Linoleic Acid Metabolism and Prostaglandin Production by Cultured Bovine Pulmonary Artery Endothelial Cells

Terry L. Kaduce, Arthur A. Spector, and Robert S. Bar

When bovine pulmonary artery endothelial cells are cultured in a medium supplemented with linoleic acid, their capacity to produce prostacyclin (PGI₂) is reduced by about 60%. This reduction occurs when PGI₂ formation is stimulated by the addition of either the calcium ionophore A23187 or arachidonic acid. In addition, supplementation with linoleic acid reduced the production of prostaglandin E₂ and F₂, from 1-14C-arachidonic acid by more than 50%. The capacity of cultured bovine pulmonary vein and aortic endothelial cells to convert extracellular arachidonic acid into PGI₂ also was reduced by about 50% when the growth medium was supplemented with linoleic acid. Although bovine pulmonary artery endothelial cells incorporated large amounts of 1-14C-linoleic acid into cellular phospholipids and triglycerides, a maximum of only 2.3% of the radioactivity was converted to arachidonic acid in 24 hours. The most prevalent radioactive metabolite was elcosadlenoic acid, the elongation product of linoleic acid. As compared with linoleic acid, the bovine endothelial cells incorporated 30% more 1-14C-arachidonic acid into phospholipids and triglycerides. When the growth medium was supplemented with linoleic acid, the percentage of this fatty acid in cellular lipids increased 3- to 4.5-fold and elcosadlenoic acid accumulated, accounting for up to 9% of the cellular fatty acids. This increase was accompanied by a 30% to 45% reduction in arachidonic acid. These findings, together with our previous results with human umbilical vein endothelium, suggest that an inability to convert large amounts of linoleic to arachidonic acid and a suppressive effect of linoleic acid enrichment on prostaglandin production may be general properties of endothelial cells. (Arteriosclerosis 2:380-389, September/October, 1982)

Endothelial cells release prostacyclin (PGI₂), a prostaglandin that is a potent vasodilator and inhibitor of platelet aggregation.1,2 Prostacyclin is synthesized from arachidonic acid,3 a member of the n-6 class* of polyunsaturated fatty acids that is derived from dietary linoleic acid. Studies with cultured human umbilical vein endothelial cells revealed several unexpected results regarding linoleic acid metabolism and its effect on PGI₂ production. These cultures were found to convert only very small amounts of linoleic acid to arachidonic acid.4 Furthermore, supplementation with linoleic acid produced a reduction in the arachidonic acid content of the cellular phospholipids and a decreased capacity of the cells to release PGI₂.5,6 Similar effects of linoleic acid supplementation on prostaglandin production have not been observed in Madin-Darby canine kidney (MDCK) or 3T3 cells, both of which release predominantly prostaglandin E₂ (PGE₂). These cells accumulate increased amounts of arachidonic acid in phospholipids when they are enriched with linoleic acid, and PGE₂ production is enhanced.5,6 MDCK cells are derived from dog kidney tubular epithelium, and 3T3 cells are from a mouse embryo. Although these cells probably have different properties than cells of vascular origin, the fact that the findings were so different made it of interest to determine whether our previous findings regarding linoleic acid metabolism

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*The notation n-6 refers to the fact that the first double bond is 6 carbon atoms removed from the methyl terminus in this class of polyunsaturated fatty acids. Linoleic and arachidonic acids are members of the n-6 class.
and PGI₂ production might represent a peculiarity of the umbilical vein endothelial cells that were tested. Metabolic differences, including differences in prostaglandin production, between venous and arterial endothelium have been demonstrated. To further explore the question of whether our previous findings concerning polyunsaturated fatty acids and PGI₂ release are representative, we have extended the studies of linoleic acid metabolism and prostaglandin production to cultured bovine endothelial cells.

