ABSTRACT  To study the effect of dietary supplementation with fish oil on endothelium-dependent responses, Yorkshire pigs were maintained on a normal diet or on a low (0.6 ml/kg/day) or a high (1.0 ml/kg/day) dose of cod-liver oil for 4 weeks. Endothelium-dependent responses were examined in vitro in rings of proximal left anterior descending coronary arteries taken from control and treated animals studied in parallel. Endothelium-dependent relaxations in response to bradykinin, serotonin, adenosine diphosphate, and thrombin were facilitated in arteries from treated but not in those from control animals, whereas the relaxations in response to A23187 were unaltered. The facilitated relaxations were not altered by indomethacin but significantly inhibited by methylene blue. Aggregating platelets from control and treated pigs induced comparable, facilitated endothelium-dependent relaxations in rings taken from treated pigs. The platelet-induced contractions were significantly reduced in rings with endothelium taken from treated pigs, and they were comparable in rings without endothelium in both groups. Aggregating platelets from control and treated pigs released comparable amounts of serotonin and thromboxane A2. Endothelium-dependent relaxations induced by arachidonic acid and eicosapentaenoic acid were unaltered, whereas transient endothelium-dependent contractions induced by arachidonic acid were significantly reduced by the treatment with cod-liver oil. Relaxations to sodium nitroprusside or isoproteanol, and contractions to potassium chloride or serotonin were not different in rings without endothelium from control or treated pigs. These results indicate that dietary supplementation with cod-liver oil facilitates endothelium-dependent relaxations and inhibits endothelium-dependent contractions in porcine coronary arteries.


EPIDEMIOLOGIC STUDIES suggest that the incidence of ischemic heart disease is significantly lower in populations consuming a diet rich in marine oils. Dietary supplementation with marine oils produces several cardiovascular protective effects, including reductions in platelet aggregability and thrombolytic tendencies and beneficial changes in plasma lipid levels. Little information is available on the effect of dietary supplementation of marine oils on vascular reactivity in general and on endothelium-dependent responses in particular. Endothelial cells play an important role in mediating interactions between blood components and vascular smooth muscle, including the release of unidentified endothelium-derived relaxing factor(s) (EDRF). The injury or dysfunction of endothelial cells, together with resultant platelet aggregation and the release of platelet products, is one of the important mechanisms of atherosclerosis. The present study was designed to examine the effect of dietary supplementation with cod-liver oil on endothelium-dependent responses in porcine coronary arteries.

Methods

Animal preparations. Twenty-six normal Yorkshire pigs, 8 to 12 weeks of age (28.0 ± 1.2 kg), were used. The pigs were randomly divided into three groups: 13 pigs were fed regular chow (50 g/kg/d), maximal intake 1800 g/day; Hog Finisher, Bedke Brothers Feed and Seed Co., Dover, MN (control group), five pigs were fed regular chow plus 0.6 ml/kg cod-liver oil per day (Squibb, Princeton, NJ) (low fish oil group), and eight more were fed regular chow plus 1.0 ml/kg cod-liver oil per day (high fish oil group). The cod-liver oil contained 9% of eicosapentaenoic acid. All animals were housed individually for 4
weeks, under the same environmental conditions. Body weight was recorded every week and the amounts of regular chow and cod-liver oil were adjusted to the body weight. With this dietary treatment, no side effects were noted. Before and after 4 weeks of diet, the following variables were measured: serum concentrations of total cholesterol and triglyceride (enzymatic method),\textsuperscript{18} platelet count (Model S-plus IV, Coulter Electronics, Inc.), bleeding time (ear immersion method),\textsuperscript{19} prothrombin time, and activated partial thromboplastin time (MLA Electra 700, Medical Laboratory Automation, Inc.). Bleeding time was obtained from both ears and the mean of the values is reported (mean bleeding time).

