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# Role of triglycerides in endothelial cell arachidonic acid metabolism

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**Abstract** Arachidonic acid was incorporated into triglycerides by cultured bovine endothelial cells in a time- and concentration-dependent manner. At 75 $\mu$M or higher, more arachidonic acid was incorporated into triglycerides than into phospholipids. The triglyceride content of the cells increased as much as 5.5-fold, cytoplasmic inclusions appeared, and arachidonic acid comprised 22% of the triglyceride fatty acids. Triglyceride turnover occurred during subsequent maintenance culture; there was a 60% decrease in the radioactive arachidonic acid contained in triglycerides and a 40% decrease in triglyceride content in 6 hr. Most of the radioactivity was released into the medium as free fatty acid. The turnover of arachidonic acid, but not oleic acid in cellular triglycerides, decreased when supplemental fatty acid was added to the maintenance medium. Incorporation and turnover of radioactive arachidonic acid in triglycerides also was observed in human skin fibroblasts, 3T3-L1 cells, and MDCK cells. Other fatty acids were incorporated into triglycerides by the endothelial cells; the amounts after a 16-hr incubation with 50 $\mu$M fatty acid were 20:3 > 20:4 > 18:1 > 18:2 > 22:6 > 16:0 > 20:5. These findings indicate that triglyceride formation and turnover can play a role in the fatty acid metabolism of endothelial cells and that arachidonic acid can be stored in endothelial cell triglycerides—**Denning, G. M., P. H. Figard, T. L. Kaduce, and A. A. Spector.** Role of triglycerides in endothelial cell arachidonic acid metabolism. *J. Lipid Res.* 1983. **24:** 993–1001.

**Supplementary key words** cultured cells • fatty acids • phospholipids • lipid turnover

Arachidonic acid, which is the substrate for prostacyclin ($PGI_2$) synthesis (1, 2), comprises about 10% of the phospholipid fatty acyl chain of endothelial cells (3, 4). Arachidonic acid is formed in animal tissues by the desaturation and elongation of linoleic acid (5). Although arachidonic acid availability appears to be important for endothelial function, cultured endothelial cells do not synthesize appreciable amounts of arachidonic acid from linoleic acid (6, 7). This suggests that the endothelium probably depends on a source of preformed arachidonic acid, either from the plasma free fatty acids or lipoproteins. Consistent with this view are the findings that cultured endothelial cells take up albumin-bound arachidonic acid and that this uptake continues even in the presence of an excess of other free fatty acids (6). Indirect evidence based on $PGI_2$ production suggests that plasma high density lipoproteins also can supply arachidonic acid to endothelial cells (8).

In their initial studies on lipid metabolism in cultured endothelial cells, Hoak, Czervionke, and Lewis (9) noted that palmitic acid was incorporated into cellular neutral glycerides. Subsequently, we also observed that arachidonic acid can be incorporated into this fraction, which is almost entirely composed of triglycerides (7). Based upon these observations, we wondered whether triglycerides might serve as a supplementary intracellular storage pool for arachidonic acid in endothelial cells. To examine this possibility, we have investigated the formation and turnover of triglycerides in cultured bovine aortic endothelial cells, with emphasis on the potential role of this process in cellular arachidonic acid metabolism.

## METHODS

### Materials

M-199 (Earle's Base) medium was obtained from KC Biologicals (Kansas City, KS) and fetal bovine serum was supplied by Sterile Systems (Logan, UT). This serum contained cholesterol (0.83 mmol/l), triglycerides (0.41 mmol/l), and free fatty acids (0.06 mmol/l). The composition of the major fatty acids present in the serum was 16% palmitic, 5% palmitoleic, 14% stearic, 28% oleic, 7% linoleic, and 13% arachidonic acid. Glutamine, BME vitamins, MEM nonessential amino acids, trypsin, and neomycin sulfate were purchased from Grand Island Biological Co. (Grand Island, NY). Fatty acids that were >98% pure as determined by gas–liquid chromatography were obtained from Nu-Chek Prep (Elysian, MN). Radiolabeled fatty acids that were at least 97% pure as determined by collection and radioactivity assay following gas–liquid chromatography were purchased from New England Nuclear (Boston, MA). Bo-

Abbreviations: $PGI_2$, prostacyclin; DPBS, Dulbecco's phosphate-buffered saline solution; GLC, gas–liquid chromatography.



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vine plasma albumin (Fraction V, fatty acid-free) was obtained from Miles Laboratories, Inc. (Elkhart, IN). All other chemicals were commercial reagent grade quality.

