Utilization of Arachidonic and Linoleic Acids by Cultured Human Endothelial Cells

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ABSTRACT When cultured human umbilical vein endothelial cells are supplemented with linoleic acid, the arachidonic acid content of the cellular phospholipids is reduced ~35%. Most of the fatty acid compositional change occurs during the first 24 h. One factor responsible for this effect is the inability of the endothelial cells to convert appreciable amounts of linoleic to arachidonic acid, due to a fatty acid Δ6-desaturase deficiency. By contrast, these endothelial cultures contain Δ5- and Δ9-desaturase activity and are able to elongate long-chain polyunsaturated fatty acids. The other factor that contributes to the decrease in arachidonic acid is that high concentrations of linoleic acid reduce the incorporation of arachidonate into cellular phospholipids. Stearic acid, a long-chain saturate, does not produce any reduction, whereas eicosatrienoic acid is an even more effective inhibitor than linoleic acid. In spite of the fact that high concentrations of these polyunsaturates produced inhibition, the endothelial cells were found to efficiently incorporate exogenous arachidonic acid into cellular phospholipids and triglycerides. This may serve to compensate for the inability of these cells to synthesize arachidonic acid from linoleic acid. These findings suggest that the endothelium obtains arachidonic acid from an extracellular source, that this cannot be provided in the form of linoleic acid and, in fact, that high concentrations of linoleic acid actually may interfere with the ability of the endothelium to maintain an adequate supply of intracellular arachidonic acid.

INTRODUCTION

Cultured human umbilical vein endothelial cells produce prostacyclin (prostaglandin I_2), a potent vasodilator and inhibitor of platelet aggregation, from arachidonic acid (1, 2). In mammals arachidonic acid is synthesized from linoleic acid, the most abundant polyunsaturated fatty acid usually present in the diet. A number of cultured cells, including human diploid fibroblasts, are able to convert linoleic to arachidonic acid (3–5). Those cultures that cannot perform this conversion are for the most part transformed rodent cell lines (3, 4), and it has been suggested that the deletion of this capacity is somehow associated with malignant transformation (3). Based upon this, one might expect that the endothelial cells, which are primary diploid cultures, would be able to convert linoleic to arachidonic acid so as to insure a readily available supply of substrate for prostacyclin production.

When cultured human umbilical vein endothelial cells are enriched with linoleic acid, however, their ability to produce prostacyclin in response to stimulation with thrombin or a calcium ionophore is reduced (6). Likewise, their capacity to inhibit platelet aggregation is decreased, presumably because of the lesser prostacyclin release (7). One factor that appears to regulate prostaglandin production by cultured cells is the amount of arachidonic acid contained in cellular lipids (8). Therefore, we have examined the factors that influence the arachidonic acid content of the endothelial cultures in an attempt to explain the apparently anomalous findings noted above (6, 7). In particular, we investigated the capacity of the endothelial cells to convert linoleic to arachidonic acid, their ability to use extracellular arachidonic acid, and the extent to which different types of fatty acid can interfere with arachidonic acid incorporation into cellular phospholipids.

METHODS

Endothelial cell cultures. Endothelial cells were obtained from human umbilical veins (9), and primary cultures were prepared according to a slight modification of the method of Jaffe et al. (10) as described (6). Briefly, the cells were suspended in a modified medium 199 containing 20% heat-inactivated fetal bovine serum, counted with a hemocytometer, and seeded in 25-cm^2 flasks at a concentration of 2.25

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$\times 10^6$ cells per flask. After incubation for 24 h at 37°C in an atmosphere of 5% CO_2, this medium was replaced with 3 ml of medium 199 containing 25 mM Hepes plus 20% fetal bovine serum, and the cultures were continued in the 5% CO_2 atmosphere.

Preparation of fatty acid-supplemented media. Fatty acids were purchased from NuCheck Prep (Elysian, Minn.) and were >99% pure as determined by gas-liquid chromatography. [1-^{14}C]Stearic acid, [1-^{14}C]linoleic acid, [1-^{14}C]eicosatrienoic acid (n-6 class), [1-^{14}C]arachidonic acid, and [5, 6, 8, 9, 11, 12, 14, 15-^{3}H]arachidonic acid (hereafter called [^{3}H]arachidonic acid) were obtained from New England Nuclear (Boston, Mass.). These isotopes were checked for purity by thin-layer and gas-liquid chromatography. Where purities were <95%, the isotopes were repurified by a combination of preparative thin-layer and gas-liquid chromatography. Fetal bovine serum was supplemented with fatty acid by adding a warm solution of the sodium salt with mechanical stirring. Before supplementation, the serum contained 0.29 μmol/ml of free fatty acid with a 17-nmol/ml concentration of free linoleic acid and a 29-nmol/ml concentration of arachidonic acid. Free fatty acid concentration was measured colorimetrically (11), and individual fatty acids were determined by gas-liquid chromatography (6). Experimental media were prepared by adding the fatty acid-supplemented fetal bovine serum to medium 199 containing 25 mM Hepes. The medium was adjusted to pH 7.4 at 37°C and sterilized by filtration through a 0.22 micron filter.

