Endothelium-Dependent Relaxation in Response to Aggregating Platelets in Porcine Femoral Veins and Its Modulation by Diet

Kimihiro Komori, MD, Hiroaki Shimokawa, MD, and Paul M. Vanhoutte, MD

The present study examined the protective role of the venous endothelium against aggregating platelets and its modulation by diet. Yorkshire pigs were fed a regular chow (control pigs), 2% high-cholesterol diet (for 10 weeks, cholesterol-fed pigs), and regular chow plus cod-liver oil (30 ml/day for 4 weeks, oil-fed pigs). Endothelium-dependent responses were examined in vitro in rings of femoral veins in the presence of the inhibitor of cyclooxgenase indomethacin. In control pigs, aggregating platelets, serotonin, and ADP caused endothelium-dependent relaxations. The platelet-induced relaxations were attenuated by methiothepin (a combined 5-HT₁ and 5-HT₂ serotoninergic blocker) or apyrase (an ADPase and ATPase) and were abolished by the combination of the two agents. In quiescent rings, platelets caused contractions, which were reduced in the presence of endothelium; the contractions were prevented by ketanserin (a 5-HT₂ serotoninergic blocker) or methiothepin but not by R 68 070 (a thromboxane A₂ receptor blocker) or dazoxiben (a thromboxane-synthetase blocker). In cholesterol-fed pigs, the platelet-induced relaxations were not altered, whereas in oil-fed pigs, the endothelium-dependent relaxations to platelets, serotonin, and ADP were augmented. Platelet-induced contractions were significantly reduced in rings with endothelium from oil-fed pigs, whereas the contractions were comparable in rings without endothelium among the three groups. Endothelium-dependent relaxations in response to the calcium ionophore A23187, direct relaxations in response to sodium nitroprusside, and direct contractions in response to potassium chloride were comparable among the three groups. These results indicate that 1) the endothelium exerts inhibitory effects against aggregating platelets in porcine femoral veins, 2) the relaxations are mediated by serotonin and ADP released from the aggregating platelets, 3) platelet-induced contractions are mediated by serotonin with little contribution of thromboxanes, and 4) hypercholesterolemia does not affect, but cod-liver oil facilitates, the endothelium-dependent relaxations to aggregating platelets because of augmented responses to released serotonin and ADP. (Circulation 1989;80:401–409)

Studies in isolated blood vessels have shown the important role of the endothelium in modulating the responsiveness of the underlying vascular smooth muscle. In particular, the endothelium of large arteries exerts a protective action against the constrictor effects of platelet products. Interactions between the platelets and venous wall are particularly important in disorders such as venous thrombosis or in the clinical use of vein grafts for bypassing arterial occlusive disease. However, no information seems available on endothelium-dependent responses to aggregating platelets in veins. Hypercholesterolemia or atherosclerosis impairs endothelium-dependent relaxations of isolated arteries in different tissues. By contrast, dietary supplementation with cod-liver oil facilitates endothelium-dependent relaxations in porcine coronary arteries. Hence, the present studies were designed to examine in porcine femoral veins the extent to which aggregating platelets cause endothelium-dependent relaxations and whether the response to platelets can be altered by long-term intake of either cholesterol or cod-liver oil.

Methods

Male Yorkshire pigs, 6–8 weeks of age (body weight, 21.5±0.7 kg, n=24), were randomly divided into three groups that were fed either a regular chow (Hog Finisher, Bedtke Brothers Feed and Seed, Rochester, Minnesota; 0.09% cholesterol; for 4–10 weeks; control group), 2% high-cholesterol
diet (TD 86019 with 19% lard and 2% cholesterol, Teklad, Madison, Wisconsin; for 10 weeks; cholesterol-fed group) or regular chow plus cod-liver oil (E.R. Squibb, Princeton, New Jersey; 30 ml/day; for 4 weeks; oil-fed group). Before and after the feeding, serum concentrations of lipids (enzymatic method\textsuperscript{15}) and the fatty acids profile of plasma lipids (gas chromatographic analysis\textsuperscript{16}) were measured. To prevent excessive weight gain, the daily food intake of regular or high-cholesterol diet was limited to an amount equal to 3% of the body weight per day.\textsuperscript{8}

