
A LONG-TERM FISH DIET MODIFIES THE TOXIC PROPERTIES OF HUMAN PARTIALLY OXIDIZED LDL ON VASCULAR PREPARATIONS IN VITRO

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Both LDL oxidation and LDL fatty acid composition affect vascular relaxation and contraction. The aim of this study was to investigate whether long-lasting dietary habits (vegetarian, fish and high saturated fat as a control group) can change those properties of partially oxidized LDL (ox-LDL) which are reflected in altered vascular responses measured with a bioassay. The effects of ox-LDL were investigated on rat mesenteric arteries.

In endothelium intact arterial rings the contractile responses to noradrenaline (NA) tended to be diminished in the presence of ox-LDL derived from the fish diet group compared with the other groups. In the endothelium denuded arterial rings the contractile responses to NA and KCl were significantly enhanced by ox-LDL from the fish diet group compared with the control group. The ox-LDL from the fish diet group increased the d甸loenaс, L-NAME resistant relaxations to ACh compared to the control diet group suggesting the role of endothelium derived hyperpolarizing factor (EDHF).

In conclusion, partially oxidized LDL from subjects living on a fish diet is biologically more vasoactive in bioassay systems than partially oxidized LDL from those living on vegetarian or saturated fatty acid containing diets. The impaired responses in vasoconstriction and improved vasodilation seem to be endothelium dependent.

Key words: low density lipoprotein, oxidized LDL, fish, vegetarian, polyunsaturated fatty acids, vascular response, mesenteric artery.

INTRODUCTION

Endothelial cells produce and release both relaxing (for review see 1) and constrictive vasoactive substances (for review see 2 and 3), which regulate the tone of underlying vascular smooth muscle (for review see 4). An attenuation of vasodilation and of relatively well maintained vasoconstriction are common features in hypertension. The endothelium dependent relaxation responses to
acetylcholine (ACh), substance P and thrombin have been shown to be decreased in various arteries of hypercholesterolemic rabbits (5,6), pigs (7) and monkeys (8). The endothelial function is impaired in hyperlipidemia mainly due to oxidized LDL (ox-LDL) which, by activating scavenger receptors, impairs the activity of the L-arginine-nitric oxide (NO) pathway (for review see 9). Ox-LDL has been reported to enhance contraction also in endothelium denuded arterial rings (10). Some conflicting results have also been published on the attenuation of endothelium dependent vasodilations by exposure to native LDL (for review see 11). Recently, Napoli and co-workers (12) reported that mildly oxidized but not native LDL from healthy subjects impaired contraction and endothelium-dependent relaxation in the carotid but not in the basilar artery in the rabbit, thus indicating important differences in vascular reactivity between different arteries. Similar findings were described by Mougenot and co-workers (13) using human internal mammary artery and rat thoracic aorta preparations.

Dietary fatty acids influence LDL oxidation by changing the fatty acid composition of LDL (14). A fish diet is rich in polyunsaturated (PUFA) long chain n—3 fatty acids, which are susceptible to oxidation (15). Supplementation with n—3 fatty acids in animals has reduced (16, 17), not affected (18), or increased (19) the vascular contraction to noradrenaline (NA) and not affected potassium chloride (KCl) -induced contraction (20, 21, 22). Vascular relaxations to acetylcholine are increased (18, 20), or not affected (16, 17) in rats fed n—3 fatty acids. All these studies have been short-term dietary interventions and the vascular responses have been tested in the animals or subjects themselves.

As far as we know, there are no reports in the literature where long lasting dietary habits affect those properties of partially oxidized LDL which are reflected in altered vascular responses measured with a bioassay system.