**Methods**

**Cell Culture**

Bovine pulmonary artery, pulmonary vein, and aortic endothelial cell cultures were initiated from vessels obtained from freshly slaughtered steers and heifers. The vessels were clamped and excised, then rinsed with 100 mM phosphate buffered saline (PBS) and filled with 0.1% crude collagenase (Worthington Biochemical Company, Freehold, New Jersey). After 20 minutes, the cell suspension was collected and pooled, with cells being obtained by a subsequent PBS rinse of the vessels. The cell suspensions were centrifuged at 250 x g for 10 minutes. Cell pellets were then resuspended in M-199 with 17% fetal bovine serum at a density of about 25,000 cells/cm² of dish surface. Cells from bovine pulmonary veins were obtained by scraping vascular walls with a sterile cotton-tipped applicator. The endothelial cells were then handled as previously described for the bovine arterial endothelium. To subculture cells, the medium was removed and replaced with PBS containing 0.1% trypsin and 0.05% EDTA (Earle's salts and 17.5% heat-inactivated fetal bovine serum). The complete medium then was adjusted to pH 7.4 at 37°C and sterilized by filtration through a 0.22 micron filter.

**Incubations**

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, 3 mM KCl, 1 mM CaCl₂, 0.5 mM MgCl₂, 8 mM Na₂HPO₄, and 2 mM KH₂PO₄, 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% CO₂ atmosphere. The incubations were terminated by aspirating the medium, followed by washing the monolayer once with ice-cold buffer containing 50 μM fatty acid-free bovine serum albumin, and then 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 minutes 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. The remainder of the suspension was extracted with 20 volumes of a mixture of chloroform and methanol (2:1, vol/vol) in order to extract the cell lipids. Following separation and isolation of the chloroform phase, the solvent was evaporated under N₂ and the lipid residue was dissolved in a measured volume of fresh chloroform.

**Lipid Analyses**

The separation of lipid classes was obtained by thin-layer chromatography on silica gel G with a solvent system containing hexane-diethyl ether-acetic acid-methanol (170:40:6:4). Standards obtained from NuChek Prep were added to each chromatogram, and the lipids were visualized by exposure to I₂ vapor. After sublimation, the outlined segments of
silica gel were scraped into liquid scintillation counting vials.

Fatty acids were isolated from the cell lipids by saponification, acidification, and extraction into hexane. After methylation with 14% BF3 in methanol, the fatty acid methyl esters were extracted into hexane and separated by gas-liquid chromatography. Separation was achieved using a 2 mm × 1.9 m glass column packed with 10% SP2330 on 100/120 mesh Chromosorb W-AW (Supelco, Incorporated, Bellefonte, Pennsylvania). The Hewlett-Packard model 5700 gas chromatograph (Hewlett-Packard Company, Palo Alto, California) 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. The carrier gas was N2 at a flow rate of 25 ml/min, and the oven temperature was programmed from 176°C 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. Preliminary studies with this collection system revealed that more than 96% of previously purified 1-14C-linoleic acid was recovered in the linoleic acid peak even when this acid comprised less than 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, Illinois). Quenching was monitored with a 226Ra external standard.

Radioimmunoassay for PGI2

PGI2 release was measured in washed monolayers of confluent endothelial cells containing 140 ± 9 µg of cellular protein. The cultures, present in 35 × 10 mm plastic dishes, were incubated for 5 minutes at 37°C in 1 ml of the buffered medium containing bovine serum albumin, thrombin, bradykinin, the calcium ionophore A23187, or arachidonic acid were added to the medium. After incubation, the medium was assayed for the stable catabolic product of PGI2, 6-keto-prostaglandin F1α (6-keto-PGF1α). The radioimmunoassay was performed using an antiserum against 6-keto-PGF1α. A measured amount (100 µl) of either standard or samples was mixed with 100 µl of 3H-6 keto-PGF1α (New England Nuclear, Boston, Massachusetts) and 10 µl of antiserum at a dilution that bound 50% of the radioactivity in the absence of standard. After a 1-hour incubation at 37°C, 50 µl IgG sorb (The Enzyme Center Incorporated, Tufts University School of Medicine, Boston, Massachusetts) was added, and the radioactivity contained in a 100 µl sample of the supernatant solution was measured in a liquid scintillation spectrometer. Assay detection limits were 0.3 pmol of 6-keto-PGF1α and 50% inhibition was obtained with 3.3 pmol of 6-keto-PGF1α. This assay had negligibly small amounts of cross-reactivity with prostaglandins other than 6-keto-PGF1α.