**Organ chamber experiments.** Experiments were performed in vitro in one control pig and one treated pig in parallel, following a blinded design. The hearts were removed after anesthesia with ketamine hydrochloride, 300 mg im (Ketaject, Bristol), and inhalation of 0.5% halothane (Fluothane, Ayerst). The proximal left anterior descending coronary artery was dissected from each pig starting at the sinus and extending distally to the left anterior descending coronary artery (LAD) confluence. After the addition of collagenase and the coronary artery was cut into segments 2 cm long. The segments were suspended from a fixed support in a 100 ml organ chamber (in 3 to 4 ml of Krebs-Ringer bicarbonate solution of the following composition (mM): NaCl, 118.3; KCl, 4.7; MgSO_{4}, 1.2; KH_{2}PO_{4}, 1.2; CaCl_{2}, 2.5; NaHCO_{3}, 25.0; Ca-EDTA, 0.016; and glucose, 11.1 (control solution). Rings (3 to 4 mm long) were cleaned of loose connective tissue, with special care taken not to touch the luminal surface. In some rings, the endothelium was removed deliberately by rubbing the luminal surface gently with a cotton swab wetted with control solution.\textsuperscript{20} A maximum of 10 rings were obtained from each coronary artery; they were numbered from proximal to distal portions and rings with the same number taken from the two pigs were studied in parallel. The rings were mounted horizontally in organ chambers filled with 25 ml of control solution (37°C, pH 7.4) gassed with 95% O2 and 5% CO2. The preparations were attached to a strain gauge (Gould UTC2) and isometric tension was recorded. The rings were then progressively stretched until the contractile response evoked by 20 mM KCl was maximal (optimal basal tension).\textsuperscript{21} They were allowed to equilibrate for 30 min before the experiments.

**Platelets.** Autologous blood (200 ml) was drawn from the femoral 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{15} The blood was centrifuged for 40 min at 55 g 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, 105 mM dextrose, and 5 mM KCl, pH 6.5) was added to the platelet-rich plasma, and the mixture was centrifuged for 40 min at 370 g. 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, Inc.) 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/μl, 50,000/μl, or 75,000/μl. Platelet aggregation on exposure to the collagen of the blood vessel wall and the calcium-containing modified Krebs-Ringer bicarbonate solution was evidenced by clearing of the initially turbid solution and formation of visible platelet clumps\textsuperscript{15} and by detection of the levels of serotonin and thromboxane B2 in the bath solution. Samples of fluid (1.5 ml) were withdrawn from the chambers 7 min after the addition of platelets (75,000/μl), divided for the determination of the concentrations of serotonin and thromboxane B2, and frozen until analysis. The concentrations of serotonin and thromboxane B2 were determined by reverse-phase high-pressure liquid chromatography and by radioimmunoassay, respectively, as reported previously.\textsuperscript{22}

**Protocol.** After 30 min of equilibration, all rings were exposed to bradykinin (concentration response curve [10^{-10}M to 10^{-7}M] or one dose [10^{-7}M]) during a contraction caused by prostaglandin F_{2α} (2 x 10^{-6}M) to confirm the presence or absence of functional endothelial cells.\textsuperscript{23} In all rings with endothelium, bradykinin caused more than 100% decrease in tension caused by prostaglandin F_{2α}. After this confirmation, in the first five pairs of pigs in which one of the pair was fed the low dose of cod-liver oil, relaxations were examined during a contraction caused by prostaglandin F_{2α} (2 x 10^{-6}M) as follows: in set A (1) serotonin (10^{-10}M to 3 x 10^{-8}M) and (2) KCl (quiescent rings, 5 to 100 mM); in set B (1) adenosine diphosphate (ADP) (10^{-9}M to 10^{-7}M) and (2) calcium ionophore A23187 (10^{-7}M to 10^{-6}M); in set C (1) sodium nitroprusside (10^{-7}M to 10^{-6}M) and (2) arachidonic acid (10^{-7}M to 10^{-6}M). In the last eight pairs of pigs in which one of the pair was fed the high dose of cod-liver oil, relaxations were examined during a contraction caused by prostaglandin F_{2α} (2 x 10^{-6}M) or contractions were examined in quiescent rings as follows: in set A (1) serotonin (10^{-10}M to 3 x 10^{-8}M), (2) platelets taken from one of the two pigs (25,000, 50,000, and 75,000/μl), and (3) thrombin (0.01, 0.03, and 0.1U/ml); in set B (1) ADP (10^{-8}M to 10^{-7}M), (2) platelets taken from another pig (25,000, 50,000, and 75,000/μl), and (3) calcium ionophore A23187 (10^{-7}M to 10^{-6}M); in set C (1) platelets taken from one of the two pigs (quiescent rings, 75,000/μl), (2) serotonin (quiescent rings, 10^{-9}M to 10^{-7}M), (3) arachidonic acid (10^{-8}M to 10^{-7}M), and (4) KCl (quiescent rings, 100 mM); in set D (1) platelets taken from another pig (quiescent rings, 75,000/μl), (2) sodium nitroprusside (10^{-8}M to 10^{-7}M), (3) isoproterenol (10^{-7}M to 10^{-6}M), (4) eicosapentaenoic acid (10^{-7}M to 10^{-6}M), and (5) KCl (quiescent rings, 5 to 100 mM).