MATERIAL AND METHODS

Subjects, dietary intake and LDL fatty acid composition

Human LDL samples were obtained from three different diet groups: a fish diet (n = 9), a vegetarian diet (n = 11), and a control diet (n = 7). The study was approved by the Ethics Committee of Helsinki University Central Hospital. The gender, age and serum lipid composition of the subjects are shown in Table 1. People were chosen by interview with a validated questionnaire on their food habits (23). The Nutrica programme with a Finnish database (24) was used for calculation of the nutrient intake recorded by means of a frequency questionnaire accompanied by an illustrated book describing the quality of food and the quantity of food portions (25). The dietary intake, serum antioxidants, fatty acid content of LDL and the oxidation of LDL have recently been reported by us (26). The LDL fatty acid component differed between the diet groups according to the PUFAs. The mean percentage LDL content of n—6 series was higher
in the vegetarian group compared to the fish group (45.6 ± 1.1 vs. 36.8 ± 2.1\%, p < 0.01) and the LDL content of n—3 series was lower in the vegetarian group compared to the fish and the control group (4.1 ± 0.4\% vs. 12.0 ± 2.4\% for the fish group (p < 0.01) and vs. 7.1 ± 0.4\% for the control group (p < 0.01)).

**Table 1.** The gender, age, BMI, exercise habits and serum lipid composition (mmol/l) of the subjects in the fish, vegetarian and control groups, Mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Fish n = 9</th>
<th>Vegetarian n = 11</th>
<th>Control n = 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>5/4</td>
<td>4/7</td>
<td>4/3</td>
</tr>
<tr>
<td>Age (y)</td>
<td>41 ± 3.9</td>
<td>34 ± 3.0</td>
<td>40 ± 2.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.0 ± 1.1*</td>
<td>20.9 ± 0.5*</td>
<td>23.1 ± 0.4</td>
</tr>
<tr>
<td>Exercise (times/wk)</td>
<td>3.1 ± 0.8</td>
<td>3.1 ± 0.8</td>
<td>3.4 ± 0.9</td>
</tr>
<tr>
<td>Tot-chol</td>
<td>5.05 ± 0.30</td>
<td>4.13 ± 0.27</td>
<td>5.15 ± 0.23</td>
</tr>
<tr>
<td>HDL-chol</td>
<td>1.39 ± 0.08</td>
<td>1.22 ± 0.09</td>
<td>1.43 ± 0.16</td>
</tr>
<tr>
<td>LDL-chol</td>
<td>3.47 ± 0.31</td>
<td>2.69 ± 0.20</td>
<td>3.32 ± 0.26</td>
</tr>
<tr>
<td>VLDL-chol</td>
<td>0.21 ± 0.04</td>
<td>0.29 ± 0.08</td>
<td>0.22 ± 0.07</td>
</tr>
<tr>
<td>ToT-trigly</td>
<td>1.04 ± 0.10</td>
<td>1.16 ± 0.16</td>
<td>1.01 ± 0.18</td>
</tr>
</tbody>
</table>

The Kruskall-Wallis test was used to compare the groups as regards to the parameters. In pairwise comparisons, significant (p < 0.05) differences were found in comparison to the vegetarian diet (v), and control diet (c).

**Preparation and oxidation of LDL, animals and experimental design**

LDL was oxidized by incubation with CuSO₄ for 2.5 h (partially ox-LDL) or when the diene conjugation curve was at its maximum (totally ox-LDL), as described previously (27). The degree of oxidation was quantified by the absorption increase at 234 nm wavelength, indicating a conjugated diene formation of the fatty acids.

Normotensive male Wistar rats (350—400 g) were used for the bioassay preparations. They were housed four rats to a cage with free access to food and drinking water and they were submitted to a 12-h dark-light cycle. The animals were anaesthetized with 40 mg/kg i.p. sodium pentobarbital and then decapitated. The superior mesenteric arteries were carefully excised and cleaned of adherent connective tissue. In each experiment, four pieces obtained from the same animal were studied simultaneously. Arterial rings were cut and randomized for different LDL incubations. Before incubation for 60 min at +4°C, LDL (native, 2.5 h oxidized i.e. partially oxidized, totally oxidized) concentrations were adjusted to 400 µg/ml and EDTA (0.2 M) to prevent any further oxidation reaction of the LDL in the cuvettes.