**Organ Chamber Experiments**

The pigs were anesthetized with ketamine hydrochloride (300 mg i.m.) and sodium pentobarbital (12.5 mg/kg i.v.). After collecting autologous blood (300 ml) from the left carotid artery into citrate anticoagulant for platelet preparation, the animals were exsanguinated. The femoral vein was removed and immersed in cold, modified Krebs-Ringer bicarbonate solution of the following composition (mM): NaCl 118.3, KCl 4.7, MgSO\textsubscript{4} 1.2, KH\textsubscript{2}PO\textsubscript{4} 1.2, CaCl\textsubscript{2} 2.5, NaHCO\textsubscript{3} 25.0, Ca-EDTA 0.016, and glucose 11.1 (control solution). The vein was cleaned of loose connective tissue under a microscope and cut into rings (4–5 mm long), with special care taken not to touch the luminal surface. In some rings, the tips of a watchmaker’s forceps were inserted into the lumen, and the endothelium was removed by gently rolling the tissue back and forth on paper towels wetted with control solution.\textsuperscript{17,18}

The rings were mounted horizontally in organ chambers filled with 25 ml control solution (37° C, pH 7.4) and gassed with 95% O\textsubscript{2}–5% CO\textsubscript{2}. The preparations were attached to a strain gauge (Statham UC2, Oxnard, California), and isometric force was recorded. The rings were progressively stretched until the contractile response evoked by norepinephrine (3×10\textsuperscript{–7} M) was maximal (optimal tension) (Table 2). They were allowed to equilibrate for 45 minutes before the experiments. The presence or absence of endothelium was confirmed functionally by the presence or absence of endothelium-dependent relaxations to bradykinin (concentration response curve [10\textsuperscript{–9} to 10\textsuperscript{–7} M] or one dose [10\textsuperscript{–7} M]). Bradykinin-induced relaxations were absent in rings without endothelium, confirming the effectiveness of the removal procedure.\textsuperscript{17,18} After the experiments, the rings were examined histologically by hematoxylin and eosin staining for general observation\textsuperscript{17,18} and Sudan IV staining for confirmation of lipid deposition in the blood vessel wall.\textsuperscript{13,19}

**Protocol**

Relaxations were examined in rings contracted with prostaglandin F\textsubscript{2\alpha} (2×10\textsuperscript{–6} M), and contractions were examined in quiescent rings. The order of the drugs tested was as follows. Set A: 1) acetylcholine (10\textsuperscript{–9} to 10\textsuperscript{–6} M), 2) ADP (10\textsuperscript{–8} to 10\textsuperscript{–4} M), and 3) thrombin (0.01–0.3 units/ml). Set B: 1) serotonin (10\textsuperscript{–9} to 3×10\textsuperscript{–6} M) and 2) platelets (25,000–75,000/μl). Set C: 1) vasopressin (10\textsuperscript{–10} to 3×10\textsuperscript{–7} M) and 2) histamine (10\textsuperscript{–6} to 10\textsuperscript{–5} M). Set D: 1) sodium nitroprusside (10\textsuperscript{–4} to 10\textsuperscript{–5} M), 2) A23187 (10\textsuperscript{–9} to 10\textsuperscript{–6} M), and 3) KCl (5–70 mM). Set E: contractions to 1) serotonin (10\textsuperscript{–9} to 3×10\textsuperscript{–6} M) and 2) platelets (75,000/μl). Pairs of rings with and without endothelium were compared. All rings were incubated with indomethacin (10\textsuperscript{–5} M) for 40 minutes to prevent the synthesis of endogenous prostanoids.

To inhibit the direct effects of platelets or serotonin on vascular smooth muscle and to examine their effects on the endothelium, the rings were incubated with ketanserin for 40 minutes before determining relaxations to aggregating platelets and serotonin.\textsuperscript{8} Preliminary experiments showed that the 5-HT\textsubscript{2} serotonergic antagonist unmasks endothelium-dependent responses to platelets and serotonin in the femoral vein (n=4, data not shown).