In organ chamber experiments with isolated rings of blood vessels, unlike under physiologic conditions, or when examining perfused segments of arteries,\textsuperscript{22} the administered drugs can reach directly the vascular smooth muscle from the adventitial sides and the cut surfaces. It is important to inhibit the direct effects of the drugs on the vascular smooth muscle to examine their effects on the endothelium. Therefore, when determining relaxations to aggregating platelets and serotonin, we first incubated the rings with ketanserin (10^{-6}M) for 40 min. Preliminary experiments demonstrated that the S2-serotonergic antagonist unmask endothelium-dependent responses to serotonin and platelets (n = 3, data not shown) by inhibiting S2-serotonergic activation of vascular smooth muscle cells. Likewise, the rings were treated with the P2-purinergic blocker theophylline (10^{-5}M) during studies of relaxation to ADP, since preliminary observations demonstrated that this drug inhibits the relaxing effect of the adenine nucleotide on smooth muscle cells without affecting the endothelium-dependent component of the action of ADP (n = 3, data not shown).

**Drugs.** The following drugs were used: ADP, arachidonic acid, bovine thrombin, bradykinin, the calcium-ionophore A23187, S, 5, 8, 11, 14, 17-eicosapentaenoic acid, 5-hydroxytryptamine creatinine sulfate (serotonin), indomethacin, isoproterenol, KCl, prostaglandin F_{2α}, sodium nitroprusside, theophylline (all from Sigma), ketanserin tartrate (Janssen Pharmaceutica), and methylene blue (Eastman-Kodak). All drugs were prepared daily with distilled water except for the calcium ionophore and indomethacin, which were dissolved in dimethylsulfoxide (1%) and Na_{2}CO_{3} (10^{-3}M), respectively. The drugs were kept on ice during the experiments; the platelet suspension was kept at room temperature. Because of the possible air and light sensitivity of eicosapentaenoic acid, the fatty acid was prepared immediately before use.

**Morphology.** The hearts were divided into five horizontal blocks and were examined macroscopically for the presence or absence of myocardial infarction. The rings used in the organ
chamber study were examined histologically by hematoxylin-eosin and van Gieson's elastic stains.

Data analysis. Results are expressed as mean ± SE. In rings contracted with prostaglandin F$_{2\alpha}$, responses are expressed as percent changes from the contracted levels, and in quiescent rings responses are expressed as percent changes of the maximal response to KCl (100 mM). Unless otherwise specified, n refers to the number of animals. Statistical evaluation of the data was performed by Student's t test for either paired or unpaired observations. When more than two means were compared, a two-way 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 at p < .05. For relaxations, the negative logarithm of the effective molar concentration of agonist causing 50% inhibition (IC$_{50}$) of the contractions to prostaglandin F$_{2\alpha}$ was calculated for each concentration-response curve and the mean of these values were presented. For contractions evoked by KCl, the effective concentration producing 50% of the maximal response (ED$_{50}$) was calculated. The relationship between IC$_{50}$ values (serotonin and adenosine diphosphate) and percent changes in other variables (total cholesterol, platelet count, and bleeding time) was examined by linear regression analysis.

