Interaction of Oxidized Low Density Lipoprotein with Macrophages in Atherosclerosis, and the Antiatherogenicity of Antioxidants

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Summary: Macrophage cholesterol accumulation and foam cell formation, the hallmark of early atherosclerosis, is the result of enhanced cellular uptake of plasma low density lipoprotein (LDL). Native LDL, however, has to undergo oxidative modifications in order to be taken up at an enhanced rate by macrophages, leading to foam cell formation. Macrophage uptake of oxidized LDL involves its binding to scavenger receptors (including cellular proteoglycans) and this is followed by an impaired cellular cholesterol metabolism. Cells of the arterial wall including macrophages can oxidize LDL in a process that involves activation of cellular oxygenases, such as NADPH oxidase and 15-lipoxygenase. This process, however, also depends on the macrophage antioxidant environment, where glutathione peroxidase and reduced glutathione play an important protective role against cell-mediated oxidation of LDL. Macrophage phospholipids peroxidation under oxidative stress can also contribute to macrophage-mediated oxidation of LDL.

Evidence for the occurrence of oxidized LDL in vivo is as follows:

1) In the atherosclerotic lesion [in humans, as well as in the transgenic, apolipoprotein E-deficient mice], LDL is oxidized (and as a result, it is also aggregated), in comparison to plasma LDL which is normally not oxidized.

2) Plasma LDL from patients at high risk for atherosclerosis (such as hypercholesterolaemic, hypertensive, diabetic and renal failure patients), as well as from the apolipoprotein E-deficient mice, demonstrates increased susceptibility to oxidation in comparison to normal LDL. In some groups of these patients LDL is minimally oxidized already in plasma.

3) Supplementation of nutritional antioxidants, which are rich in polyphenols (red wine, licorice, olive oil), or of selenium to humans or to the apolipoprotein E-deficient mice, as well as therapy with β-hydroxy-β-methyl-glutaryl-CoA reductase inhibitors (so-called "statins") in hypercholesterolaemic patients, were shown to reduce the susceptibility of LDL to oxidation. This effect could be associated with a reduction in the size of the atherosclerotic lesion and may thus contribute to attenuation of the atherosclerotic process.

Introduction

Atherosclerosis is the major cause of morbidity and mortality in the Western world and its pathogenesis involves complicated interactions among cells of the arterial wall, blood cells, and plasma lipoproteins (1). Atherogenesis is associated with endothelial dysfunction (2), platelet activation (3, 4), lipoprotein retention (5), lipoprotein oxidation (6–17), lipoprotein aggregation (18, 19), macrophage foam cell formation (2, 8, 14), inflammation (20, 21) and thrombosis (1, 4). These processes are closely related and interact among themselves, finally leading to the formation of the atherosclerotic lesion. The present article summarizes our contribution to the understanding of the roles of oxidatively modified low density lipoprotein (LDL) in atherogenesis. We studied the role of modified forms of LDL in the conversion of macrophages to cholesterol-loaded foam cells, and present evidences for the presence of oxidized LDL in the atherosclerotic lesion. Increased susceptibility to oxidation of LDL from patients with increased risk for atherosclerosis, enhanced uptake of oxidized LDL by macrophages, and macrophage-mediated oxidation of LDL were shown to be important contributors to accelerated atherosclerosis. Finally, we present evidences for the role of antioxidants in the inhibition of LDL oxidation and in the attenuation of the atherosclerotic processes.

Modified LDLs and Atherogenesis

High plasma LDL concentration is a major risk factor for atherosclerosis. Thus, increasing LDL receptor activ-
ity (by using potent β-hydroxy-β-methyl-glutaryl-CoA reductase inhibitors like Flu-, Lo-, Pra-, Sim-vastatin, the so-called "statins") which block cellular cholesterol synthesis), contributes to the reduction in plasma LDL levels and to attenuation of atherosclerosis. The hypocholesterolaemic effect of statins is exerted mainly in liver cells, which can excrete excess cholesterol as bile acids, and thus prevents cellular cholesterol accumulation. In contrast, enhanced lipoprotein uptake by cells of the arterial wall, and mainly the uptake of oxidized LDL via the macrophage scavenger receptor can contribute to foam cell formation, the hallmark of early atherosclerosis (1, 2, 8, 14). Most of the cholesterol in these cells is derived from plasma LDL. Native LDL however must first become modified (22), in order to cause extensive macrophage cholesterol accumulation. Chemical, as well as enzymatic modifications of LDL have been demonstrated (22–31).