**Drugs**

The following drugs were used: acetylcholine chloride (ACh), ADP, apryase (an ADPase and ATPase, grade V from potato), arginine vasopressin, bradykinin, the calcium ionophore A23187, indomethacin, prostaglandin F\textsubscript{2\alpha}, pyrilamine maleate, 5-hydroxytryptamine creatinine sulfate (serotonin), sodium nitroprusside (all from Sigma Chemical, St. Louis, Missouri); dazoxiben HCl (Pfizer, Groton, Connecticut); ketanserin tartrate, R 68 070 (Janssen Pharmaceutica, Beerse, Belgium); and methiothepin maleate (Hoffmann-La Roche, Nutley, New Jersey). Unless otherwise specified, drugs were prepared daily in distilled water, kept on ice, and added to the organ chambers in volumes less than 250 μl. The calcium ionophore A23187 was dissolved in dimethyl sulfoxide (final bath concentration, 8.2×10\textsuperscript{–7} M) and diluted with distilled water; the tissues did not respond to the solvent alone. Indomethacin was dissolved in an equal molar concentration of Na\textsubscript{2}CO\textsubscript{3} (10\textsuperscript{–5} M). Inhibitors were added to the bath 40 minutes before experiments. Drug concentrations are reported as the final molar concentration (M) in the bath solution.

**Platelets**

A platelet-rich solution was prepared from autologous blood taken from each animal group and used for experiments within each group. Previous studies\textsuperscript{14,19} have shown that the responses (relaxations and contractions) of porcine coronary arteries to aggregating platelets are comparable irrespective of the sources of platelets (control or treated pigs). Autologous blood (300 ml) was drawn from the carotid artery of the pig into citrate anticoagulant to yield final concentrations of 9.3 mM sodium citrate/0.7 mM citric acid/14 mM dextrose.\textsuperscript{4,6–8} The blood was centrifuged for 40 minutes at 55g at room temperature, and the platelet-rich plasma was pipetted off. An equal volume of cold citrate anticoagulant solution (93 mM sodium citrate, 7 mM citric acid) was added to the blood.
Table 1. Baseline Data

<table>
<thead>
<tr>
<th></th>
<th>Control (n=10)</th>
<th>Cholesterol (n=8)</th>
<th>Fish oil (n=10)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>23.5±1.2</td>
<td>45.5±2.6*</td>
<td>19.8±0.8</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>99.1±7.5</td>
<td>110.0±4.3</td>
<td>91.5±7.6</td>
</tr>
<tr>
<td>Triglyceride (mg/ml)</td>
<td>28.7±3.2</td>
<td>32.0±3.6</td>
<td>27.3±4.4</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM. *p<0.05 compared with before.

Acid, 105 mM dextrose, and 5 mM KCl, pH 6.5) was added to the platelet-rich plasma, and the mixture was centrifuged for 20 minutes at 570g. The supernatant was discarded, and the remaining platelet pellet was resuspended in a small volume of the second citrate anticoagulant mixture. A platelet count of this suspension was then obtained (Model S-plus IV, Coulter Electronics, Denver, Colorado) and the volume of the suspension was adjusted so that when added to the organ chamber (in a dilution of 1:40 or higher) the resulting platelet concentration in the bath was 25,000, 50,000 or 75,000/μl. Platelet aggregation on exposure to the collagen of the blood vessel wall and the oxygenated calcium-containing Krebs-Ringer bicarbonate solution was evidenced by clearing of the initially turbid solution and formation of visible platelet clumps. Apyrase was suspended in control solution and added to the organ chamber in a concentration of 33.4 ADPase and 25.0 ATPase activity per 25 ml (as defined by supplier, 1 U activity liberates 1 μmol PO4/min). The concentration of apyrase is reported as a final concentration of ADPase activity in the organ chamber. In some cases, platelets were incubated with dazoxiben (10^-5 M), a selective inhibitor of thromboxane A2 synthetase, for 60 minutes before addition to the organ chamber.

Calculations and Statistical Analysis

The results are expressed as mean±SEM. Unless otherwise specified, n is the number of animals from which rings were taken. In rings contracted with prostaglandin F2α (2×10^-6 M), responses are expressed as percent changes from the contracted levels, and in quiescent rings, responses are expressed as percentage of the contractions to 60 mM KCl (which caused a maximal contraction); the KCl was added to the rings according to the concentration-response curves. For relaxations, the effective concentration of vasodilators causing 50% inhibition (IC50) of the contractions to prostaglandin F2α (2×10^-6 M) was calculated from each concentration-response curve, and the means of these values were presented as the negative logarithm of the molar concentration. For contractions evoked by serotonin, the effective concentration producing 30% (ED30) of the contractions to 60 mM KCl was calculated. Statistical evaluation of the data was performed by Student's t test for paired or unpaired observations. When more than two means were compared, an analysis of variance was used. If a significant value was found, Scheffe's test for multiple comparisons was used to identify differences among groups. Values were considered to be statistically different when p was less than 0.05.

