Free radical production in aortic rings from rats fed a fish oil-rich diet

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The modulation of vascular smooth muscle tone involves the release of potent vasorelaxants such as nitric oxide (NO) (26) and prostacyclin (27), which counterbalance the vasoconstrictor properties of thromboxane A2 (38) and the superoxide anion (O2−) (15) among other agents. It has also been shown that ·NO has antiatherogenic properties both in vitro and in vivo (18). However, it has been demonstrated at a vascular level that ·NO reacts with O2− to give peroxynitrite, reducing its relaxant effect (40).

Fish oils are the main source of human dietary long chain ω-3 polyunsaturated fatty acids. A fish oil-rich diet, by replacing arachidonic acid by eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) in plasma and in phospholipid membranes (29, 32), impairs the release of free radicals by different types of cells and induces changes in eicosanoid metabolites (3, 24).

These oils are used in the prevention of cardiovascular disease such as atherosclerosis (12). Several studies have been carried out on the effect of ω-3 polyunsaturated fatty acids on the release of relaxing factors (2, 5, 33, 34) and on the reduction of blood pressure in healthy volunteers (35) and in patients with mild hypertension (37). Also, its supplementation increases coronary artery vasodilation in response to acetylcholine infusion in heart transplant patients (10).

Little is known about the production of O2− and ·NO, their interaction, or about the impairment of the activity of cyclooxygenase at a vascular level by a fish oil-rich diet. The purpose of this study was to test the effect of a fish oil-rich diet on vascular smooth muscle reactivity, especially through O2− and ·NO production and through cyclooxygenase stimulation by acetylcholine in rat aortic rings.

METHODS

Animals and diets. After weaning, two groups of male Sprague-Dawley rats were fed for 8 wk with semipurified diets prepared in our laboratory (Table 1), which contained 5% lipids. The lipids were either corn oil (CO), rich in 18:2 ω-6, or menaden oil (MO), rich in 20:5 ω-3 (EPA) and 22:6ω-3 (DHA). The content of some of the fatty acids in the diets was evaluated according to the technique of Haan et al. (13) and is shown in Table 2. The oils provided −2 mg α-tocopherol per kilogram diet. Diets were supplemented with 90 mg/kg of all rac-α-tocopherol acetate (equivalent to 60 IU/kg of α-tocopherol). Diets were prepared weekly and stored at −20°C to prevent oxidation. The peroxide value of the MO diet was <10 meq/kg when ready for consumption. Food was provided daily, and uneaten food was also removed daily. Body weight was recorded every week. At the end of the feeding period, rats were anesthetized with sodium urethane (1.5 g/kg ip) and exsanguinated.

Aortic preparation. The thoracic and proximal-abdominal aortas were excised and placed in Krebs-Ringer bicarbonate solution at pH 7.4, which contained (in mM) 118.07 NaCl, 4.70 KCl, 1.77 CaCl2·2H2O, 1.17 KH2PO4, 1.17 MgSO4·7H2O, 24.04 NaHCO3, and 12.2 glucose. The proximal-ab-

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dominal and thoracic aortas were carefully cleaned of debris and blood, with care taken not to touch the luminal surface, and they were then cut into three to four rings of 3–4 mm in length, respectively. The endothelium of some rings was removed mechanically by gentle rubbing of the intimal surface and blood, with care taken not to touch the luminal surface. The chemiluminescence by integrating the values obtained over 2.5 min (25). The results obtained with lucigenin were subtracted from the ones obtained in the absence of lucigenin. After the luminometer assay, the rings were opened and extended on a board, and a photo was taken. The images were processed to calculate the surface area of the aortic rings. The results are expressed as arbitrary units per millimeter squared. Peroxynitrite formation was assessed in a similar protocol using 250 μM luminol (28).