Incubation and analyses. The maintenance medium was removed, and the endothelial cell monolayers were washed twice with 2.5 ml of a buffer solution containing 137 mM NaCl, 5 mM KCl, 8 mM Na_2HPO_4, and 2 mM KH_2PO_4, pH 7.4. After addition of 2.5 ml of the fatty acid-supplemented medium, the incubations were carried out at 37°C with a 5% CO_2 atmosphere. The incubations were terminated by aspirating the medium, followed by washing the monolayer twice with 2 ml of ice-cold buffer solution. After harvesting by scraping, the cells were suspended in the cold buffer, sedimented at 600 *g* for 10 min at 4°C, and suspended in 1 ml of fresh cold buffer. A portion of the suspension was removed for determination of the cell protein content (12). The remainder of the suspension was extracted with 20 vol of a mixture of chloroform and methanol (2:1, vol/vol) in order to isolate the cell lipid content (13). Following separation and isolation of the chloroform phase, the solvent was evaporated under N_2, and the lipid residue was dissolved in a measured volume of fresh chloroform.

In an attempt to validate this method for terminating the incubations and isolating the cells, especially with regard to studies involving radioactive arachidonic acid, we carried out an experiment in which the cells were incubated with a mixture of [^{3}H]arachidonic acid and [1-^{14}C]stearic acid. One set of incubations was terminated by the scraping procedure described above whereas in the other, the incubations were terminated by addition of trichloroacetic acid. Lipids were extracted from each set of cultures, and the ratio of ^{14}C/^{3}H in the cell phospholipids was measured. When the scraping procedure was used, the value obtained was 0.192±0.002. A value of 0.195±0.005 was obtained using the trichloroacetic acid procedure. These results indicate that the cell isolation procedure involving scraping did not lead to an excessive loss of arachidonic acid from the cell phospholipids. It was concluded that this procedure was valid within the limits of currently available methodology.

Separation of phospholipids and neutral lipids was obtained by thin-layer chromatography on silica gel G with a solvent system containing hexane-diethyl ether acetic acid-methanol (170:40:4:4). Standards obtained from NuCheck Prep were added to each chromatogram, and the lipids were visualized by exposure to I_2 vapor. After sublimation, the outlined segments of silica gel were scraped into liquid scintillation counting vials. Phospholipids were separated by thin-layer chromatography on silica gel H using a modification of the method of Skipski et al. (14). This modification, which separates the inositol and serine phosphoglycerides, uses a solvent system containing chloroform-methanol-acetic acid-water (100:50:16:8). The R_f values for the major phospholipid classes in these chromatograms are: lysophosphatidylcholine, 0.08; sphingomyelin, 0.22; phosphatidylcholine, 0.40; phosphatidylinositol, 0.65; phosphatidylserine, 0.72; phosphatidylethanolamine, 0.90.

The phospholipid content of the cell lipid extract or of the individual fractions separated by thin-layer chromatography was measured colorimetrically with the malachite green reagent (15). A micromodification of the fluorometric method that utilizes sodium periodate and acetylacetone was used to measure the triglyceride content (16).

Fatty acids were isolated from the various lipid classes by saponification, acidification, and extraction into hexane (17). After methylation with 14% BF_3 in methanol (18), the fatty acid methyl esters were extracted into hexane and separated by gas-liquid chromatography (17). Separation was achieved using a 2 mm × 1.9-m glass column packed with 10% SP2330 on 100/120 mesh Chromosorb W-AW (Supelco, Inc., Bellefonte, Pa.). The Hewlett-Packard model 5700 gas chromatograph (Hewlett-Packard Co., Palo Alto, Calif.) was equipped with a 9:1 stream splitter so that a portion of the effluent could be collected when radiolabeled material was chromatographed. A flame ionization detector was used. N_2 served as the carrier gas at a flow rate of 25 ml/min, and the oven temperature was programmed from 176° to 220°C. Peak areas were determined with a Hewlett-Packard model 3380S integrator-recorder, and areas are reported as weight percentages. In several experiments radioactivity present in the effluent stream was diverted by the stream splitter through a heated collecting port and trapped in a 5-cm length of Teflon tubing immersed in liquid scintillation solution (19). Preliminary studies with this collection system revealed that >96% of previously purified [1-^{14}C]linoleic acid was recovered in the linoleic acid peak even when this acid comprised <20% of a fatty acid mixture.

Radioactivity was measured in a liquid scintillation spectrometer using counting vials containing 10 ml of Budget Solve scintillator solution (Research Products International, Elk Grove Village, Ill.). Quenching was monitored with a ^{226}Ra external standard.