Results

Baseline data. Body weight significantly increased after 4 weeks of feeding; at this time there was no difference in body weight (kg) among the three groups (43.6 ± 8.0 in control, 46.0 ± 3.4 in low fish oil, and 42.4 ± 2.6 in high fish oil groups). There also was no difference in serum concentrations of total cholesterol (mg/dl) or triglyceride (mg/dl) among the three groups (91 ± 4 and 26 ± 2 in control, 76 ± 11 and 16 ± 2 in low fish oil, and 85 ± 4 and 19 ± 3 in high fish oil groups, respectively). No difference was noted in platelet count (× 10$^3$/mm$^3$) among the three groups (446 ± 24 in control, 455 ± 34 in low fish oil, and 420 ± 45 in high fish oil groups). No specific effect of fish oil was noted on bleeding time (sec), prothrombin time (sec), or activated partial thromboplastin time (sec) between control (n = 8) and fish oil groups (n = 8; n = 5 from low fish oil and n = 3 from high fish oil groups) (139 ± 19, 21.4 ± 0.8, and 25.0 ± 0.4 in control and 162 ± 24, 22.2 ± 0.8, and 24.2 ± 0.9 in fish oil groups, respectively).

Morphology. No macroscopically visible region of myocardial infarction was present in any of the 26 hearts studied. In the 224 rings used for organ chamber experiments, the presence or absence of endothelium was confirmed histologically. No intimal thickening was noted in any of the rings studied.

Organ chamber experiments. There was no statistically significant differences in optimal basal tension (g) or contractions (g) evoked by prostaglandin F$_{2\alpha}$ (2 × 10$^{-4}$)M among the three groups studied (8.7 ± 0.2 and 5.3 ± 0.2 in control, 8.5 ± 0.2 and 5.9 ± 0.6 in low fish oil, and 8.9 ± 0.3 and 5.2 ± 0.3 in high fish oil groups, respectively). KCl caused comparable, concentration-dependent contractions in rings without endothelium in the three groups (ED$_{50}$ values [mM] were 10.49 ± 1.12 in control, 9.52 ± 1.01 in low fish oil, and 9.76 ± 0.77 in high fish oil groups, respectively). Sodium nitroprusside and isoproterenol caused comparable relaxations in rings without endothelium in both control and fish oil groups (IC$_{50}$ values [−log M] were 7.92 ± 0.07 and 7.49 ± 0.07 in control and 7.88 ± 0.09 and 7.61 ± 0.07 in high fish oil groups, respectively).

Bradykinin, serotonin, and ADP caused endothelium-dependent and concentration-dependent relaxations, which were dose (of cod-liver oil)–dependently augmented in the oil-fed groups (figures 1 and 2) (IC$_{50}$ values [−log M] to bradykinin were 8.4 ± 0.11 in control, 8.63 ± 0.09 in low fish oil [p < .05], and 9.24 ± 0.06 in high fish oil groups [p < .05], respectively. These relaxations were not significantly altered by indomethacin in either group (figures 1 and 2) but were significantly inhibited by methylene blue (figures 1 and 2). The maximal relaxations in response to bradykinin and ADP were not statistically different among the three groups, but those to serotonin were dose-dependently greater in the fish oil groups (figure 1). In contrast, ADP caused comparable relaxations in rings without endothelium in the three groups (figure 2).

In quiescent rings without endothelium, serotonin caused comparable, concentration-dependent contractions in both control and high fish oil groups (figure 3). These contractions were significantly smaller in rings with endothelium and were significantly reduced in the high fish oil group compared with the control group (figure 3).

Thrombin caused endothelium-dependent and concentration-dependent relaxations in both control and treated groups (figure 4). These relaxations were significantly augmented in the high fish oil group; they were not altered by indomethacin in either group (figure 4).

Aggregating platelets caused endothelium-dependent relaxations, which were significantly augmented in the high fish oil group compared with the control group, regardless of whether the platelets were taken from the control group or the fish oil–fed group (figure 5). These relaxations were not altered by indomethacin (n = 6, data not shown). In quiescent rings, without endothelium, aggregating platelets caused comparable contractions in both control and high fish oil groups, regardless of whether the platelets were taken from control or fish oil–treated pigs (figure 6). In quiescent rings with endothelium, platelets caused significantly
smaller contractions compared with those in rings without endothelium, and these contractions were significantly reduced in the high fish oil group compared with control, regardless of the source of platelets (figure 6). Aggregating platelets taken from control and high fish oil groups released serotonin into the bath solution, at a concentration of $322 \pm 29$ ng/ml ($1.9 \times 10^{-9}$M) and $360 \pm 27$ ng/ml ($2.1 \times 10^{-9}$M) respectively (not statistically different; n = 6). The levels of thromboxane B$_2$ measured in the bath solution after addition of platelets taken from control and high fish oil groups were $232 \pm 55$ pg/ml ($6.2 \times 10^{-10}$M) and $240 \pm 60$ pg/ml ($6.4 \times 10^{-10}$M), respectively (not statistically different, n = 6).