Formation of Radioactive Prostaglandins

After washing, the endothelial cell monolayers were incubated for 30 minutes at 37°C with either the buffered salt solution or this solution containing 2 mM acetylsalicylic acid. These media were removed, and the endothelial cell monolayers were incubated for 5 minutes at 37°C with 2.5 ml of medium containing 8.75 nmol of 1-14C-arachidonic acid 55 Ci/mmol, 140 mM NaCl, 5.8 mM KCl, 2.7 mM CaCl2, 1.1 mM Na2CO3, and 16.3 mM Tris HCl adjusted to pH 7.4. Corresponding control incubations were done in empty dishes. After incubation, the medium was removed quantitatively, acidified to pH 3.5 with 1 M citric acid, and extracted twice with 2 volumes of ethyl acetate after addition of NaCl. Authentic prostaglandin standards were added, and aliquots of the ethyl acetate extract then were chromatographed on silica gel thin layer plates with the organic phase of a mixture containing ethyl acetate-acetic acid-2,2,4-trimethyl pentane-H2O (110:20:50:100). After staining with I2 vapor and sublimination, the radioactivity contained in the outlined segments of silica gel corresponding to the separated prostaglandin standards was measured in a liquid scintillation spectrometer. The small amount of background radioactivity recovered from the empty dish controls was subtracted from the quantity recovered in the prostaglandin region of the chromatograms. An initial experiment indicated that the pulmonary artery endothelial cultures produced very little radioactive lipooxygenase products from 1-14C-arachidonic acid in 5 minutes, and this aspect was not pursued.

Results

Prostaglandin Formation

To determine the distribution of prostaglandins produced by the bovine pulmonary artery cells, cultures were incubated for 5 minutes with 1-14C-arachidonic acid, and the radioactive prostaglandin products were separated by thin-layer chromatography. PGI2 accounted for 60% ± 5% (mean ± sem, n = 3) of the radioactive products formed. (PGI2 was measured as 6-keto-PGF1α.) The other most abundant products were PGE2, 12% ± 1%, and prostaglandin F2α (PGF2α), 11% ± 1%. Because PGI2 was the major product, most of our subsequent studies involved quantitative measurements of only this prostaglandin, using a radioimmunoassay.

The amount of PGI2 released by the bovine pulmonary artery endothelial cell cultures was found to be roughly linear relative to the extracellular arachidonic acid concentration. In these experiments, the medium contained 50 µM bovine albumin and the amount
of arachidonic acid added was varied from 50 to 200 
μM. PGI₂ production during a 5 minute incubation, as 
measured by radioimmunoassay, increased from 7 ± 1 pmol/ml when 50 μM arachidonic acid was add-
ted to 200 ± 25 pmol/ml in the presence of 200 μM arachidonic acid (mean ± SEM, n = 3).

Additional studies were done to determine wheth-
er PGI₂ production by the bovine pulmonary artery 
endothelial cultures could be stimulated in the ab-
ence of any extracellular arachidonic acid. In every 
case, cells incubated in buffer solution without any 
stimulating agent were included as controls. These 
cultures released only 5 pmol/ml of PGI₂ or less. This 
small release was subtracted from the values ob-
tained with the corresponding stimulated cultures. 
Bovine thrombin (1 U/ml) did not stimulate PGI₂ re-
lease. Bradykinin (10 μg/ml) caused the release of 5 ± 0 pmol/ml of PGI₂ during a 5 minute incubation 
(mean ± SEM n = 3). Greater release was observed 
with calcium ionophore A23187 (10 μM), the values 
ranging from 20 ± 2 to 70 ± 7 pmol/ml in 5 minutes. 
The latter values are considerably less than those 
found under similar conditions when primary cultures 
of human umbilical vein endothelium were exposed 
for 5 minutes to the calcium ionophore, the release 
being 164 to 246 pmol PGI₂/ml in that system.5

