Biotechnol. Bioprocess Eng. **2000**, 5: 313-319

Low Density Lipoprotein (LDL), Atherosclerosis and Antioxidants

Beung Ho Ryu*

Department of Food Science and Biotechnology, Kyungsung University, Busan 608-736, Korea

Abstract A crucial and causative role in the pathogenesis of atherosclerosis is believed to be the oxidative modification of low density lipoprotein (LDL). The oxidation of LDL involves released free radical driven lipid peroxidation. Several lines of evidence support the role of oxidized LDL in atherogenesis. Epidemiologic studies have demonstrated an association between an increased intake of dietary antioxidant vitamins, such as vitamin E and vitamin C and reduced morbidity and mortality from coronary artery diseases. It is thus hypothesized that dietary antioxidants may help prevent the development and progression of atherosclerosis. The oxidation of LDL has been shown to be reduced by antioxidants, and, in animal models, improved antioxidants may offer possibilities for the prevention of atherosclerosis. The results of several on going long randomized intervention trials will provide valuahle information on the efficacy and safety of improved antioxidants in the prevention of atherosclerosis. This review a evaluates current literature involving antioxidants and vascular disease, with a particular focus on the potential mechanisms.

Keywords: low density lipoprotein (LDL), atherosclerosis, antioxidant

INTRODUCTION

An increased concentration of plasma low density lipoprotein (LDL) cholesterol constitutes a major risk factor for atherosclerosis. Epidemiological, clinical, and genetic studies convincingly demonstrate that LDL promotes atherosclerosis [1,2]. However, the precise mechanism(s) by which LDL promotes the development of the early fatty-streak lesion still remains to be elucidated. Several lines of evidence support the role of oxidatively modified LDL in atherosclerosis and its *in vivo* existance. The mechanisms for LDL oxidation have been previously explored as a result, explored the dozens of products of this oxidation have been partially identified, along with the description of numerous effects of oxidized LDL on cultured cells [3,4]. Among the latter are a number of cell-altering phenomena that appear to be atherogenic in nature and do not appear duplicable by exposing cells to native LDL. Therefore, even based on *in vitro* studies alone, it can be speculated that LDL oxidation may well play a causal role in atherogenesis and even in later lesion development [3-5].

Epidemiologic studies have demonstrated an association between an increased intake of antioxidant vitamins, such as vitamin E and C, and reduced morbidity and mortality from coronary artery disease [6]. This association has been explained on the basis of the "oxidative-modification hypothesis" of atherosclerosis, which proposes that atherogenesis is initiated by the oxidation of the lipids in low-density lipoprotein (LDL), also termed lipid peroxidation. As a corollary to this hypothesis, the antioxidants that inhibit lipid peroxidation in LDL should also limit atherosclerosis and its clinical manifestations, such as myocardial infarction and strokes [7]. Recent studies demonstrating the congruence of the actions of oxidized LDL cells with the known features of atherosclerotic lesions plus the evidence that LDL in lesions is oxidized strongly suggest that antioxidant administration retards the progression of atherosclerosis [8]. Although many antioxidants are known to have other actions that could explain their antiatherosclerotic activities, decreased atherosclerosis has been observed with a wide variety of antioxidants [9,10]. Nevertheless, the available data on antioxidants remains unconvincing and whether oxidized LDL is the real perpetrator of atherosclerosis stil needs to be confirmed. To date, researchers have established that oxidized lipoproteins reside in atherosclerotic lesions and that antioxidant data *in vivo* and culture cell function data indirectly support the hypothesis that oxidized LDL is a mediator of the disease [8,9].

The focus of previous literature on oxidized lipoproteins has centered on LDL because it serves as the principal carrier of cholesterol in normal human plasma and because of the strong correlations between elevated plasma LDL or cholesterol levels and increased atherosclerosis risk.

OXIDATIVE MODIFICATION OF LDL

Human LDL is defined as the population of lipopro-

*Corresponding author
Tel: +82-51-620-4712 Fax: +82-51-622-4986
e-mail: bhryu@star.kyungsung.ac.kr

teins that can be isolated by ultracentrifugation within a density range of 1.019-1.063 kg/L [11]. Each LDL particle contains approximately 1,600 molecules of cholesteryl ester and 170 molecules of triglycerides, which form a central lipophilic core. This core is then surrounded by a monolayer which is consists of about 700 phospholipid molecules, consisting mainly of lecithin and small amounts of sphingomyelin and lysolecithin and 600 molecules of free cholesterol. Embedded materials in the outer layer is a large protein, apolipoprotein (apo) B-100, consisting of 4,536 amino acid residues. The total number of fatty acids bound in different classes of an LDL molecule is approximately 2,700, half of these being polyunsaturated fatty acids (PUFAs), mainly linoleic acid. Variations in the PUFAs content contribute to the difference in the oxidation behavior between different LDL. The PUFAs in LDL have protection against free radical damage by several antioxidants, the predominant one being α-tocopherol.

