Membrane Active Lipids in Remnant Lipoproteins Cause Impairment of Endothelium-Dependent Vasorelaxation

Hideki Doi, Kiyotaka Kugiyama, Masamichi Ohgushi, Seigo Sugiyama, Toshiyuki Matsumura, Yasutaka Ohta, Hideki Oka, Nobuhiko Ogata, Akira Hirata, Yorihiro Yamamoto, Hirofumi Yasue

Abstract—We have recently found that remnant lipoproteins (RLPs) and their lipid fractions impair endothelium-dependent vasorelaxation (EDR). This study was aimed at clarifying mechanisms responsible for RLP-induced endothelial dysfunction in isolated rabbit aortas. RLPs were isolated from plasma in hyperlipidemic subjects by use of the immunoaffinity gel mixture of anti-ApoA1 and anti-ApoB100 monoclonal antibodies and ultracentrifugation. Organ chamber experiments showed that EDR impairment was restored by addition of reduced glutathione (GSH) or N-acetylcysteine, antioxidants, into the incubation buffer containing isolated rabbit aortas and RLPs (0.75 mg of triglyceride/mL). Furthermore, the incubation of isolated human red blood cells (RBCs) with RLP and its lipids converted the normal shape of RBCs to echinocytes, but coinubcation with antioxidants suppressed the RLP-induced EDR transformation, suggesting that they exerted oxidative damage on RBC surface membranes. Studies with HPLC and the postcolumn chemiluminescence method showed that RLPs contain a substantial amount of phosphatidylcholine hydroperoxides. Peroxidized phosphatidylcholine also impaired EDR and had echinocytic action, both of which were suppressed by N-acetylcysteine. RLPs isolated from plasma of patients under treatment with α-tocophorol, an antioxidant, had a lower level of phosphatidylcholine hydroperoxides (15% of the amount in nontreated patients), which was associated with a lack of the inhibitory action on EDR and with lesser effect on RBC transformation. Oxidative damage caused by lipid components in RLPs, especially peroxidized phospholipids, deteriorates cell surface membrane and may be at least partly responsible for RLP-induced impairment of EDR. (Arterioscler Thromb Vasc Biol. 1999;19:1918-1924.)

Key Words: endothelium ■ lipoproteins ■ antioxidants ■ endothelial-derived factors ■ hyperlipoproteinemia

We recently found that lipids in remnant lipoproteins (RLPs), remnant-like lipoprotein particles in the strict sense of the word, from postprandial plasma caused impairment of endothelium-dependent vasorelaxation (EDR) in an apoprotein receptor–independent manner.1 In a clinical study, we further showed that levels of RLPs were an independent predictor of coronary endothelial vasomotor dysfunction, an early event leading to the formation and development of atherosclerosis.2 Thus, the endothelial dysfunction caused by RLPs may play a role in the pathogenesis of atherosclerotic coronary artery disease in postprandial hypertriglyceridemic patients. However, the precise mechanisms underlying RLP-induced endothelial dysfunction remain to be clarified.

It was previously difficult to isolate RLPs because they have heterogeneous properties.3 However, Nakajima et al have recently developed a simple and reliable method to isolate RLPs in the unbound fraction by an immunoaffinity gel mixture of human anti-ApoA1 and anti-ApoB100 monoclonal antibodies.1-5 The unique anti-ApoB100 antibody has been shown to recognize ApoB100 in LDLs and most VLDLs but not in ApoE-enriched VLDLs.1-5 The current method with anti-ApoB100 antibody is capable of isolating remnants of VLDLs and chylomicron, neither of which binds to the gel. Our previous reports confirmed that lipoproteins isolated from plasma taken 5 hours after meals by using this immunoaffinity gel consisted mainly of VLDL remnants.1,2 Oxidative stress is a common feature of various coronary risk factors for atherosclerosis.6 Further, several clinical studies showed that antioxidant therapy improved endothelial dysfunction in patients with hyperlipidemia and in normcholesterolenic subjects after a single fatty meal.7,8 In this study, we thus tested the hypothesis that peroxidized lipids in RLPs may have a causative role in EDR impairment by RLPs. The present study shows that antioxidants, added to the incubation mixture containing isolated rabbit aortas and RLPs, suppressed EDR impairment. RLPs were found to contain peroxidized phospholipids that were effective components for the inhibitory action of RLPs, and they caused perturbation of cell surface membranes that was also attenuated by antioxidants.