The $Ca^{2+}$-ionophore A23187 caused comparable, endothelium-dependent relaxations in all three groups, which were not significantly altered by indomethacin ($IC_{50}$ values [-log M] were $7.59 \pm 0.06$ in control, $7.63 \pm 0.11$ in low fish oil, and $7.58 \pm 0.09$ in high fish oil groups, respectively).

Arachidonic acid induced biphasic responses in rings with endothelium during a contraction caused by prostaglandin F$_{2\alpha}$; transient contractions followed by relaxations. The relaxations were slightly but significantly

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**FIGURE 1.** Cumulative concentration-response curves to serotonin during a contraction evoked by prostaglandin F$_{2\alpha}$ ($2 \times 10^{-6}$M) in the presence of ketanserin ($10^{-6}$M). The relaxation responses are expressed as percent decrease in tension from the contraction evoked by prostaglandin F$_{2\alpha}$. Data shown as means ± SEM. Left, Relaxations in the three different groups. Right, Relaxations in control and high fish oil groups under control conditions and in the presence of indomethacin ($10^{-6}$M) or methylene blue ($10^{-5}$M).

**FIGURE 2.** Cumulative concentration-response curves to ADP during a contraction evoked by prostaglandin F$_{2\alpha}$ ($2 \times 10^{-6}$M), in the presence of theophylline ($10^{-6}$M). The relaxations are expressed as percent decrease in tension from the contraction evoked by prostaglandin F$_{2\alpha}$. Data shown as mean ± SEM. Left, Relaxations in the three different groups. Right, Relaxations in control and high fish oil groups in control conditions and in the presence of indomethacin ($10^{-6}$M) or methylene blue ($10^{-5}$M).
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FIGURE 3. Cumulative concentration-response curves to serotonin in quiescent rings in control and high fish oil groups. The contractions are expressed as percent increase in tension compared with maximal contractions caused by KCl (100 mM). Data shown as mean ± SEM.

FIGURE 4. Cumulative concentration-response curves to thrombin during a contraction evoked by prostaglandin F$_{2a}$ (2 × 10⁻⁶M) in control and high fish oil groups. The responses are expressed as percent change in tension from the contraction level evoked by prostaglandin F$_{2a}$. Data shown as mean ± SEM.

FIGURE 5. Cumulative concentration-response curves to aggregating platelets during a contraction evoked by prostaglandin F$_{2a}$ (2 × 10⁻⁶M) in control and high fish oil groups. The responses are expressed as percent change in tension from contraction evoked by prostaglandin F$_{2a}$. Data shown as mean ± SEM.

FIGURE 6. Effects of aggregating platelets (75,000/μl) in quiescent rings from control and high fish oil groups. The responses are expressed as percent change in tension from the resting level compared with maximal contractions caused by KCl (100 mM). LAD = left anterior descending coronary artery. *Statistically significant difference (p < .05) compared with control.

augmented in rings with vs those without endothelium in the three groups (figure 7). However, there was no difference in the relaxations among the three groups. These relaxations were not altered by indomethacin (n = 5, data not shown). In contrast, the transient contractions to the fatty acid were abolished by removal of the endothelium, markedly inhibited by indomethacin, and significantly reduced by feeding with cod-liver oil (figure 8).

Eicosapentaenoic acid caused relaxations at higher concentrations, which were greater in rings with than in those without endothelium (figure 7). The relaxations were comparable in control and high fish oil groups (figure 7) and were not altered by indomethacin (n = 3, data not shown).

Correlation between endothelium-dependent responsiveness and other variables. There was no significant correlation between the IC$_{50}$ value to serotonin and changes in other variables (cholesterol, platelet count, and bleeding time) among all pigs (r = .47, r = .16, r = .18, respectively) or among oil-fed pigs (r = .14, r = .23, r = .11, respectively). No significant correlation was noted between the IC$_{50}$ value to ADP and changes in other variables mentioned above among all pigs (r = .07, r = .14, r = .18, respectively) or among oil-fed pigs (r = .02, r = .07, r = .27, respectively).
in both groups. (5) Arachidonic acid and eicosapentaenoic acid cause endothelium-dependent relaxations at higher concentrations, which are not altered with the supplementation with cod-liver oil. In contrast, the endothelium-dependent contractions induced by arachidonic acid are significantly reduced by the dietary treatment.

