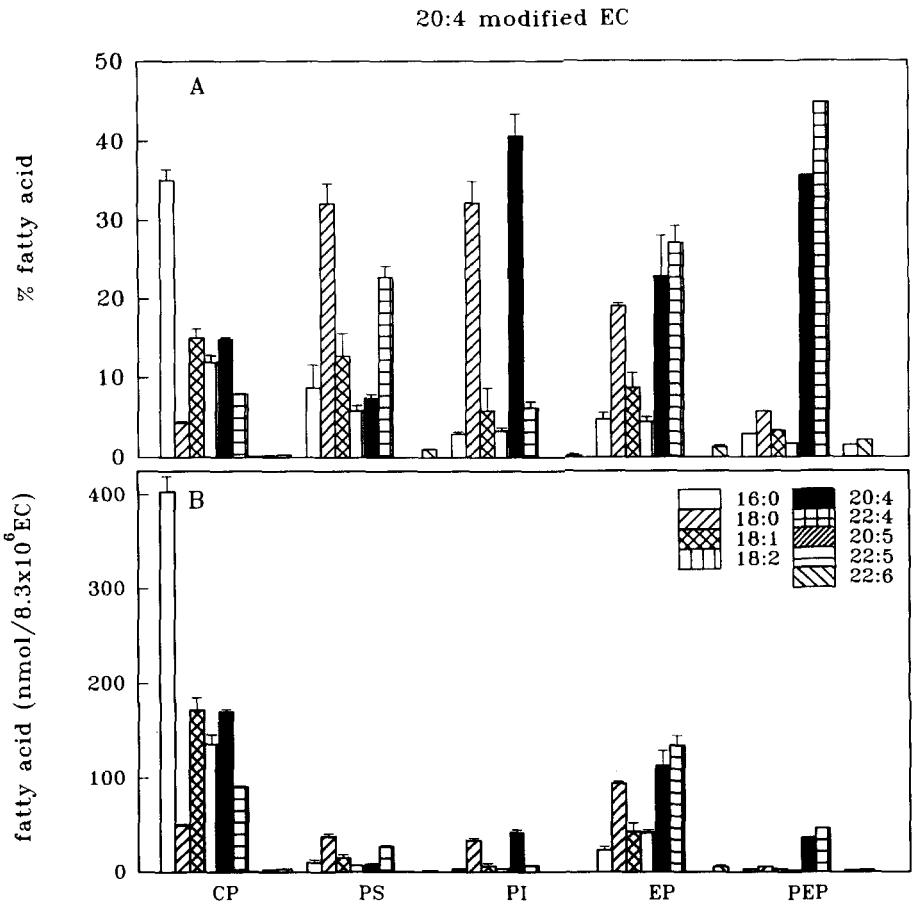

Fig. 2. Fatty acid composition of phospholipid classes in 20:4-modified endothelial cells. Endothelial cells were cultured from passage 2 to 4 in standard culture medium supplemented with 20:4(n-6) and fatty acid compositions of phospholipid classes were analyzed. Only the major fatty acids are depicted. A: The amounts of fatty acids are expressed as a percentage of total fatty acids per phospholipid class. B: Shows the fatty acid mass contents, expressed as nmol fatty acid per 8.3 × 10^6 cells (equivalent to 1 μmol cellular phospholipid). These values are calculated from data given in Table 1 and Fig. 2A. The mean ± SEM of three independent experiments are represented. Abbreviations as in Fig. 1.

CP and PS have an intermediate fatty acid group distribution. While there are only small differences in fatty acid group distributions of cells modified with saturated and polyunsaturated fatty acids, 18:1-modified cells show a remarkable increase in monounsaturated fatty acids at the expense of both saturated and polyunsaturated fatty acids in every phospholipid class.