**Mesenteric arterial responses in vitro**

Four successive 3 mm-long sections of the mesenteric artery from each animal were cut. One of the rings was denuded by gently rubbing with a jagged injection needle (28). Each prepared ring was placed carefully between stainless steel hooks and suspended in an organ bath chamber (volume 8 ml) in a medium of the following composition (mM): NaCl 119.0, NaHCO₃ 25.0, glucose 11.1, CaCl₂ 1.6, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, and aerated with 95\% O₂ and 5\% CO₂, pH 7.4.
The rings were initially equilibrated for 1 h at 37 °C with a resting tension of 1.0 g. The force of contraction was measured with an isometric force displacement transducer and registered on a polygraph (FT03 transducer and model 7E Polygraph; Grass Instrument Co., Quincy, MA, U.S.A). The absence of endothelium in vascular preparations was confirmed by the lack of relaxation response to acetylcholine (ACh) after precontraction with 1 μM noradrenaline (NA), which resulted in approximately 60% of the maximal contractile response attained in each group. The concentration relaxation responses to ACh were then obtained and repeated in the presence of 3 μM diclofenac with or without 0.1 mM N\textsuperscript{\textcircled{O}}-nitro-L-arginine methyl ester (L-NAME) to prevent endothelial prostacyclin and nitric oxide production, respectively. Relaxation responses were also tested for two endothelium independent vasodilators i.e. sodium nitroprusside (SNP) and a β-adrenergic agonist isoprenaline (ISO). After washing and a 30-min pause, concentration response curves for NA, and 30 min pause for KCl, were cumulatively determined. The next concentration of contracting or relaxing compound was added only after the previous level was stable.

Drugs

The following drugs were used: acetylcholine, potassium chloride, sodium nitroprusside, L-noradrenaline, isoprenaline hydrochloride, N\textsuperscript{\textcircled{O}}-nitro-L-arginine methyl ester, diclofenac (Sigma Chemical Co., St.Louis, MO, U.S.A). The stock solutions of the compounds used in the in vitro studies were dissolved in distilled water. All solutions were freshly prepared before use and protected from light.

Analysis of results and statistics

The NA- and KCl-induced contractile responses are expressed in grams. The relaxations in response to ACh, SNP and isoprenaline are presented as a percentage of the pre-existing contractile force induced by 1μM NA. pD\textsubscript{2}-values are the negative logarithm of the concentration of agonist required to produce a half-maximal contractile response. The EC\textsubscript{25} for ACh and ISO were calculated because maximal relaxations to these agonists did not reach 50% relaxation in every diet group. pD\textsubscript{2} values are calculated from the figures. EC\textsubscript{25} and EC\textsubscript{50} values were used in the statistical analysis. The Kruskall-Wallis test was used to compare the differences between the groups. For multiple comparison of means, the Mann-Whitney U test was used. Pairwise comparisons were Bonferroni adjusted. ANOVA for repeated measurements was applied for data consisting of repeated observation at different time points. Results are expressed as mean ± SEM, with p < 0.05 considered statistically significant.

RESULTS

LDL oxidation in the three different dietary group

After 2.5 h of CuSO\textsubscript{4} treatment the LDL oxidation percentage was highest in the fish group compared to the vegetarian group (44 ± 6% vs. 22 ± 3%, p = 0.01). In the control group the oxidation percentage was between the two other groups 31 ± 5%. The length of the lag phase and the rate of oxidation have been reported elsewhere (26).
Arterial responses in the presence of native or totally oxidized LDL

In the case of native LDL, the maximal relaxation to acetylcholine was smallest after preincubation with native LDL from the fish group (83.7 ± 5.6 vs. 98.2 ± 0.8% for the control group (p < 0.05) and vs. 91.8 ± 3.4% for the vegetarian group (p = 0.07)) (Table 2).

When the endothelial prostacyclin and nitric oxide productions were inhibited with diclofenac alone or in combination with L-NAME, this difference was not found (Table 2).

Table 2. Effects of native, and totally oxidized LDL from three different dietary groups on acetylcholine and nitroprusside induced maximal relaxations of rat endothelium intact mesenteric artery in vitro. Sixty min preincubation at 4°C with LDLs before the experiment. Maximal relaxation values for relaxing substances are given. Mean ± SEM (n = 5–12).