RESULTS

Supplementation with linoleic acid. To follow the time-dependent changes in cellular fatty acid composition, the endothelial cell cultures were exposed to 150 μmol/liter supplemental linoleic acid during a 72-h culture period. The changes in fatty acyl composition of the cellular phospholipid and neutral lipid fractions during this time of culture are shown in Fig. 1. Most of the changes that occurred in both fractions took place during the first 24 h. In the cell phospholipids (upper panel), linoleic acid (18:2)[1] increased

[1] Fatty acids are abbreviated as number of carbon atoms: number of double bonds. Therefore, 18:2 represents a fatty acid containing 18 carbon atoms and 2 double bonds, i.e., linoleic acid.

from 6 to 22% of the fatty acyl groups during the 72-h period. This increase was compensated for primarily by a reduction in the oleic acid (18:1) content of the phospholipids from 25 to 15%. Docosadienoic acid (20:2), the direct elongation product of 18:2, increased from 0.4 to 2.5% as the phospholipids became enriched with 18:2. On the other hand, arachidonic acid (20:4) decreased from 13.4 to 8.7% of the phospholipid fatty acyl groups as this fraction became enriched with 18:2. The cellular phospholipid content did not change appreciably during the 72-h period: 557±12 μg/mg cell protein initially and 538±20 at the end of the supplementation period. Therefore, the percentage changes shown in Fig. 1 represent differences in the fatty acid composition of the usual complement of cellular phospholipids, rather than an accumulation of excess phospholipid in the cells.

Cell neutral lipids also became enriched with 18:2, the increase being from 6 to 35% over the 72-h period. This was accompanied by an increase in 20:2 from 0.1 to 3.7%. However, there was very little change in 20:4, this varying from 2.9 to 3.9%. Stearic acid (18:0) was the only other neutral lipid fatty acid to change appreciably, this decreasing from 23 to 9%. As opposed to the cellular phospholipids, however, the quantity of triglycerides increased from 14±3 to 71±11 μg/mg cell protein during the supplementation period. Therefore, the changes in neutral lipid fatty acyl composition were associated with considerable triglyceride accumulation in the cells.

Utilization of [1-^{14}C]linoleic acid The ability of the cultured endothelial cells to use labeled linoleic acid was examined in an attempt to determine why the large enrichment with 18:2 was not associated with any increase in the 20:4 content of the cells, especially in the cell phospholipids. When the cultures were supplemented with 150 μmol/liter [1-^{14}C]linoleic acid under conditions identical to those used in Fig. 1, the cells incorporated 200±21 nmol/mg cell protein over the 72-h period. Phospholipids contained 35% and neutral lipids 65% of the incorporated radioactivity. Table I shows the distribution of the incorporated radioactivity among the major polyunsaturated fatty acids of the linoleic acid series. In both the phospholipid and neutral lipid fractions, most of the radioactivity remained as 18:2, even after 72 h of incubation. Only trace quantities of radioactivity were present in 18:3. Very little of the incorporated radioactivity was converted to either 20:3 or 20:4, the maximum amount being 2.0 and 2.9%, respectively, in the phospholipids after 72 h. There was a brisk elongation of 18:2 to 20:2, however, and 20:2 contained about 10% of the radioactivity contained in both fractions after 72 h. These isotopic fiindings are consistent with the fatty acid composition data presented in Fig. 1 which show an accumulation of 18:2 and 20:2 but not 20:3 or 20:4 in the cell lipids when the cultures are supplemented with linoleic acid.

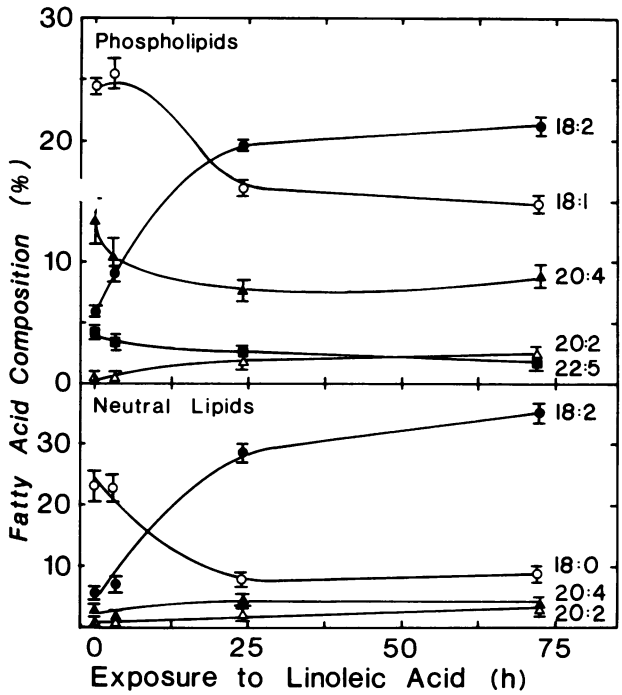

FIGURE 1 Changes in the fatty acid composition of endothelial cell lipids during incubation with supplemental linoleic acid. Endothelial cell cultures in a medium containing 20% fetal bovine serum were exposed to 150 μmol/liter linoleic acid for the times indicated in the abscissa. Lipids were extracted from the washed cell monolayer with a chloroform-methanol mixture, and phospholipids were separated from neutral lipids by thin-layer chromatography. After saponification and methylation, the fatty acids were separated by gas-liquid chromatography. Each point is the mean±SE of four separate cultures.