Incorporation of [1-^{14}C]arachidonic acid into total phospholipids and neutral lipids of fatty acid-modified endothelial cells

As not all phospholipid classes contribute to the same extent to liberation of 20:4(n-6) for production of eicosanoids, it is of interest to know whether the incorporation, distribution, and modification of exogenously added 20:4(n-6) in different phospholipid classes was influenced by membrane fatty acid modification. Therefore, fatty acid-modified endothelial cells at passage 4 were either incubated for 2 h with [1-^{14}C]arachidonic acid in RPMI1640 containing human serum albumin (5 g/l) or were cultured for 3 days with [1-^{14}C]arachidonic acid in fatty acid-supplemented media. The incorporation of radiolabel into phospholipids and neutral lipids is shown in **Fig. 4.** No differences in total [1-^{14}C]arachidonic acid incorporation were found between differently modified endothelial cells at 2 h incubation or 3 days culture. However, after 3 days culture the incorporation of radiolabel into phospholipids was increased with increasing amount of unlabeled 20:4(n-6) already present in the phospholipids of the differently modified cells, as could be expected. The incorporation into neutral lipids (predominantly triglycerides) was much lower than in phospholipids, except for 20:4-modified endothelial cells, which showed a relatively high increase of radiolabel into neutral lipids.

It should be noted that during 3 days culture of en-

dothelial cells in 20:4-modified media, dilution of radiolabel occurred already in the culture medium. Therefore, the actual mass incorporation of 20:4(n-6) is much higher than would be concluded from the dpm values of

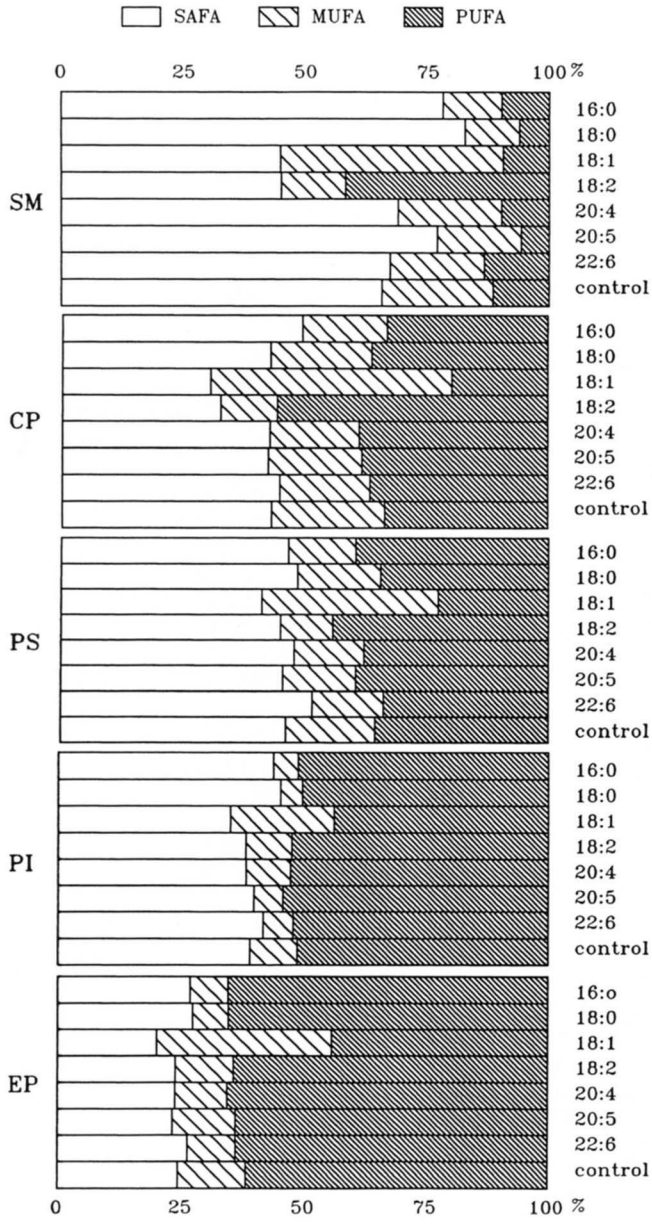

Fig. 3. Distribution patterns of fatty acid groups in phospholipid classes of differently modified endothelial cells. Endothelial cells were cultured from passage 2 to 4 in standard culture medium supplemented with different fatty acids and fatty acid compositions of phospholipid classes were analyzed. The fatty acids were divided among three fatty acid groups: saturated (SAFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids expressed as percentage of total fatty acids in a phospholipid class. Values represent the mean of three independent experiments. Abbreviations: control indicates control cells cultured without supplemented fatty acid; 16:0, 18:0, 18:1, 18:2, 20:4, 20:5, and 22:6 indicate endothelial cells cultured with palmitic, stearic, oleic, linoleic, arachidonic, eicosapentaenoic, and docosahexaenoic acid, respectively.