Phenylephrine-induced vasoconstriction. By blocking either the O2•− released by exogenous SOD or the NO production by L-NNA, we can modulate the vasoconstriction. This allows us to indirectly assess the production of NO and O2•− in basal conditions, respectively. After the equilibration period, randomized rings were exposed to cumulative concentrations of the α1-adrenergic agonist phenylephrine (from 10−9 to 10−4 M). The phenylephrine-induced contractions took place in some rings in the absence of exogenous SOD and in the presence of 60 U/ml SOD. The organ bath solution was changed three times between the two assays, and rings were allowed to equilibrate for at least 15 min to return to baseline. Other rings were used to study the effect of the addition of 60 U/ml SOD plus 0.1 mM L-NNA. L-NNA was added 20 min before the first concentration of phenylephrine. Endothelium-denuded rings were also exposed to the cumulative concentrations of phenylephrine mentioned above. Phenylephrine-induced contractions of aortic rings are expressed in milligrams of force. The effective molar concentration producing 50% of the maximal response (EC50) was also calculated by linear curve-fitting data and expressed as a negative logarithm.

Acetylcholine-induced vasorelaxation. To assess the effect of MO on NO-mediated vasorelaxation, endothelium-intact randomized rings were precontracted with 2 × 10−5 M phenylephrine and relaxed with cumulative concentrations of acetylcholine (from 10−8 to 10−4 M). This process was repeated in some rings in the absence of exogenous SOD; 2) in the absence of SOD and in the presence of 0.1 mM L-NNA, added 20 min before the precontraction; 3) in the presence of 60 U/ml SOD, added immediately before the precontraction; and 4) in the presence of both SOD and L-NNA. To assess the implication of the cyclooxygenase pathway on acetylcholine-induced vasorelaxation, endothelium-intact randomized rings were precontracted with phenylephrine and relaxed with acetylcholine as described above. This process was repeated in the same ring 1) in the presence of 60 U/ml SOD, added immediately before the precontraction; 2) in the presence of both 60 U/ml SOD and 0.1 mM L-NNA, added 20 min before the precontraction; and 3) in the presence of 60 U/ml SOD, 0.1 mM L-NNA, and 0.1 mM indomethacin, added 20 min before the precontraction. The organ bath solution was changed three times after each assay, and rings were allowed to equilibrate for at least 15 min to recover to baseline. Acetylcholine-induced relaxations are expressed as a percentage of the level of precontraction. The EC50 of acetylcholine was also calculated for each concentration-response

### Table 1. Composition of semipurified diets

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount, g/kg diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>225</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>446</td>
</tr>
<tr>
<td>Sucrose</td>
<td>223</td>
</tr>
<tr>
<td>α-Cellulose</td>
<td>31</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>1</td>
</tr>
<tr>
<td>Mineral mix†</td>
<td>14</td>
</tr>
<tr>
<td>Vitamin mix‡</td>
<td>10</td>
</tr>
<tr>
<td>Oil§</td>
<td>50</td>
</tr>
<tr>
<td>α-Tocopherol acetate</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*Mixture provided the following (in g/kg diet): 12.5 calcium carbonate, 8.75 monopotassium phosphate, 2.59 sodium chloride, 1.63 potassium sulfate, 0.98 potassium citrate monohydrate, 0.84 magnesium oxide, 0.058 zinc carbonate, 0.022 manganese carbonate, 0.011 copper carbonate, 0.0035 potassium iodate, 0.00036 sodium selenite anhydrous, 0.00028 ammonium molybdate·4H2O, 0.051 sodium metasilicate·9H2O, 0.000097 chromium potassium sulfate·12H2O, 0.0029 boric acid, 0.0022 sodium fluoride, 0.0011 nickel carbonate, 0.00061 lithium chloride, and 0.00023 ammonium vanadate. †Mixture provided the following (in g/kg diet): 0.03 niacin, 0.016 n-calcium pantothenate, 0.007 pyridoxine·HCl, 0.006 thiamine·HCl, 0.002 folic acid, 0.0002 biotin, 0.025 vitamin B-12 (0.1% trit), 0.016 vitamin A palmitate (250,000 U/g), 0.0025 vitamin D-3 (400,000 U/g), and 0.00075 menadione. ‡Oil was either corn oil (CO) or menhaden oil (MO).
curves by linear curve-fitting data and expressed as a negative logarithm.