Further experiments in which the fetal bovine serum content of the medium was varied from 2.5 to 20% indicated that a maximum of only 2.1% of the added [1-^{14}C]linoleic acid was converted to 20:4. Therefore, the relatively high serum concentration routinely used in the culture medium was not responsible for the very low conversion of 18:2 to 20:4 by the endothelial cells. Additional experiments in which the [1-^{14}C]-linoleic acid concentration of the culture medium was varied from 3 to 100 μmol/liter indicated that only 2 to 4% of the radioactivity was converted to 20:4, even at the lowest concentration of supplemental linoleate. These results indicate that the failure of the endothelial cells to convert large amounts of 18:2 to 20:4 was not due to the relatively high concentrations of supplemental 18:2 that were used. Finally, the relationship between cell density and the conversion of 18:2 to 20:4 was investigated. A maximum of 1.1% of the [1-^{14}C]linoleic acid taken up by the cells was converted to 20:4 over the range of cell densities that were

TABLE I
*Time-dependent Modifications of [1-^{14}C]Linoleic Acid**

Fatty acid	Distribution of radioactivity					
	Phospholipids			Neutral lipids		
	3 h‡	24 h	72 h	3 h	24 h	72 h
	%					
18:2	95.5±0.9	90.6±1.2	75.8±6.8	93.5±2.2	81.5±3.6	81.0±1.6
18:3	ND§	ND	0.8±0.4	0.9±0.5	0.9±0.5	0.1±0.1
20:2	3.7±0.2	6.7±0.6	10.0±2.9	3.0±0.2	7.5±1.5	9.7±1.1
20:3	0.8±0.2	0.3±0.1	2.0±0.5	1.1±0.7	1.3±0.7	1.3±0.7
20:4	0.1±0.1	0.4±0.1	2.9±0.9	0.2±0.1	0.7±0.3	0.6±0.1

* The culture medium contained 20% fetal bovine serum and 150 μmol/liter [1-^{14}C]linoleic acid. After extraction of the cell lipid content, the phospholipids and neutral lipids were separated by thin-layer chromatography. The isolated lipid fractions were saponified, methylated, and separated by thin-layer chromatography using an effluent 9:1 stream splitter. Liquid scintillation counting was used to measure the radioactivity contained in each fatty acid methyl ester fraction. Each value is the mean±SE of four separate cultures. The values do not add up to 100% because small quantities of radioactivity were recovered in several other fractions.
‡ Time of incubation with [1-^{14}C]linoleic acid.
§ Not detected.

tested, 54 to 378 μg cell protein. Therefore, the inability of the endothelial cultures to convert appreciable amounts of 18:2–20:4 was not due to the relatively high cell densities that were routinely used.

Modification of other fatty acids. The ability of the endothelial cells to modify other long-chain fatty acids was examined to determine whether the very small conversion of linoleic to arachidonic acid might be due to a generalized defect in the desaturation mechanism. Table II shows the distribution of radioactivity from [1-^{14}C]stearic, [1-^{14}C]eicosatrienoic, and [1-^{14}C]arachidonic acids recovered in the cell phospholipids. For comparison, [1-^{14}C]linoleate also was tested in this series of experiments. As observed previously, 6% of the linoleate radioactivity was recovered in 20:2, only 1% was converted to 20:4, and no radioactivity was detected in either 18:3 or 20:3. With [1-^{14}C]-stearate, 4.6% of the radioactivity incorporated into phospholipids was converted to 18:1. With [1-^{14}C]-eicosatrienoic acid, 14% of the radioactivity incorporated into phospholipids was converted to 20:4 plus 22:4. These results indicate that the fatty acid Δ9-desaturase, which converts 18:0 to 18:1, and the Δ5-desaturase, which converts 20:3 to 20:4, are much more active in the endothelial cell than is the Δ6-desaturase, which uses 18:2. [1-^{14}C]Arachidonic acid was elongated to 22:4, but it was not further desaturated.