### Cultured cells

Endothelial cell cultures were isolated from bovine aorta (7, 10), and all experiments were performed with cells below passage 20. The cells were maintained on M-199 (Earle's Base) medium supplemented with 10% fetal bovine serum, BME vitamins, MEM nonessential amino acids, 2 mM glutamine, 10 mM HEPES (pH 7.4), and 100 μg/ml neomycin sulfate in an atmosphere of 95% air and 5% $CO_2$. Seeding was performed following detachment of the cells from the flask with 0.25% trypsin and 0.02% EDTA in a solution containing 150 mM NaCl, 5 mM KCl, 8 mM $Na_2HPO_4$, and 2 mM $KH_2PO_4$, pH 7.4. The cells were suspended in maintenance medium and seeded at a concentration of 5–7 × $10^3$ cells/$cm^2$ in T-25 Falcon flasks. Cultures were fed every 3 days and reached confluence within 5–7 days after seeding.

Other cultured cell lines were grown and passaged as described previously; Madin-Darby canine kidney epithelial cells (MDCK) (11), albino mouse embryo 3T3-L1 cells (12), and human foreskin fibroblasts (13).

Photomicrographs were taken with a Leitz Leica camera adapted for attachment to a Leitz Diavert polarized microscope. The film employed was Kodak Technical Pan 2415 (ASA 100).

### Fatty acid incorporation

Fatty acids were dissolved in ethanol solution. After adding 1–2 drops of 1 N NaOH, the material was dried under high-purity $N_2$ (99.997% pure), redissolved in a small amount of warm distilled water, and added to M-199 medium supplemented with 10% fetal bovine serum. The pH was adjusted immediately to 7.4.

Confluent monolayers were washed with Dulbecco's phosphate-buffered saline (DPBS) (137 mM NaCl, 2.7 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 1.5 mM $KH_2PO_4$, and 8.0 mM $Na_2HPO_4$, pH 7.4), and then with DPBS containing 50 μM fatty acid-free albumin. The cells were incubated for the indicated time at 37°C with 5 ml of M-199 containing 10% fetal bovine serum supplemented with $^{14}C$-labeled fatty acids (3–5 × $10^5$ dpm/flask). After incubation, the cells were washed with ice-cold DPBS, scraped into tubes, and sedimented at 600 *g* for 10 min. The cells were resuspended in DPBS and aliquots were taken for protein determination by a modification of the Lowry method (14). The remaining cells were extracted with $CHCl_3$–$CH_3OH$ 2:1 (v/v) (15), and the phases were separated with a solution of 9 mM NaCl containing 0.04 N HCl.

To measure the turnover of the incorporated radioactive fatty acid, confluent monolayers were exposed initially for 16 hr at 37°C to 150 μM $^{14}C$-labeled fatty acid (4 × $10^5$ dpm) in M-199 containing 10% fetal bovine serum. After incubation, the cells were washed with warm DPBS and DPBS containing 50 μM fatty acid-free albumin. Three of the cultures were extracted at this time in order to measure the amount and distribution of the incorporated radioactivity. The remaining cultures were incubated for various times at 37°C with 5 ml of M-199 medium containing 10% fetal bovine serum or this medium supplemented with fatty acid. After incubation, the cells were washed and harvested to determine the amount and distribution of the remaining radioactivity. In some experiments, the medium was collected following the second incubation and assayed for radioactivity.

### Lipid analysis

To determine incorporation of radioactivity into total cell lipids, aliquots of the chloroform extract were dried under $N_2$ and dissolved in 10 ml of Budget-Solve (Research Products International, Inc.). The distribution of radioactivity in the cellular lipid fractions was determined by spotting additional aliquots of the lipid extract on silica gel G plates (Analabs, North Haven, CT) along with a standard lipid mixture, and developing the chromotogram in heptane–ethyl ether–acetic acid–methanol 170:40:4:4 (v/v) (13). The plates were stained with $I_2$ and after sublimation, bands corresponding to the different lipid fractions were scraped into scintillation vials containing 10 ml of Budget-Solve. All radioactivity measurements were made with a Beckman LS7000 liquid scintillation spectrometer. Quenching was monitored with the $^{137}Cs$ external standard.

### Triglyceride assay

Endothelial cells were grown to confluence in T-75 Falcon flasks. The cultures were then washed with cold buffer, scraped into tubes submerged in ice, and sedimented by centrifugation at 0°C. Aliquots were assayed for protein content by a modification of the Lowry method (14). Other aliquots were extracted with $CHCl_3$–$CH_3OH$ 2:1 (v/v) (15). The organic phase was dried under $N_2$, and the triglyceride content was determined by a micromodification of a spectrophotofluorometric method (13, 16).