To assess the effect of enrichment with n-6 poly-
unsaturated fatty acids, the bovine pulmonary artery 
endothelial cultures were supplemented for 24 hours 
with 100 μM linoleic acid in a medium containing 
17.5% fetal bovine serum. These media contained 
80 μM bovine plasma albumin so that the molar ratio 
of supplemental fatty acid to albumin was 1:2. Under 
these conditions, the supplemental fatty acid did not 
influence the rate of cell growth, cell morphology, or 
viability as assessed by dye exclusion. As shown in 
table 1, supplementation of the cells with linoleic acid 
reduced subsequent PGI₂ production in response to 
the calcium ionophore A23187 by about 60%, a re-
duction similar to that produced in human endothelial 
cells.5,6 Supplementation with linoleic acid also re-
duced PGI₂ production from added arachidonic acid 
by 55%, a slightly larger decrease than noted with 
human endothelial cells under similar conditions.5

Previous work indicated that enrichment with lino-
leic acid had the opposite effect on prostaglandin 
production in MDCK and 3T3 cultures. These cells 
release predominantly PGE₂, and supplementation 
with linoleic acid causes a considerable stimulation 
of prostaglandin production.7,8 Therefore, we wished 
to determine whether enrichment of the bovine endo-
thelial cells with linoleic acid might possibly have a 
different effect on the formation of PGE₂ or the other 
prostaglandins that they produce as compared with 
PGI₂. After supplementation for 24 hours with linoleic acid followed by washing, the cultures were incu-
batated for 5 minutes with 1-14C-arachidonic acid. The 
amount of the various prostaglandins produced was 
calculated from the specific radioactivity of the added 
arachidonate. As seen in figure 1, the reduction in 
radioactive PGI₂ production when the cultures were 

![Table 1. Effect of Supplementation with Linoleic Acid on PGI₂ Production by Bovine Pulmonary Artery Endothelial Cells](http://atvb.ahajournals.org/content/full/2/6/383/F1.large.jpg)

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Control</th>
<th>Linoleic acid†</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Calcium ionophore</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A23187‡</td>
<td>69 ± 8</td>
<td>29 ± 6</td>
</tr>
<tr>
<td>Arachidonic acid§</td>
<td>204 ± 26</td>
<td>89 ± 4</td>
</tr>
</tbody>
</table>

*Measured by radioimmunoassay as 6-keto-PGF₁α, mean ± SEM, three separate cultures.
†Cultures supplemented for 24 hours with 100 μM linoleic acid in a medium containing 17.5% fetal bovine serum. The medium of the control cultures contained the serum, but no supplemental fatty acid.
‡10 μM. §200 μM complexed with 50 μM bovine serum albumin.

supplemented with linoleic acid was 55%, a value 
similar to that found using the radioimmunoassay 
table 1. There was also a 60% decrease in PGE₂ 
production and a 65% decrease in PGF₂α production 
when the cultures were enriched with linoleic acid. 
These results indicate that the reduction produced 
by linoleate supplementation of the bovine pulmo-

![Figure 1. Conversion of 1-14C-arachidonic acid to radioactive prostaglandins by cultured bovine endothelial cells.](http://atvb.ahajournals.org/content/full/2/6/383/F2.large.jpg)

**Figure 1.** Conversion of 1-14C-arachidonic acid to radioactive prostaglandins by cultured bovine endothelial cells. The supplemented cells were incubated with 100 μM linoleic acid in 17.5% fetal bovine serum for 24 hours. After removal of the medium and washing, the cultures were incubated with a buffered salt solution containing 3.5 μM 1-14C-arachidonic acid for 5 minutes at 37°C. Each value is the mean ± SEM of three separate cultures.
nary artery endothelial cultures is not confined to PGI₂. The distribution of radioactivity in the cells enriched with linoleic acid was 68% PGI₂, 10% PGE₂, and 7% PGF₂α. These values are only slightly different than those noted for the unsupplemented cells.

Figure 1 also shows the effect of linoleic acid supplementation in cultured bovine aortic and pulmonary vein endothelial cells. PGI₂ is the main prostaglandin released by these cultures, accounting for about 80% of the total radioactive prostaglandins formed by the aortic endothelial cultures and 60% by the pulmonary vein endothelial cultures. In both cases, however, linoleic acid supplementation considerably reduced PGI₂ production by about 40%. Therefore, the inhibitory effect of linoleic acid supplementation on PGI₂ production appears to be similar in endothelium from different anatomic locations, as well as in both the bovine and human species. For each type of endothelium, the unsupplemented and linoleic acid-supplemented cultures were from the same passage. However, the three types of endothelial cells that were tested were from different passage numbers. Since previous studies have shown that passage number can influence the quantity of PGI₂ produced, the differences in PGI₂ formation noted between the aortic, pulmonary artery, and pulmonary vein cells could be due to the difference in passage number rather than to intrinsic differences in these three types of endothelium.