The changes in phospholipid fatty acyl composi-

TABLE II
*Incorporation and Modification of Supplemental Radioactive Fatty Acids**

Supplemental fatty acid	Distribution in cell phospholipid fatty acids							
	18:0	18:1	18:2	18:3	20:2	20:3	20:4	22:4
	nmol/mg protein							
[1-^{14}C]Linoleic acid			71.2±0.8	ND‡	5.0±0.5	ND	0.4±0.1	
[1-^{14}C]Stearic acid	202±3	9.8±1.1						
[1-^{14}C]Eicosatrienioic acid						80.6±3.2	6.0±1.0	7.2±1.2
[1-^{14}C]Arachidonic acid							27.2±2.9	8.4±0.9

* The culture medium contained 150 μmol/liter of the supplemental fatty acid and 20% fetal bovine serum. After 24 h of culture, the lipids were isolated from the cells by extraction with chloroform and methanol, saponified, and methylated. Fractions containing individual fatty acid methyl esters were collected from the effluent of a gas-liquid chromatography column fitted with a 9:1 stream splitter. The radioactivity contained in each effluent fraction was measured by liquid scintillation spectrometry. Each value is the mean±SE of three separate cultures.
‡ Not detected.

tion when the cultures were supplemented with fatty acids other than linoleic acid are given in Table III. As compared with endothelial cells cultured in media containing no supplemental fatty acid, those exposed for 24 h to stearic acid contained more 18:0 and considerably less 16:0. In spite of the conversion of some 18:0 radioactivity to 18:1, there was no appreciable change in the 18:1 content of the cell phospholipids. The cultures supplemented with eicosatrienoic acid exhibited a very large increase in the 20:3 content of the cell phospholipids, in agreement with the large uptake of this isotope that was noted. An appreciable decrease in the phospholipid 18:1 content occurred. Likewise, the 20:4 and 22:4 contents decreased even though 20:3 radioactivity was converted to these metabolites. The cultures supplemented with arachidonic acid exhibited a large increase in 20:4 and 22:4 in cellular phospholipids, in agreement with the isotopic results.

Use of [1-^{14}C]arachidonic acid. Because of the apparent ability of the endothelial cells to use extracellular arachidonate, we investigated this process more intensively. The incorporation of [1-^{14}C]arachidonic acid into the cell triglycerides and phospholipids during a 4-h incubation with a medium containing 20% fetal bovine serum and 150 μmol/liter arachidonic acid was roughly linear, with the triglycerides containing about twice as much radioactivity. The diglyceride fraction, which contained only about 1–5% of the radioactivity, reached a steady-state level within the first hour of incubation. The free fatty acid fraction, which also reached a steady state level during the first hour, contained about 5% of the radioactivity. There was also a progressive increase in the radioactivity contained in each of the individual phospholipid fractions over the 4-h incubation.

To determine the extent to which these results were representative, we examined the effect of lower arachidonic acid concentrations that are more in the range of those occurring in the plasma free fatty acid fraction. The concentrations tested were 5–52 μmol/liter, and the time of incubation was 4 h. As seen in the upper part of Fig. 2, incorporation of arachidonate radioactivity into each of the major cell lipid classes increased as the extracellular concentration was raised, but the concentration dependence varied among the different fractions. Incorporation into triglycerides showed the greatest concentration dependence, the increase being 4.2-fold. At the lowest arachidonic acid concentration tested, 5 μmol/liter, 33% more radioactivity was recovered in cell phospholipids as compared with triglycerides. By contrast, more radioactivity was recovered in triglycerides than phospholipids at each of the higher arachidonate concentrations. Only small amounts of radioactivity were incorporated into the cell diglyceride and free fatty acid fractions at all of the concentrations tested. The distribution of radioactivity in the individual phospholipid fractions is shown in the lower part of Fig. 2. Increasing amounts of radioactivity were recovered in the choline and ethanolamine phosphoglycerides as the arachidonic acid concentration was raised, with the choline fraction exhibiting the most marked concentration dependence. Little concentration dependence was noted for the inositol phosphoglycerides, and the incorporation into the serine fraction actually appeared to decrease as the arachidonic acid concentration was raised. Except at the lowest concentration tested, the incorporation of radioactivity into the choline phosphoglycerides was considerably greater than into any of the other phospholipid fractions.

Competition between fatty acids. Studies were done to determine the extent to which other long-chain fatty acids compete with arachidonic acid for incorporation into cellular phospholipids. In these experiments, the [^{3}H]arachidonic acid concentration was held constant at 10 μmol/liter, whereas the concentration of the second fatty acid was raised to as much as 180 μmol/liter. The second fatty acid was labeled

TABLE III
*Effect of Fatty Acid Supplementation on the Fatty Acid Composition of Cell Phospholipids**

Fatty acid	Composition			
	None‡	Stearic acid§	Eicosatrienoic acid	Arachidonic acid
	%			
16:0	19.1	14.6	19.8	17.1
18:0	17.5	27.3	13.9	15.8
18:1	22.4	21.8	14.0	18.8
18:2	3.9	3.8	2.2	2.7
18:3	1.0	0.5	0.2	0.2
20:2	ND‖	ND	ND	ND
20:3	1.9	2.0	25.7	1.7
20:4	12.8	14.2	7.5	21.0
22:4	3.4	4.0	2.8	11.5
22:5	3.1	3.2	2.1	2.3
22:6	2.2	3.2	1.3	1.2

* After 24 h incubation with culture media containing 100 μmol supplementary fatty acid, the fatty acid composition of the cell phospholipids was determined by gas-liquid chromatography. Each value is the average of two separate cultures which varied by <5%. The values do not add up to 100% because the cell phospholipids contain small amounts of a number of additional fatty acids that are not listed.
‡ Medium containing 20% fetal bovine serum but no supplemental fatty acid.
§ Fatty acid supplement (150 μmol/liter) added to the medium containing 20% fetal bovine serum.
‖ Not detected.