We have previously shown that cells of the arterial wall can modify LDL via the action of several lipases, including lipoprotein lipase (25, 26), cholesterol esterase (27), and phospholipase D (28), C (18, 19) and A2 (29) (tab. 1). In addition, blood platelets are also able to modify the LDL molecule (32, 33). Atherosclerosis involves LDL oxidation and platelet activation (fig. 1, A and 1, B) and both of these processes can affect each other. Platelet activation is increased by oxidative stress (4), and oxidized LDL was shown (4) to enhance platelet activation (fig. 1, C). Activated platelets, in turn can increase LDL oxidizability (fig. 1, D).

Oxidative modification of LDL was shown to result in enhanced uptake of the modified lipoprotein via the macrophage scavenger receptor. Of interest is that angiotensin II can also modify LDL to a non-oxidized form, which is taken up by macrophages via the scavenger receptor at an enhanced rate (34).

Oxidized LDL, in addition to its contribution to macrophage cholesterol accumulation, possesses several other atherogenic properties which can also contribute to the development of the atherosclerotic lesion (tab. 2).

**Oxidized LDL-Induced Macrophage Cholesterol Accumulation**

Cholesterol in the atherosclerotic lesion accumulates both as cholesteryl ester droplets and as unesterified cholesterol (35). This effect may be secondary to the uptake of oxidized LDL via scavenger receptors (36–40). Previous studies have demonstrated that heparan sulphate proteoglycans participate in the cellular uptake of LDL, in the presence of lipoprotein lipase (41). Recently, we analysed the contribution of the macrophage surface proteoglycans (heparan sulphate and chondroitin
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Fig. 1 Oxidized LDL, platelet activation and atherosclerosis. LDL oxidation (A) and platelet activation (B) are two key events in atherogenesis, which lead to the formation of the atherosclerotic lesion. These processes are interrelated, in that oxidized LDL can activate platelets (C), and activated platelets increase the susceptibility of LDL to oxidation (D).

Tab. 2 Atherogenicity of oxidized LDL.

1. Increase uptake by macrophages (foam cell formation).
2. Act as chemoattractant for circulating monocytes.
3. Inhibit the movement of tissue macrophages back to the circulation.
4. Act as chemoattractant for T-lymphocytes.
5. Is cytotoxic to cells of the arterial wall.
6. Alter gene expression of neighbouring cells (monocyte chemotactic protein 1 (MCP-1), colony stimulating factors (CSF)).
7. Is immunogenic and can elicit autoantibody formation.
8. Inhibit nitric oxide-stimulation of vasodilation.
10. Increase platelet activation.

Among the various atherogenic properties of oxidized LDL, macrophage cholesterol accumulation is of major importance to the development of atherosclerosis. Macrophage uptake of oxidized low density lipoprotein resulted in lysosomal accumulation of unesterified cholesterol (42-46). We have recently shown that 7-oxocholesterol, which is present in oxidized LDL, inhibits lysosomal sphingomyelinase, leading to lysosomal accumulation of sphingomyelin. The sphingomyelin, known for its high affinity to unesterified cholesterol, binds the unesterified cholesterol avidly and inhibits its further cellular processing outside the lysosome (47).