**Incorporation of Radioactive Fatty Acids**

The uptake of linoleic acid by the bovine pulmonary artery endothelial cells was assessed during the course of a 72-hour culture period. In this experiment, 100 μM of 1-14C-linoleic acid was complexed with a medium containing 17.5% fetal bovine serum. About 75% of the total uptake occurred during the first 4 hours. A slower but progressive rate of 1-14C-linoleic-acid accumulation in the cells occurred between 4 and 48 hours, but no net accumulation of radioactivity took place between 48 and 72 hours. The effect of linoleic acid concentration on uptake was then assessed in a 24-hour incubation as shown in figure 2. These media also contained 17.5% fetal bovine serum and 100 μM 1-14C-linoleic acid. The differences in PGI₂ formation noted between the aortic, pulmonary artery, and pulmonary vein cells could be due to the difference in passage number rather than to intrinsic differences in these three types of endothelium.

**Elongation and Desaturation of Fatty Acids**

Table 2 shows the conversion of 1-14C-linoleic acid into elongation and desaturation products during the course of a 72-hour culture period. The medium contained 17.5% fetal bovine serum and 100 μM 1-14C-linoleic acid. Even after 72 hours, most of the radioactivity present in the cell lipids remained as linoleic acid (18:2). (The fatty acids are abbreviated as number of carbon atoms:number of double bonds.) Most of the conversion was into 20:2, the direct elongation product of linoleic acid, and a maximum of only about 2% of the radioactivity was recovered as 20:4. The effect of linoleic acid concentration on uptake was then assessed in a 24-hour incubation as shown in figure 2. These media also contained 17.5% fetal bovine serum and 100 μM 1-14C-linoleic acid. The differences in PGI₂ formation noted between the aortic, pulmonary artery, and pulmonary vein cells could be due to the difference in passage number rather than to intrinsic differences in these three types of endothelium.

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Figure 3. Comparison of the incorporation of several fatty acids by bovine pulmonary artery endothelial cells. In each case, 100 μM of radioactive fatty acid was complexed with a medium containing 17.5% fetal bovine serum. The values are the mean ± SEM of three separate cultures.

Then incubated in the usual growth medium for the ensuing 24 hours. At the end of the 24-hour chase period, 85% ± 2% (mean ± SEM, n = 4) of the radioactivity present in the cells remained as 18:2, and only 0.5% ± 0.6% of the radioactivity was converted to 20:4.

To determine whether the low rates of desaturation and elongation were specific for linoleic acid, other long-chain fatty acids were tested. A 24-hour incubation was carried out in a medium containing 100 μM of the radioactive fatty acid and 17.5% fetal bovine serum. Table 3 shows that, except in the case of arachidonic acid, most of the radioactivity taken up by the bovine pulmonary artery endothelial cells remained in the form of the fatty acid that was presented, indicating that little elongation or desaturation occurred. Almost 20% of the arachidonic acid uptake, however, was converted to 22:4, its direct elongation product. Furthermore, the conversion of 20:3 to 20:4, although still small, was considerably greater than the conversion of 18:2 to 20:4.

Cellular Fatty Acid Composition

The effect of supplementation with linoleic acid on the fatty acid composition of the bovine pulmonary artery endothelial cells was also assessed. Although values for all the fatty acids were obtained, for clarity only those that changed appreciably are presented in figure 4. As seen in the left half of figure 4, there was a three-fold increase in the percentage of 18:2 contained in cellular lipids when the medium was supplemented with 100 μM linoleic acid. Most of the increase was manifest within the first 24 hours. The 20:2 content also increased consistent with the isotopic results, indicating that 18:2 was converted primarily to 20:2. These increases were associated with appreciable reductions in the percentages of 18:1 and 20:4 in the cellular lipids. In particular, the percentage of 20:4 was reduced by 50% after 72 hours of exposure to supplemental linoleic acid. As shown in the right half of figure 4, these changes in cellular fatty acid composition were dependent on the concentration of linoleic acid added to the culture medium when this varied between 15 and 150 μM.