Results

Body weight increased significantly in the three groups. The plasma concentration of total cholesterol significantly increased only in the cholesterol-fed group. The plasma concentration of triglyceride was unchanged in the control and the cholesterol-fed group, whereas in the oil-fed group, a significant decrease was noted (Table 1). In the oil-fed group, the plasma levels (expressed as percentage of total content) of eicosapentaenoic acid (EPA) increased.

Table 2. Responsiveness in Smooth Muscle of Porcine Femoral Veins

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cholesterol</th>
<th>Fish oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Optimal tension (g)</td>
<td>1.7±0.1 (48)</td>
<td>1.9±0.1 (48)</td>
<td>1.6±0.1 (48)</td>
</tr>
<tr>
<td>Contraction to KCl (n=5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ED50 (mM)</td>
<td>9.4±1.0</td>
<td>10.2±0.8</td>
<td>12.4±1.9</td>
</tr>
<tr>
<td>Maximum contraction (g)</td>
<td>5.1±0.5</td>
<td>5.1±0.7</td>
<td>5.0±0.6</td>
</tr>
<tr>
<td>Contraction to 2×10^-4 M PGF2α (g)</td>
<td>3.7±0.3 (36)</td>
<td>3.8±0.3 (36)</td>
<td>3.8±0.3 (36)</td>
</tr>
<tr>
<td>Relaxations to sodium nitroprusside (n=6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC50 (−log M)</td>
<td>7.5±0.1</td>
<td>7.3±0.2</td>
<td>7.3±0.2</td>
</tr>
<tr>
<td>Maximum relaxation (%)</td>
<td>108±2</td>
<td>108±3</td>
<td>118±6</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM.

Numbers in parentheses are the number of rings tested in each group. For the relaxations to sodium nitroprusside and contractions to KCl, data obtained in rings without endothelium are reported.

PGF2α, prostaglandin F2α; IC50, effective concentration causing 50% inhibition of the contractions to prostaglandin F2α (2×10^-6 M); Maximum relaxation, maximal relaxation in percentage of the response to prostaglandin F2α (2×10^-6 M).
significantly (from 0.5±0.2% to 6.9±0.5%; p<0.05, n=6), and those of arachidonic acid decreased significantly (from 8.1±0.7% to 3.4±0.4%; p<0.05, n=6). As a result, the ratio of EPA to arachidonic acid increased significantly (from 0.1±0.1 to 2.3±0.4; p<0.05, n=6). Hematoxyline and eosin and Sudan IV staining revealed no morphologic differences among the experimental three groups.

All in vitro studies were performed after incubation (40 minutes) in the presence of indomethacin (10−5 M).

Characteristics of the Smooth Muscle

There was no statistically significant difference in optimal tension or contractions evoked by prostaglandin F2α (2×10−6 M) among the three groups. KCl (5–70 mM) caused comparable, concentration-dependent contractions in rings without endothelium in the three groups. Sodium nitroprusside (10−9 to 10−5 M) caused comparable concentration-dependent relaxations in rings without endothelium in the three groups.

Endothelium-Dependent Relaxations

**Control group.** Aggregating platelets caused endothelium-dependent relaxations (Figures 1 and 2). These relaxations were significantly inhibited by methiothepin (10−6 M) or apyrase (1.0 unit/ml) and practically abolished by the combination of the two agents (Figure 2). Serotonin caused endothelium-dependent relaxations that were blocked by methiothepin (Figure 3). ADP caused endothelium-dependent relaxations that were attenuated by apyrase (Figure 4).

Acetylcholine, bradykinin, thrombin, and A23187 caused concentration-dependent, endothelium-dependent relaxations (Table 3). Vasopressin (10−10 to 3×10−7 M) and histamine (10−9 to 10−5 M), in the presence or absence of pyrilamine (an H1-histaminergic blocker, 10−6 M), did not induce endothelium-dependent or independent relaxations (n=4, data not shown).

**Cholesterol-fed group.** Platelets, serotonin, ADP, acetylcholine, bradykinin, thrombin, and A23187 caused endothelium-dependent relaxations that did not differ significantly from those obtained in veins from the control group (Figures 2–4 and Table 3).