The oxidation of LDL is a free radical-mediated process, resulting in the numerous structural changes, all of which depend on a common initiating event, the peroxidation of the PUFAs in LDL. The oxidation of a LDL, as shown in Fig. 1, is initiated by reactive oxygen species that abstract a hydrogen from a double bond in a PUFA, which is followed by a molecular rearrangement, leading to the formation of conjugated double bonds, referred to as conjugated dienes [12]. During this initiation phase of LDL oxidation, the rate of oxidation is decreased by the presence of endogenous antioxidants within the LDL particle, which results in the lag phase of oxidation. The lag phase is followed by a rapid propagation phase, which occurs when the antioxidants are depleted and involves the abstraction of another protium hydrogen by a PUFA-peroxyl radical (LOO) from another PUFA, thereby resulting in the formation of lipid peroxides. A typical time course of copper-catalyzed LDL oxidation, depicting both lag and propagation phases, and including measures of both lipid and protein oxidation, is shown in Fig. 2. The cholesterol in LDL can be oxidized to oxysterols, such as 7-keto-cholesterol. The propagation phase is followed by the decomposition of the degradation phase, during which there is cleavage of the double bonds, resulting in the formation of aldehydes. The major aldehydes produced include malondialdehyde (MDA), 4-hydroxyno-nenal (HNE), and hexanal, which can cross-link with amino groups on apo B-100 [12]. The oxidation of LDL can be initiated by a number of different mechanisms. However, the cell-mediated oxidation of LDL would appear to be the most relevant to understanding LDL oxidation *in vivo*. In a culture, all those cells normally present in the artery wall, including endothelial cells, smooth muscle cells, macrophages, and lymphocytes can oxidize LDL [12]. The mechanisms by which cells initiate the oxidation of LDL are still poorly defined. Since cells can produce reactive oxygen species and secrete thiols into a medium they may therefore participate, either directly or indirectly, in the initiation of LDL oxidation [13]. Cell-mediated LDL oxidation *in vitro* in most

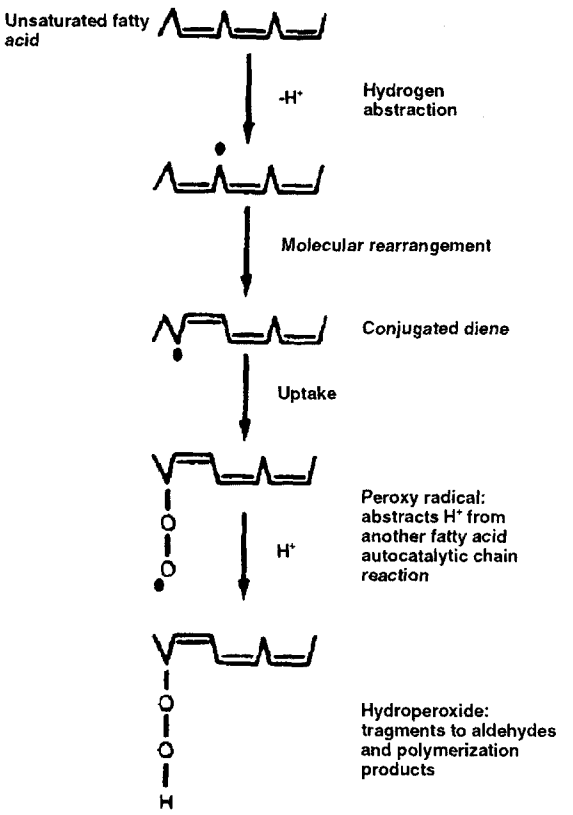

Fig. 1. Peroxidation of PUFA [12].