Sodium nitroprusside-induced vasorelaxation in endothelium-denuded rings. The functional removal of the endothelium was assessed by either a precontraction response or absence of relaxation on exposure to $10^{-4}$ M acetylcholine after a precontraction with $2 \times 10^{-7}$ M phenylephrine. After the rings were washed, they were exposed to cumulative concentrations of phenylephrine, and when the maximum contraction was attained, rings were exposed to cumulative concentrations of sodium nitroprusside (from $10^{-10}$ to $10^{-7}$ M), an exogenous NO donor. The sodium nitroprusside-induced relaxation was studied twice in the same ring in the absence and presence of SOD, changing the order of treatment in different rings. The organ bath solution was changed three times after each assay, and rings were allowed to equilibrate for at least 15 min to recover to baseline. Sodium nitroprusside-induced relaxations were expressed as a percentage of the level of precontraction. The EC$_{50}$ of sodium nitroprusside was calculated for each concentration-response curve by linear curve-fitting data and expressed as a negative logarithm.

Materials. Oils, casein, sucrose, $\alpha$-cellulose, DL-methionine, all-rac-$\alpha$-tocopherol acetate, phenylephrine hydrochloride, acetylcholine chloride, L-NNA, l-NMMA, sodium nitroprusside, indomethacin, and SOD were purchased from Sigma (St. Louis, MO). Starch and FeSO$_4$·7H$_2$O were purchased from Panreac (Barcelona, Spain). Mineral mix (without iron) and vitamin mix (without vitamin E) were obtained from ICN Biomedicals (Aurora, OH).

Statistical analysis. Data are expressed as means ± SE. Data were analyzed by Student’s t-test for unpaired or paired observations. Differences were considered to be significant when $P$ values were <0.05.

RESULTS

Animals. The increase in body weight over the 8-wk period was the same in the three groups of rats, and there was no difference in the final body weight: 343 ± 5 and 343 ± 8 g for CO- and MO-fed rats, respectively.

$O_2^·$ and peroxynitrite production. Aortic rings from rats fed CO or MO produced a similar basal level of $O_2^·$. Values increased after stimulation with NADPH, and no significant difference was also observed between the two dietary groups (Table 3). SOD reduced $O_2^·$ production by 60% under all conditions. The addition of l-NMMA significantly increased $O_2^·$ production, and this increase was greater in aortic rings from rats fed the MO diet incubated without NADPH. Luminal-mediated chemiluminescence as an indicator of peroxynitrite formation was hardly detectable in the basal condition and after the addition of NADPH.

Phenylephrine-induced vasoconstriction. Phenylephrine induced concentration-dependent contractions in endothelium-intact aortic rings from rats fed CO (Fig. 1A) or MO (Fig. 1B) diets. Incubation with SOD significantly reduced the efficacy of phenylephrine at low concentrations (from $5 \times 10^{-9}$ to $2 \times 10^{-8}$ M) in the two groups of rats (Fig. 1, A and B). The maximal aortic ring contraction (Table 4) was attained in rats fed the MO diet when incubated with SOD plus l-NNA. The EC$_{50}$ in the absence of SOD (Table 4) was similar in control aortic rings from rats fed CO and MO diets. Addition of SOD increased the EC$_{50}$ ($-\log M$), by 154% when expressed in nanomolars, in the two dietary groups ($P < 0.05$), and l-NNA blocked SOD-induced relaxation. A single phenylephrine-induced contraction at a concentration of $2 \times 10^{-7}$ M in endothelium-intact rings from the two groups of rats gave the same

Table 3. Superoxide anion production in endothelium-intact aortic rings from rats fed CO or MO diets