Macrophage-Mediated Oxidation of LDL

While oxidation of LDL almost certainly occurs within the arterial wall, it is not known with certainty which of the arterial cells are most important for LDL oxidation, nor to what extent other tissue sites contribute to LDL oxidation in vivo (48-50). The action of arterial wall cells on LDL can result in the formation of oxidized LDL, and this process depends on the activation of cellular oxygenases. Arterial wall macrophages were shown to accumulate oxidized lipids in areas of the atherosclerotic lesion (51) and these cells can oxidize LDL in the vascular interstitial space. Macrophage-mediated oxidation of LDL is probably a key event in early atherosclerosis and it was recently shown in some types of macrophages and under certain oxidative conditions to require LDL binding to the cellular LDL receptor.
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Polyphenols
Antioxidants (glutathione)
Oxygenases, (NADPH oxidase, lipoxygenase)

Macrophage
Oxidized LDL in atherosclerosis

LDL oxidation by macrophages is affected by both the LDL-associated antioxidants (vitamin E, carotenoids, polyphenols), and by the balance (→) between cellular antioxidants [such as glutathione (GSH)] and cellular oxygenases [such as NADPH oxidase and lipoxygenases].

(52). The susceptibility of LDL to oxidation by cells of the arterial wall depends both on the activities of cellular oxygenases and on the cellular antioxidants (fig. 2).

Cellular oxygenases

LDL oxidation by arterial wall cells was suggested to involve the activation of 15-lipoxygenase and that of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (53—55). We sought to identify the role of these oxygenases in macrophage-mediated oxidation of LDL under oxidative stress. When LDL was incubated for six minutes with J-774 A.1 macrophage-like cell line or with human monocyte-derived macrophages, in the presence of 1 μmol/l CuSO₄, Superoxide anions were released into the medium. Under these conditions, the cytosolic protein components of the NADPH oxidase complex, P-47 and P-67, translocated to the plasma membrane, indicating LDL-mediated activation of the NADPH oxidase complex. Under the above mentioned experimental conditions, the macrophage 15-lipoxygenase was also activated as shown by the release of 15-hydroxy-5,8,11,13 eicosatetraenoic acid (15-HETE) and of 13-hydroxy-octadecadienoic acid (13-HODE) into the medium. Inhibition of the macrophage NADPH oxidase with apocynin, or dismutation of the Superoxide anions with Superoxide dismutase, significantly inhibited macrophage-mediated oxidation of LDL (by 61—89%) under the above conditions. Phorbol myristate acetate caused NADPH oxidase activation in J-774 A.1 macrophages with no significant effect on 15-lipoxygenase activity, but still resulted in cell-mediated oxidation of LDL. Finally, monocyte-derived macrophages from two patients with chronic granulomatous disease, which were shown to lack active NADPH oxidase, but to possess normal 15-lipoxygenase activity, failed to oxidize LDL (56). We thus conclude that LDL-induced NADPH oxidase activation (under oxidative stress) is required for macrophage-mediated oxidation of LDL, whereas activation of 15-lipoxygenase may not be sufficient for LDL oxidation.

Cell membrane lipid peroxidation

While the lipid peroxidation of LDL is under intensive investigation, little is known about lipid peroxidation of cells in the arterial wall. Macrophage lipid peroxidation (induced by iron ions, by angiotensin II or by deoxycholate) can contribute to cell-mediated oxidation of LDL. When cultured macrophages were exposed to ferrous ions (50 μmol/l FeSO₄) for 4 h at 37 °C, cellular lipid peroxidation [measured by analyses of malondialdehyde, conjugated dienes, and lipid peroxides], increased 2—4 fold in comparison with non-treated cells. Incubation of LDL with the oxidized macrophages resulted in LDL lipid peroxidation, as evidenced by an 8-fold increase in the LDL-associated malondialdehyde, in comparison with LDL that was incubated under similar conditions with non-oxidized cells. Incubation of LDL with the oxidized macrophages resulted in LDL lipid peroxidation, as evidenced by an 8-fold increase in the LDL-associated malondialdehyde, in comparison with LDL that was incubated under similar conditions with non-oxidized macrophages. Incubation of LDL for 24 hours at 37 °C with either deoxycholic acid or with angiotensin II, or with FeSO₄, induced a significant macrophage lipid peroxidation. Upon incubation of these oxidized cells with LDL, the lipoprotein was oxidized, as evidenced by increased malondialdehyde formation (by 50%, 105%, and 257%, respectively) and by increased macrophage uptake of the lipoprotein (by 60%, 166% and 230%, respectively). A positive correlation (r = 0.88) was found between the extent of the cellular peroxidation and the increment in the cellular uptake of the lipoprotein.