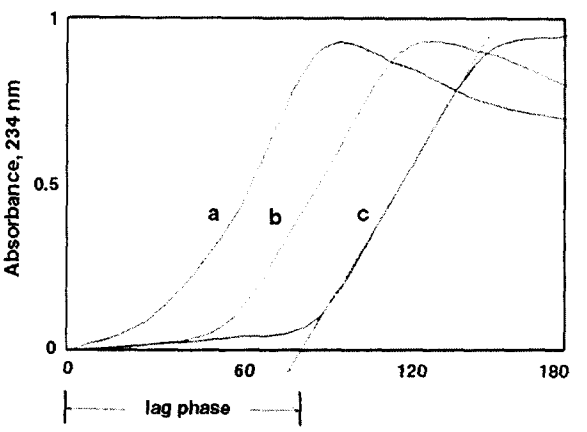

Fig. 2. Determination of oxidation resistance of LDL samples. To LDL solutions in phosphate buffered saline (0.25 mg total LDL/mL) in 1 cm quartz cuvettes, $CuCl_2$ was added to a final concentration of 1.67 μM Cu^{2+}. The oxidation was followed by recording the 234 nm absorption in intervals of 1 min using an UV-spectrophotometer with automatic cuvette changing. The oxidation resistance was defined as the length of the lag-phase, which was determined as the intercept of the tangent at the first turning point. The curves represent LDL samples from three different donors(measured in parallel), and the lag-phases were 43, 69, and 90 min for donor a, b, and c respectively.

Table 1. Mechanisms through which oxidized LDL may be atherogenic

1	It has an enhanced uptake by macrophages leading to cholesteryl ester enrichment and foam cell formation.
2	It is chemotactic for circulating monocytes and T-lymphocytes.
3	It inhibits the motility of tissue macrophages.
4	It is cytotoxic.
5	It renders LDL more susceptible to aggregation, which leads to an enhanced macrophage uptake.
6	It can alter the gene expression of neighboring arterial cells such as the induction of MCP-1, colony-stimulating factors, IL-1, and endothelial expression of adhesion molecules.
7	It can adversely alter coagulation pathways, for example, by the induction of a tissue factor.
8	It can adversely alter the vasomotor properties of the coronary arteries.
9	It is immunogenic and can elicit autoantibody formation and reactive T-cells.

cases requires the presence of transition metals to facilitate the decomposition of the preformed lipoperoxides to more reactive peroxy radicals [14]. Accordingly the removal of transition metals, such as iron or copper, by the addition of chelators, such as EDTA, will inhibit LDL oxidation. It has not yet been demonstrated whether there is normally sufficient unbound copper or iron in the artery wall to allow oxidation to proceed. However, in areas of inflammation or injury, when the tissue pH is lower, the release of iron from various binding proteins undoubtedly occurs. The mechanisms of oxidation most relevant to *in vivo* oxidation are still unknown and under active investigation.

OXIDIZED LDL AND ATHEROSCLEROSIS

Atherosclerosis is a major cause of morbidity and mortality in the Western world. According to the oxidative-modification hypothesis, LDL initially accumulates in the extracellular subendothelial space of arteries and, through the action of resident vascular cells, is mildly oxidized to a form known as minimally modified LDL (Table 1) [3,4]. This minimally modified LDL induces local vascular cells to produce monocyte chemotactic protein, granulocyte and macrophage colony-stimulating factors, which stimulate monocyte recruitment and differentiation to macrophages in the arterial walls (Fig. 3). The accumulating monocytes and macrophages then stimulate further peroxidation of LDL [15]. The products of this reaction make the protein component of LDL (apolipoprotein B-100) more negatively charged. By virtue of its increased negative charge, this completely oxidized LDL is recognized by scavenger receptors on macrophages and internalized to form so-called foam cells [4]. In contrast to the uptake of native (unoxidized) LDL by the LDL (apolipoproteins B) receptor on macrophages, the uptake of oxidized LDL by the scavenger-receptor pathway is not subject to negative-feedback regulation and thus results in a massive uptake of cholesterol (from oxidized LDL) by the macrophages [3,5]. In addition to promoting the formation of foam cells, oxidized LDL includes direct chemotactic activity for monocytes and stimulates the binding of monocytes to the endothelium. Once monocytes cross the endothelial layer, they become trapped in the subendothelial space, partly because oxidized LDL inhibits their progress from the arterial wall. Oxidized LDL is also cytotoxic to vascular cells, thereby promoting the release of lipids and lysosomal enzymes into the intimal extracellular space and enhancing the progression of atherosclerotic lesions (Fig. 4) [16].