**Mechanisms of facilitated relaxations.** One of the striking findings was that endothelium-dependent relaxations to bradykinin, serotonin, ADP and thrombin were facilitated in oil-fed animals. These facilitations were not altered by indomethacin but were significantly inhibited by methylene blue, an inhibitor of guanylate cyclase. These results are consistent with the interpretation that the augmented relaxation is caused by EDRF and indicate that the facilitations to those agonists cannot be attributed to vasodilator prostaglandins. In contrast, endothelium-dependent relaxations were unaltered in response to A23187, which is thought to activate the synthesis and release of EDRF while bypassing cell membrane receptors. Thus treatment with cod-liver oil appears to facilitate only the receptor-operated release of EDRF from the endothelium.

Endothelium-dependent relaxations are achieved through several processes and several possibilities could explain the facilitated endothelium-dependent relaxations observed in the animals treated with cod-liver oil. First, the characteristics of smooth muscle cells and in particular their sensitivity to EDRF may be augmented. This possibility is unlikely because in rings without endothelium the relaxations to sodium nitroprusside (which induces relaxations through activation

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**FIGURE 7.** Relaxations of porcine coronary arteries of control and high oil-fed groups to $3 \times 10^{-3}$M arachidonic acid (AA, top) or $3 \times 10^{-5}$M eicosapentaenoic acid (EPA, bottom) during a contraction evoked by prostaglandin $F_2\alpha$ ($2 \times 10^{-6}$M). *Statistically significant difference (p < .05) between rings with and without endothelium.

**FIGURE 8.** Contractions to arachidonic acid during a contraction evoked by prostaglandin $F_2\alpha$ ($2 \times 10^{-6}$M). The contraction responses are expressed as percent increase in tension compared with the contraction evoked by prostaglandin $F_2\alpha$.

**Discussion**

The major effects of dietary supplementation with cod-liver oil on vascular reactivity were as follows: (1) The endothelium-dependent relaxations to bradykinin, serotonin, ADP, and thrombin are facilitated with the dietary supplementation, whereas the responses to the Ca$^{2+}$ ionophore A23187 are unaltered. (2) These facilitations are not altered by indomethacin but are significantly inhibited by methylene blue. (3) These facilitations occur at a time when the ability of underlying smooth muscle to relax or contract is unchanged and when no intimal thickening was noted in the coronary arteries. (4) The endothelium-dependent relaxations to aggregating platelets are also facilitated by the dietary treatment, regardless of whether the platelets are taken from control animals or oil-fed animals. Those platelets taken from the two different groups release comparable amounts of serotonin and thromboxane $A_2$ and cause comparable contractions of underlying smooth muscle.
of guanylate cyclase as EDRF does\textsuperscript{11}) and isoprotrenol and the contractions to KCl and serotonin were unaltered. In addition, the optimal basal tension and the contractile responses to prostaglandin F\textsubscript{2}\alpha were unaltered. Second, the diffusion of EDRF from the endothelium to the underlying smooth muscle may be facilitated or the half-life of EDRF\textsuperscript{24} prolonged. This possibility seems to be unlikely because the endothelium-dependent relaxations to A23187, arachidonic acid, and eicosapentaenoic acid were unaltered. Third, the synthesis and/or release of EDRF by the endothelium may be facilitated. Fatty acids may change the fluidity of the endothelial cell membrane.\textsuperscript{25} Administered acutely, eicosapentaenoic acid, the dominant fatty acid in cod-liver oil, caused endothelium-dependent relaxations. Therefore, it is reasonable to postulate that dietary consumption of cod-liver oil, rich in fatty acids, especially eicosapentaenoic acid, changes the membrane fluidity of endothelial cells, promoting the synthesis and/or release of EDRF in response to several agonists.