Table 2. Elongation and Desaturation of 1-14C-Linoleic Acid Taken Up by Bovine Pulmonary Artery Endothelial Cells

<table>
<thead>
<tr>
<th>Time of incubation</th>
<th>Total incorporation* (nmol/mg cell protein)</th>
<th>Distribution of radioactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18:2†</td>
<td>18:3</td>
</tr>
<tr>
<td>4</td>
<td>140 ± 0.8</td>
<td>97.1 ± 0.6</td>
</tr>
<tr>
<td>12</td>
<td>154 ± 0.5</td>
<td>93.3 ± 0.3</td>
</tr>
<tr>
<td>24</td>
<td>171 ± 4.1</td>
<td>90.0 ± 2.4</td>
</tr>
<tr>
<td>48</td>
<td>191 ± 7.0</td>
<td>83.6 ± 3.7</td>
</tr>
<tr>
<td>72</td>
<td>187 ± 3.7</td>
<td>84.3 ± 2.0</td>
</tr>
</tbody>
</table>

*The cultures were incubated with 100 μM 1-14C-linoleic acid complexed with a medium containing 17.5% fetal bovine serum. Each value is the mean ± SEM from three separate cultures.
†Fatty acid fraction eluted from the gas chromatogram. No radioactivity was detected in 20:3.
ND = not detected.
Table 3. Comparison of the Elongation and Desaturation of Fatty Acids by Bovine Pulmonary Artery Endothelial Cells

<table>
<thead>
<tr>
<th>Fatty acid*</th>
<th>Total incorporation† (nmol/mg cell protein)</th>
<th>Distribution of radioactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18:0</td>
<td>18:1</td>
</tr>
<tr>
<td>1-14C-stearic</td>
<td>155±11.4</td>
<td>95.8±0.8</td>
</tr>
<tr>
<td>1-14C-eicosatrienoic (n-6)</td>
<td>309±4.0</td>
<td>—</td>
</tr>
<tr>
<td>1-14C-arachidonic</td>
<td>407±19.1</td>
<td>—</td>
</tr>
<tr>
<td>1-14C-linoleic</td>
<td>268±2.0</td>
<td>—</td>
</tr>
</tbody>
</table>

*100 μM of the radioactive fatty acid was complexed with a culture medium containing 17.5% fetal bovine serum. The incubations were for 24 hours.
†Values are means ± SEM for three separate cultures.

In an attempt to further explore the reduction in 20:4 noted when the cells were exposed to linoleic acid, experiments were done using media supplemented with 100 μM of either linoleic acid, eicosatrienoic acid (n-6), or arachidonic acid. A comparison of the cellular fatty acid compositions following 24 hours of culture is presented in table 4. All cultures utilized in this set of experiments were of the same passage number. Although supplementation with eicosatrienoic acid produced a very large increase in the percentage of 20:3 in cellular lipids, there was no change in the percentage of 20:4. By contrast, a 40%
Table 4. Fatty Acid Compositions of Bovine Pulmonary Artery Endothelial Cells Supplemented with Polyunsaturated Fatty Acids