### Fatty acid separation

Confluent cell monolayers were washed with warm DPBS followed by DPBS containing 50 μM albumin. The cells were incubated for 16 hr with 5 ml of M-199 containing 10% fetal bovine serum supplemented with 150 μM [1-$^{14}C$]arachidonic acid (4.5 × $10^5$ dpm/flask)



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TABLE 1. Effect of fatty acid concentration on incorporation into endothelial cell triglycerides and phospholipids[a]

| Fatty Acid Concentration | Incorporation | | | |
|---|---|---|---|---|
| | Triglycerides | | Phospholipids | |
| | [1-$^{14}$C]Arachidonic Acid | [1-$^{14}$C]Oleic Acid | [1-$^{14}$C]Arachidonic Acid | [1-$^{14}$C]Oleic Acid |
| *μM* | *nmol / mg protein* | | | |
| 25 | 24 ± 2 | 13 ± 3[b] | 102 ± 5 | 103 ± 5 |
| 50 | 81 ± 2 | 58 ± 5[c] | 133 ± 7 | 129 ± 3 |
| 75 | 148 ± 7 | 95 ± 8[c] | 157 ± 12 | 150 ± 5 |
| 100 | 181 ± 5 | 153 ± 8 | 145 ± 12 | 195 ± 13 |
| 150 | 290 ± 7 | 329 ± 8[b] | 205 ± 7 | 234 ± 8 |

[a] The time of incubation was 16 hr. The media contained 10% fetal bovine serum. Each value is mean ± SE of three separate cultures.

[b] $P < 0.05$ as compared with the results obtained for cultures incubated with [1-$^{14}$C]arachidonic acid.

[c] $P < 0.001$ as compared with the results obtained for cultures incubated with [1-$^{14}$C]arachidonic acid.

or [1-$^{14}$C]oleic acid ($3.4 \times 10^6$ dpm/flask). The cells were then washed with ice-cold buffer, scraped, and sedimented, and the cell pellet was extracted with $CHCl_3$–$CH_3OH$ 2:1 (v/v) (17). After the extract was dried under $N_2$, 1 ml of 14% $BF_3$ in $CH_3OH$ was added, the samples were heated at 100°C for 10 min, and the methyl esters were extracted into n-heptane. The fatty acid methyl esters were separated by GLC using a 2 mm × 1.9 m glass column packed with 10% SP2330 on 100/120 mesh Chromasorb W-AW. A Hewlett-Packard model 5700 gas chromatograph was equipped with a 9:1 stream splitter so that most of the column effluent could be diverted, collected, and assayed for radioactivity by liquid scintillation spectrometry (18). In additional experiments, the fatty acid composition was determined as described above without the use of the GLC stream splitter (13).

## RESULTS

### Triglyceride formation

Initial experiments indicated that [1-$^{14}$C]arachidonic acid was incorporated into the triglycerides and phospholipids in a time-dependent manner. When the arachidonic acid concentration was 30 μM in a medium containing 10% fetal bovine serum, the uptake was essentially completed within 8 hr. Although uptake continued over a 24-hr incubation period when the medium contained 100 μM arachidonic acid, there was only a relatively small increase in cell lipid radioactivity between 16 and 24 hr. Based on this finding, a 16-hr incubation period was employed in most experiments as an approximation of the steady state arachidonic acid incorporation.

The effect of arachidonic acid concentration on its incorporation into the endothelial cell triglycerides and phospholipids after a 16-hr incubation is shown in **Table 1.** A 12-fold increase in incorporation of radioactivity into triglycerides occurred when the arachidonic acid concentration was raised from 25 to 150 μM. While more radioactivity also was incorporated into phospholipids over this concentration range, the maximum increase was only 2-fold. For comparison, the incorporation of [1-$^{14}$C]oleic acid over this concentration range also is shown in Table 1. As observed with arachidonic acid, the increase in radioactive oleic acid incorporation into triglycerides was much greater than into phospholipids. Except at the highest concentration tested, however, considerably more radioactive arachidonic than oleic acid was incorporated into triglycerides. Moreover, at the lower concentrations, 50 to 100% more arachidonic acid than oleic acid was incorporated into triglycerides. By contrast, there was no significant difference in the amounts of arachidonic and oleic acid radioactivity incorporated into phospholipids.

After incubation for 16 hr with 150 μM [1-$^{14}$C]arachidonic acid, 75% of the radioactivity incorporated into the endothelial cell triglycerides was recovered in the 20:4 fraction isolated by GLC.[1] The only other fatty acid fraction that contained an appreciable amount of radioactivity was 22:4, which accounted for 12%. When the endothelial cultures were incubated similarly with 150 μM [1-$^{14}$C]oleic acid, 83% of the radioactivity incorporated into triglycerides was recovered following GLC separation in the 18:1 fraction, and

[1] The fatty acids are abbreviated as number of carbon atoms: number of double bonds.