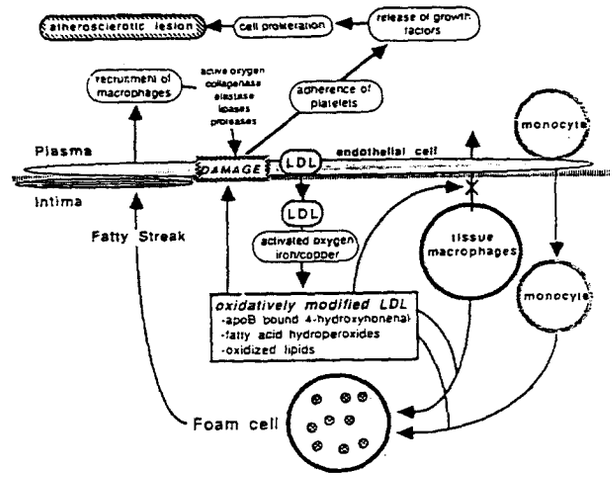

Fig. 3. Free radicals and atherosclerosis. In addition to cholesterol, LDLs contain proteins and polyunsaturated fatty acids. The oxidation of these fatty acids and modification of the apoB protein make LDLs highly susceptible to phagocytosis by tissue macrophages and monocytes. These phagocytic cells adhere to the endothelium and then penetrate into subendothelial spaces, perhaps in response to chemotactic factors including oxidized LDLs. The presence of large amounts of lipid within macrophages and monocytes yields foam cells. which appear to be necessary to generate fatty streaks in the vessel wall. These fatty streaks recruit additional macrophages that can damage endothelial cells, releasing additional factors. The net result of these reactions is the eventual production of an atherosclerotic lesion [16].

Investigators have also shown *in vitro* that oxidized LDL is toxic to a variety of cell types [17,18]. Several soluble polar sterols formed during oxidation have been implicated in this cytotoxicity [18]. As macrophages and other artery wall cells oxidize LDL and in turn internalize or are exposed to these toxic oxidation products they may also induce their own death [19]. These dying macrophages may then release toxic products that can damage and perhaps contribute to the disruption of the artery wall structure and rupture of the overlying intact endothelium. Damage to the endothelium can also have several direct atherogenic and

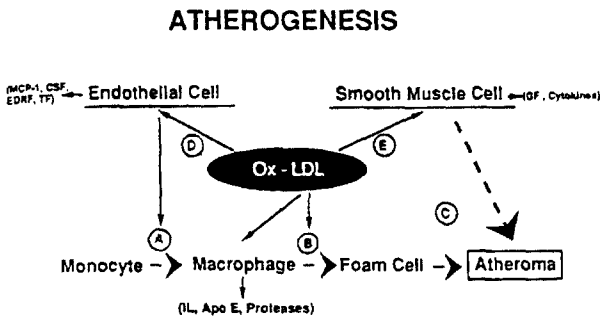

Fig. 4. Some roles of oxidized LDL in atherogenesis [15]. (A) The conversion of blood manocytes into macrophages involves the effect of Ox-LDL on the production and secretion of endothelial cell derived factors. MCP-1, monocyte chemoattractant protein 1; CSF, colony stimulating factor, EDRF, endothelium derived relaxation factor, TF, tissue factor; (B) Ox-LDL-mediated macrophage cholesterol accumulation and foam cell formation. Macrophages release interleukins (IL), apolipoprotein E (apoE) and proteases; (C) The formation of atheroma involves smooth muscle cell proliferation and the secretion of growth factors (GF) and cytokines.

Table 2. Sources of dietary antioxidants

Antioxidant	Sources
Vitamin E (tocopherols, tocotrienols)	Vegetable oils, nuts, whole grains (germ), other seeds, sweetpotatoes, butter, liver, egg yolk and some fruits/vegetables
Carotenes (>600 compounds)	Orange fruits and vegetables, spinach, broccoli, green beans, peas and peppers
Ascorbic acid	Fresh fruits, cruciferous vegetables, potaoes and other vegetables
Flavonoids (>3000 compounds)	Colored fruit and vegetable skins, apples, citrus, onions, potatoes, other root and tuber skins and tea
Ubiquinone-10 (ubiquinol/ubiquinone)	Soybean oil, meats, sardines, mackerel, muts, wheat germ, beans, garlic, spinach and other vegetables
Selenium	Grains, meat, fish, some wild waterfowl, some garlic (content varies geographically)

thrombogenic effects [20]. LDL extracted from human atherosclerotic lesions, yet not plasma-derived LDL, resembles intermediated low density lipoprotein that has been oxidatively modified *in vitro*.

Thus, the oxidative modification of LDL would appear to have an important role in foam-cell formation and atherogenesis. This link between the oxidation of LDL and atherogenesis provides a convenient and simple rationale for the beneficial effect of antioxidants on the incidence of coronary artery disease. A number of clinical and animal studies have also explored this link between LDL oxidation and atherogenesis.

ATHEROSCLEROSIS AND DIETARY ANTIOXIDANTS

There is a wealth of epidemiologic data linking the dietary and supplemental intake of antioxidant vitamins with a reduction in the clinical manifestations of atherosclerosis. Initially, these data were limited to descriptive studies in European and North American populations [23]. However, subsequent case-control studies have indicated that patients with angina pectoris have lower plasma concentrations of vitamin E, and also reduced concentrations of vitamin C in the leukocytes are predictive of angiographically evident coronary artery disease (CAD) [21]. Although these reports would seem to provide evidence for a contributory role of lipid and lipoprotein oxidation in atherogenesis, further studies are still required to confirm this relationship. Epidemiologic studies do however suggest a relationship between CAD and dietary intake and/or serum levels of natural antioxidants such as vitamin C, vitamin E, and β-carotene. This was noted in a cross sectional study of heart disease risk factors among European countries with diverse rates of coronary heart diseases.