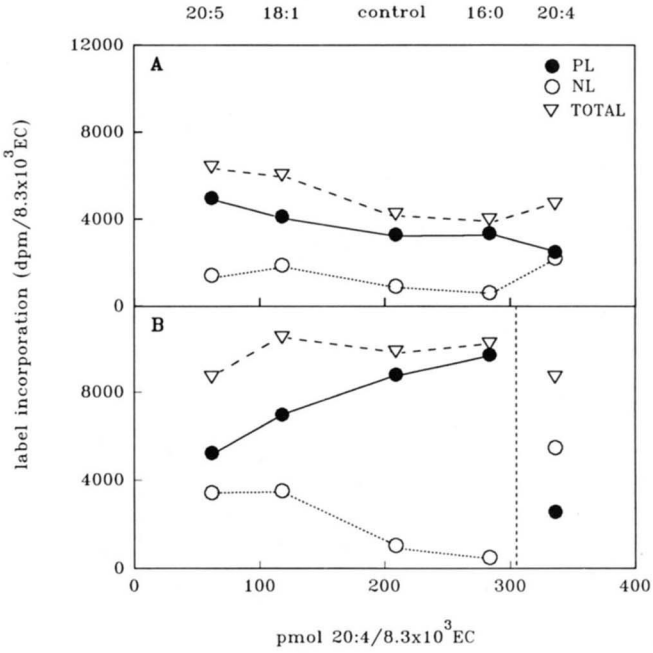

Fig. 4. Incorporation of radiolabel in phospholipids and neutral lipids of differently modified endothelial cells. At passage 4 endothelial cells, modified with different fatty acids, were either incubated for 2 h or cultured for 3 days with [1-^{14}C]arachidonic acid. Lipids were analyzed by TLC. At the y-axis incorporation of radiolabel is depicted for the 2-h incubation (A) or the 3 days culture (B), expressed as dpm per 8.3 × 10^3 cells. The x-axis shows the content of unlabeled 20:4(n-6) in the total phospholipids of fatty acid-modified endothelial cells (after 7 or 8 days culture) (8). The top of the figure indicates with fatty acid the endothelial cells were modified with. Values are from one experiment, representative of two independently performed experiments. Abbreviations are as in Fig. 3. For 20:4-modified endothelial cells after 3 days culture, the dpm values represent a much larger 20:4(n-6) mass incorporation because of dilution of radiolabel in the 20:4-modified culture medium.

20:4-modified cells shown in **Fig. 5B.** This, however, does not influence the ratio of radiolabel incorporation into phospholipids to that into neutral lipids.

Elongation of [1-^{14}C]arachidonic acid in differently modified endothelial cells

By means of HPLC analyses, modification of incorporated [1-^{14}C]arachidonic acid was determined for the differently modified endothelial cells (**Table 4**). After 2 h incubation, a large amount of 20:4(n-6) was already elongated to 22:4(n-6). No desaturation products or further elongation of 22:4(n-6) were found. The percentage elongation in total phospholipids after 2 h incubation was between 17% and 22% for all fatty acid-modified cells, except for 20:5-modified cells (7%). After 3 days culture the percentage elongation was further increased in all cells, but still rather low in 20:5-modified endothelial cells (Table 4). 20:4- and 18:1-modified cells showed the highest percentage elongation after 3 days culture. **Table 5** shows the incorporation of the elongated product into various phospholipid classes of 20:4-modified endothelial cells.