The degree of facilitation of the endothelium-dependent relaxations depended on the dose of cod-liver oil but were not correlated to variables such as serum cholesterol level, platelet count, or bleeding time. These findings suggest that the dietary supplementation with cod-liver oil affected endothelial function directly, rather than indirectly, through changes in blood components.

The reduction of serotonin-induced contractions in rings with endothelium in the high fish oil group also can be explained in part by the facilitated release of EDRF in response to the monoamine.\textsuperscript{22}

Aggregating platelets. A previous study demonstrated that in porcine coronary arteries, endothelium-dependent relaxations to aggregating platelets were achieved by a combination of a purinergic mechanism (ADP and ATP) and an S\textsubscript{3}-serotonergic mechanism and that the contractions to the platelets were achieved by an S\textsubscript{2}-serotonergic mechanism with little contribution of thromboxanes.\textsuperscript{21} In the present study, aggregating platelets released comparable amounts of serotonin and thromboxane A\textsubscript{2} in control and oil-fed groups. Hence, since in the absence of endothelium the contractions to the platelets were comparable in control and oil-fed groups, the augmented endothelium-dependent responses to ADP and serotonin on treatment with cod-liver oil must explain the facilitated relaxations to aggregating platelets.

Fatty acids. The endothelium-dependent relaxations to arachidonic acid and eicosapentaenoic acid were unaltered with the dietary supplementation of cod-liver oil. These results seem to support the proposed mechanism that fatty acids are not precursors of EDRF but rather promote its synthesis and/or release by changing the fluidity of the endothelial cell membrane.\textsuperscript{25} In porcine coronary arteries, unlike canine coronary arteries,\textsuperscript{26} the relaxations to arachidonic acid were unaltered by indomethacin, indicating that the contribution of vasodilator prostaglandins was minimal.

In contrast, the transient contractions induced by arachidonic acid were significantly reduced by the dietary supplementation with cod-liver oil. Since these contractions are endothelium-dependent and indomethacin-sensitive,\textsuperscript{20} arachidonic acid and eicosapentaenoic acid must compete for the endothelium cyclooxygenase, as they do in platelets.\textsuperscript{27, 28} Eicosapentaenoic acid is enzymatically converted to trienoic prostaglandins, such as prostaglandin I\textsubscript{3} with antiaggregatory and vasodilator actions and thromboxane A\textsubscript{3} with no proaggregatory action.\textsuperscript{6, 29}

In isolated aortic strips from rats maintained for 3 weeks on a diet supplemented with menhaden-fish oil, the concentration-contraction curves to norepinephrine shifted to the right and the contractions to arachidonic acid were reduced.\textsuperscript{30} In contrast, there were no differences in contractions to KCl or prostaglandin F\textsubscript{2}\alpha or in relaxations to sodium nitroprusside between control and treated groups. This suggested that the changes in vascular responsiveness to norepinephrine and arachidonic acid were caused by a change in prostaglandin metabolism in the blood vessel wall.\textsuperscript{30} The depressed contractions to norepinephrine in the treated group could be explained by the facilitated release of EDRF, since norepinephrine causes endothelium-dependent relaxations by activating A\textsubscript{2}-adrenergic receptors on the endothelial cells.\textsuperscript{31, 32} The reduction in arachidonic acid–induced contractions and the unaltered characteristics of the smooth muscle after treatment with fish oil are consistent with the present observations.

Clinical implications. Our findings demonstrated that dietary supplementation with fish oil resulted in a marked augmentation of endothelium-dependent relaxations to aggregating platelets, platelet products, and thrombin. Endothelial cells possess several protective actions, including the release of EDRF,\textsuperscript{12–15} against platelet aggregation. Injury or dysfunction of endothelial cells allows platelet aggregation and the release of platelet products that may cause vasospasm.\textsuperscript{33} Impaired endothelium-dependent responses have been reported in blood vessels from atherosclerotic monkeys\textsuperscript{34} and humans.\textsuperscript{35, 36} Dietary supplementation with fish oil prevents the process of atherosclerosis in hyperlipidemic pigs\textsuperscript{37} and the present results suggest
that long-term treatment with fish oil may be beneficial in preventing or reducing vasospasm that occurs in diseased vessels with endothelial injury or dysfunction (e.g., atherosclerosis). This is the first report that clinically available maneuvers could facilitate endothelium-dependent relaxations.

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