**Oil-fed group.** The endothelium-dependent relaxations to platelets, serotonin, and ADP were significantly augmented in veins taken from oil-fed pigs (Figures 2–4). Treatment with cod-liver oil augmented the maximal relaxations evoked by acetylcholine. Relaxations induced by bradykinin, thrombin, and A23187 were comparable with control (Table 3).

Contractions

**Control group.** Aggregating platelets caused contractions, which were significantly inhibited in rings with endothelium compared with those without

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**Figure 1.** Recordings of effects of aggregating platelets in the presence of indomethacin (10−5 M) and ketanserin (10−8 M) during a contraction to prostaglandin F2α (2×10−6 M) in femoral veins from control. W₀ is washout with control solution.

**Figure 2.** Left panel: plot of effects of methiothepin or apyrase on platelet-induced relaxation in rings with endothelium from porcine femoral veins. Right panel: plot of responses to platelets in control, cholesterol-fed, and oil-fed groups. The relaxations were examined during contractions to prostaglandin F2α (2×10−6 M) in the presence of indomethacin (10−5 M) and ketanserin (10−6 M). The relaxations are expressed as percent decrease in tension from the contractions evoked by prostaglandin F2α. Data shown as mean±SEM. (+) and (−) indicate veins with and without endothelium, respectively. *Significant difference (Scheffe’s test; p<0.05) compared with control.
endothelium (Figure 5). The platelet-induced contractions were significantly inhibited by ketanserin (10⁻⁶ M) or methiothepin (10⁻⁶ M) but not by R 68 070 (5×10⁻³ M, a thromboxane A₂ receptor and synthetase blocker), 20 dazoxiben, or pyrilamine (10⁻⁶ M) (Figure 5). Serotonin also caused concentration-dependent contractions, which were significantly smaller in preparations with than in those without endothelium (Figure 6). In rings without endothelium, the serotonin-induced contractions were almost abolished by ketanserin (Figure 6).

**Cholesterol-fed group.** The contractions induced by platelets and serotonin were comparable to those observed in veins from control pigs (Figures 5 and 6).

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** Plot of cumulative concentration-response curves to serotonin in femoral veins from control, cholesterol-fed, and oil-fed groups during a contraction evoked by prostaglandin F₂α (2×10⁻⁶ M) in the presence of ketanserin (10⁻⁶ M) and indomethacin (10⁻⁴ M). The effects of methiothepin (10⁻⁶ M) on serotonin-induced relaxations in control veins are also shown. The relaxations are expressed as percent decrease in tension from the contractions evoked by prostaglandin F₂α. Data shown as mean±SEM. *Significant difference (Scheffe’s test; p<0.05) compared with control.

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Plot of cumulative concentration-response curves to ADP in femoral veins from control, cholesterol-fed, and oil-fed groups during a contraction evoked by prostaglandin F₂α (2×10⁻⁶ M) in the presence of indomethacin (10⁻⁵ M). The effects of apyrase on ADP-induced relaxations in control veins are also shown. The relaxations are expressed as percent decrease in tension from the contractions evoked by prostaglandin F₂α. Data shown as mean±SEM. *Significant difference (Scheffe’s test; p<0.05) compared with control.

**Oil-fed group.** The contractions evoked by platelets and serotonin were significantly reduced in rings with endothelium from the oil-fed group, whereas comparable contractions were observed in rings without endothelium from control and oil-fed pigs (Figures 5 and 6).

**Discussion**

The present experiments were initiated because little information is available on endothelium-dependent responses to aggregating platelets in peripheral veins. They showed that in porcine femoral veins 1) aggregating platelets cause endothelium-dependent relaxations and endothelium-independent...
contractions, 2) the endothelium-dependent relaxations to aggregating platelets are mediated mainly by serotonin and ADP, 3) the platelet-induced contractions are mediated mainly by activation of 5-HT₂ serotonin receptors with little contribution of thromboxane A₂, and 4) hypercholesterolemia does not affect the platelet-induced relaxations, but a diet rich in cod-liver oil facilitates these relaxations because it enhances the effect of the released serotonin and ADP.

Endothelium-derived relaxing factor is not a product of cyclooxygenase, and its release is not affected by indomethacin.² In addition, in the porcine coronary artery, prostacyclin has synergistic interaction with endothelium-derived relaxing factor and stimulates the release of the factor.²¹ Therefore, in the present studies, the endothelium-dependent responses were examined in the presence of indomethacin to focus on endothelium-derived relaxing factor(s).