<table>
<thead>
<tr>
<th></th>
<th>CO, U/mm$^2$</th>
<th>MO, U/mm$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>3.75 ± 0.45</td>
<td>2.68 ± 0.21</td>
</tr>
<tr>
<td>Basal + SOD</td>
<td>1.50 ± 0.18</td>
<td>1.07 ± 0.09</td>
</tr>
<tr>
<td>Basal + l-NMMA</td>
<td>5.38 ± 0.68</td>
<td>4.44 ± 0.35</td>
</tr>
<tr>
<td>Basal + NADPH</td>
<td>79.78 ± 6.06</td>
<td>80.08 ± 4.66</td>
</tr>
<tr>
<td>Basal + NADPH + SOD</td>
<td>31.91 ± 2.42</td>
<td>32.03 ± 1.86</td>
</tr>
<tr>
<td>Basal + NADPH + l-NMMA</td>
<td>119.68 ± 9.08</td>
<td>120.11 ± 6.99</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 4$–6 rats. Values are the average of 2 rings from each rat. Results are expressed in arbitrary units per millimeter squared of aortic surface. SOD, superoxide dismutase; l-NMMA, N$^\circ$-monomethyl-l-arginine. *$P < 0.05$ vs. basal or basal + NADPH.
response (Table 5). Phenylephrine-induced responses in endothelium-denuded rings in the absence of SOD (the EC$_{50}$ was 7.62 ± 0.05 and 7.54 ± 0.04 for rats fed the CO and MO diets, respectively) were similar to responses in intact rings. The maximal contraction was significantly higher ($P$ < 0.05) in rats fed the MO diet (1,798 ± 124 mg) than in rats fed the CO diet (1,450 ± 100 mg), and, in contrast to intact rings, the addition of 60 U/ml SOD produced no relaxation.

**Acetylcholine-induced vasorelaxation.** Relaxation studies took place after precontraction of endothelium-intact aortic rings with $2 \times 10^{-7}$ M phenylephrine. Acetylcholine induced cumulative concentration-dependent relaxations in precontracted rings. There was no significant difference in maximal relaxation (46.4 ± 3.9% in rats fed the CO diet vs. 51.9 ± 4.0% in rats fed the MO diet) in the absence of exogenous SOD, but the EC$_{50}$ ($-\log$ M) increased in rats fed the MO diet (6.91 ± 0.08) versus the CO diet (6.60 ± 0.08) ($P$ < 0.05), and the addition of L-NNA eliminated this difference (the EC$_{50}$ was 6.91 ± 0.08 and 7.02 ± 0.1 for rats fed the CO and MO diets, respectively). The acetylcholine-induced relaxation curve in the presence of 60 U/ml SOD shifted leftward in rats fed the MO diet (Fig. 2, A and B), and the maximal relaxation attained was 68.5 and 85.9% for CO- and MO-fed rats, respectively (Table 6). These values represent an increase of 48 and 66%, respectively, when compared with values from rings incubated in the absence of SOD. The blockade of endothelial $\cdot$NO synthesis with 0.1 mM L-NNA (Table 6) reduced the maximal relaxation by ~25% in rings from the two groups of rats. This means that the $\cdot$NO-dependent relaxation accounted for 40.2 and 62.6% of the maximal relaxation in rings from rats fed CO and MO, respectively. The blockade of endogenous prostanoid-induced relaxation with 10 μM indomethacin gave values of 39.1 and 61.3% in rings from rats fed CO and MO, respectively. Therefore, the prostanoid-dependent relaxation accounted for 29.4 and 24.6% of the maximal relaxation in rings from rats fed CO and MO, respectively. The $\cdot$NO-independent relaxation plus prostanoid-independent relaxation equaled the relaxation observed in rings in the control condition. The MO diet increased the EC$_{50}$ ($-\log$ M) in aortic rings, a 68% increase ($P$ < 0.05) when expressed in millimolars (Table 6). The inhibitory effect of L-NNA was reversed by $10^{-4}$ M L-arginine (above 85% of the original relaxation).

Table 4. Cumulative phenylephrine-induced contractions in endothelium-intact aortic rings from rats fed CO or MO diets

<table>
<thead>
<tr>
<th></th>
<th>Maximal contraction, mg</th>
<th>EC$_{50}$, $-\log$ M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO</td>
<td>MO</td>
</tr>
<tr>
<td>Control</td>
<td>1,270 ± 158</td>
<td>1,567 ± 130</td>
</tr>
<tr>
<td>Control + SOD</td>
<td>1,228 ± 109</td>
<td>1,287 ± 111</td>
</tr>
<tr>
<td>Control + SOD + L-NNA</td>
<td>1,240 ± 121</td>
<td>1,778 ± 148†</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 8$–16 rats. *$P < 0.05$ vs. control; †$P < 0.05$ vs. CO.

