Effects of antioxidants and fatty acids on low-density-lipoprotein oxidation[1-3]

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ABSTRACT Evidence continues to accumulate that implicates the oxidative modification of low-density lipoprotein (LDL) in the pathogenesis of atherosclerosis. Numerous studies have indicated the existence of oxidized LDL in vivo. Supplementation of animals and humans with antioxidants such as α-tocopherol have shown promise in reducing the extent of LDL oxidation. However, another possible means of preventing LDL oxidative modification may be by reducing the amount of oxidizable polyunsaturated fatty acids in the LDL particle. Monounsaturated fatty acids have been shown to decrease the susceptibility of LDL oxidation in human studies. It remains to be seen whether saturated fatty acids can do the same. Stearic acid, found in cocoa butter, would be an ideal saturated fatty acid to test because it has a neutral effect on the plasma lipid profile. *Am J Clin Nutr* 1994;60(suppl):1010S–3S.

KEY WORDS Oxidized LDL, fatty acids, antioxidants, atherosclerosis

Introduction

Evidence continues to accumulate that implicates the oxidative modification of low-density lipoprotein (LDL) in the pathogenesis of atherosclerosis. The most compelling data come from animal studies that show regression of atherosclerotic lesions after feeding of antioxidants. In addition, studies in humans have shown a reduction in LDL oxidation after antioxidant supplementation. However, the fatty acid composition of LDL may also play a role in its susceptibility to oxidation. This article examines the literature on LDL oxidation and how the stearic acid in cocoa butter may reduce the risk of LDL oxidation without adversely affecting the plasma lipid profile.

LDL oxidation and atherogenesis

A high concentration of plasma LDL cholesterol is a primary risk factor for atherosclerosis; however, it is unknown how LDL promotes the development of the fatty streak lesion. The classic LDL-receptor pathway is down-regulated by an increase in cellular cholesterol, thus the LDL receptor cannot be responsible for foam cell formation (1). On the other hand, the macrophage scavenger receptor is able to recognize LDL that has been modified (2). It is not down-regulated by intracellular cholesterol, so it can result in substantial cholesterol ester accumulation in cells such as macrophages (1).

The most plausible and biologically relevant modification of LDL is oxidation. The three main cell types of the arterial wall—endothelial cells, smooth muscle cells, and macrophages—are able to oxidize LDL in vitro (3, 4). LDL can also be modified in cell-free systems by transition metals (usually copper), or a free radical initiator (5, 6). When LDL is oxidized, the amount of polyunsaturated fatty acids (PUFAs) is reduced, accompanied by an increase in aldehydes and lipid peroxides (3). Cholesterol in LDL is oxidized to oxysterols (7). The phosphatidylcholine content of LDL decreases on oxidation, concomitant with an increase in lysophosphatidylcholine (LPC) (3). Extensive oxidation of LDL results in a binding of radical intermediates to lysine residues on apolipoprotein (apo) B, thus increasing its negative charge. Fragmentation of apo B is also seen (8). These changes in apo B decrease LDL uptake by the LDL receptor but increase its uptake by the scavenger receptor (3).

Oxidatively modified LDL has several effects on cultured cells that could result in atherogenesis in vivo. Uptake of oxidized LDL by the macrophage via the scavenger receptor stimulates increased cholesterol esterification (8). Aldehydes, LPC, and oxysterols formed by LDL oxidation are cytotoxic (9). This cytotoxicity may induce endothelial cell dysfunction and may promote the development of a more advanced lesion.

Mild oxidation of LDL results in the formation of minimally modified LDL (MM-LDL). MM-LDL is taken into cell by the LDL receptor instead of by the scavenger receptor (10). Cushing et al (11) showed that MM-LDL stimulated monocyte attachment to endothelial cells and production of monocyte chemotactic protein-1 by smooth muscle and endothelial cells, which promotes differentiation of monocytes into macrophages. These macrophages could in turn further modify MM-LDL, resulting in more severely oxidized LDL. LPC in oxidized LDL stimulates production of monocyte adhesion molecules by endothelial cells (12). Oxidized LDL and LPC are chemoattractants for monocytes (13); oxidized LDL also inhibits macrophage chemotaxis (14). The net effect of these phenomena may be the trapping of mac-

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rophages in the subendothelial space and could lead to foam cell formation as the macrophages take up more oxidized LDL.

Several lines of evidence support the in vivo existence of oxidized LDL. Immunostaining of rabbit atherosclerotic lesions shows positive reactions to antibodies directed against oxidized LDL (15). LDLs isolated from human and rabbit atheromas show biological and physicochemical properties of oxidized LDL (16). Oxidatively modified and fragmented apo B has been isolated from the plasma of both normal and atherosclerotic individuals (17). Autoantibodies against oxidized LDL epitopes have been isolated from human and rabbit plasma and correlated with the progression of carotid artery stenosis (18). Regnström et al (19) showed that the duration of the lag phase of LDL oxidation was correlated with the coronary lesion score in humans. The most compelling evidence for the existence of oxidized LDL in vivo comes from animal studies showing that supplementation with antioxidants such as probucol, α-tocopherol, butylated hydroxytoluene (BHT), and *N,N'*-diphenyl-*P*-phenylenediamine (DPPD) can reduce the degree of LDL oxidation and the area of atheromatous lesions in animal models of atherosclerosis (20–22). Although these studies are not conclusive, they do support the hypothesis that oxidized LDL may be crucial in the early events of atherogenesis.