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TABLE 2. Comparison of fatty acid incorporation into endothelial cell lipids[a]

| [1-$^{14}$C]Fatty Acid | Incorporation | |
|---|---|---|
| | Triglycerides | Phospholipids |
| | *nmol/mg protein* | |
| Arachidonic (20:4 n-6) | 78 ± 1 | 102 ± 2 |
| Palmitic (16:0) | 39 ± 3[b] | 274 ± 21[b] |
| Linoleic (18:2 n-6) | 59 ± 1[b] | 107 ± 2 |
| Eicosatrienoic (20:3 n-6) | 127 ± 4[b] | 77 ± 2[b] |
| Eicosapentaenoic (20:5 n-3) | 24 ± 1[b] | 95 ± 3 |
| Docosahexaenoic (22:6 n-3) | 53 ± 2[b] | 55 ± 3[b] |

[a] The time of incubation was 16 hr. Each of the media contained 10% fetal bovine serum supplemented with 50 μM of the fatty acid, labeled with $^{14}$C. The values are the mean ± SE of three separate cultures.

[b] $P < 0.01$ as compared with the results obtained for the cultures incubated with [1-$^{14}$C]arachidonic acid.

6% was recovered in a fraction that contained 20:1, 18:3, and 20:3.

The incorporation of a number of other radioactive fatty acids into endothelial cell lipids was compared with that of arachidonic acid, and the data are shown in **Table 2.** Except for eicosatrienoic acid, the immediate precursor of arachidonic acid, all of the other radioactive fatty acids were incorporated into triglycerides to a lesser extent than arachidonic acid. With regard to phospholipids, however, three of the other fatty acids were incorporated to either about the same or a greater extent than arachidonic acid.

### Triglyceride turnover

To assess the turnover of arachidonic acid incorporated into cellular triglycerides, cultures were labeled with [1-$^{14}$C]arachidonic acid for 16 hr under conditions where about 60% of the radioactivity taken up was present in triglycerides. After washing with solutions containing albumin to remove any adherent free fatty acid radioactivity, the cultures were incubated in a maintenance medium containing 10% fetal bovine serum without any supplemental fatty acid. As seen in **Fig. 1** (left side), there was a rapid decline in triglyceride radioactivity, a 25% decrease occurring in 1 hr, and a 55% decrease in 6 hr. By contrast, there was no significant change in the amount of radioactivity contained in the cell phospholipids. A similar experiment with [1-$^{14}$C]oleic acid is shown on the right side of Fig. 1. As opposed to the results with arachidonic acid, no significant change in triglyceride radioactivity occurred during the first hour following transfer to the maintenance medium. Triglyceride radioactivity decreased subsequently and after 6 h, the percentage reduction was about as large as had occurred with [1-$^{14}$C]arachidonic acid. A small but continuous reduction in the amount of [1-$^{14}$C]oleic acid radioactivity in phospholipids was observed after transfer to the maintenance medium, the decrease being 27% after 6 hr.

In an additional experiment, the maintenance medium was collected for assay of radioactivity released from the cells. More than 80% of the released radioactivity was found to be present as free fatty acid.

Turnover experiments also were done to assess the effect of extracellular fatty acid availability on the loss of radioactive arachidonic acid from cellular triglycerides. As seen in **Table 3,** 49% of the initial arachidonic acid radioactivity present in triglycerides was depleted during a subsequent 4-hr incubation in the absence of any supplemental fatty acid. Considerably less arachidonate radioactivity was depleted when either oleic or arachidonic acid was added to the maintenance medium. In similar experiments with radioactive oleic acid, only 24% of the initial triglyceride radioactivity was depleted when the maintenance medium contained no supplemental fatty acid. The addition of oleic acid to the medium did not significantly change the loss of triglyceride radioactivity. When arachidonic acid was added, however, the amount of oleic acid radioactivity that was lost from the cellular triglycerides more than doubled.

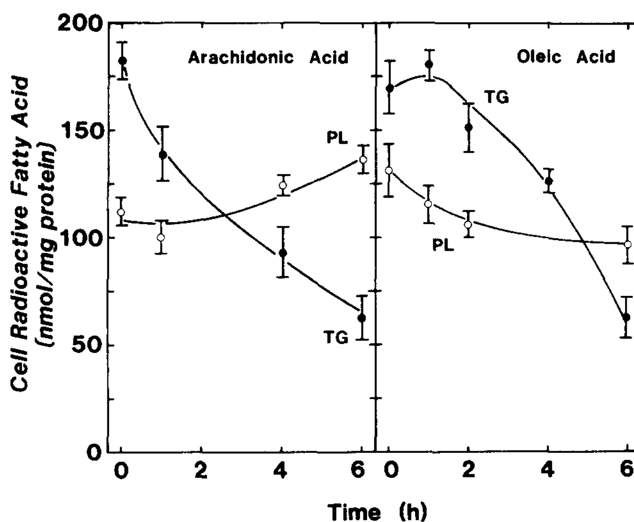


**Fig. 1.** Turnover of radioactive triglyceride fatty acids in endothelial cells. Confluent monolayers of endothelial cells were incubated for 24 hr with 150 μM of either [1-$^{14}$C]arachidonic or [1-$^{14}$C]oleic acid. After the cultures were washed, the radioactivity contained in the cellular triglycerides and phospholipids was determined. These values are plotted at 0-time. The remaining cultures were then incubated with a maintenance medium containing 10% fetal bovine serum but no supplemental fatty acid for up to 6 hr, and the radioactivity remaining in triglycerides and phospholipids after 2, 4, and 6 hr of incubation was determined. Results for cells labeled with arachidonic acid are plotted on the left side; those for cells labeled with oleic acid are plotted on the right side. Each point is the mean ± SE of results obtained from three separate cultures.