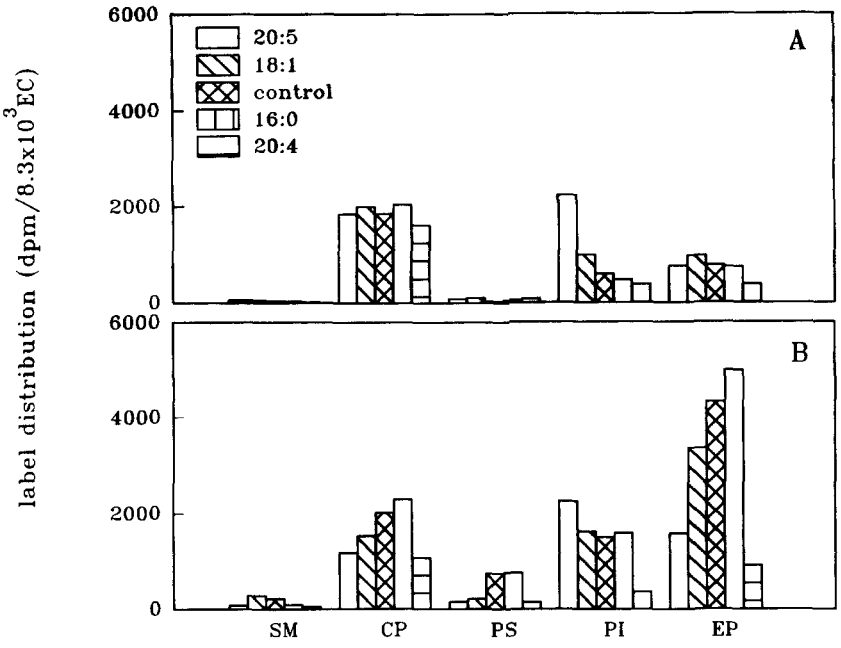

Fig. 5. Distribution of radiolabel among phospholipid classes in differently modified endothelial cells. Procedure as described in Fig. 4. Radiolabel distribution among phospholipid classes of differently modified endothelial cells is shown after 2 h incubation in A and after 3 days culture in B, expressed as dpm per 8.3 × 10^3 cells. Values are from one experiment, representative of two independently performed experiments. Abbreviations are as in Fig. 3. For 20:4-modified endothelial cells after 3 days culture, the dpm values represent a much larger 20:4(n-6) mass incorporation because of dilution of radiolabel in the 20:4-modified culture medium.

These data are consistent with the previous observations that 22:4(n-6) is incorporated preferentially into PS and EP.

It cannot be excluded that part of the [1-^{14}C]arachidonate may release ^{14}C through beta-oxidation, followed by reincorporation of the label into de novo synthesized fatty acids including 22:4(n-6) in a 3-day culture period. However, we examined radiolabel incorporation into all major fatty acids by HPLC and detected radiolabel incorporation almost exclusively in 20:4(n-6) and 22:4(n-6), which always accounted for more than 97% of total radiolabel measured (including minor unidentified peaks). Most probably, other fatty acids would be labeled as well, when ^{14}C would have been released from arachidonate and reused in measurable amounts in de novo lipid synthesis.

TABLE 4. Percentage of elongated [1-^{14}C]arachidonic acid in differently modified endothelial cells

	% 22:4(n-6)	
Endothelial Cells Modified with	2 Hours	3 Days
Control	17.3	35.6
16:0	20.7	34.1
18:1	21.6	43.6
20:4	17.5	41.1
20:5	7.0	12.7

Endothelial cells were cultured from passage 2 to 4 in various fatty acid-modified media. Cells were then either incubated for 2 h or cultured for 3 days with [1-^{14}C]arachidonic acid. Lipids were extracted and analyzed by TLC and HPLC as described in Experimental Procedures. Percent elongation is calculated as dpm 22:4/dpm (20:4 + 22:4) × 100. Values are from one experiment representative of two independently performed experiments.

Distribution of [1-^{14}C]arachidonic acid among phospholipid classes of differently modified endothelial cells

The radiolabel distributions among phospholipid classes of differently modified endothelial cells after 2 h incubation and 3 days culture are shown in Fig. 5A and 5B, respectively. The distribution patterns of radiolabel among the various phospholipid classes were similar for differently modified endothelial cells. However, 20:5-modified endothelial cells formed an exception. These cells showed a relatively high incorporation of radiolabel into PI compared to other cells. Also, the incorporation in total phospholipids after 2 h incubation was higher in these cells than that in other modified cells.