Methods

Lipoprotein Preparations

EDTA plasma (1 mg/mL EDTA) was obtained at 5 hours after the test meal from 14 hypertriglyceridemic subjects who had >5 mg of
cholesterol/dL (or 20 mg of triglyceride/dL, ~75th percentile of the distribution of the RLP levels in the population) of RLP levels in a plasma sample after overnight fasting. The energy content of the test meal was 490 kcal/m² of body surface area (86.8% from fat, 8.7% from carbohydrate, 4.5% from protein, and the ratio of polysaturated fatty acids to saturated fatty acids was 0.51). They had no other serious diseases and did not take cardiovascular medications, antioxidants, and estrogen for >7 days before the overnight fast. The mean level of RLPs in the postprandial plasma was 84.2±15 mg of triglyceride/dL (21.5±5 mg of cholesterol/dL) in these subjects.

Plasma from each subject was applied to the immunoaffinity-mixed gel containing anti-ApoA1 and anti ApoB100 monoclonal antibodies (Japan ImmunoResearch Laboratories). The epite for ApoB100 monoclonal antibody has been localized to an amphiphilic helical region of ApoB100 encompassing residues 2291 to 2318, ie, at about ApoB51. This antibody can recognize ApoB100 in LDL and most VLDLs but not in ApoE-enriched VLDLs. The unbound fraction containing ApoE-enriched lipoproteins and albumin was eluted with PBS (mmol/L: 138 NaCl, 2.7 KCl, 8.1 NaH₂PO₄, 1.1 KH₂PO₄, pH 7.4). The bound fraction was then eluted with 3 mol/L NaSCN containing 1 mg/mL BSA (fatty acid-free BSA). The unbound fraction was then ultracentrifuged (d<1.006) to isolate RLPs from the unbound fraction containing albumin. Bound VLDL was also isolated from the bound fraction by ultracentrifugation (d<1.006). The fraction in the d<1.006 g/mL was also obtained from the EDTA plasma by ultracentrifugation, and then the fraction in the d<1.006 g/mL was subjected to the immunoaffinity gel column to isolate RLPs. These 2 protocols for isolation of RLPs were aimed at testing the effect of the presence of albumin during RLP elution on the vasoactivity of RLP. The prepared lipoproteins were extensively dialyzed for 24 hours at 4°C against PBS (mmol/L: 137 NaCl, 2.7 KCl, 1.4 NaH₂PO₄, 4.3 NaHPO₄, pH 7.4) containing EDTA (50 μmol/L) and then sterilized by filtration (filter pore size, 0.22 μm; Millipore). According to analyses with SDS–polyacrylamide gel electrophoretograms, elution profiles with HPLC, agarose amide gel electrophoretograms, electron photomicrographs, and composition of lipids and apoproteins, the lipoproteins isolated from the unbound fraction after ultracentrifugation consisted mainly of VLDL RLPs, as shown in our previous reports. The affinity of this fraction containing ApoE-enriched lipoproteins and albumin was eluted with 3 mol/L NaSCN containing 1 mg/mL BSA (fatty acid-free BSA). The unbound fraction was then ultracentrifuged (d<1.006) to isolate remnants from the unbound fraction. Lipids were extracted from RLPs and bound VLDL with chloroform/methanol (2:1, vol/vol). Total cholesterol and triglycerides were measured enzymatically by commercially available kits (Nos. 439-17501 and 274-69802, Wako Chemicals). Final concentrations of the prepared lipoproteins were 25 to 35 mg of triglyceride in lipoproteins per milliliter. The α-tocopherol level of lipoproteins was measured by HPLC. Twelve subjects who had fasting RLP levels of >30 mg of triglyceride/dL were treated orally with α-tocopherol (300 mg/d, n=6) or placebo (n=6) for 4 weeks. The 2 treatment groups of subjects were matched with the respect to age, sex, and other coronary risk factors. They were advised to adhere to their usual diet and exercise activity throughout the 4-week treatment. Before and after the 4-week treatment, EDTA plasma was obtained at 5 hours after the test meal. Postprandial levels of RLP were comparable between the α-tocopherol and placebo treatment groups both before and after the treatment (85.4±2.7 and 80.3±2.8 mg of triglyceride/dL, respectively) and after the 4-week treatment (85.4±12 and 84.2±8 mg of triglyceride/dL, respectively).