Numerous naturally occurring compounds found in foods possess antioxidant properties that may influence the development of atherogenesis (Table 2). Several descriptive studies have indicated that the consumption of fruit and vegetables, which are excellent sources of several antioxidants, is associated with reduced cardiovascular disease [22]. Furthermore, in a randomized trial, the consumption of a low fat diet enriched with fruit and vegetables compared to a standard low fat diet was associated with an apparent 40% reduction in cardiac events and 45% less mortality in 406 men with myocardial infarction [23]. These findings underscore the notion that fruit and vegetables contain many other compounds, in addition to common antioxidant vitamins, that can inhibit the development of atherosclerosis.

These results have been confirmed in recent prospective cohort studies. In the Nurses' Health Study [24] and Health Professionals' Follow-up Study [25], there was a 35 to 40 percent reduction in the incidence of major coronary events (nonfatal myocardial infarction and death from cardiac causes) among the subjects in the highest quontile of vitamin E intake over a four-to-eight-year follow-up period, when compared with those in the lowest quontile. The benefit was greatest in those subjects taking 100 to 250 IU of supplemental vitamin E per day, with little further benefit at higher doses. There was no relation between the vitamin C intake and major coronary events in either study, yet in another study, those subjects whose vitamin E intake exceeded 50 mg per day had a lower rate of death from all cardiovascular diseases [26].

The results of recent randomized trials to investigate

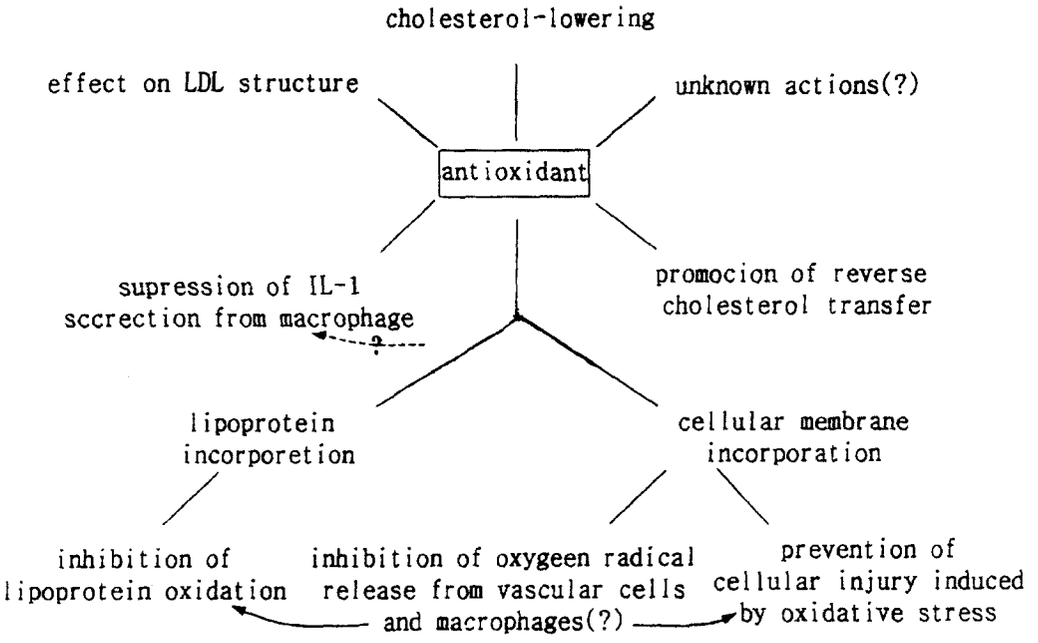

Fig. 5. Antioxidant and atherosclerosis.

whether there is a cause and effect relation between antioxidant intake and a reduction in coronary artery disease have been mixed. In the α-Tocopherol, β-Carotene, and Cancer Prevention Study, Finnish smokers were treated with beta carotene, α-tocopherol (vitamin E), both, or neither daily for five to eight years. There was no benefit with respect to coronary artery disease for either compound [29], however, the dose of α-tocopherol (50 mg per day) was below the protective range suggested by both the Nurses' Health Study and the Health Professionals' Follow-up Study [24,25]. There was no reduction in deaths from cardiovascular causes among physicians receiving supplemental β-carotene over a 12-year period in the Physicians' Health Study [26]. In contrast, in the Cambridge Heart Antioxidant Study, in which 2,002 patients with angiographically evident coronary artery disease were treated with α-tocopherol (400 to 800 IU per day) or a placebo, there was a 77 percent reduction in nonfatal myocardial infarction in the group receiving α-tocopherol during a median follow-up period of 510 days [27].