After 3 days culture, incorporation of radiolabel into all classes was increased, especially into EP. In fact, the overall pattern shows a shift in radiolabel incorporation from

TABLE 5. Percentage of elongated [1-^{14}C]arachidonic acid in phospholipid classes of 20:4-modified endothelial cells

Phospholipid in 20:4 EC	% 22:4(n-6) 3 Days
PS	66.7
EP	50.7
CP	36.7
PI	30.3
SM	nd
NL	40.7

Procedure and calculations as described in Table 4. Values are from one experiment, representative of two independently performed experiments. Abbreviations: NL, neutral lipids; nd, not detected; 20:4 EC, endothelial cells modified with arachidonic acid.

CP to EP when the 2-h and 3-day data were compared. The distribution patterns after 3 days culture resembled those of unlabeled fatty acids. For 20:5-modified endothelial cells, again a relatively high incorporation of radiolabel into PI was found, which was not increased, however, compared to that of 2 h.

The efficiency of radiolabel incorporation for each phospholipid class per se is depicted in **Fig. 6A and 6B** for 2 h incubation and 3 days culture, respectively. The results after 2 h incubation showed a high labeling efficiency of PI for all modified endothelial cells, whereas 20:5-modified cells showed a relatively higher labeling efficiency of PI, which was still found after 3 days culture.

DISCUSSION

Several studies (3, 4, 8) have shown that culturing of endothelial cells in media supplemented with a particular fatty acid results in increased levels of that fatty acid and its elongated product in total membrane phospholipids. We have reported previously that such an increase of a particular fatty acid is always balanced by a change in the level of other fatty acids, and this apparent homeostatic control is also found at the level of cellular functional activity upon long-term fatty acid modification (8). The present data are consistent with these observations and show, in addition, that such a phenomenon is not only present in the total phospholipids, but is also apparent within each individual phospholipid class. The present results show that, in general, long-term fatty acid modifications do not appreciably influence the mean levels of saturated and unsaturated fatty acids per phospholipid class. A notable exception was found in 18:1-modified cells, which show an increase in monounsaturated fatty acids at the expense of both saturated and unsaturated fatty acids. This indicates subtle homeostatic control of membrane unsaturation in each phospholipid class.

As essential fatty acids exert special functions, it could be hypothesized that 20:4(n-6) has a dual role in the membrane. It is not only involved in the regulation of membrane physico-chemical parameters, but it also

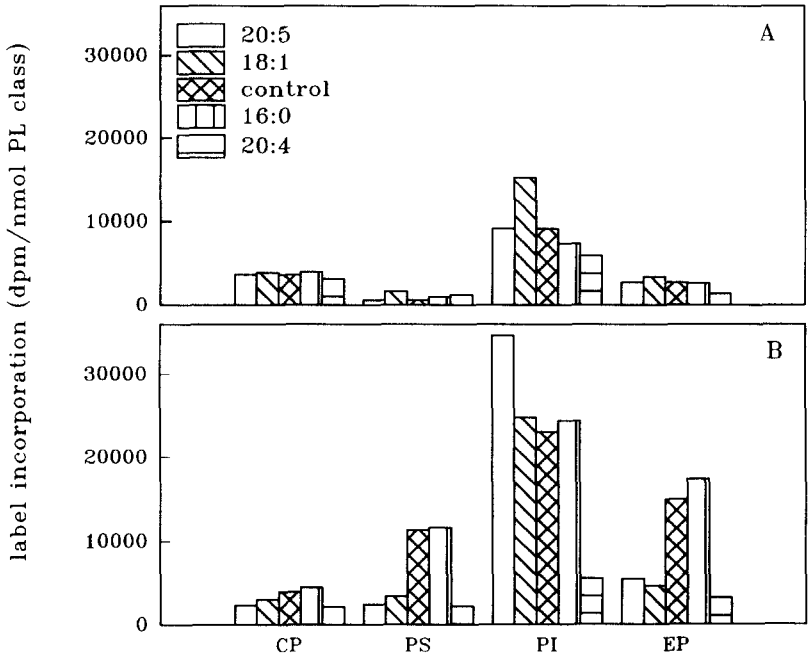

Fig. 6. Efficiency of radiolabel incorporation per phospholipid class in differently modified endothelial cells. Procedure as described in Fig. 4. In order to show the efficiency of label incorporation for the different phospholipid classes per se, radiolabel incorporation for each phospholipid class was calculated as dpm incorporated radiolabel in a phospholipid class divided by the pmol amount of that phospholipid class. Values are from one experiment, representative of two independently performed experiments. Abbreviations are as in Fig. 3.