Prevention of LDL oxidation

Role of antioxidants

Although BHT, probucol, and DPPD are effective in preventing atherosclerosis in animals, they are not benign agents. BHT can cause renal and hepatic damage (23) and DPPD is a mutagen (22). These toxicity issues effectively preclude their use in humans. Probucol can cause a decrease in high-density-lipoprotein (HDL) cholesterol and may prolong the Q-T interval in some patients (24). In addition, it has an elimination half-life of 47 d (24). These side effects cloud prospects for its long-term use in humans.

There are two other possible ways to reduce the susceptibility of LDL to oxidation by dietary means. One is by supplementation with the antioxidant nutrients α-tocopherol, β-carotene, or ascorbic acid. The advantages of these agents are their relative safety at doses above the recommended dietary allowances (25) and that oral supplementation significantly increases plasma concentrations. All have been shown to reduce LDL oxidation in vitro (4, 9, 26, 27). Alpha-tocopherol, the most potent and prevalent form of vitamin E, is the predominant antioxidant for LDL (28). Two animal studies indicated that α-tocopherol may slow the progression of atherosclerosis (29, 30). The results of epidemiologic studies also indicate that high intakes of α-tocopherol are inversely related to the incidence of heart disease in both men and women (31, 32) and to self-reported angina pectoris (33). Alpha-tocopherol was shown to reduce the susceptibility of LDL to oxidation in clinical trials (28, 34).

Beta-carotene is a member of the carotenoid family of plant pigments that is also carried in the blood within the LDL particle (35). It has antioxidant effects at low pressures of oxygen (36), which presumably exist within arterial walls. A recent study indicated that β-carotene may be useful for treating angina (37). Supplementation with β-carotene has shown mixed results in reducing the extent of LDL oxidation. Princen et al (34) showed that β-carotene supplementation slightly reduced the susceptibility of LDL to oxidation but Reaven et al (38) found that it was ineffective when compared with α-tocopherol. Methodological differences may account for the discrepancies between the study results.

Ascorbate is not carried within the LDL particle because of its high water solubility; however, ascorbate has been shown to protect LDL from oxidation in vitro (39). In addition, ascorbate can regenerate α-tocopherol from its radical form (40), thus sparing this lipophilic antioxidant. Plasma ascorbate concentrations have been inversely correlated with coronary disease mortality (41). Harats et al (42) showed that supplementation with 1–1.5 g ascorbate could reduce the extent of LDL oxidation produced by acute cigarette smoking.

Altering the LDL fatty acid content

The other dietary means by which LDL can be protected from oxidation is to feed fatty acids that are not easily oxidized, which enriches the LDL particle with these fatty acids. PUFAs, although they reduce plasma cholesterol (43), may increase LDL susceptibility to oxidation. They may also increase the body's requirements for antioxidants (44). Three studies have compared the effects of diets high in PUFA vs diets high in monounsaturated fatty acids (MUFAs) on LDL oxidative susceptibility of humans. Reaven et al (45) reported that feeding a high-PUFA diet for 8 wk to hypercholesterolemic subjects increased the LDL oxidative susceptibility over that of subjects fed an oleic acid–rich diet. The percentage of 18:2 fatty acids in LDL was strongly correlated with two measure of LDL oxidation: formation of conjugated dienes and degradation of oxidized LDL by macrophages. Abbey et al (46) found that a diet high in linoleic acid increased the rate but not the lag phase of oxidation in a randomized crossover study. Similar results were seen in a study by Bonanome et al (47).

To date the relative effects of saturated fatty acids on LDL oxidation have not been examined. Stearic acid would be an ideal fatty acid to test in an experiment of this type, because it theoretically should not increase LDL oxidative susceptibility. In addition, it would not increase plasma cholesterol concentrations as do other saturated fatty acids (43, 48). It appears to be rapidly converted to oleic acid within the body, which may explain its neutral effect on plasma lipids (49). Cocoa butter contains an average of 33% stearic acid (50) and thus would make a good vehicle to test stearic acid's effects on LDL oxidizability.

To test the hypothesis that a diet containing stearic acid could reduce LDL oxidation, a blinded crossover study could compare cocoa butter, olive oil, or another abundant MUFA source with a high-PUFA source in a mixed real-food diet with healthy volunteers. The test fats should be incorporated into a foodstuff where the differences in fatty acid melting points would not be easily detected, such as baked goods. Because cocoa butter will probably never be a predominant fat source in the typical Western diet, it is important to incorporate it at a modest percentage (20–30%) of total dietary fat. The results of such an experiment could further advance the oxidized LDL theory of atherogenesis. In addition, it would allow the cholesterol-conscious consumer an occasional treat of chocolate without guilt.

Conclusions

Although the body of evidence linking oxidized LDL to atherogenesis is growing rapidly, many questions remain to be an-

swered before the oxidized LDL theory is firmly established. One question concerns the role of saturated fatty acid sources such as cocoa butter on the susceptibility of LDL to oxidative modification. If the results of human studies show that cocoa butter (or stearic acid) can reduce the oxidation of LDL without concurrent adverse effects on the plasma lipid profile, the current recommendations for saturated fatty acid intake could be altered to reflect these findings.

An alternative possibility would be to supplement the cocoa butter used in chocolate products with α-tocopherol or other tocopherols to offer another means of protection from LDL oxidation. Such supplementation would engender much scrutiny from the nutrition community, and may not be sufficient to protect the PUFA in LDL from oxidative modification in the amounts of chocolate generally consumed. Much research needs to be done to establish the role of cocoa butter and chocolate in a "heart healthy" diet.

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