Measurement of Susceptibility of RLP to Oxidative Modification
The susceptibility of RLP to oxidative modification was determined by measuring Cu²⁺-induced formation of conjugated dienes. The conjugated dienes formation in RLP (0.1 mg of triglyceride/mL) was monitored by spectrophotometric change in absorbance at 234 nm, as described previously.

Measurement of Phosphatidylcholine Hydroperoxides and LysoPhosphatidylcholine in RLP
Lipids in RLP were extracted with methanol and hexane in sequence as described previously. The hexane layer was removed and evaporated under vacuum and then redissolved in HPLC eluent. Measurement of phosphatidylcholine hydroperoxides (PC-OH) was performed by using the HPLC and isoluminol chemiluminescence detection system as described in our previous reports. Concentration of lyso phosphatidylcholine (lysoPC) in RLP was measured by using thin layer chromatography as described in our recent report.

Transformation of Red Blood Cell (RBC) Shape
The erythrocyte surface membranes are known to be susceptible to peroxidation and serve as a good model for the oxidative damage of biological membrane. To test whether RLPs may have membrane active molecules, RLPs were incubated with RBCs devoid of lipoprotein receptors. RBCs freshly obtained from healthy volunteers
TABLE 1. Effects of Lipoproteins on Relaxation of Rabbit Aortas

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Bound VLDL</th>
<th>RLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. relaxation (%)</td>
<td>75.8±2</td>
<td>72.6±2</td>
<td>36.9±4*</td>
</tr>
<tr>
<td>EC50 (nmol/L)</td>
<td>33.8±5</td>
<td>33.4±4</td>
<td>532.2±138†</td>
</tr>
<tr>
<td>A23187</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. relaxation (%)</td>
<td>92.6±1</td>
<td>91.4±1</td>
<td>67.1±3*</td>
</tr>
<tr>
<td>EC50 (nmol/L)</td>
<td>21.1±1</td>
<td>22.5±1</td>
<td>112.5±34†</td>
</tr>
<tr>
<td>SNP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. relaxation (%)</td>
<td>99.0±1</td>
<td>98.1±1</td>
<td>97.8±1</td>
</tr>
<tr>
<td>EC50 (nmol/L)</td>
<td>26.3±1</td>
<td>30.0±1</td>
<td>36.8±2</td>
</tr>
</tbody>
</table>

Isolated rabbit thoracic aortas were incubated with RLP (0.75 mg of triglyceride/mL) or bound VLDL (0.75 mg of triglyceride/mL). After the incubation, the aortas were precontracted with phenylephrine (1 μmol/L) or bound VLDL (0.75 mg of triglyceride/mL). After the curves was analyzed by unpaired Student’s t test. A value of P<0.05 was considered statistically significant.

Figure 2. Line graphs showing effects of RLPs (0.75 mg of triglyceride/mL) on the relaxation of rabbit thoracic aortas in response to graded concentrations of ACh in the presence or absence of NAC (1 mmol/L) or GSH (1 mmol/L). Results consisted of 10 separate experiments using RLPs from 10 different subjects. Probability values for comparison of the 2 curves by 2-way ANOVA for repeated measures are shown.
the oxidation of RLP isolated from patients under treatment with α-tocopherol was longer than that of RLP before treatment (267.2±23 minutes before α-tocopherol versus 456.1±111 minutes after α-tocopherol, P<0.05, n=6). In the present study, RLP was eluted with or without the albumin fraction during the isolation procedure. However, the inhibitory effect of RLP eluted with the albumin fraction on vasorelaxation in response to ACh was comparable with that of RLP without the albumin fraction (maximum relaxations were 38.4±4% with albumin fraction versus 35.8±3% without albumin fraction, P=NS, n=6), suggesting that the presence of albumin during isolation of RLP and bound VLDL did not affect the inhibitory action of RLP on vasorelaxation. In the present study, we were very careful to prevent intraexperimental autoxidation of RLP during preparation of the lipoproteins. It is unlikely that intraexperimental artifacts including autoxidation of RLP would cause EDR impairment because another lipoprotein, bound VLDL, which was prepared in the same manner as RLP, had no effect on EDR, and TBARS, an indicator of lipid peroxidation, was not detected in the incubation mixture after incubation with the aortas.