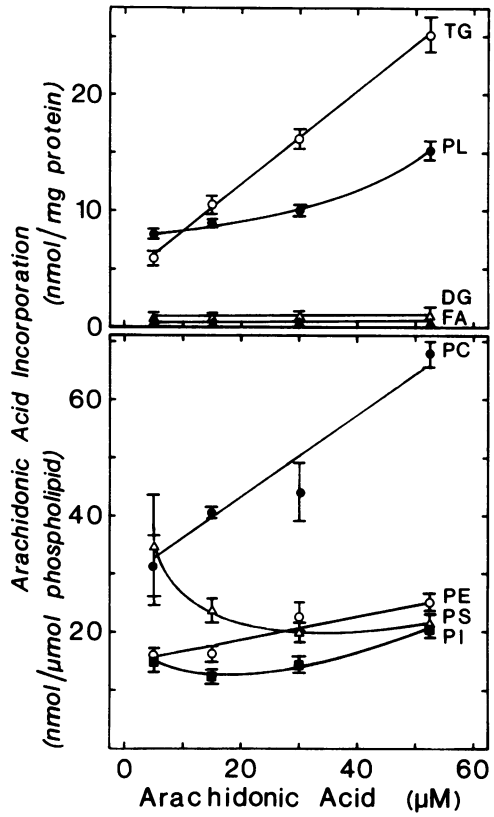

FIGURE 2 Effect of arachidonic acid concentration on its incorporation into endothelial cell lipids. The culture medium contained 20% fetal bovine serum supplemented with 5–52 μmol/liter [^{3}H]arachidonic acid. After washing, the cell monolayers were extracted with a mixture of chloroform and methanol, and the lipid fractions were separated by thin-layer chromatography. Incorporation of radioactivity was measured by liquid scintillation counting. The time of incubation was 4 h. Each point is the mean±SE of four separate cultures. The abbreviations used are: TG, triglycerides; PL, total phospholipids; FA, free fatty acids; DG, diglycerides; PC, choline phosphoglycerides; PE, ethanolamine phosphoglycerides; PI, inositol phosphoglycerides; and PS, serine phosphoglycerides.

with ^{14}C, and the amounts of each fatty acid incorporated was determined by double label scintillation counting. When only 10 μmol/liter of [^{3}H]arachidonic acid was available, about 11 nmol/mg cell protein was incorporated into cell phospholipids as seen in Fig. 3. When increasing amounts of [1-^{14}C]stearic acid (top panel), [1-^{14}C]linoleic acid (middle panel), or [1-^{14}C]-eicosatrienoic acid (lower panel) were added, considerably more total fatty acid was incorporated into the cell phospholipids. With stearic acid (18:0), the amount of arachidonic acid incorporated remained relatively constant even though increasing amounts of stearate were incorporated. By contrast, the amount of arachidonic acid incorporated decreased up to 40% as increasing amounts of linoleic acid (18:2) were incorporated, indicating that some competition for esterification into phospholipids exists between these two polyunsaturated acids. Even more marked competition was observed with eicosatrienoic acid (20:3), for arachidonic acid incorporation into cell phospholipids decreased as much as 80% in this case.

An additional experiment was done with a mixture of [^{3}H]arachidonate and [1-^{14}C]linoleate in order to determine whether the apparent competition for incorporation might be localized to a single phospholipid class. The results are shown in Table IV. In this experiment the arachidonic acid concentration was maintained at 10 μmol/liter and the linoleic acid concentration was either 86 or 150 μmol/liter. At the highest linoleate concentration used, the incorporation of arachidonate into the total phospholipid fraction was re-