Since macrophage-conditioned medium from the oxidized cells (but not from non-oxidized cells) induced a

Fig. 2 Lipoproteins and antioxidants.
LDL oxidation by macrophages is affected by both the LDL-associated antioxidants (vitamin E, carotenoids, polyphenols), and by the balance (→) between cellular antioxidants (such as glutathione (GSH)) and cellular oxygenases (such as NADPH oxidase and lipoxygenases).

Oxidized LDL but not native LDL lead to the conversion of macrophages to cholesterol [cholesteryl ester and unesterified cholesterol]-loaded foam cells.
significant LDL oxidation, it is concluded that oxidative modification of LDL by oxidized macrophages can be induced by cellular factors secreted into the medium. These results suggest that oxidized lipids released from lipid-peroxidized macrophages can interact with LDL, leading to its subsequent oxidation (57–60).

**Cellular antioxidants**

The susceptibility of the vascular wall to oxidative stress is also influenced by the balance between the activities of cellular oxygenases and the integrity of the cellular antioxidants machinery (fig. 2). We have recently studied the possible role of the macrophage glutathione system in LDL oxidation by modulating the cellular glutathione content. Incubation of J-774 A.1 macrophages with bothionine sulphoximine, a glutamate analogue that inhibits glutathione synthesis (by the inhibition of γ-glutamyl cysteine synthetase), caused a substantial decrease (8-fold) in cellular glutathione content. In parallel, the ability of these cells to oxidatively modify LDL increased by 3-fold. Thus, a reduced content of macrophage glutathione contributes to enhanced cell-mediated oxidation of LDL. This phenomenon was found to be associated with increased production of superoxide anions by the glutathione-depleted cells.

In contrast, when the macrophage glutathione content was increased (1.6 fold) by exposing the cells to 2-oxo-4-thiazolidine carboxylic acid (a 5-oxoproline analogue that is converted by 5-oxoprolinase to 5-carboxy cysteine which is a source for cellular glutathione), cell-mediated oxidation of LDL was reduced by 44%. Similar results were obtained with selenium-treated macrophages in tissue cultures and in mice. Thus, these results suggest that the glutathione system plays a major role in the protection of cells against oxidative stress in humans.

**Oxidized LDL in the Atherosclerotic Lesion**

Macrophage foam cell formation involves the transport of both LDL and monocytes into the arterial wall, followed by LDL modification(s) by arterial wall cells including macrophages (fig. 3). Secondary to this process, the oxidative modification of LDL in the arterial wall can lead to acceleration of the atherosclerotic process.

The involvement of oxidized LDL in atherosclerosis is suggested from its presence in the atherosclerotic lesion (51, 61–63). We studied modifications of LDL derived from lesions that were obtained from humans endarterectomies, as well as from apolipoprotein E-deficient (E°) transgenic mice. In apolipoprotein E-deficient mice, the absence of plasma apolipoprotein E is associated with high plasma LDL (and VLDL) cholesterol concentrations, and these mice develop atherosclerosis as early as at 6 months of age. In lesion LDL from atherosclerotic humans or from the apolipoprotein E-deficient mice, the concentration of lipoprotein-associated thiobarbituric acid-reactive substances was 3-fold higher than in plasma LDL (64). In addition, these lesion LDL contained aggregated particles. We found that plasma LDL, even in young, still non-atherosclerotic apolipoprotein E-deficient mice (6 weeks of age) was already oxidized, but it was not yet aggregated. At 6 months of age, plasma LDL oxidation increased by 40% and the extent of plasma LDL aggregation also progressively increased with age. In contrast to plasma LDL, lesion LDL was already oxidized and aggregated in the young apolipoprotein E-deficient mice. During aging of the apolipoprotein E-deficient mice, atherosclerosis progressively increased, paralleled by the increment in the oxidation and aggregation rates of lesion LDL. In the apolipoprotein E-deficient mice, macrophage proteoglycans may play a role in the binding and uptake of oxidized LDL. Proteoglycans can form complexes with...
Increased LDL Oxidizability in Atherosclerotic Patients