In summary, descriptive, case-control, and prospective cohort studies have all found inverse associations between the frequency of coronary artery disease and the dietary intake of antioxidant vitamins. Randomized therapeutic trials have thus far shown no benefit with β-carotene and possible benefit with vitamin E.

ATHEROSCLEROSIS AND ANTIOXIDANTS

With the increasing evidence that lipoprotein oxidation is fundamental to the development of atherosclerosis, there is a need to understand the factors which influence the susceptibility of LDL to oxidation.

Extrinsic factors refer primarily to cellular prooxidant activity, plasma and extracellular fluid pro-oxidants (trace metals), and antioxidants (*e.g.*, ascorbate, bilirubin, urate, HDL), as well as a variety of other factors which may increase the residence time of LDL in the intima. The inherent susceptibility of LDL particles to oxidation also undoubtedly plays an important role in its overall rate of oxidation. Similarly/Equally, the antioxidant content of the LDL particle itself must also play an important role in protecting it from oxidation. There are a number of antioxidant compounds, both natural and synthetic, that have been demonstrated *in vitro* to inhibit LDL oxidation [8,28].

Interrestingly, some of the same lipophilic antioxidants shown to inhibit atherosclerosis progression also appear to limit vascular regrowth after a balloon injury. Probucol [29], dibutyl hydroxytoluene (BHT) [30] and vitamin E [31] have each been shown to significantly reduce intimal thickening following a balloon injury of the arteries in cholesterol fed rabbits. Probucol was also reported to be of benefit after angioplasty in a limited human study [32]. Vitamins E and C together, yet neither alone, were able to limit luminal narrowing in porcine coronary arteries after a balloon injury, independent of any inhibition of intimal thickening [33]. In addition, desferal, a chelator of iron, has been shown to inhibit stenosis in a vascular injury model and to inhibit smooth muscle cell proliferation *in vitro* [34]. Ebselen [35], sylimarine [36] and sylibin [37], a compound that reduces lipid hydroperoxides, has been reported to limit restenosis after angioplasty in a small group of humans.

Plus lipophilic substances such as vitamin E and probucol would be expected to require a significant time to reach elevated antioxidant levels in tissue. Different approaches have been taken to examine the effects of antioxidants on atherosclerosis in humans. Among multi-national populations, the indices of cardiovascular mortality and ritamin consumption revealed a significant correlation between reduced cardiovascular disease and vitamin E consumption that was independent of other risk factors. The correlation apperared stronger than that with reduced plasma cholesterol. In the majority of studies linking antioxidants with decreased atherosclerosis in animals, an atherogenic diet and antioxidant therapy were introduced simultaneously or at a very early age. Such studies focus on the initiating events in atherogenesis, as such, the decrease in LDL oxidation by antioxidants could explain the reduction in the early formation of foam cells and plaque (Fig. 4).

Antioxidants may limit the clinical expression of coromary artery disease by causing the regression of and slowing the progression of coronary atherosclerotic lesions. Studies in humans and animals indicate that supplementation with lipid-soluble antioxidants increases the resistance of LDL to oxidation in *in vivo* assays. However, a precise cause-and-effect relation between the resistance of LDL to oxidation and the potential benefits of antioxidant therapy with respect to either atherosclerosis or its clinical expression has yet to be established.

CONCLUSION

It has become increasingly evident that oxidation of LDL is a key step in atherosclerosis. The observations rewiewed here strongly indicate that free radical process lipid peroxidation is involved in the pathogenesis of atherosclerosis through the formation of oxidized LDL. Various studies have been performed and analyzed using diverse criteria for atherosclerosis progression, from which certain comparisions and generalizations have been made. The data hawever invites speculation that lipophilic scavengers of lipid radicals that can associate with LDL and that interfere with the propagation of lipid peroxidation in lipoprotein particles appear to retard fatty streak formation.

There are a number of antioxidant compounds, both natural and synthetic, that have also been demonstrated *in vitro* to inhibit LDL oxidation. However, most supplementation studies, have focused on the lipid-solubed antioxidant naturally present in LDL

Epidemiological and *in vitro* evidence of antioxidant and atherosclerosis protective effects support the hypothesis that natural and synthetic antioxidative compounds benefit health. The inhibition of LDL oxidation by antioxidants suggests that regular consumption of foods containing vitamin E or C and moderate consumption of food may protect against atherosclersis. The data regarding the antiatherosclerosis effects of antioxidants is strong enough to warant continued research of a lipoprotein oxidation theory atherosclerosis. However, caution needs to be applied to avoid embracing the concept without proof.