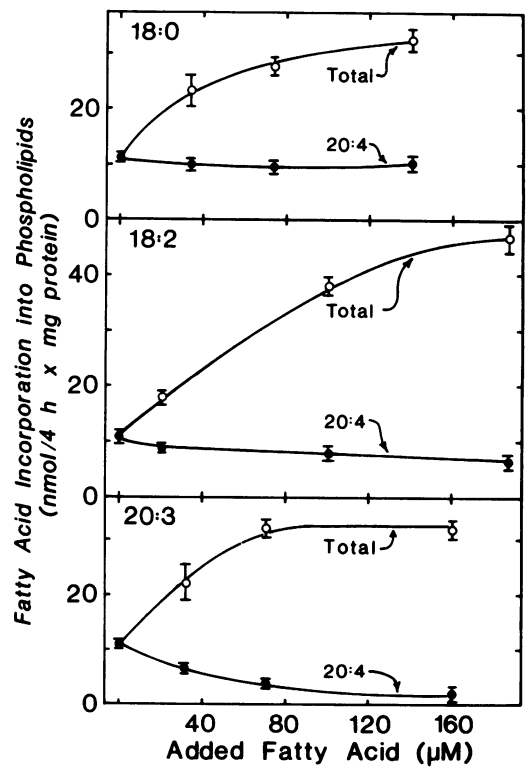

FIGURE 3 Incorporation of fatty acid mixtures into endothelial cell phospholipids. The incubation medium contained 20% fetal bovine serum, 10 μmol/liter [^{3}H]arachidonic acid, and varying amounts of a second fatty acid labeled with ^{14}C. Three fatty acids were tested, stearic (18:0), linoleic (18:2), and eicosatrienoic (20:3) acids, each in a different culture of cells. A 4-h incubation was used. After the medium was removed and the cell monolayers washed, the cell lipids were extracted with a mixture of chloroform and methanol and the total cell phospholipids isolated by thin-layer chromatography. The amount of ^{3}H and ^{14}C present in the phospholipid fraction was determined by double label liquid scintillation counting. Each point is the mean±SE of four separate cultures, the "total" representing the sum of the [^{3}H]arachidonic acid and 1-^{14}C-fatty acid incorporation.

TABLE IV

*Effect of Linoleic Acid on Arachidonic Acid Incorporation into Cell Phospholipids**

Linoleic acid	Incorporation							
	Choline phosphoglycerides		Ethanolamine phosphoglycerides		Inositol phosphoglycerides		Serine phosphoglycerides	
	20:4‡	18:2§	20:4	18:2	20:4	18:2	20:4	18:2
mM	*nmol/mg protein*							
0	4.2±0.1		1.2±0.1		1.3±0.1		1.0±0.1	
86	3.8±0.2	17.0±1.3	1.1±0.2	3.7±0.7	1.0±0.1	1.3±0.1	0.9±0.1	0.7±0.1
150	3.7±0.1	22.3±0.6	0.8±0.1	4.6±0.1	0.9±0.1	1.4±0.1	0.6±0.1	0.8±0.1

* Endothelial cell monolayers were incubated for 4 h with either 10 μmol/liter [^{3}H]arachidonic acid or this fatty acid plus [1-^{14}C]linoleic acid. After incubation and washing, the cell lipids were extracted with a mixture of chloroform and methanol, and the individual phospholipid fractions were isolated by thin-layer chromatography. The amount of ^{3}H and ^{14}C contained in each fraction was determined by double-label liquid scintillation counting. Each value is the mean±SE of four separate cultures.

‡ Incorporation of [^{3}H]arachidonic acid.

§ Incorporation of [1-^{14}C]linoleic acid.

duced by 22%, a somewhat lesser value than that observed in Fig. 3. Some decrease in arachidonate incorporation was noted in each of the four phosphoglyceride fractions, indicating that the competition for incorporation between linoleate and arachidonate was not localized to one phospholipid class.

DISCUSSION

Our results indicate that two factors are responsible for the decrease in arachidonic acid that occurs in cell phospholipids when cultured human endothelium is enriched with linoleic acid. One is that the endothelial cell has a limited capacity to convert linoleic acid to arachidonic acid. The other is that the incorporation of arachidonic acid into all of the major cellular phosphoglycerides is reduced as the availability of linoleic acid increases, presumably through competitive inhibition. These two factors appear to operate concurrently. Because of the limited conversion capacity, most of the linoleic acid that is taken up by the endothelial cell remains in the dienoic form. As the intracellular linoleic acid concentration increases, more of it is esterified into cellular phospholipids, to the exclusion of arachidonic acid. Most of these changes occurred within the first 24 h, indicating that the depletion of arachidonic acid from the intracellular phospholipids can occur rapidly following exposure of the endothelial cells to elevated concentrations of linoleic acid.

The conversion of linoleic to arachidonic acid requires two desaturations and a single elongation. These reactions occur in the microsomes with a pathway of 18:2 → 18:3 → 20:3 → 20:4 (20). The endothelial cells elongated both linoleic and arachidonic acids, forming 20:2 and 22:4, respectively. While the conversion of 18:2 to 20:2 is not identical to the elongation step in the arachidonic acid synthetic pathway, studies with rat liver microsomes indicate that 18:2 and 18:3 are elongated at roughly comparable rates (21). Therefore, it is unlikely that the conversion of linoleic to arachidonic acid is limited by the elongation step. Each of the two desaturations in the pathway is mediated by a separate microsomal enzyme (22). A fatty acid Δ6-desaturase converts 18:2 to 18:3, whereas the conversion of 20:3 to 20:4 is mediated by a Δ5-desaturase. Because the endothelial cells converted radioactive 20:3 to 20:4, the Δ5-desaturase is operative. This leads to the conclusion that the inactive step in the pathway is the Δ6-desaturase reaction. Some Δ9-desaturase activity, which converts 18:0 to 18:1 also was present in these cells. These findings indicate that the cells do not have a generalized desaturase deficiency. Like the endothelial cultures, several other cell lines are reported to have a Δ6-desaturase deficiency. These include the L-fibroblast (3), LM cell (3, 4, 23), HSDM fibrosarcoma (3), and Chang liver cell (4). However, a number of other cultured cells contain this activity, including mouse neuroblastoma (24), human skin fibroblasts (3, 5), and human lung fibroblasts (3, 4). Therefore, the Δ6-desaturase deficiency appears to be unrelated to the fact that the endothelial cells were cultured.