serves as a substrate pool for local eicosanoid mediators. As fatty acid modification of endothelial cells considerably changes the 20:4(n-6) content of individual phospholipid classes, the availability of 20:4(n-6) for the formation of bioactive mediators upon cell stimulation may also be changed. Therefore, the incorporation, distribution, and modification of [1-^{14}C]arachidonic acid into phospholipids and neutral lipids of differently modified endothelial cells were studied after 2 h incubation and after 3 days culture.

After a 2-h incubation, [1-^{14}C]arachidonic acid is incorporated to about the same extent into the phospholipid fraction of differently modified endothelial cells. This indicates that the initial radiolabel incorporation is not influenced by fatty acyl modification. After 3 days culture, radiolabel incorporation into total phospholipids reflects its actual 20:4(n-6) mass content. [1-^{14}C]arachidonic acid is incorporated to a large extent, however, into the neutral lipid fraction (mostly triglycerides) of 20:4-modified endothelial cells after 3 days, while incorporation into triglycerides is relatively low in other cells. It is possible that in 20:4-modified cells the membrane phospholipids as well as the culture medium are already "saturated" with 20:4(n-6), so that newly incorporated 20:4(n-6) will be "stored" in triglycerides. Consistently, in 16:0-modified cells only a limited amount of 20:4(n-6) is available from the culture medium (in 20% human serum) and is therefore incorporated directly into phospholipids for compensatory reasons. Indications for "storage" regulation of excess 20:4(n-6) into triglycerides of cultured cells has been reported also by others (10, 18). In addition, Cunnane (27) reported retention of 20:4(n-6) in triglycerides of plasma and liver of fasting rats.

Another mechanism for "storage" of sufficient 20:4(n-6) into endothelial cells might be the elongation of 20:4(n-6) to 22:4(n-6), which is subsequently "stored" in PS and EP (including plasmalogen EP), phospholipid classes with a relatively low turnover rate (2). If necessary, 20:4(n-6) can be replenished by retroconversion of the "stored" 22:4(n-6). Rosenthal et al. (20) have recently reported that efficient retroconversion can occur in both fibroblasts and endothelial cells. Elongation products of other fatty acids are also selectively incorporated into specific phospholipid classes, for example 24:1(n-9) and 24:2(n-6) into SM of 18:1- and 18:2-modified cells, respectively. In the present study as well as in that of Rosenthal et al. (20), prolonged elongation of 20:4(n-6) is found in endothelial cells after 3 days modification with 20:4(n-6). For 20:5-modified cells, less elongation of 20:4(n-6) but more elongation of 20:5(n-3) to 22:5(n-3) is found, which is incorporated preferentially into PS and EP (including plasmalogen EP). This points to the importance of long time culture for achieving equilibrium conditions when studying the fate of exogenously added fatty acids.

Analysis of the fatty acid modifications in plasmalogen EP show a slightly increased content of 22-carbon elongation products in cells modified with different polyunsaturated fatty acids, and an increased content of 20:4(n-6) in saturated fatty acid-modified cells compared to that of diacyl EP (including alkyl-acyl EP). However, no gross deviations from the general pattern are found, taking into account that the fatty acid composition of plasmalogen EP is derived only from the *sn*-2 position, in contrast to that of diacyl EP.

For all modified endothelial cells, a rapid incorporation of [1-^{14}C]arachidonic acid is found into CP and PI after 2 h, followed by a redistribution from CP to EP in 3 days without stimulation of the cells. This time-dependent transesterification of 20:4(n-6), also reported by others (5, 15, 18, 19), seems not to be influenced by fatty acid modification and membrane 20:4(n-6) content. Takayama et al. (15) showed that this remodeling is highly selective for 20:4(n-6) and 20:5(n-3).