In conclusion, these results show that, *in vitro*, antioxidants increase LDL resistance to oxidation by decreasing the consumption of vitamin E and this mechanism could contribute to protective effects against cardiovascular diseases linked to the high intake of antioxidants.

REFERENCES

[1] R. J. Havel, H. A. Eder, and J. H. Bragdon (1995) The distribution and chemical composition of ultracentrifuglly separated lipoproteins in human serum. *J. Clin. Invest.* 34: 1345-1535.

[2] Stenbrecher, U. P., H. Zhang, and M. Lougheed (1990) Role of oxidatively modified LDL in atherosclerosis. *Free Radic. Biol. Med.* 9: 155.

[3] Steinverg, D., S. Parthasarathy, T. E. Carew, J. C. Khoo, and J. L. Wiztum (1989) Beyond cholesterol: Modifications of low density lipoprotein that increases its atherogenicity. *N. Engl. J. Med.* 320: 915-924

[4] Wiztum, J. L. and D. Steinverg (1991) Role of oxidized low density lipoprotein in atherogenesis. *J. Clin. Invest.* 88: 1985-1972

[5] Steinvrecher, U. P., H. Zhang, and M. Lougheed (1990) Role of oxidatively modified LDL in atherosclerosis. *Free Radic. Bile. Med.* 9: 155-168.

[6] Renaud, D. and M. De Lorgiril (1992) Wine, alcohol, platelets, and the French Paradox for coronary heart disease. *Lancet* 339: 1523-1525.

[7] Yla-Hertuala, S., W. Palinski, M, E. Rosenfeld, S. Parhasrathy, T. E. Carew, S. Butler, J. L. Wiztum, and D. Steinberg (1989) Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J. Clin. Invest.* 84: 1086-1095.

[8] Esterbauer, H., J. Gebiki, H. Puhl, and G. Jurgens (1992) The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radic. Biol. Med.* 13: 341-390.

[9] Jiaial, I. and S. M. Grundy (1992) The effect of dietary supplementation with alpha-tocopherol in the oxidative modification of LDL. *J. Lipid Res.* 33: 889-906.

[10] Verlangieri, A. J., J. C. Kapeghian, S. el-Dean, and M. Bush (1985) Fruit and vegetable consumption and cardiovascular disease mortality. *Med. Hypotheses* 16: 7-15.

[11] Gey, K. F. and P. Puska (1989) Plasma vitamins E and A inversely correlated to mortality from ischemic heart disease in cross-cultural epidemiology. *Ann. NY Acad. Sci.* 570: 268-282.

[12] Sinclair, A. J., A. H. Barnett, and J. Lunec (1993) Free radicals and antioxidant systems in health and disease *Br. J. Hosp. Med.* 94: 350-356.

[13] Esterbauer, H., H. Puhl, M. Cieber-Rotheneder, G. Waeg, and H. Rabl (1991) Effect of antioxidants on oxidative modification of LDL. *Ann. Med.* 23: 573.

[14] Esterbauer, H., J. Gebicki, H. Puhl, and G. Jurgens (1992)

The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Rad. Biol. Med.* 13: 341-390.

[15] Chisolm G. M. (1991) Cytotoxidity of oxidized lipotroteins. *Curr Opin Lipidol.* 2: 215-232.

[16] Jaffe, E. A., R. L. Nachman, C. G., Becker, and C. R. Minick (1973) Culture of human endothelial cells derived from human umbilical rein. *J. Clin. Invest.* 27: 2745-2756.

[17] Yla-Hertuala, S., W. Palinski, D. Steinverg, D. Steinberg, and J. L. Wiztum, (1991) Rabbit and human atherosclerotic lesions contain IgG that recognizes MDA-LDL and copper-oxidized LDL. *Atherosclerosis* 11: 1426-1433.

[18] Avogaro, P., G. Bittolo-Bon, and F. Cazzolato (1998) Presence of a moditied low density lipoprotein in human. *Atherosclerosis* 8: 78-97.

[19] Henriksen T., S. A. Evensen, and B. Carlande (1979) Injury to human endothelial cells in culture induced by low density lipoproteins. *Scand. J. Clin. Lib. Invest.* 39: 361-370.

[20] Gey, K. D., P. Prska, P. Jordan, and U. K. Moser (1991) Inverse correlation between plasma vitamin E and Mortality from ischemic heart disease in cross-cultural epidemiology. *Am. J. Clin. Nutr.* 53: 5265-5272.