Table 5. Phenylephrine ($2 \times 10^{-7}$ M)-induced contraction in endothelium-intact aortic rings from rats fed CO or MO diets

<table>
<thead>
<tr>
<th></th>
<th>CO, mg</th>
<th>MO, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,164 ± 107</td>
<td>1,172 ± 72</td>
</tr>
<tr>
<td>Control + SOD</td>
<td>919 ± 94</td>
<td>935 ± 45</td>
</tr>
<tr>
<td>Control + L-NNA</td>
<td>1,322 ± 116</td>
<td>1,515 ± 85</td>
</tr>
<tr>
<td>Control + SOD + L-NNA</td>
<td>1,321 ± 129</td>
<td>1,545 ± 81*</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 8$–10 rats. *$P < 0.05$ vs. control.
Sodium nitroprusside-induced vasorelaxation. The studies on sodium nitroprusside-induced relaxation took place in endothelium-denuded aortic rings after preconstriction with phenylephrine in the absence or in the presence of 60 U/ml SOD. Sodium nitroprusside-induced relaxation was concentration-dependent (Fig. 3), which was potentiated by SOD only at 3 × 10⁻² and sodium nitroprusside at 10⁻⁸ M. The maximal relaxation attained (~100%) and the EC₅₀ (~log M) were the same in rings from the two groups of rats.

DISCUSSION

Early observations have indicated that dietary manipulation with ω-3 polyunsaturated fatty acids increases endothelium-dependent relaxation in isolated porcine coronary arteries (34) and cerebral arteries (16). The mechanisms underlying the regulation of vascular tone are complex and involve free radicals and products of the cyclooxygenase pathway (15, 26, 27, 38). However, we show here, in aortic rings from rats fed a MO-rich diet and in conditions that prevent interactions between O₂⁻ and ·NO, the following: 1) there is no impairment in the release of O₂⁻ by the vascular wall; 2) the endothelium-dependent ·NO relaxation induced by acetylcholine is increased, and there is no facilitated response of the smooth muscle to ·NO; and 3) despite a reduction in arachidonic acid and an increase in EPA in phospholipid membranes, the resultant cyclooxygenase-dependent vascular response to acetylcholine is not modified.

There is increasing evidence of O₂⁻ production in the vascular wall by NAD(P)H oxidase (22, 25, 41), and the concentration of lucigenin is a critical parameter affecting the validity of the O₂⁻ measurement because it generates O₂⁻ at 250 μM but not at 5 μM (36). Our results of both basal and NADPH-stimulated production of O₂⁻ in aortic rings showed that there were no differences between the two dietary groups. The O₂⁻ production by aortic vessels is consistent with the observations of Pagano et al. (25). They described an increase in O₂⁻ release by extracellular NADPH related to a plasma membrane-associated NADPH oxidase. The ·NO depresses the baseline chemiluminescence signal, and the evaluation of this signal in the presence of a NO synthase inhibitor is an indirect probe to estimate ·NO release (25, 36). In this condition, basal ·NO was higher in aortic rings from rats fed the fish oil diet, and, in addition, we observed similar results by electronic paramagnetic resonance (unpublished data).

The release of ·NO by endothelium-intact aortic rings exerts a tonic vasodilator action opposing the effects of vasoconstrictor agents such as O₂⁻ (15). The favorable kinetics of the reaction between O₂⁻ and ·NO intrinsically make vascular O₂⁻ levels an important determinant of ·NO biological activity (23). Thus the reduction of ·NO-induced vasodilation associated with peroxynitrite generation may contribute to pathological situations such as hypertension. Our results with exogenous SOD are indicative of O₂⁻ production, which would reduce ·NO bioavailability. The absence of SOD effect on endothelium-denuded rings indicates that the vasoconstrictor effect of O₂⁻ is mainly due to chemical inactivation of ·NO. Therefore, SOD was used in further experiments to reach optimal ·NO-mediated responses. L-NNA potentiation of phenylephrine-induced contraction in rings from rats fed MO (38%) implied a greater ·NO production in animals fed MO than those fed CO.