Typically, a relatively high efficiency of radiolabel incorporation is found in PI of 20:5-modified endothelial cells. With regard to the high turnover rate of PI (13, 28, 29), this indicates a very efficient and specific incorporation mechanism for 20:4(n-6) into PI. This effect is most evident in 20:5-modified cells, which are relatively poor in 20:4(n-6) because of competition by 20:5(n-3). The fast incorporation, relatively high level, and low extent of elongation of 20:4(n-6) in PI of 20:5-modified cells could be interpreted as control of a critical 20:4(n-6) level in the PI class of endothelial membranes, possibly important for effective signal transduction upon cellular stimulation.

Finally, the results of the present study can be put into physiological context. One functional role of fatty acids, as part of phospholipids in biomembranes, is to provide a suitable physico-chemical environment for membrane-associated processes. This may allow extensive variations in fatty acid content of all phospholipid classes, within homeostatic control of physico-chemical properties, as determined by all phospholipid classes together. A second function of the essential fatty acid 20:4(n-6) is to provide a precursor pool for local mediator formation during cell stimulation. For this cellular response, however, not all phospholipid classes participate to the same extent (2, 21). For the rapid initial response, the PI-class (including 20:4(n-6) release mediated by PI-specific phospholipase C and diacylglycerol lipase) is important, while for the further amplification of the activation response, liberation of arachidonic acid by phospholipase A_2 from other classes (CP and EP) also plays a role. The present results indicate that the cells carefully maintain a critical 20:4(n-6) level in PI irrespective of adaption to different fatty acid-containing media. This presumably explains why the functional cellular reactivity remains virtually unaltered in the endothelial cells modified with different fatty acids (8). However, the observation that the 20:4(n-6) content of other phospholipid classes can vary considerably may

imply that alterations in membrane fatty acid content, which influence eicosanoid formation (8), have consequences for the amplification of the cellular response, for example in inflammatory reactions and hemostatic processes. ■

The authors wish to thank Marianne Simonis for technical assistance with gas-liquid chromatography, Mariet Molenaar for typing the manuscript, the Department of Obstetrics and Gynaecology (De Wever Hospital, Heerlen) for supply of umbilical cords, and the Red Cross Bloodbank Limburg (Maastricht) for supply of human serum.

Manuscript received 31 March 1992 and in revised form 19 August 1992.