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control*</th>
<th>Linoleic</th>
<th>Eicosatrienoic (n-6)</th>
<th>Arachidonic</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>18.0 ± 2.1</td>
<td>20.6 ± 0.8</td>
<td>13.7 ± 0.6</td>
<td>16.3 ± 0.1</td>
</tr>
<tr>
<td>16:1</td>
<td>4.0 ± 0.1</td>
<td>3.6 ± 0.2</td>
<td>1.7 ± 0.4</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>18:0</td>
<td>18.5 ± 0.4</td>
<td>10.9 ± 1.5</td>
<td>16.3 ± 1.8</td>
<td>15.5 ± 0.3</td>
</tr>
<tr>
<td>18:1</td>
<td>24.1 ± 0.6</td>
<td>21.4 ± 2.7</td>
<td>14.9 ± 1.0</td>
<td>14.4 ± 1.2</td>
</tr>
<tr>
<td>18:2</td>
<td>6.6 ± 0.7</td>
<td>19.8 ± 0.4</td>
<td>2.2 ± 0.2</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>20:2</td>
<td>ND</td>
<td>8.6 ± 0.4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>20:3</td>
<td>0.5 ± 0.1</td>
<td>ND</td>
<td>34.5 ± 2.0</td>
<td>ND</td>
</tr>
<tr>
<td>20:4</td>
<td>10.9 ± 1.0</td>
<td>6.9 ± 0.3</td>
<td>11.1 ± 0.5</td>
<td>31.3 ± 1.1</td>
</tr>
<tr>
<td>22:4</td>
<td>1.3 ± 0.3</td>
<td>1.3 ± 0.2</td>
<td>2.3 ± 1.0</td>
<td>8.7 ± 0.4</td>
</tr>
<tr>
<td>22:5</td>
<td>4.5 ± 2.5</td>
<td>1.7 ± 0.7</td>
<td>1.6 ± 0.5</td>
<td>0.4 ± 0.4</td>
</tr>
<tr>
<td>22:6</td>
<td>4.6 ± 0.1</td>
<td>1.1 ± 1.1</td>
<td>0.8 ± 0.5</td>
<td>0.3 ± 0.3</td>
</tr>
</tbody>
</table>

*The culture medium contained 17.5% fetal bovine serum. No supplemental fatty acid was added to the control cultures. In the experimental cultures, 100 μM of the respective fatty acid was added. The incubations were for 24 hours.

Values are means ± SEM of three separate cultures. The values do not add up to 100% because unidentified fatty acids, and those present in only trace amounts were not included. ND = not detected.

Discussion

Like human umbilical vein endothelial cells, cultured bovine pulmonary artery endothelium has only a very limited capacity to convert linoleic acid to arachidonic acid. Less arachidonic acid remains in cellular lipids when the bovine endothelial cultures are supplemented with linoleic acid. Furthermore, bovine pulmonary artery, pulmonary vein, and aortic endothelial cells have a lesser capacity to produce PGI2 when enriched with linoleic acid, a response similar to that obtained with the human umbilical vein endothelial cultures.

Linoleic acid can be converted to arachidonic acid in a number of mammalian tissues. Because of the central role of arachidonic acid in endothelial function, we thought that endothelium would probably contain this metabolic pathway. Our concern regarding the previous findings with the human cultures was heightened by the fact that both MDCK and 3T3 cells were observed to readily convert linoleic acid to arachidonic acid. Furthermore, enrichment with linoleic acid greatly enhanced the capacity of the MDCK and 3T3 cells to produce prostaglandins. Since the human endothelial cells were derived from the maternofetal circulation, we wondered whether the utilization and effects of linoleic acid in these cultures might not be representative of other types of endothelium. These concerns are obviated by the results of this study. It is now evident that a similar inability to convert linoleic acid to arachidonic acid occurs in cultured bovine endothelium. Moreover, endothelium from three different anatomic locations in the adult animal produces less PGI2 when it is enriched with linoleic acid.

This study indicates that the suppressive effect of linoleic acid on prostaglandin production in endothelium is not confined to PGI2, the only product assayed in the previous studies with human cultures. PGE2 and PGF2α, production by bovine pulmonary artery endothelial cells was also reduced when the cultures were enriched with linoleic acid. This is an important finding concerning mechanistic considerations. PGE2 is the main prostaglandin formed by the MDCK and 3T3 cells, systems in which linoleic acid enrichment enhances, rather than inhibits, prostaglandin production. Based on the information available previously, it was possible that the differences in prostaglandin production between the various cells could be explained by the fact that PGI2 was measured in human endothelial cells, whereas PGE2 was measured in MDCK and 3T3 cells. Since the results of this study indicate that PGE2 production is also reduced in bovine endothelial cells supplemented with linoleic acid, an explanation based upon differences in the major type of prostaglandin formed appears to be excluded.

Reductions in prostaglandin production in the bovine endothelial cells enriched with linoleic acid occurred when the cells were either stimulated with the calcium ionophore A23187 or exposed to external
arachidonic acid. The latter finding suggests that the effect occurs at a step after arachidonic acid release and tends to exclude any mechanism based solely on the lesser amount of arachidonic acid in cellular lipids. Although these results do not precisely locate this step, the fact that the percentage decreases in arachidonic acid. The latter finding suggests that the reduction in cyclooxygenase activity in microsomal preparations from rat stomach, sheep vesicular gland, and human skin. Since the percentage of 18:2 and 20:2 are increased considerably when the cultures are supplemented with linoleic acid, such a mechanism is possible in the endothelium.