<table>
<thead>
<tr>
<th>Maximal relaxation to acetylcholine (%)</th>
<th>Native LDL</th>
<th>Totally oxidized LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Precontraction with NA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetarian diet</td>
<td>91.8 ± 3.41</td>
<td>94.4 ± 1.87</td>
</tr>
<tr>
<td>Fish diet</td>
<td>83.7 ± 5.61</td>
<td>93.5 ± 3.60</td>
</tr>
<tr>
<td>Control diet</td>
<td>98.2 ± 0.82</td>
<td>95.2 ± 1.23</td>
</tr>
<tr>
<td><strong>Response in the presence of diclofenac</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetarian diet</td>
<td>93.0 ± 2.53</td>
<td>95.8 ± 1.87</td>
</tr>
<tr>
<td>Fish diet</td>
<td>87.0 ± 5.64</td>
<td>93.9 ± 4.61</td>
</tr>
<tr>
<td>Control diet</td>
<td>95.6 ± 1.68</td>
<td>93.7 ± 2.12</td>
</tr>
<tr>
<td><strong>Response in the presence of diclofenac and L-NAME</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetarian diet</td>
<td>44.6 ± 6.68</td>
<td>42.9 ± 9.68</td>
</tr>
<tr>
<td>Fish diet</td>
<td>28.4 ± 11.4</td>
<td>57.6 ± 5.93</td>
</tr>
<tr>
<td>Control diet</td>
<td>40.7 ± 7.58</td>
<td>28.5 ± 6.94</td>
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<tr>
<td><strong>Maximal relaxation to nitroprusside (%)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Vegetarian diet</td>
<td>97.0 ± 2.05</td>
<td>99.8 ± 0.17</td>
</tr>
<tr>
<td>Fish diet</td>
<td>99.6 ± 0.28</td>
<td>96.9 ± 2.56</td>
</tr>
<tr>
<td>Control diet</td>
<td>98.5 ± 0.46</td>
<td>99.8 ± 0.15</td>
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</table>

The Kruskall-Wallis test was used to compare the groups as regards to the parameters:
In pairwise comparisons, significant (p < 0.05) differences were found in comparison to the fish diet (f), and control diet (c).

Preincubation with totally oxidized LDL from the fish diet group enhanced the relaxation even after the blockade of prostacyclin and nitric oxide formation (57.6 ± 5.9 vs. 28.5 ± 6.9% for the control group (p < 0.05) and 42.9 ± 9.7% for the vegetarian group (p < 0.05)) (Table 2). No differences between the dietary groups were seen when no preincubation or preincubation with diclofenac alone were used.
In the *endothelium intact* mesenteric arterial rings maximal contractile responses to NA and KCl in grams were generally smaller than those of the endothelium denuded arteries (*Table 3*).

*Table 3. Effects of partially oxidized LDL from three different dietary groups on noradrenaline and potassium chloride induced maximal contractions and acetylcholine and nitroprusside induced maximal relaxations of endothelium intact and denuded rat mesenteric artery in vitro. Sixty min preincubation at 4°C with LDLs before the experiment. Mean ± SEM (n = 5—12).*

<table>
<thead>
<tr>
<th></th>
<th>Vegetarian diet</th>
<th>Fish diet</th>
<th>Control diet</th>
</tr>
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<tbody>
<tr>
<td><strong>Endothelium intact</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Maximal contraction (g) to noradrenaline</td>
<td>1.43 ± 0.46</td>
<td>0.95 ± 0.13</td>
<td>1.28 ± 0.47</td>
</tr>
<tr>
<td></td>
<td>1.75 ± 0.31</td>
<td>1.25 ± 0.12</td>
<td>1.70 ± 0.37</td>
</tr>
<tr>
<td>Maximal relaxation (%) to acetylcholine</td>
<td>95.9 ± 1.01</td>
<td>96.1 ± 2.29</td>
<td>90.3 ± 3.55</td>
</tr>
<tr>
<td>with diclofenac</td>
<td>90.1 ± 2.75</td>
<td>97.8 ± 1.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>83.2 ± 5.24</td>
</tr>
<tr>
<td>with diclofenac and N-NAME</td>
<td>44.3 ± 9.47</td>
<td>57.3 ± 8.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>31.9 ± 5.47</td>
</tr>
<tr>
<td>nitroprusside</td>
<td>99.3 ± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>93.3 ± 5.57</td>
<td>96.0 ± 1.71</td>
</tr>
<tr>
<td><strong>Endothelium denuded</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal contraction (g) to noradrenaline</td>
<td>2.72 ± 0.47</td>
<td>2.63 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.01 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>2.20 ± 0.43</td>
<td>2.24 ± 0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.77 ± 0.24</td>
</tr>
<tr>
<td>Maximal relaxation (%) to isoprenaline</td>
<td>59.1 ± 6.66</td>
<td>63.4 ± 8.83</td>
<td>48.5 ± 10.2</td>
</tr>
<tr>
<td>nitroprusside</td>
<td>97.1 ± 1.78</td>
<td>98.9 ± 1.01</td>
<td>98.5 ± 0.65</td>
</tr>
</tbody>
</table>