REFERENCES

1. Gimbrone, M. A. 1986. Vascular endothelium: nature's blood container. *In* Vascular Endothelium in Hemostasis and Thrombosis. M. A. Gimbrone, editor. Churchill Livingstone, New York. 1–13.
2. Stubbs, C. D., and A. D. Smith. 1984. The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim. Biophys. Acta.* **779:** 89–137.
3. Spector, A. A., and M. A. Yorek. 1985. Membrane lipid composition and cellular function. *J. Lipid Res.* **26:** 1015–1035.
4. Spector, A. A., T. L. Kaduce, F. C. Hoak, and G. L. Fry. 1981. Utilization of arachidonic and linoleic acids by cultured human endothelial cells. *J. Clin. Invest.* **68:** 1003–1011.
5. Wey, H. E., J. A. Jakubowski, and D. Deykin. 1986. Incorporation and redistribution of arachidonic acid in diacyl and ether phospholipids of bovine aortic endothelial cells. *Biochim. Biophys. Acta.* **878:** 380–386.
6. Dicorletto, P. E., and G. M. Chisolm. 1986. Participation of the endothelium in the development of the atherosclerotic plaque. *Prog. Lipid Res.* **25:** 365–374.
7. Irvine, R. F. 1982. How is the level of free arachidonic acid controlled in mammalian cells? *Biochem. J.* **204:** 3–16.
8. Vossen, R. C. R. M., M. C. E. van Dam-Mieras, P. J. M. R. Lemmens, G. Hornstra, and R. F. A. Zwaal. 1991. Membrane fatty acid composition and endothelial cell functional properties. *Biochim. Biophys. Acta.* **1083:** 243–251.
9. Hong, S. L., and D. Deykin. 1982. Activation of phospholipases A_2 and C in pig aortic endothelial cells synthesizing prostacyclin. *J. Biol. Chem.* **257:** 7151–7154.
10. Denning, G. M., P. H. Figard, T. L. Kaduce, and A. A. Spector. 1983. Role of triglycerides in endothelial cell arachidonic acid metabolism. *J. Lipid Res.* **24:** 993–1001.
11. Hong, S. L., N. J. McLaughlin, C. Tzeng, and G. Patton. 1985. Prostacyclin synthesis and deacylation of phospholipids in human endothelial cells: comparison of thrombin, histamin and ionophore A_{23187}. *Thromb. Res.* **38:** 1–10.
12. Brown, M. L., J. A. Jakubowski, L. L. Leventis, and D. Deykin. 1987. Ionophore-induced metabolism of phospholipids and eicosanoid production in porcine aortic endothelial cells: selective release of arachidonic acid from diacyl and ether phospholipids. *Biochim. Biophys. Acta.* **921:** 159–166.
13. Takayama, H., M. A. Jr. Gimbrone, 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.
14. Ragab-Thomas, J. M. F., F. Hullin, H. Chap, and L. Douste-Blazy. 1987. Pathways of arachidonic acid liberation in thrombin and calcium ionophore A_{23187}-stimulated endothelial cells: respective roles of phospholipids and triacylglycerol and evidence for diacylglycerol generation from phosphatidylcholine. *Biochim. Biophys. Acta.* **917:** 388–397.
15. Takayama, H., M. H. Kroll, M. A. Gimbrone, and A. I. Schafer. 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. Martin, T. W., and K. C. Michaelis. 1990. Ca^{2+}-dependent synthesis of prostaglandin I_2 and mobilization of arachidonic acid from phospholipids in cultured endothelial cells permeabilized with saponin. *Biochim. Biophys. Acta.* **1054:** 154–168.
17. Thomas, J. M. F., F. Hullin, H. Chap, and L. Douste-Blazy. 1984. Phosphatidylcholine is the major phospholipid providing arachidonic acid for prostacylcin synthesis in thrombin-stimulated human endothelial cells. *Thromb. Res.* **34:** 117–123.
18. Sivarajan, M., E. R. Hall, K. K. Wu, M. E. Rafelson, and C. Manner. 1984. Regulation of intracellular arachidonate in normal and stressed endothelial cells. *Biochim. Biophys. Acta.* **795:** 271–276.
19. Hall, E. R., C. E. Manner, J. Carinkas, R. Snopko, and M. Rafelson. 1987. Selective internalization of arachidonic acid by endothelial cells. *Biochem. J.* **245:** 151–157.
20. Rosenthal, M. D., M. C. Garcia, M. R. Jones, and H. Sprecher. 1991. Retroconversion and Δ^4 desaturation of docosatetraenoate (22:4(n–6)) and docosapentaenoate (22:5(n–3)) by human cells in culture. *Biochim. Biophys. Acta.* **1083:** 29–36.
21. Nozawa, Y., S. Nakashima, and K. Nagata. 1991. Phospholipid-mediated signaling in receptor activation of human platelets. *Biochim. Biophys. Acta.* **1082:** 219–238.
22. Maciag, T., I. Cerundole, S. Istey, P. R. Kelly, and R. Foriand. 1979. An endothelial cell growth factor from bovine hypothalamus: identification and partial characterization. *Proc. Natl. Acad. Sci. USA.* **76:** 5674–5678.
23. Bligh, E. R., and W. J. Dyer. 1959. A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–918.
24. Böttcher, C. J. F., C. M. van Gent, and C. Pries. 1961. A rapid and sensitive sub-micro phosphorus determination. *Anal. Chim. Acta.* **24:** 203–204.
25. Rand, M. L., A. A. H. M. Hennissen, and G. Hornstra. 1986. Effects of dietary sunflower seed oil and marine oil on platelet membrane fluidity, arterial thrombosis, and platelet responses in rats. *Atherosclerosis.* **62:** 267–276.
26. Touchstone, J. C., J. C. Chen, and K. M. Beaver. 1980. Improved separation of phospholipids in thin-layer chromatography. *Lipids.* **15:** 61–62.
27. Cunnane, S. C. 1990. Differential utilization of long chain fatty acids during fasting-induced triacylglycerol depletion. III. Comparison of n-3 and n-6 fatty acids in rat plasma and liver. *Biochim. Biophys. Acta.* **1036:** 64–70.
28. Holub, B. J. 1987. The cellular forms and functions of the inositol phospholipids and their metabolic derivatives. *Nutr. Rev.* **45:** 65–71.
29. 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.* **87:** 211–215.