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TABLE 3. Effect of fatty acid availability on the utilization of cellular triglyceride radioactivity[a]

| Medium Fatty Acid Supplement[b] | Decrease in Cell Triglyceride Radioactivity | |
|---|---|---|
| | [1-$^{14}$C]Arachidonic Acid[c] | [1-$^{14}$C]Oleic Acid[c] |
| | % | |
| None | 49 ± 7 | 24 ± 1 |
| Oleic | 16 ± 3 | 27 ± 1 |
| Arachidonic | 26 ± 4 | 51 ± 4 |

[a] Cells were labeled for 16 hr with 100 μM radioactive fatty acid complexed with 10% fetal bovine serum. After washing with solutions containing albumin, the cultures were incubated for 4 hr in a maintenance medium containing 10% fetal bovine serum with or without 150 μM supplemental fatty acid. The radioactivity contained in the cellular triglycerides following this incubation was measured and compared to the radioactivity contained in the triglycerides of corresponding cultures before the start of the 4-hr incubation. Each value is the mean ± SE of results obtained from three separate sets of cultures.

[b] Fatty acid added to maintenance medium.

[c] Radioactive fatty acid with which the cultures were labeled. The cultures incubated with [1-$^{14}$C]arachidonic acid contained 148 ± 9.5 nmol of this fatty acid in triglycerides before the start of the maintenance period; those incubated with oleic acid contained 132 ± 13 nmol.

TABLE 4. Incorporation of fatty acid into triglycerides[a]

| Cultured Cells | Incorporation | |
|---|---|---|
| | [1-$^{14}$C]Arachidonic Acid | [1-$^{14}$C]Oleic Acid |
| | *nmol/mg protein* | |
| Bovine aortic endothelium | 124 ± 6 | 56 ± 4[b] |
| Human skin fibroblasts | 306 ± 11 | 204 ± 12[b] |
| Mouse 3T3-L1 preadipocytes | 144 ± 5 | 140 ± 10 |
| Canine MDCK kidney epithelium | 62 ± 3 | 86 ± 6[c] |

[a] Cells were incubated for 16 hr with 50 μM of radioactive fatty acid in a medium containing 10% fetal bovine serum. Each value is the mean ± SE of results from three separate cultures.

[b] $P < 0.01$ as compared with the results obtained with [1-$^{14}$C]arachidonic acid.

[c] $P < 0.05$ as compared with the results obtained with [1-$^{14}$C]arachidonic acid.

## Comparison with other cultured cells

The ability of human skin fibroblasts, mouse 3T3-L1 preadipocytes, and canine MDCK kidney epithelial cells to incorporate arachidonic and oleic acids into triglycerides also was examined. As shown in **Table 4,** the fibroblasts exhibited the highest rates of incorporation and like the endothelial cells, they incorporated more arachidonic than oleic acid into triglycerides. By contrast, the 3T3-L1 cells incorporated equal amounts of both fatty acids into triglycerides, and the MDCK cells incorporated less arachidonic than oleic acid.

The depletion of triglyceride radioactivity was compared when the cultures labeled with [1-$^{14}$C]arachidonic acid were maintained subsequently for 6 hr in a medium containing no supplemental fatty acids. **Fig. 2** illustrates the results. In this experiment, 64% of the initial triglyceride radioactivity contained in the endothelial cells was lost within 2 hr, and 85% after 6 hr. By contrast, the triglyceride radioactivity contained in the fibroblasts was not reduced after 2 hr, and it decreased only 48% after 6 hr. Reductions in triglyceride radioactivity were noted after both 2 and 6 hr with the 3T3-L1 and MDCK cells, but the percentage decreases in both cases were considerably smaller than those observed with the endothelial cultures.

## Triglyceride content and fatty acid composition

The triglyceride content of the endothelial cells was measured spectrophotofluorometrically, and the results are shown in **Table 5.** After incubation for 16 hr with 150 μM arachidonic or oleic acid, the cell triglyceride content increased by 540 and 340%, respectively. The cultures then were transferred to a maintenance medium containing 10% fetal bovine serum without supplemental fatty acid. In both cases, there was a 40% decrease in the triglyceride content of the cells after 6 hr. Some additional decrease occurred after 24 hr in the cells enriched with arachidonic acid, but not in the cells enriched with oleic acid.