Relaxation of blood vessels is subject to complex control. There is a basal production of ·NO that exerts a tonic vasodilator action that plays an important role in the local control of vascular tone. This basal production is stimulated by a large number of biological mediators such as acetylcholine (11). The release of ·NO under basal and agonist-stimulated conditions was measured in the presence of SOD in the organ bath, although some authors (1, 21) have observed no SOD effect on the acetylcholine-mediated relaxation in ar-

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Table 6. Cumulative acetylcholine-induced relaxations in endothelium-intact aortic rings from rats fed CO or MO diets

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>CO Maximal Relaxation %</th>
<th>CO EC₅₀, −log M</th>
<th>MO Maximal Relaxation %</th>
<th>MO EC₅₀, −log M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control + SOD</td>
<td>68.5 ± 4.4</td>
<td>6.93 ± 0.08</td>
<td>85.9 ± 3.1†</td>
<td>7.40 ± 0.05†</td>
</tr>
<tr>
<td>Control + SOD + L-NNA</td>
<td>28.3 ± 2.9*</td>
<td>6.88 ± 0.11</td>
<td>23.3 ± 3.9*</td>
<td>6.89 ± 0.11*</td>
</tr>
<tr>
<td>Control + SOD + indomethacin</td>
<td>39.1 ± 1.8*</td>
<td>6.79 ± 0.06</td>
<td>61.3 ± 3.3†</td>
<td>7.01 ± 0.05*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8–15 rats. *P < 0.05 vs. control + SOD; †P < 0.05 vs. CO.

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![Fig. 3](http://example.com/fig3.png)

Fig. 3. Cumulative sodium nitroprusside-induced relaxation in endothelium-denuded aortic rings from rats fed CO or MO diets. Rings were incubated with or without SOD (60 U/ml). Values are means ± SE; n = 7 rats.
terial rings. We ruled out that differences in relaxations in precontracted rings were due to a facilitated relaxation of vascular smooth muscle because the concentration-response curves to sodium nitroprusside were similar in rings from the CO and MO groups. Moreover, the endothelium-dependent relaxation to acetylcholine involves ·NO and cyclooxygenase products (4, 39). The incorporation of ω-3 polyunsaturated fatty acids into phospholipids of cell membranes may affect endothelial ·NO synthase, and ω-3 polyunsaturated fatty acids decrease the synthesis of series 2 and 4 eicosanoids and increase the synthesis of series 3 and 5. However, Fischer and Weber (9) observed similar effects with prostaglandin I2 and prostaglandin I2 (prostacyclin). The ·NO-mediated relaxation increased 58%, whereas prostanooid-mediated relaxation was reduced by 16%, in rats fed the MO diet. Thus we have shown that, in basal and in agonist-stimulated ·NO production, the MO diet elicited a vasorelaxant activity, which was mediated by ·NO.

The imbalance between O2·− and ·NO production with enhanced activation of ·NO by a fish oil diet may, in addition to the reported beneficial effects in blood vessels, also have lead to vasorelaxant activity. The oxidation of low-density lipoproteins (LDL) is the first step in the development of atherosclerotic lesions. The acceleration of LDL oxidation may be brought about by peroxynitrite production (7), by incorporation of ω-3 polyunsaturated fatty acids to LDL (20, 24, 42), or by reduction of lipid-soluble antioxidants such as α-tocopherol (8) among others. However, in vivo studies (6, 12, 42) have demonstrated that fish oil prevents the development of coronary diseases. It has been observed that ·NO is a potent antioxidant (31) that prevents the oxidation of LDL in vitro (14, 17) by inhibiting radical chain propagation reactions (30) and thus acts as an antiatherogenic agent (18). More work is needed to substantiate the protective role of fish oil on LDL oxidation.

New studies on the effects of dietary fatty acids in the prevention and treatment of cardiovascular disease are emerging. We can conclude from the present study that a long-term MO-rich diet has a beneficial effect on blood vessels by potentiating the production of endothelial ·NO, a potent vasodilator with antiatherogenic properties, without affecting either the production of O2·− or the relaxation due to the activation of the cyclooxygenase pathway.

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