Measurement of Peroxidized PC and its Effects on Vasorelaxation

RLPs contained a substantial amount of PC-OH, detected by HPLC and isoluminol chemiluminescence detection system, as shown in Figure 3. LysoPC, an active component of oxidized LDL, was not detectable in RLP (≤5 pmol/mg of protein). The level of PC-OH contained in RLPs was significantly decreased in RLPs isolated from subjects under treatment with α-tocopherol compared with that level before treatment (PC-OH in RLP, 292.5±66 pmol/mg of triglyceride in RLP before α-tocopherol versus 49.6±6 pmol/mg of triglyceride in RLP after α-tocopherol, P<0.01, n=5). TBARS were not detectable in the isolated RLPs.

Incubation of the artery with peroxidized PC but not untreated PC significantly impaired vasorelaxation in response to ACh, as shown in Table 2. Incubation of the artery with 5-CHO-PC (10 μmol/L) also impaired vasorelaxation in response to ACh (maximum relaxations were 77.1±2% for control versus 53.9±3% for 5-CHO-PC, P<0.001, n=6). Furthermore, the combined incubation with NAC (1 mmol/L) significantly suppressed the impairment of vasorelaxation induced by peroxidized PC, as shown in Table 2. On the other hand, vasorelaxation to sodium nitroprusside was fully preserved after incubation with peroxidized PC, as shown in Table 2.

Transformation of RBC Shape

RLPs (0.5 mg of triglyceride/mL) and their lipid extracts (0.5 mg of triglyceride/mL) transformed RBC shape to echinocytes, whereas bound VLDL (0.5 mg of triglyceride/mL) had less effect on the shape of RBCs, as shown in Figures 4 and 5. Coincubation with GSH (1 mmol/L) or NAC (1 mmol/L) suppressed the RLP-induced RBC transformation (Figure 5). Furthermore, RLPs from patients under treatment with α-tocopherol had lower effect on RBC transformation, as shown in Figures 4 and 5. Incubation of RBCs with peroxidized PC but not untreated PC significantly transformed the shape of RBCs (Figure 5). Coincubation with NAC (1 mmol/L) also

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**TABLE 2. Effects of Peroxidized Phosphatidylcholine on Relaxation of Rabbit Aortas**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Untreated PC</th>
<th>Peroxidized PC</th>
<th>Peroxidized PC+NAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. relaxation (%)</td>
<td>67.4±2</td>
<td>64.8±2</td>
<td>27.0±5*†‡</td>
<td>62.4±2</td>
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<tr>
<td>EC50 (nmol/L)</td>
<td>33.6±2</td>
<td>47.3±11</td>
<td>377.2±50*†‡</td>
<td>72.6±3</td>
</tr>
<tr>
<td>SNP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. relaxation (%)</td>
<td>99.9±1</td>
<td>99.9±1</td>
<td>99.7±1</td>
<td>99.8±1</td>
</tr>
<tr>
<td>EC50 (nmol/L)</td>
<td>62.0±5</td>
<td>61.9±3</td>
<td>71.1±3</td>
<td>73.8±2</td>
</tr>
</tbody>
</table>

Isolated rabbit thoracic aortas were incubated with untreated phosphatidylcholine (Untreated PC) (15 μmol/L) or peroxidized phosphatidylcholine (Peroxidized PC) (15 μmol/L) in the presence or absence of NAC. After the incubation, the aortas were precontracted with phenylephrine (1 μmol/L) and tested with acetylcholine (ACh). The control study (Control) was performed to expose the aortic strips in the organ chambers to the same volume of PBS (as a vehicle); otherwise the protocol was the same as for phosphatidylcholine. The maximum (Max.) relaxation is expressed as a percentage of the reduction from precontraction elicited by phenylephrine. EC50 and EC20 were calculated as the concentrations causing 50% and 20% decreases, respectively, of the contraction with phenylephrine.