Competition between linoleic and arachidonic acids for incorporation into phospholipids, the second factor that contributes to the reduction in arachidonic acid content, could occur at the level of either the acyl coenzyme A synthetase or acyltransferase reactions. Stearic acid, which was incorporated into phospholipids to about the same extent as linoleic acid, did not inhibit arachidonic acid incorporation. Because stearic acid also had to be converted to the acyl coenzyme

A derivative before esterification, competition at the level of fatty acid activation appears unlikely. On the other hand, there is some evidence that separate microsomal acyl coenzyme A synthetases activate long-chain saturated and polyunsaturated fatty acids (25), so that competition at this step might possibly occur between arachidonate and linoleate but not stearate. The present results, however, are much more consistent with a mechanism in which competition occurs at the acyltransferase step. Arachidonate should be incorporated primarily into position 2 of the glycerophospholipids (26), and polyunsaturates are known to compete much more effectively than saturates for this 2-acyltransferase reaction (27). Our findings that linoleic and eicosatrienoic acids, but not stearic acid, reduce arachidonate incorporation into the endothelial cell phospholipids, are in complete agreement with these previous observations.

Assuming that these results with umbilical vein cultures are representative of the physiologic situation, it appears that the endothelium requires a source of preformed arachidonic acid in order to maintain its intracellular arachidonate stores. This could be provided either as plasma free fatty acid or in the form of esterified fatty acid contained in the plasma lipoprotein. In spite of the observed competition, our results demonstrate that arachidonic acid incorporation into cellular phospholipids persists even in the face of a large excess of stearic or linoleic acids. The endothelium apparently has evolved so that it can continue to incorporate some arachidonic acid into phospholipids even though the extracellular arachidonic acid concentration is low relative to other fatty acids. Although eicosatrienoic acid effectively inhibited arachidonic acid incorporation into phospholipids, appreciable amounts of this fatty acid normally are not present in the plasma. Furthermore, the endothelial cultures are able to convert eicosatrienoic to arachidonic acid, providing some protection against arachidonic acid depletion should the eicosatrienoate level begin to build up. Another mechanism that probably contributes to the maintenance of the intracellular arachidonic acid content is its ready incorporation into triglycerides, even when the extracellular concentration is only 5–50 μmol/liter. With palmitic acid, much higher extracellular concentrations were required to obtain similar incorporations into endothelial cell triglycerides (28). Taken together, these findings suggest that the human endothelial cell has developed efficient mechanisms for incorporating arachidonic acid into cellular lipids, perhaps to compensate for its inability to convert linoleate to arachidonate.

Whereas these mechanisms probably insure an adequate intracellular supply of arachidonic acid under most conditions, the present results demonstrate that they may not be able to compensate adequately when a large excess of linoleic acid is available to the endothelium. Such a situation might arise when humans ingest a diet highly enriched in polyunsaturated fat. Linoleic acid is the predominant polyunsaturate when the diet is supplemented with either corn or safflower oil. When humans consume this type of diet, a large increase in linoleic acid occurs in all of the plasma lipoproteins within 7–14 d (29, 30). Moreover, a single meal rich in these polyunsaturated fats can greatly increase the linoleic acid content of the chylomicron triglycerides and plasma free fatty acids within 4–6 h (31–34), and the plasma linoleic acid concentration can remain elevated for as long as 2 d (35). Within 6 h after feeding safflower oil, the plasma free fatty acid concentration increased from a base-line value of 0.32 to 0.55 μmol/ml (34). Linoleic acid comprised 45% of the plasma free fatty acids after 6 h, or 0.25 μmol/ml, as opposed to only 15%, or 0.048 μmol/ml in the fasting state (34). There is no increase in arachidonic acid under these conditions; in fact, the plasma arachidonic acid content decreased in several instances after polyunsaturated fats rich in linoleic acid were fed (34, 36). The present findings suggest that the endothelium may not be able to compensate sufficiently for this type of circulating fatty acid compositional imbalance and that the amount of arachidonate present in cellular phospholipids may decrease under these conditions. Furthermore, previous studies with this cultured endothelial model system suggest that such modifications produced in intracellular fatty acid composition may be sufficient to decrease the capacity of the cells to form prostacyclin (6). Because such a change could reduce the protective action of the endothelium against arterial vasoconstriction or thrombosis, it becomes important to determine whether a similar effect will occur in vivo in response to feeding large quantities of polyunsaturated fat.

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