The platelet-induced relaxations were attenuated by the serotonin antagonist (methiothepin) or the ADPase and ATPase (aprase). In addition, endothelium-dependent relaxations to serotonin and ADP were attenuated by methiothepin and apyrase, respectively. These results indicate that platelet-induced relaxations in porcine femoral veins are mediated by both serotonin and ADP. A similar conclusion has been reached for the porcine coronary artery,⁸ whereas in canine coronary and po-

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**FIGURE 5.** Bar graphs of effects of aggregating platelets (75,000/μl) in quiescent rings in femoral veins from control, cholesterol-fed, and oil-fed groups in the presence of indomethacin (10⁻⁵ M). Effects of R 68 070, dazoxiben, ketanserin, methiothepin, and pyrilamine on platelet-induced contractions in rings (without endothelium) of control veins are shown also. The contractions are expressed as percent increase in tension compared with maximal contractions caused by KCl (60 mM). Data shown as mean±SEM. *Statistically significant difference (Student’s t test for paired observations; p<0.05) between rings with and without endothelium. **p<0.05 compared with control (Scheffe’s test).

**FIGURE 6.** Plots of cumulative concentration-response curves to serotonin in quiescent rings in femoral veins from control, cholesterol-fed, and oil-fed groups in the presence of indomethacin (10⁻³M). The effects of ketanserin on serotonin-induced contractions of rings without endothelium obtained from control veins are also shown. The contractions are expressed as percent increase in tension compared with maximal contractions caused by KCl (60 mM). Data shown as mean±SEM. *Statistically significant difference (Student’s t test for paired observations; p<0.05) between rings with and without endothelium. The ED₉₀ for serotonin-induced contractions was not significantly different in rings without endothelium from control, cholesterol-fed, and oil-fed groups (−7.2±0.2, −7.4±0.3, and −7.7±0.1 M, respectively; n=6).
cine basilar arteries, the platelet-induced relaxations are mediated mainly by ADP.\textsuperscript{6,13} Human platelets contain vasopressin, which evokes endothelium-dependent relaxations of canine basilar arteries,\textsuperscript{5,22} and histamine released from platelets plays a role in platelet-induced contraction in rabbit coronary and porcine pulmonary arteries.\textsuperscript{23,24} However, vasopressin and histamine did not induce any relaxations in the porcine femoral vein, making a contribution of these substances to platelet-induced relaxations most unlikely.

The platelet-induced contractions were significantly inhibited by ketanserin or methiothepin but not by dazoxiben or pyrilamine. These results indicate that these contractions are mediated mainly by activation of 5-HT\textsubscript{2} serotonergic rather than thromboxane or histamine receptors. Similar conclusions have been reached in porcine coronary arteries.\textsuperscript{8} By contrast, platelet-induced contractions are mediated mainly by histamine in the pig pulmonary arteries\textsuperscript{24} and by 5-HT\textsubscript{1} serotonergic receptors in the canine coronary and pig basilar arteries.\textsuperscript{5,13}

Impaired endothelium-dependent relaxations in atherosclerotic arteries have been reported in animals\textsuperscript{9-11} and in humans.\textsuperscript{25} Endothelium-dependent relaxations to aggregating platelets are impaired also by hypercholesterolemia in the coronary and basilar arteries of the pig.\textsuperscript{12,13} However, in femoral veins, the endothelium-dependent responses to aggregating platelets were not altered by the long-term intake of excess cholesterol. These results are in agreement with the observations that in jugular veins from hypercholesterolemic monkeys endothelium-dependent relaxations to acetylcholine or thrombin are not altered.\textsuperscript{10} The reasons underlying the different effect of hypercholesterolemia in arteries and veins are unknown. Endothelium-dependent relaxations are less pronounced in venous tissues than in arterial tissue.\textsuperscript{18,26,27} These differences are due more to differences in the ability of the endothelium to release endothelium-derived relaxing factor than to the ability of the venous smooth muscle to respond to the factor.\textsuperscript{28,29} The different responsiveness of the endothelium of the venous wall may determine the differential responses to hypercholesterolemia. Indeed, endothelial cells of arteries and veins are exposed to different environments, such as different blood flow, distending pressure, and partial pressure of O\textsubscript{2} and CO\textsubscript{2}, which are factors that can modulate endothelium-dependent responses.\textsuperscript{30} Changes in shear stress and partial pressure of oxygen modulate the enzyme activity of endothelial cells.\textsuperscript{31-33} In the arterial circulation, hypercholesterolemia accelerates the endothelial turnover rate.\textsuperscript{34} Regenerated endothelial cells exhibit a reduced endothelium-dependent relaxations to platelets and serotonin.\textsuperscript{8} Thus, another possible explanation for the absence of impairment of endothelium-dependent relaxations in the veins could be that hypercholesterolemia does not alter the turnover rate of the venous endothelium to the same extent as that of the arterial endothelium.\textsuperscript{34}