Increased susceptibility to oxidation of LDL from patients with increased risk for atherosclerosis (hypertensives, hypercholesterolaemics, diabetics, patients with renal failure, or patients with xanthelasma), and from the atherosclerotic apolipoprotein E-deficient transgenic mice was demonstrated, further suggesting the importance of LDL oxidation in atherosclerosis. Increased risk for atherosclerosis was shown not only in patients with increased plasma LDL concentration (fig. 4, I), but also in normocholesterolaemic subjects. This may result from either reduced plasma HDL cholesterol levels (Fig. 4, II) or from the presence of LDL which is highly susceptible to oxidation (fig. 4, III). Drug therapy aimed at each specific disease ( -hydroxy- -methyl-glutaryl-CoA reductase inhibitors (so-called “statins”) in hypercholesterolaemia and angiotensin II converting enzyme (ACE) inhibitors in hypertension), resulted in a substantial reduction in LDL oxidation (table 3, (66-74)). The effect of lovastatin therapy (20 mg/d for 8 weeks) on the susceptibility of LDL to oxidation was studied in patients with hypercholesterolaemia. In all patients, oxidized LDL prepared from LDL obtained during lovastatin treatment, showed reduced susceptibility to lipid peroxidation, an increased fluidity, and an impaired uptake by macrophages. Even more impressive was the effect of fluvastatin on the susceptibility of LDL to oxidation. Fluvastatin, administered at low concentration (1 μmol/l) to 10 hypercholesterolaemic patients, was found to be a very potent inhibitor of LDL oxidation.
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(induced by CuSO₄, as well as by the free radical generator 2,2'-azobis (2-amidinopropane) hydrochloride (AAPH). As β-hydroxy-β-methyl-glutaryl-CoA reductase inhibitors (so-called “statins”) increase LDL receptor activity in the liver, it is suggested that inhibitor-mediated removal of “aged LDL” (which is more prone to oxidation than “new LDL”) from the circulation of hypercholesterolaemic patients, is responsible for the inhibitory effect of β-hydroxy-β-methyl-glutaryl-CoA reductase inhibitors (so-called “statins”) on the susceptibility of the newly produced LDL to oxidation. The inhibitory effect of fluvastatin on LDL oxidation was also shown in vitro, and it did not involve chelation of copper ions, or free radical scavenging capacity (as determined by the inability of fluvastatin to reduce linoleic acid peroxidation). Binding of fluvastatin to the LDL surface, as shown for other so-called “statins” (68, 69, 75), can cause structural changes in the lipoprotein surface, and this changes can affect subsequent LDL oxidation. In hypertensive patients, angiotensin converting enzyme inhibitor therapy significantly reduced the oxidizability of their LDL (71).

Recently, in apolipoprotein E deficient mice, we have also found that administration of captopril or fosinopril, as well as vitamins E and C, significantly reduced the susceptibility of LDL to oxidation and also attenuated the progression of the atherosclerotic lesion. As the atherosclerotic lesion in these mice is very similar to that found in humans, these results hold promise for the possible attenuation of atherosclerosis also in humans (tab. 3).

Antioxidants against LDL Oxidation and Atherosclerosis

Any means of intervention that favorably affects the balance between the activity of the macrophage oxygenases and cellular antioxidants, as well as elevation of LDL-associated intrinsic antioxidants or extrinsic antioxidants in plasma or extracellular space, can possibly contribute to the inhibition of foam cell formation and the atherosclerotic process (76, 77). Recently, we analysed the effect of dietary selenium (found as selenocysteine in glutathione peroxidase) on cellular glutathione content, concentration, 200 mg/l) incubation at 37 °C in the presence of 10 μmol/l CuSO₄.