The Kruskall-Wallis test was used to compare the groups as regards to the parameters: In pairwise comparisons, significant (p<0.05) differences were found in comparison to the control diet (c).

In *endothelium denuded* mesenteric arteries relaxations were tested to isoprenaline and nitroprusside. In the presence of native LDL from the control diet the isoprenaline relaxation was the weakest (44.1 ± 5.5 vs. 69.8 ± 6.3% for the fish group (p<0.05) and vs. 70.3 ± 9.3% for the vegetarian group (p<0.05)). Nitroprusside induced, endothelium independent maximal relaxations were not influenced by native or totally oxidized LDLs from any of the diet groups.

**Arterial responses in the presence of partially oxidized LDL**

Because partially oxidized LDL (ox-LDL) in the different dietary groups seemed to behave differently from the native and totally oxidized LDL it was investigated in more detail.

In the *endothelium-intact* arterial rings the contractile responses to NA seemed to be reduced but the difference was not statistically significant with the LDL from the fish diet group compared with the other groups (*Fig. 1*). The
arteries incubated with ox-LDL from the fish diet group also tended to show smaller maximal contractions to KCl (Table 3).

**Fig. 1.** Concentration-response curves of endothelium intact (E+) and endothelium denuded (E-) mesenteric arterial rings to noradrenaline and potassium chloride after 60 min preincubation at +4°C with partially oxidized LDL from subjects living on fish diet, vegetarian diet, and control diet rich in saturated fat. Mean±SEM, n = 5—9 in each group.
In endothelium denuded arterial rings the contractile responses to NA and KCl were reduced in the presence of ox-LDL from the control diet group compared with ox-LDL from the fish diet group (Table 3). The contractile responses induced by NA in the fish diet group were enhanced about 3-fold in endothelium denuded arterial rings in comparison to the endothelium intact arterial rings (Fig 1a and 1b). The increase was higher with ox-LDL from the fish group than with ox-LDL from the other diet groups.

There were no significant differences in the relaxation responses induced by ACh in NA-precontracted endothelium-intact arterial rings in the presence of ox-LDL from the three dietary groups (Fig. 2a). In the presence of diclofenac (Fig. 2b), the relaxation elicited by ACh in the control diet group was reduced (p<0.05), and the concentration-response curve when the arteries were preincubated with ox-LDL from the fish group was shifted to the left in comparison to ox-LDL from the control and vegetarian groups (Fig. 2b).

![Graph showing concentration-response curves](image-url)

Fig. 2. Concentration-response curves of isolated endothelium intact mesenteric arterial rings to a) acetylcholine after 60 min preincubation at +4°C with partially oxidized LDL from subjects living on fish, vegetarian, and control diet. The relaxations were induced after precontraction with 1 μM noradrenaline in the presence of b) 3 μM diclofenac c) diclofenac and 0.1 mM N-nitro-O-L-arginine methyl ester (L-NAME). Mean ± SEM, n = 5—9 in each group. *p<0.05 compared with a control diet group, †p<0.05 compared with a vegetarian group. *p<0.05 a fish diet vs. a control diet group, ANOVA for repeated measurements.)
The addition of the NO synthase inhibitor L-NAME to the organ bath effectively reduced the ACh-induced relaxations of NA-precontracted rings in all groups (p < 0.01), and almost completely abolished them in the control group (Fig. 2c). Interestingly, the fish diet group showed increased diclofenac, L-NAME resistant relaxations to ACh compared to the control diet group, suggesting the role of the endothelium derived hyperpolarizing factor (EDHF) (Fig. 2c).