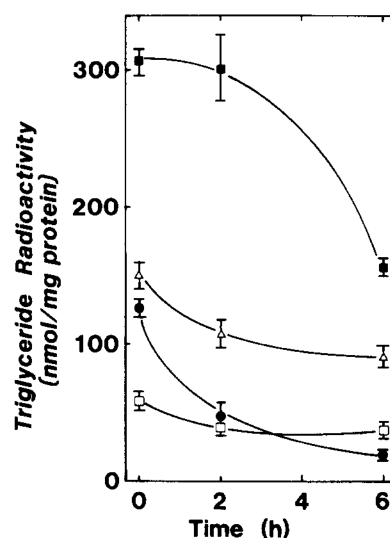


**Fig. 2.** Comparison of the turnover of radioactive triglycerides in different types of cultured cells. Each set of cultures was labeled with 50 μM of [1-$^{14}$C]arachidonic acid for 16 hr. The cells tested were bovine aortic endothelial cells (●), human foreskin fibroblasts (■), mouse 3T3-L1 preadipocytes (△), and canine MDCK kidney epithelial cells (□). Radioactivity contained in the cellular triglycerides at the end of the labeling period is plotted as the 0-time value. The cultures subsequently were incubated in a maintenance medium containing 10% fetal bovine serum but no supplemental fatty acid, and the radioactivity remaining in cellular triglycerides after 2 and 6 hr was measured. Each value is the mean ± SE of values obtained from three separate cultures.



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TABLE 5. Changes in the triglyceride content of the endothelial cells[a]

| Fatty Acid | Triglyceride Content | | |
|---|---|---|---|
| | After Supplementation | After Transfer to Maintenance Medium | |
| | | 6 hr | 24 hr |
| | *μg/mg protein* | | |
| Arachidonic acid | 125 ± 6 | 75 ± 6 | 62 ± 5 |
| Oleic acid | 78 ± 4 | 46 ± 4 | 45 ± 3 |

[a] Before supplementation, the triglyceride content of representative cultures was 23 ± 2 μg/mg cell protein. Cultures were supplemented with media containing 150 μM of either arachidonic or oleic acid for 16 hr. Each value is the mean ± SE of three determinations.

**Table 6** shows the fatty acid composition of the triglyceride fraction during this experiment. Prior to exposure to the fatty acid-supplemented media, the endothelial cell triglycerides contained <1% 20:4 and 10% 18:1. After incubation with supplemental arachidonic acid, 20:4 accounted for 22% of the triglyceride fatty acids, and 22:4 accounted for 6%. The percentage of 20:4 in triglycerides decreased to 5.4% 24 hr after transfer to a maintenance medium, but no other triglyceride fatty acid exhibited an appreciable percentage decrease. In the cultures supplemented with oleic acid, 18:1 accounted for 31% of the triglyceride fatty acids. There was a 14% reduction in the 18:1 content of the triglycerides when these cultures were incubated in maintenance medium for 24 hr, a value much smaller than the 75% reduction noted for 20:4 in the cultures initially supplemented with arachidonic acid.

## Morphologic observations

Few cytoplasmic inclusions are observed when monolayers of cultured bovine aortic endothelial cells are maintained in a medium containing 10% fetal bovine serum. Cytoplasmic inclusions become apparent, however, when the medium is supplemented with fatty acid. **Fig. 3A** illustrates the presence of many cytoplasmic inclusions, which appear as dark granules, in a culture incubated for 16 hr with a medium containing 10% fetal bovine serum supplemented with 150 μM arachidonic acid. The number and distribution of these inclusions are similar to what has been observed when mouse L fibroblasts, human skin fibroblasts, and murine L1210 leukemic lymphoblasts are exposed to media enriched with fatty acids (19–21). Fig. 3B, which is at lower magnification and shows a wider area of the culture plate, demonstrates that very few of the cytoplasmic inclusions remain after the cultures are transferred to the maintenance medium for 24 hr.

## DISCUSSION

These findings demonstrate that triglyceride formation and turnover can occur in endothelial cells. Accumulation of fatty acids in triglycerides has been observed in many other cultured cells, including mouse strain L fibroblasts (19), human skin fibroblasts (13, 22, 23), guinea pig aortic smooth muscle cells (24), L1210 murine leukemic lymphoblasts (25), rabbit liver cells (26), and mouse Ehrlich ascites cells (27). Exposure to very low density lipoproteins also leads to triglyceride accumulation in human skin fibroblasts (28, 29), rat