Control values given as malondialdehyde were 29 ± 11 nmol/mg LDL protein.

Tab. 4 Antioxidative properties against LDL oxidation of various nutritional antioxidants in vivo (in humans) and in vitro.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Duration (weeks)</th>
<th>Dose</th>
<th>LDL enrichment with active component (fraction of control)</th>
<th>LDL oxidation (fraction of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. In vivo dietary supplementation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Olive oil</td>
<td>2</td>
<td>50 g/d</td>
<td>1.64 ± 0.10 (oleic acid)</td>
<td>0.68 ± 0.07 †</td>
</tr>
<tr>
<td>2. Red wine, 11% alcohol</td>
<td>2</td>
<td>400 ml/d</td>
<td>3.80 ± 0.45 (polyphenols)</td>
<td>0.54 ± 0.07 †</td>
</tr>
<tr>
<td>3. White wine, 11% alcohol</td>
<td>2</td>
<td>400 ml/d</td>
<td>1.10 ± 0.12 (polyphenols)</td>
<td>1.15 ± 0.21 †</td>
</tr>
<tr>
<td>4. Licorice ethanolic extract</td>
<td>2</td>
<td>100 mg/d</td>
<td>1.15 ± 0.14 (glabridin)</td>
<td>0.65 ± 0.10 †</td>
</tr>
<tr>
<td>5. Selenium (Renal transplanted patients)</td>
<td>12</td>
<td>0.2 mg/d</td>
<td>1.30 ± 0.09 (glutathione in erythrocytes)</td>
<td>0.69 ± 0.11 †</td>
</tr>
<tr>
<td>6. β-Carotene, synthetic, all-trans</td>
<td>2</td>
<td>180 mg/d</td>
<td>1.58 ± 0.20 (β-carotene)</td>
<td>0.85 ± 0.08 †</td>
</tr>
<tr>
<td>7. β-Carotene, natural, Dunaliella Bardawill</td>
<td>2</td>
<td>180 mg/d</td>
<td>1.29 ± 0.11 (β-carotene)</td>
<td>0.80 ± 0.11 †</td>
</tr>
<tr>
<td>8. β-Carotene, Dunaliella, (in diabetic patients)</td>
<td>3</td>
<td>60 mg/d</td>
<td>1.32 ± 0.10 (β-carotene)</td>
<td>0.75 ± 0.07 †</td>
</tr>
<tr>
<td>9. Lycopene (tomato’s oleoresin)</td>
<td>4</td>
<td>20 mg/d</td>
<td>1.44 ± 0.10 (lycopene)</td>
<td>0.78 ± 0.07 †</td>
</tr>
<tr>
<td><strong>B. In vitro addition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Oleic acid</td>
<td></td>
<td>100 μmol/l</td>
<td>0.38 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>2. Sitosterol</td>
<td></td>
<td>100 μmol/l</td>
<td>0.35 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>3. Red wine</td>
<td></td>
<td>1 mg/l</td>
<td>0.35 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>4. White wine</td>
<td></td>
<td>1 mg/l</td>
<td>0.90 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>5. Licorice extract</td>
<td></td>
<td>1 mg/l</td>
<td>0.40 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>6. Licorice glabridine</td>
<td></td>
<td>1 mg/l</td>
<td>0.42 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>7. β-Carotene, synthetic</td>
<td></td>
<td>10 mg/l</td>
<td>0.68 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>8. β-Carotene, natural</td>
<td></td>
<td>10 mg/l</td>
<td>0.75 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>9. Lycopene</td>
<td></td>
<td>10 mg/l</td>
<td>0.68 ± 0.07</td>
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<tr>
<td>10. Vitamin E</td>
<td></td>
<td>1 mg/l</td>
<td>0.87 ± 0.07</td>
<td></td>
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<tr>
<td>11. Vitamin C</td>
<td></td>
<td>1 mg/l</td>
<td>0.75 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>12. Butylated hydroxytoluene</td>
<td></td>
<td>10 mg/l</td>
<td>0.31 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>13. Deinococcus radiophilus bacteria</td>
<td></td>
<td>10 mg/l</td>
<td>0.55 ± 0.10</td>
<td></td>
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</table>