[21] Riemersas, R. A., D. A. Wood, C. C. A. Macintyre, R. A. Elton, K. F. Gey, and M. F. Oliver (1991) Risk of angina pectoris and plasma concentrations of vitamins A, C, and E and carotene. *Lancet* 337: 1-6.

[22] Trevor-ME, L., A. Chait, W. Raines, and R. Ross (1991) The influence of oxidatirely modified low density lipoproteins on expression of latelet-derived growth factor by human monocyte-derived macrophages. *J. Biol. Chem.* 226: 13901-13901.

[23] Singh, R. B., M. A. Niaz, S. Ghosh, and S. S. Rastogi (1993) Effect on mortality and reinfarction of adding fruits and vegetables to a prudent diet in the Indian experiment of infact Survival. *J. Am. Coll. Nutr.* 12: 225-261.

[24] Stampfer, M. J., C. H. Hennekens, J. E. Manson, G. A. Colditz, B. Rosner, and W. C. Willett (1993). Vitamin E consumption and the risk of coronary disease in women. *N. Engl. J. Med.* 328: 1444-1449.

[25] Rimm, E. B., M. J. Stampfer, A. Ascherio, E. Giovannucci, G. A. Colditz, and W. C. Willett (1993) Vitamin E consumption and the risk of coronary heart disease in men. *N. Engl. J. Med.* 328: 1450-1456.

[26] Losonczy, K. G., T. B. Harris, and R. J. Havlik (1996). Vitamin E and vitamin C supplement use and risk of all-cause and coronary beart disease mortality in older persons: The established populations for epidemiologic studies of the elderly. *Am. J. Clin. Nutr.* 64: 190-196.

[27] Stephens, N. G., A. Parsons, P. M. Schofield, F. Kelly, K. cheeseman, and M. J. Mitecenson (1996), Randomized controlled trial of vitamin E in patients with coronary disease: Cambridge heart antioxidant study (GHAO). *Lancet* 347: 781-786.

[28] Jialal, I. and C. Scaccini (1992) Antioxidants and atherosclerosis. *Curr. Opin. Lipidol.* 3: 324-328.

[29] Ferns, G. A. A., L. Forster, A. Stewart-Lee, M. Konneh, J. Nourooz-Zadeh, and E. E. Anggard (1992) Probucol inhibits neointimal thickening and macrophage accumulation after balloon injury in the cholesterol-fed rabbit. *Proc. Natl. Acad. Sci. USA* 89: 11312-11316.

[30] Freyschuss, A., A. Stiko-Rahm, J. Swedenborg, P. Henriksson, I. Bjorkhem, L. Berglund, and J. Nilsson (1993) Antioxidant treatment inhibits the development of intimal thickening after balloon injury of the aorta in hypercholesterolemic rabbits. *J. Clin. Invest.* 91: 1282-1288.

[31] Enstrom, J. E., L. E. Kanim, and M. A, Klein (1992) Vitamin C intake and mortality among a sample of the United States population. *Epidemiology* 3: 194-202.

[32] Lee, Y. J., H. Yamaguchi, H. Daida, H. Yokoi, J. Miyano, J. Takaya, H. Sakurai, and Noma (1991) A. PTCA: Pharmacological interventions to modify restenosis. *Circulation* 84: II-298 (abstr).

[33] Nunes, G. L., D. S. Sgoutas, S. R. Sigman, B. Britt, M. B. Gravanis, King III, and B. C. Berk (1993) Vitamins C and E improve the response to coronary balloon injury in the pig : Effect of vascular remodeling. *Circulation* 88: I-372 (abstr).

[34] Porreco, E., S. Ucchino, C. Di Febbo, N. Di Bartolomeo, D. Angelucci, A. M. Napolitano, A. Mezzetti, and F. Cuccutullo (1994) Antiproliferative effect of desferrioxamine on vascular smooth muscle cells *in vitro* and *in vivo*. *Atheroscler. Thromb.* 14: 299-304.

[35] Hirayama, A., S. Nanto, T. Ohara, K. Nishida, Y. Okuyama, and K. Kodama (1992) Preventive effect on restenosis after PTCA by Ebselen: A newly synthesized anti-inflammatory agent. *J. Am. Coll. Cardiol.* 19: 259 (Abstr).

[36] Lee, B. C. and B. H. Ryu (1997) Antioxidant of silymarin low density lipoprotein on macrophages. *Kor. J. Appl. Microbiol. Biotechnol.* 25: 286-292.

[37] Ryu, B. H., H. S. Kim, B. C. Lee, and H. S. Lee (1988) Antioxidative effect of lilybin purified *silybum marianum* on modification of human low density lipoprotein. *Korean J. Life Sci.* 8: 35-359.

[Received August 25, 2000; accepted October 6, 2000]