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OXIDIZED LOW DENSITY LIPOPROTEINS IN ATHEROGENESIS: Role of Dietary Modification

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ABSTRACT

The development of atherosclerosis is a complex and multistep process. There are many determinants in the pathogenesis of this condition, with different factors presumably playing key roles at different times in the evolution of the atherosclerotic plaque. It has been suggested that oxidation of low density lipoproteins (LDL) by cells in the artery wall leads to a proatherogenic particle that may help initiate early lesion formation. For this reason, understanding the determinants of LDL susceptibility to oxidation is essential for developing therapeutic strategies to inhibit this process. Oxidation of LDL begins with the abstraction of hydrogen from polyunsaturated fatty acids; thus, LDL fatty acid composition undoubtedly contributes to the process of LDL oxidation. Since dietary fatty acids influence the fatty acid composition of LDL and cell membranes, the amount and type of fat in the diet may effect susceptibility of LDL and cells to oxidative damage. Additionally, since cell membrane fatty acid composition also influences cellular formation of reactive oxygen species, dietary fatty acids may help determine the prooxidant activity of artery wall cells. Both cells and lipoproteins contain a variety of antioxidants that provide protection against oxidative stress. A major source of these antioxidants is the diet. Enrichment of the diet with foods high in such antioxidants as vitamin E, β-carotene, or vitamin C, or supplementation of the diet with antioxidant vitamins, may inhibit oxidation and the process of atherosclerosis.

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BACKGROUND OF CONCEPT OF OXIDIZED LOW DENSITY LIPOPROTEINS

Hypercholesterolemia is a dominant, if not obligatory, risk factor for the development of atherosclerosis. Hypercholesterolemia may be thought of as any plasma cholesterol level above 160 mg/dl. Below this level, it is distinctly unusual for a patient to develop atherosclerosis. Above this level, other risk factors become increasingly important in the development of atherosclerotic lesions and in the expression of coronary artery disease (CAD). Many factors, both genetic and environmental, "go beyond cholesterol" (82) and contribute in important ways to the atherogenic process. Indeed, it is likely that different factors dominate in different individuals and that even in the same individual different factors are involved in the initiation and progression of the disease process and in the expression of CAD. A detailed discussion of the pathogenesis of atherosclerosis is beyond the scope of this chapter, but several excellent reviews are available (76, 83). In this chapter, we focus on the oxidation hypothesis of atherosclerosis (89, 91), and on the potential role nutritional factors could play in modulating this process (71, 82). However, it is important to emphasize that oxidation of low density lipoproteins (LDL), or other lipoproteins, is probably only one of many different determinants in the pathogenesis of atherosclerosis and in the development of clinical sequellae.

The fatty streak is the first lesion in the atherogenic process and almost certainly the precursor of the transitional and more complicated fibrous plaques that rupture and give rise to ischemic events. Therefore, understanding the etiology of the fatty streak, with its characteristic lipid-laden monocyte-derived macrophages, should enable one to develop effective strategies to inhibit their formation and the subsequent development of complex lesions. In experiment animals with hypercholesterolemia, one of the earliest detectable events is the binding of circulating monocytes to endothelium overlying a localized accumulation of cholesterol, presumably mediated via several adhesion molecules. Subsequently, monocytes enter the artery wall through gap junctions, attracted by a variety of chemoattractants. Within the subendothelial space, the monocytes differentiate into arterial wall macrophages and express a whole new set of gene products, including receptors for oxidized (or otherwise modified)

lipoproteins. Presumably, these macrophages then take up modified lipoproteins, forming the characteristic foam cells that typify the fatty streak lesion. Through a series of as yet poorly understood steps, the fatty streak progresses through a transitional lesion into the typical complex atherosclerotic plaque. Although the growing lesion may compromise the vascular lumen, it is likely that most clinical sequellae result from plaque rupture, initiation of thrombosis, and vessel occlusion.

Although much evidence supports the broad outlines noted above, it is only in the past 15 years that an explanation for the formation of foam cells has emerged. There was strong evidence that macrophage uptake of native LDL via LDL receptors could not account for foam cell formation. First, macrophages in cell culture could not be converted into foam cells by incubation with native LDL, and second, patients, or rabbits, who completely lacked LDL receptors typically developed the most severe and progressive atherosclerosis. Because the primary function of macrophages is to remove damaged material, Goldstein and colleagues proposed that LDL had to be modified in order to be recognized by this differentiated cell (33). They first demonstrated that LDL modified by acetylation was rapidly taken up by a novel receptor, which they termed the acetyl, or scavenger, receptor (33). Although there is no evidence that acetylation of LDL occurs in vivo, studies by our group and others have demonstrated that analogous modifications of LDL do occur in vivo. Preincubation of LDL with endothelial or smooth muscle cells or with macrophages leads to LDL oxidation and its subsequent rapid uptake by way of the acetyl LDL receptor (13, 40, 66, 84). Indeed, there is probably a family of scavenger receptors whose function is to remove modified lipoproteins, and other modified proteins as well (2, 24, 49, 61). Although the following discussion focuses on oxidized LDL, other modifications of LDL may potentially lead to enhanced macrophage uptake, including modification by nonenzymatic glycation and aggregation of LDL, and formation of immune complexes of modified or aggregated LDL that has enhanced uptake by way of the F_c receptor pathway.