Data are expressed as mean±SEM values; n=6 to 8.

*P<0.001 versus control; †P<0.001 versus untreated PC; ‡P<0.001 versus peroxidized PC+NAC.
suppressed the RBC transformation induced by peroxidized PC (Figure 5). Incubation of RBCs with 5-CHO-PC (10 μmol/L) also transformed RBC shape to echinocytes (transformation of RBC shape was 4.3±1% for control versus 93.6±2% for 5-CHO-PC; P<0.001, n=6). Transformed RBCs on stages I to III according to Brecher and Bessis,18 were considered echinocytes.

Discussion

The present study showed that RLPs and their extracted lipids impaired EDR of isolated rabbit aortas at the same concentrations of RLP as in postprandial plasma in patients with coronary artery disease (50 to 200 mg of triglyceride in RLP/dL), whereas the bound VLDL fraction, which is the remaining VLDL after removing RLP, had no effect on EDR. These results are compatible with those in our previous report showing that lipid components of RLP from postprandial plasma inhibited EDR in an apoprotein receptor–independent manner.1 This study further showed that coincubation with NAC and GSH, antioxidants that were added to incubation mixtures containing isolated rabbit aortas and RLP, almost completely suppressed EDR impairment, suggesting that oxygen radicals may play a significant role in the impairment of EDR by RLP.

The study with HPLC and the isoluminol chemiluminescence detection system showed that RLPs contained a substantial amount of PC-OOH. The peroxidized phospholipids are known to be a reactive oxygen species capable of initiating and propagating a chain of free radical reactions, especially in polyunsaturated fatty acids, in lipoproteins and cell surface membranes, leading to deterioration of the cell surface membrane and intracellular thiol depletion both of which result in cellular dysfunction.17,19,20 In fact, the present study showed that the incubation of RBCs with RLP or peroxidized PC including 5-CHO-PC produced a quantifiable transformation of the erythrocytes from their discoidal to echinocytic configuration. Further, the present study showed that peroxidized PC including 5-CHO-PC also caused EDR impairment. For all lipoproteins and lipid preparations tested, there was an apparent concordance between echinocytogenic action and EDR inhibition. Thus, oxidative damage, which induced perturbation of RBC surface membranes, could occur in surface membranes and in other constituents such as sulfur-containing enzymes of the endothelium, and may lead to the endothelial dysfunction. This is supported by the present results that the echinocytogenic action and EDR impairment by RLPs and peroxidized PC were both inhibited by GSH and NAC, antioxidants that improve the intracellular redox state and terminate the chain reaction of peroxidation by scavenging chain propagation radicals and reducing lipid hydroperoxides.17,20–23 Furthermore, the present study showed that RLPs isolated from plasma of patients under treatment with a usual pharmacological dose of α-tocopherol, an antioxidant, had a lower level of phospholipid hydroperoxides and resistance of the RLPs to Cu2+-induced oxidation. These effects of α-tocopherol were associated with a lack of inhibitory action on EDR and of echinocytogenic action. Taken together, these results suggest that oxidative damage mediated by the peroxidized phospholipids, primarily contained in RLPs, may be at least partly responsible for the impairment of EDR and RBC transformation by RLP. Nonspecific cytotoxicity of the lipoproteins is unlikely to cause dysfunction at the present dose of remnants because our previous experiments1 showed that incubation of the cultured endothelial cells with RLPs caused neither LDH release into the medium nor cell death, and cell lining was preserved after incubation of the aortas with RLPs by electron micrograph. One possibility is that RLPs may perturb surface receptor–mediated signal transduction as proposed for oxidized LDLs,9 which also caused EDR impairment and echinocytogenic effect.

This study cannot exclude the possibility that other oxidized lipid products possibly contained in remnants, such as degradation products of sphingomyelin, short chains of free fatty acids, peroxidized and fragmented

**Figure 4.** Electron micrograms (magnification, ×3000) showing effects of RLP (0.5 mg of triglyceride/mL) on the shape of human RBCs. The control study (Control) was performed to expose the human RBCs to the same volume of PBS (as vehicle) in otherwise the same protocol as performed with RLPs. Echinocytes of stages I to III, according to Brecher and Bessis,18 were shown after incubation with RLPs.