The underlying difference between the endothelial cells, as compared with the MDCK and 3T3 cells which are not of vascular origin, appears to be their inability to desaturate large amounts of linoleic acid. When either the MDCK or 3T3 cultures are supplemented with linoleic acid, there is only a relatively small increase in 18:2 in cellular lipids, no accumulation of 20:2, and a major increase in 20:4. Since 18:2 and 20:4 are readily converted by the endothelial cells to 20:2 and 22:4, respectively, the deficiency in the endothelium probably is not due to fatty acid chain elongation. High desaturation activities were not observed with any of the fatty acids tested. However, activities for the conversion of 18:0 to 18:1, 20:3 to 20:4, and 20:4 to 22:5 were four to seven times greater than the activity for 18:2 conversion to 20:4. This suggests that the fatty acid desaturase which acts on 18:2, the Δ6-desaturase, is less active in bovine pulmonary artery endothelial cells than those mediating the other fatty acid desaturation steps. A similar finding was made with cultured human umbilical vein endothelial cells. The possibility that the low Δ6-desaturase activity might be a result of the cell culture procedure cannot be excluded. However, the fact that it occurs in cells derived from two different species and from different types of blood vessels indicates that this deficiency may be a fairly widespread property of endothelium.

These findings suggest that endothelial cells probably obtain most of their arachidonic acid from an extracellular source, rather than by synthesis from linoleic acid. Consistent with this is the fact that unesterified arachidonate is readily taken up and incorporated into cellular lipids by the bovine pulmonary artery endothelial cells (figure 3 and table 4). Since arachidonic acid comprises about 5% of the plasma free fatty acids, this fraction could be an important source of arachidone for the endothelium under physiological conditions. About 30% more arachidonate than other fatty acids was recovered in cellular phospholipids, confirming the considerable ability to utilize this fatty acid that was observed in the human cultures. Even more striking is the greater ability of the bovine pulmonary artery cells to incorporate arachidonate as opposed to other fatty acids into triglycerides (figure 3). As compared with arachidonate, 40% less linoleate, 50% less oleate, and 80% less stearate was incorporated into the triglyceride fraction. A similar enhancement in triglyceride incorporation has not been observed in the two other cell types in which arachidonic acid was compared with other fatty acids, Ehrlich ascites, and L1210 lymphocytic leukemia. This difference might be accounted for by the fact that these are transformed murine cell lines. Alternatively, it is possible that the enhanced capacity to incorporate arachidonate into triglycerides may be a special property of endothelium which facilitates storage of any excess arachidonic acid available in the plasma.

Since PGI₂ is a potent vasodilator and inhibitor of platelet aggregation, modifications that decrease its production may reduce the protective effect of the endothelium against ischemia and thrombosis. In this regard, a sustained increase in the circulating linoleic acid concentration might be harmful, especially if associated with a reduction in arachidonic acid concentration. Such changes in the plasma free fatty acid composition have been reported following ingestion of large amounts of safflower oil. When this plant oil, which is high in linoleic acid, is administered to rats, the plasma linoleic acid concentration increases so that it comprises 50% to 70% of the free fatty acids between 4 hours and 16 hours after feeding is begun. Arachidonic acid values are not reported in this study. However, similar work in humans indicates that 8 hours after feeding safflower oil, the unesterified linoleic acid concentration increases from 48 to 330 nEq/ml, whereas the unesterified arachidonic acid concentration decreases from 12 to 8.5 nEq/ml. While the results of this study indicate that PGI₂ production by several types of endothelium might be compromised by such changes, the question of whether the effects produced in these cell cultures actually occur in the intact animal is not dealt with. Studies involving polyunsaturated fatty acid modifications will have to be extended to an appropriate in vivo experimental model in order to determine the physiologic applicability of these findings.

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References


Index Terms: prostaglandins • endothelial cells • prostacyclin • linoleic acid • fatty acids • arachidonic acid
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