WHAT IS OXIDIZED LDL?

The chemical events that occur when LDL is oxidized are indeed complex and have been reviewed elsewhere (26, 71, 91). Oxidation presumably begins when a reactive radical abstracts a hydrogen atom from a polyunsaturated fatty acid (PUFA) on the LDL particle. Lipid peroxyl and alkoxyl radicals are formed, which in turn can initiate oxidation in neighboring fatty acids. In the absence of sufficient concentrations of antioxidants, this results in the propagation of lipid peroxidation. Although this process presumably begins in the PUFA of surface phospholipids, it then spreads to involve the bulk of lipids in the core of the LDL

particle. Not only are PUFA in phospholipids involved, similar fatty acids in cholesteryl esters and triglycerides are as well. The cholesterol moiety itself also undergoes oxidation. In this process, breakdown of PUFA occurs, leading to formation of highly reactive aldehydes, ketones, and other products, and these in turn may bind to lysine residues of the apolipoprotein B moiety of LDL. These lipid-lysine adducts change the charge on the LDL and are believed to form the epitopes that are recognized by scavenger receptors on macrophages. All of these reactions lead to marked alteration of the LDL structure. They also lead to the generation of a variety of new compounds, many of which are sufficiently polar to leave the LDL particle and which appear to have many diverse biological effects, including many atherogenic properties.

In vitro, oxidation of LDL is most often initiated by incubation with copper, which breaks down existing lipid hydroperoxides and initiates the propagation reactions. However, little is known about mechanisms by which LDL becomes oxidized in vivo. In cell culture, all of the cells normally present in the artery wall can initiate oxidation of LDL, and, presumably, similar events must occur in vivo. However, the mechanisms by which cells initiate oxidation of LDL are poorly understood. Reactive oxygen species (13, 26, 40, 66), or thiols (41, 63), or both, may be released and thus participate directly in the initiation of LDL oxidation. 15-Lipoxygenase, which can introduce molecular oxygen into PUFA, may also contribute by transferring lipid hydroperoxides from cells to LDL (5, 69). Myeloperoxidase has recently been shown to be released by macrophages and may mediate oxidation by a metal-independent process (16). This is important, as in vitro cell-mediated LDL oxidation in most cases requires the presence of a transition metal. Currently, it is unknown whether or not free metals exist in the artery wall in vivo, although complexes of free metals, such as copper in ceruloplasmin, have been shown to be prooxidant (23). Other processes may participate as well. For example, glucose, particularly in the presence of reactive oxygen species, may undergo autooxidation, further accelerating lipid peroxidation (45). Similarly, the advanced glycation end products, which occur with chronic hyperglycemia, may form reactive free radical intermediates, which independently may promote lipid peroxidation (11). Finally, it should be appreciated that modification of LDL probably does not occur to any significant extent in plasma because of the presence of many water-soluble antioxidants. Similarly, the extracellular fluid of the arterial intima itself most likely contains sufficient antioxidant protection to prevent modification of LDL. Therefore, it seems likely that modification must occur in microdomains within the intima where LDL would be isolated from the many antioxidants present in the aqueous environment. In a manner similar to that in which macrophages may kill bacteria, "black holes" presumably can be formed adjacent to cells, creating microenvironments in which localized conditions sufficient to initiate lipid peroxidation can occur. Understanding the mechanisms by which LDL become oxidized in vivo is an

important area of future research that could determine the focus of new therapeutic approaches.