TABLE 6. Fatty acid composition of endothelial cell triglycerides[a]

| Fatty Acid | Composition of Triglycerides[b] | | | | |
|---|---|---|---|---|---|
| | Unsupplemented Cultures[c] | Arachidonic Acid[d] | | Oleic Acid[d] | |
| | | After 16 hr in Supplemented Medium | 24 hr after Transfer to Maintenance Medium | After 16 hr in Supplemented Medium | 24 hr after Transfer to Maintenance Medium |
| | % | | | | |
| 16:0 | 26.6 | 16.1 ± 1.6 | 20.3 ± 2.7 | 19.1 ± 2.3 | 22.2 ± 0.4 |
| 16:1 | 4.6 | 2.7 ± 1.1 | 6.6 ± 1.0 | 5.5 ± 1.7 | 4.7 ± 1.3 |
| 18:0 | 20.8 | 14.6 ± 1.0 | 18.9 ± 2.8 | 18.9 ± 3.3 | 26.0 ± 2.7 |
| 18:1 | 10.3 | 11.8 ± 0.4 | 14.3 ± 0.8 | 31.0 ± 4.8 | 26.6 ± 2.6 |
| 18:2 | 1.4 | 3.2 ± 0.8 | 2.0 ± 0.8 | 3.6 ± 1.0 | 2.2 ± 0.3 |
| 20:4 | 0.2 | 22.4 ± 3.1 | 5.4 ± 0.7 | 2.7 ± 0.8 | 2.1 ± 1.6 |
| 22:4 | 1.5 | 6.0 ± 0.8 | 5.5 ± 0.7 | 1.7 ± 0.6 | 2.1 ± 0.8 |

[a] The experimental design was the same as that described in Table 5.
[b] The values do not add up to 100% because fatty acids present in small amounts are not listed.
[c] Average value of results obtained from two separate cultures.
[d] Mean ± SE of results obtained from three separate cultures.



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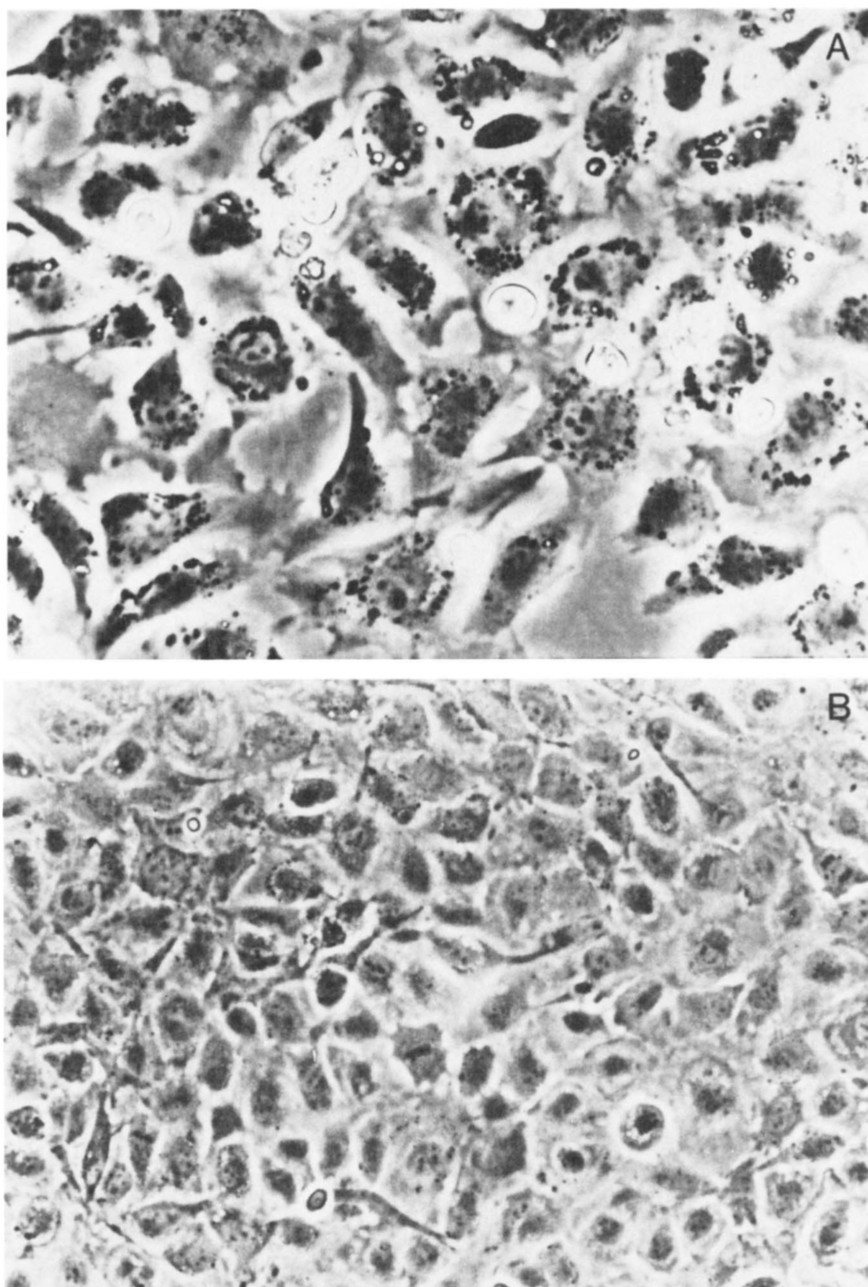