**Figure 5.** Bar graphs showing effects on echinocytogenic effect of RLP, lipid extracts of RLP, and preparations of phosphatidylcholine (PC). The isolated human RBCs were incubated with one of the preparations of lipids and lipoproteins (0.5 mg of triglyceride/mL), RLPs isolated from patients under treatment with α-tocopherol (RLP-α-tocopherol) (0.5 mg of triglyceride/mL), bound VLDLs (0.5 mg of triglyceride/mL), lipid extracts of RLPs (0.5 mg of triglyceride/mL) (RLP-lipids), untreated PC (10 μmol/L), and peroxidized PC (10 μmol/L) in the presence or absence of GSH (1 mmol/L) or NAC (1 mmol/L). The mean value in each group was determined by 6 independent experiments. The mean values were compared by using 1-way ANOVA, and then the difference between the 2 mean values was analyzed by Fisher’s protected least significant difference test. *P<0.001 versus control; †P<0.001 versus RLP; #P<0.001 versus peroxided PC.
polysaturated fatty acid–containing triglycerides, and oxysterols, could also play a role in the remnants-induced endothelial dysfunction. In fact, we previously reported that sphingosine, a sphingomyelin-hydrolyzed product, induced impairment of endothelium-dependent relaxation of the isolated rabbit aortas. During peroxidation of PC, phospholipid hydroperoxides are produced as a first step, then they are instantly decomposed to phospholipids with a short chain of aldehydes in the presence of transition metals. In the present study, we quantified the amount of only phospholipid hydroperoxides, precursors of phospholipids including aldehyde residues, in remnants. We did not determine the species of fatty acids and the degree of their degradation in the peroxidized phospholipids responsible for the endothelial dysfunction. 5-CHO-PC, a peroxidized PC, is reported to have various biological effects and it is contained in oxidized LDL and in atherosclerotic arterial walls. 5-CHO-PC was found to impair endothelium-dependent relaxation of the isolated rabbit aortas, as shown in the present study. Thus, 5-CHO-PC may be a candidate of peroxidized phospholipids responsible for endothelial dysfunction. TBARS are known to be more hydrophilic and transferable compared with peroxidized phospholipids. Thus, TBARS produced in remnants in vivo can be transferred to plasma, albumin, other lipoproteins, or phospholipid layers of cell surface membranes in the circulation or in the arterial walls and they are dialyzed during the isolation procedure. These are reasons why TBARS were not detectable in the isolated RLPs. It remains to be evaluated whether peroxidized phospholipids taken up intracellularly through the phospholipid receptors may also cause the injurious effects of remnants on endothelial function. Lipoproteins are believed to be oxidized in the subendothelial space that is sequestered from plasma antioxidants, but they are not oxidized in the circulation. There is a possibility that some of the circulating RLPs may enter into the subendothelial space and reemerge from the intima into the circulation and that lipid hydroperoxides may be produced in RLPs in the subendothelial space in the meantime. In patients with high RLP levels, the prolonged retention of RLPs in the circulation, as a result of the delayed hepatic uptake and/or the increased hepatic secretion of VLDL, may augment susceptibility of RLPs to oxidative modification in the arterial intima. Oxidative damage against endothelial cells, as observed in the present organ chamber experiment, could be induced by RLPs entering into the subendothelial space, leading to endothelial dysfunction in postprandial hyperlipidemic patients. In the present analysis, RLPs had very little lysoPC compared with oxidized LDLs. Thus, the extent of the oxidation of RLPs seems to be minimal. However, the inhibitory effect on EDR was comparable between RLPs and oxidized LDLs. This is the case in the minimally oxidized LDLs. Minimally oxidized LDLs have multiple atherogenic effects on vascular cells, and some peroxidized phospholipids also contribute to these effects. In conclusion, oxidative damage caused by lipid components in RLPs, especially peroxidized phospholipids, deteriorates cell surface membranes and may be at least partly responsible for RLP-induced impairment of EDR.

References
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