The relaxation of NA-precontracted *endothelium intact* rings to nitroprusside, an exogenous NO-donor, was significantly (p < 0.01) impaired, by the ox-LDL from the fish diet group compared with ox-LDL from the control diet group measured either by the relaxation curve or by the EC$_{50}$ value (Fig. 3a). The relaxation of NA-precontracted *endothelium denuded* rings to nitroprusside was improved in the fish diet group (Fig. 3b). Relaxation responses to isoprenaline also was increased in presence of ox-LDL from the fish diet group when compared to the control diet group (p < 0.05) (Fig. 3c). The concentration response curve to isoprenaline in the fish diet group shifted to the left, indicating increased sensitivity to relaxation in comparison to the other two diet groups.

*Fig. 3. Concentration-response curves of isolated a) endothelium intact (E+), b) endothelium denuded (E-) mesenteric arterial rings to nitroprusside and c) E-to isoprenaline after 60 min preincubation at +4°C with partially oxidized LDL from a fish diet group, a vegetarian diet group, and a control diet group. The relaxations were induced after precontraction with 1 μM noradrenaline. Mean ± SEM, n=5—9 in each group. *p<0.05 compared with a control diet group. **p<0.01, a fish diet vs. a control diet group, ANOVA for repeated measurements.*
DISCUSSION

In the present study, we investigated the possible biological effects of human ox-LDL, separated from plasma, of subjects who had for some years been on diet containing different fats (fish, vegetarian or saturated fats as in the control group). We described earlier the high content of n-3 fatty acids and the highest oxidation rate of LDL in a fish diet group. In the present study, the ox-LDL from the fish diet group reduced noradrenaline induced contractions and improved endothelium dependent relaxations, suggesting the role of the endothelium derived hyperpolarizing factor (EDHF).

Mesenteric arterial rings from normotensive rats were used as the biological assay system. This in vitro preparation has been widely used as a valuable tool for testing new drugs, endogenous substances and toxic compounds on vasculature without reflectory and compensatory mechanisms activated in vivo (29, for review see 3).

Endothelial cells produce and release both relaxing and constrictive vasoactive substances, which regulate the tone of underlying vascular smooth muscle (30, for review see 1 and 4). Ox-LDL has a selective effect on endothelium to inhibit vascular relaxation to some agonists. Cox and Cohen (31) concluded that the procontractile effects of ox-LDL are a consequence of its inhibitory effects on the release or activity of NO rather than a direct effect on vascular smooth muscle, and that it may also enhance arterial contraction by stimulating the release of contractile factors from the endothelium. There are several mechanisms that have been proposed to account for the impairment of endothelium dependent arterial relaxations. These include increased production of endothelin (32) a reactive oxygen radical-induced decrease of prostacyclin synthesis (33), reduced NO formation due to endothelial NO-synthesis inhibition (34), and inactivation of NO by free radicals (35).

In the present study, the sensitivity of endothelium intact arterial rings to NA and KCl was reduced by partially oxidized LDL (ox-LDL) from subjects of the fish diet group in comparison to the control diet group. Mougenot et al. (13) reported that vasoconstriction of both rat and human arteries induced by KCl was not significantly modified in relation to the degree of LDL oxidation but that the NA-induced contraction was enhanced by increasing the degree of LDL oxidation. In our recent studies, we did not find any clear correlation between the degree of oxidation of LDL and vasoactive properties in rat mesenteric artery preparations in vitro although partially ox-LDL seemed to be most reactive and was studied therefore in more detail. Also the partially ox-LDL was the most potent inducer of the DNA synthesis compared to native and totally ox-LDL in smooth muscle cell culture (36).
Acetylcholine (ACh) relaxes arteries by releasing NO, prostacyclin, and EDHF from the endothelial cells. NO stimulates soluble guanylate cyclase, elevating intracellular cGMP in smooth muscle; prostacyclin acts via adenylate cyclase and cAMP, and EDHF dilates arteries by opening of K+ channels (37, 38).