Dietary supplementation with fish oils may be beneficial in the prevention of coronary artery disease.\textsuperscript{35-37} The present measurements of plasma levels of fatty acids confirm those measurements obtained in a previous study reporting that after treatment with cod-liver oil EPA markedly increases and arachidonic acid decreases.\textsuperscript{19,38} The increase in EPA, which is a major component among the fatty acids contained in fish oil,\textsuperscript{39} probably plays a major role in the augmentation of endothelium-dependent relaxations.\textsuperscript{40} As in the coronary arteries of pigs,\textsuperscript{14} in the femoral veins of the same species, the dietary supplementation with cod-liver oil facilitated endothelium-dependent relaxations to platelets, serotonin, and ADP. These augmented relaxations cannot be attributed to vasodilator prostaglandins because all experiments were performed in the presence of indomethacin. The sensitivity of smooth muscle cells to endothelium-derived relaxing factor may be augmented by the treatment. However, this possibility is not likely because in rings without endothelium the relaxations to sodium nitroprusside (which induces relaxations through activation of guanylate cyclase as does endothelium-derived relaxing factor\textsuperscript{41,42}) and the contractions to prostaglandin F\textsubscript{2\alpha} serotonin, and KCl were unaltered. The diffusion of endothelium-derived relaxing factor from the endothelium to the underlying smooth muscle may be facilitated or the half-life of endothelium-derived relaxing factor\textsuperscript{43} may be prolonged by cod-liver oil. This possibility appears also unlikely because the endothelium-dependent relaxations to the calcium ionophore A23187 were unaltered. Finally, the oil supplementation may facilitate the production or release of the endothelium-derived relaxing factor.\textsuperscript{14} This interpretation seems the most likely. Fatty acids can change the fluidity of the endothelial cell membrane leading to larger release of endothelium-derived relaxing factor.\textsuperscript{44} The release of endothelium-derived relaxing factor is augmented in coronary arteries from oil-fed pigs.\textsuperscript{40} The reduction of contractions induced by platelet or serotonin in rings with endothelium in the oil-fed group can be explained also in part by a facilitated release or augmented production of endothelium-derived relaxing factor.\textsuperscript{7,8,45} However, the mechanisms for selectively augmenting serotonin -, ADP-, and acetylcholine-mediated relaxations while preserving the bradykinin-, thrombin-, and A23187-induced relaxations are unclear.

The present experiments show that the endothelium of veins and of arteries can curtail the vasoinstinction induced by aggregating platelets. Although the mechanisms underlying deep vein thrombosis are still unclear, the present results suggest that dysfunction or injury of the endothelium in the vein contributes to the pathogenesis of this disorder. When peripheral veins are used as vascular grafts for arterial occlusive disease,\textsuperscript{46,47}
spasm of vein bypass grafts has been reported.48–50 In addition, graft failure can occur because of thrombosis or intimal hyperplasia that may be platelet-mediated.51–53 It is likely that impairment of the response of the endothelium to aggregating platelets may be one of the causes of vein graft spasm or late graft failure. The present results imply also that dietary supplementation with fish oil is beneficial in preventing venous disorders in association with endothelial injury or dysfunction because supplementation could prevent the impairment of endothelium-dependent relaxations in hypercholesterolemia and in atherosclerosis.19 In addition, the fact that long-term treatment with cod-liver oil facilitates endothelium-dependent responses to aggregating platelets may be the reason why treatment with cod-liver oil decreases the intimal hyperplasia in canine venous autografts.54

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Key Words • adenosine diphosphate • cod-liver oil • endothelium-derived relaxing factor • hypercholesterolemia • serotonin
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