**Fig. 3.** Photomicrographs of endothelial cells at various times following incubation with supplemental arachidonic acid. The cells were incubated in a medium containing 10% fetal bovine serum and 150 $\mu$M arachidonic acid for 16 hr. A, magnified 200×, a representative culture at the end of this incubation period after the cell monolayer was washed with a buffer solution containing albumin. The cultures then were continued in a medium containing 10% fetal bovine serum but no supplemental fatty acid. B, a representative culture after 24 hr of maintenance, magnified 100×.

preadipocytes (30) and Ehrlich ascites cells (31). Several lines of evidence indicate that the fatty acid stored as triglyceride in cultured cells can be subsequently utilized. First, the triglyceride droplets that accumulate in the cytoplasm disappear rapidly when the lipid-rich medium is removed (26), and the chemically measured cellular triglyceride content also rapidly declines (32). In addition, fatty acids contained in triglycerides are transferred to phospholipids in L fibroblasts when the supply of extracellular fatty acid is removed (33). The present studies indicate that all of these processes occur in cultured bovine aortic endothelial cells. To our



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knowledge, this is the first demonstration that triglycerides may play a role in the metabolism of fatty acids in the endothelium, and there is no information regarding the pathway for triglyceride synthesis in endothelial cells.

The majority of the work dealing with cellular triglyceride formation and turnover has been done with palmitate, oleate, or linoleate, the most abundant long-chain fatty acids in biological systems. However, fibroblasts have been previously reported to incorporate unsaturated fatty acids more readily than palmitate into triglycerides (22). Moreover, neuroblastoma and glioma cells readily incorporate arachidonic acid, as well as other polyunsaturates including eicosatrienoic (n-6) acid, into triglycerides (34). Of the seven fatty acids tested, only eicosatrienoic (n-6) acid accumulated in triglycerides to a greater extent than arachidonic acid. In this regard, endothelial cells are able to convert to eicosatrienoic (n-6) acid to arachidonic acid (6, 7). Guinea pig aortic smooth muscle cells, which like endothelial cells release prostaglandins, also can incorporate a considerable amount of arachidonic acid into triglycerides (24). Likewise, we found that two other cell lines that produce prostaglandins, MDCK (35) and 3T3-L1 cells (36), incorporate an appreciable amount of arachidonic acid into triglycerides. The relationship of this pathway to prostaglandin production is questionable, however, because human skin fibroblasts exhibited the largest capacity to incorporate arachidonic acid into triglycerides. A more likely explanation, which has been suggested for neuroblastoma and glioma cells, is that triglycerides serve as a temporary storage site when the influx of arachidonic acid is large, and they subsequently play a role in the synthesis and turnover of membrane phospholipid acyl groups (34).

Several findings suggest indirectly that triglycerides could play a role in endothelial cell arachidonic acid metabolism under physiologic conditions. One is that more arachidonic than oleic acid is incorporated into triglycerides when the fatty acid concentration is low. Ordinarily, only relatively small amounts of arachidonic acid are contained in the plasma, especially as free fatty acid (37). Therefore, if the process were to have any physiologic relevance, the cells probably would have to be able to incorporate arachidonic acid more effectively at low concentrations than an abundant fatty acid such as oleic acid. This was observed (Table 1). Second, there was some preferential turnover of the arachidonic acid stored in triglycerides (Table 6). By contrast, preferential turnover of oleic acid did not occur when the triglycerides were enriched with this fatty acid. In addition, when the extracellular fluid contains an abundance of fatty acid, the cells are able to conserve the arachidonic acid present in triglycerides more readily than oleic acid (Table 3). Furthermore, they release more oleic acid from triglycerides when extracellular arachidonic acid is available for storage. It seems unlikely that the endothelial cells would exhibit such selective conservation or turnover of triglyceride fatty acids, even under the artificial conditions of culture, unless this process had some biologic relevance.

While the ability to incorporate arachidonic acid into triglycerides is not unique to endothelial cells, it may have an especially important function in this tissue. Endothelial cells cannot desaturate linoleic acid, and they require a preformed supply of arachidonic acid to replenish their phospholipid substrate pools (6, 7). Only extracellular free fatty acid has been shown conclusively to provide arachidonic acid to endothelial cells (6, 7). Although plasma high density lipoproteins also may serve as a source, the evidence for this is as yet indirect (8). If plasma free fatty acid actually is the main source, availability could be limited under certain conditions because arachidonic acid ordinarily comprises only about 4% of this fraction (37). The ability to store some arachidonic acid in triglycerides even when it is available at relatively low concentrations may enable the endothelial cell to resupply its phospholipid pools, if necessary, from an intracellular source.

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