In our study, inhibition of prostacyclin and NO-synthesis diminished relaxations of NA-precontracted rings to ACh more effectively in the arteries pretreated with ox-LDL from the control diet group than that from the fish diet group. This diclofenac and L-NAME resistant relaxation suggests that an endothelial product other than NO was responsible for the maintenance of relaxations to ACh in the case of ox-LDL from the fish diet group. Electrophysiological studies performed side by side with relaxation experiments have indicated that endothelium mediated relaxations, which remain resistant to both NO synthase and cyclo-oxygenase inhibition, are linked to EDHF. The chemical characteristics of EDHF are unknown, but it has been suggested that it is the nonprostanoid product of the metabolism of arachidonic acid (for review see 39, 40). EDHF is functionally an endogenous potassium channel opener, an action which can be inhibited by depolarizing the cell membrane with high concentrations of KCl (for review see 39). In pathological states which may be associated with reduced bioavailability of endothelium-derived NO, EDHF-mediated vasorelaxation could be enhanced (41).

In the endothelium-intact preparations, SNP-induced concentration relaxation curves did not differ significantly between the diet groups. However, in endothelium denuded preparations, ox-LDL originating from the fish diet sensitized the arteries to SNP. This was even more marked to isoprenaline. Isoprenaline relaxes smooth muscle predominantly via the stimulation of β-adrenoceptors and the subsequent increase in cyclic AMP in smooth muscle cells (42). On the other hand, the mechanism is also dependent on hyperpolarization (43). The present findings show that ox-LDL from fish-eating subjects can influence both the endothelium intact and endothelium denuded arteries by improving acetylcholine, SNP and isoprenaline induced relaxations. Its effects on contractions in endothelium intact and denuded preparations were reversed: impairment of the former and augmentation of the latter. This suggests different mechanisms of action in the endothelial and smooth muscle cells, possibly via the release of EDHF from the endothelium and some direct membrane effects in the smooth muscle cells.

Arterial responsiveness to ox-LDL derived from the fish group is possibly due to the reactive radicals in originated from ox-LDL. It has been suggested that ox-LDL induced contraction or inhibition of relaxation may be due to oxysterols or lysophosphatidylcholine (13, 44, 45). Subsequent studies revealed that only long-chain lysophosphatidylcholine was effective in impairing
endothelium dependent relaxation (46). Deckert et al. (47) found that unlike pretreatment with native LDL, pretreatment with ox-LDL significantly reduced the acetylcholine mediated relaxation of rabbit aortic segments compared with control segments, but disparities appeared in the ability of individual ox-LDL preparations to act as inhibitors of the endothelium dependent relaxation. Deckert et al. (47) found that values obtained with oxidized and native LDL correlated significantly with the formation of 7-ketocholesterol, 7α-hydroxycholesterol, and 7β-hydroxycholesterol but not with the amount of lipoperoxides of lysophosphatidylcholine formed. The contradictory findings between the above studies and ours may be due to different animal species and vascular preparations as described by Napoli et al (12).

Of the LDL fatty acids, oleic acid has been reported to have similar vascular effects as ox-LDL (48). MacLeod et al. (17) noticed that n-3 but not n-6 PUFA supplements attenuated contractile responses of rat femoral resistance arteries to noradrenaline. In vivo fish oil supplementation may alter vascular reactivity in a number of ways. A high concentration of n-3 PUFA in LDL may affect the vascular response by affecting fluidity of the cell or the balance of the synthesis rate of vasoactive eicosanoids. Malis et al. (20) found that the decrease in contractile response to noradrenaline and vasopressin in the preanoxic de-endothelialized fish oil rings suggested a possible direct effect of fish oil on the contractility of vascular smooth muscle. Yanagisawa and Lefer (49) found, in the isolated perfused coronary artery of cats, that eicosapentaenoic acid exerted a vasodilator effect that was endothelium independent and inhibited by the lipoxygenase antagonist.

In conclusion, we found that ox-LDL from subjects living on fish diet for many years have beneficial effects on endothelium intact rat mesenteric arteries in comparison to ox-LDL from vegetarians or the control diet group. The effects might be related to increased EDHF formation. On the other hand, ox-LDL from fish-eating persons have direct effects on vascular smooth muscle, such as augmented contractions but improved relaxations of endothelium denuded arteries to nitroprusside and isoprenaline, possibly due to membrane changes.

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