Control LDL represents lipoprotein derived prior to the dietary administration of the food additives (A. in vivo studies) or LDL incubated with no additions (B. in vitro studies).

LDL oxidation was measured after 4 h of lipoprotein (LDL protein concentration, 200 mg/l) incubation at 37 °C in the presence of 10 μmol/l CuSO₄. Control values given as malondialdehyde were 29 ± 11 nmol/mg LDL protein.
LDL oxidizability, and the development of atherosclerosis. In renal transplanted patients (which are under oxidative stress, and thus at high risk for atherosclerosis), dietary selenium (0.2 mg/d for 3 months) resulted in the elevation of red blood cell glutathione peroxidase and glutathione reductase activities, as well as in a 30% elevation in total glutathione content. In parallel, this selenium treatment resulted in a 50% reduction in 2’-azobis (2-aminodipropane) hydrochloride-induced plasma lipid peroxidation, and also in a 20% reduction in CuSO₄-induced LDL oxidation. Similarly, in the atherosclerotic apolipoprotein-E deficient transgenic mice, dietary selenium resulted in a 74% reduction in the resistance of LDL to oxidation, paralleled by a 30% reduction in the size of their atherosclerotic lesion.

In addition to cellular antioxidants, such as the glutathione system, LDL substrates which are prone to oxidation (polyunsaturated fatty acids and cholesterol) and the antioxidants in the LDL environment (intrinsic constituents such as vitamin E, β-carotene, lycopene and polyphenols, and extrinsic components such as vitamin C), also affect LDL oxidation (tab. 4). We have recently demonstrated that the oleic acid and polyphenols in olive oil, as well as the polyphenols in red wine and licorice (Glycyrrhiza glabra) root extract (78—85) act as antioxidants of LDL oxidation. In addition, the synthetic and the Dunaliella β-carotene (79—81) as well as tomatos’ lycopene possess antioxidative properties, though to a limited extent and probably only in certain oxidative systems, such as those producing singlet oxygen radicals and only in LDL samples with high vitamin E content.

The antiatherosclerotic potency of carotenoids however is questionable and more studies are required in groups of patients with cardiovascular diseases (86, 87). Flavonoids are present in some fruits and vegetables and their consumption has been shown to be inversely associated with atherosclerosis (88—90). Several flavonoids were shown to be very potent antioxidants against LDL oxidation (79—85).

Vitamin E is probably antiatherogenit in atherosclerotic patients but the data concerning primary prevention are still controversial (91—93). Not only dietary antioxidants, but also high density lipoprotein (HDL) can contribute to the attenuation of LDL oxidation and this phenomenon involves the antioxidative effect of apolipoprotein A-I against LDL oxidation (94). We conclude that several mechanisms associated with oxidative stress on cells of the arterial wall can contribute to LDL oxidation during early atherosclerosis (95). Strategies to reduce LDL oxidation thus involve the use of various antioxidants which act on the cells and/or on the LDL molecules (tab. 5).

Tab. 5 Strategies to reduce LDL oxidation.

1. Reduce cellular oxygenases (NADPH oxidase, lipoxygenase).
2. Increase cellular antioxidants (glutathione, superoxide dismutase, catalase).
3. Enrich LDL with monounsaturated fatty acids.
4. Enrich LDL environment with antioxidants (vitamin E, vitamin C, β-carotene, lycopene, polyphenols, vitamin C).
5. Use transition metal ion chelators.
6. Use angiotensin converting enzyme inhibitors.

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