PROPERTIES OF OXIDIZED LDL THAT MAKE IT PROATHEROGENIC

Although the original impetus for the study of oxidized LDL was the observation that uptake of this modified lipoprotein by macrophages converted them into foam cells, it is now apparent that oxidation of LDL changes it in many ways that make it proatherogenic (Table 1). As described above, when LDL undergoes oxidation, a variety of different products are formed, some of which are quite polar, and which presumably leave the LDL particle to effect changes on neighboring cells. For example, lysolecithin, generated by the oxidation of LDL, has been shown to be capable of inducing adhesion molecules for monocytes on the surface of endothelial cells (51), and to be a chemoattractant for monocytes and T-cells (56, 67). In addition, other products of oxidized LDL can cause expression of monocyte chemoattractant protein (MCP-1) and monocyte colony stimulating factor by cells (15, 68). These cytokines will both attract monocytes to the subendothelium and affect their growth and differentiation into macrophages. Other products of oxidized LDL are cytotoxic (13, 42) and may contribute to disruption of endothelial cell integrity, and metalloproteinases released by activated macrophages may result in matrix degradation and disorganization (30). Minimally oxidized LDL can induce expression of MCP-1, as noted above, but it can also induce a variety of other inflammatory gene products, such as hemoxygenase, possibly mediated by nuclear factor-κB–mediated signal transduction pathways (52). A variety of other potential proatherogenic properties are listed in Table 1. Thus, minimally modified forms of LDL, which are insufficiently modified to have accelerated macrophage uptake, may nevertheless release a variety of polar compounds that may be highly injurious to the artery wall and proatherogenic. It is possible that by the time LDL becomes so extensively modified that it is recognized by macrophage scavenger receptors, it may already have adversely affected the artery wall. From this perspective, uptake by macrophages may now be seen as protective, albeit at the expense of increased foam cell formation (90). If this formulation is correct, then it implies that preventing oxidation of LDL in the first place will be of primary therapeutic importance, and, as a corollary, interventions that inhibit macrophage uptake of modified LDL may be counterproductive.

EVIDENCE THAT OXIDATION OF LDL IS IMPORTANT IN ATHEROGENESIS

When LDL is oxidized in vitro by incubation with endothelial cells or with copper, if the medium is supplemented with 5% serum or albumin, oxidation

Table 1 Potential mechanisms by which OX-LDL may be atherogenic[a]

It has enhanced uptake by macrophages leading to cholesteryl ester enrichment and foam cell formation
It is chemotactic for circulating monocytes and T-lymphocytes
It inhibits the motility of tissue macrophages and endothelial cells
It is cytotoxic
It can alter gene expression of neighboring arterial cells such as induction of MCP-1, colony-stimulating factors, IL-1, and endothelial expression of adhesion molecules
It can induce a variety of proinflammatory genes and their products such as hemoxygenase, SAA, and ceruloplasmin
It is immunogenic and can elicit autoantibody formation and reactive T-cells
It renders LDL more susceptible to aggregation, which independently leads to enhanced macrophage uptake
It can adversely alter coagulation pathways, such as by induction of tissue factor and alteration of platelet aggregation
It can adversely alter vasomotor properties of coronary arteries

[a] Modified from Reference 71.

is completely inhibited. Thus, when the oxidation hypothesis was first proposed, there was much skepticism that it could occur in vivo because of the seemingly ubiquitous presence of serum proteins and aqueous antioxidants. However, there are now many lines of evidence that oxidation of lipoproteins does occur in vivo and is probably quantitatively very important. This evidence, extensively reviewed elsewhere (71, 89, 91), can be summarized as follows. 1. Immunocytochemical evidence using antibodies directed against epitopes of oxidized LDL demonstrates the presence of such epitopes in atherosclerotic lesions of animals and humans but not in normal arterial tissue. 2. LDL extracted from atherosclerotic tissue of rabbits and humans has all the physical, immunologic, and biologic properties observed with LDL oxidized in vitro. 3. Small amounts of LDL isolated from plasma can be shown to have changes consistent with minimal degrees of oxidative modification, such as the presence of oxidized lipids. 4. Oxidized lipids are also found in atherosclerotic tissue, and, in particular, products of 15-lipoxygenase can be found in early atherosclerotic lesions, implying enzymatic modification of PUFA. 5. Oxidized LDL is immunogenic, and autoantibodies to epitopes of oxidized LDL can be found in sera of animals and humans. In studies in humans and mice, such autoantibody titers are related to the presence of atherosclerosis and/or to the rate of progression of disease. 6. Antibodies to oxidized LDL can be found in atherosclerotic lesions of rabbits and man, and, in part, such immunoglobulins are part of immune complexes with oxidized LDL in these lesions. 7. Most importantly, treatment of hypercholesterolemic rabbits and nonhuman primates with a variety of antioxidants leads to inhibition of the

progression of atherosclerosis, independent of any effects on plasma lipoprotein levels (Table 2). In most of these studies, the inhibition of atherosclerosis varied from 40–80%. It is important to point out that, in each of these studies, very potent lipophilic antioxidants were used. Although it is possible this inhibition occurred via mechanisms unrelated to their antioxidant properties, the recent study of Sasahara et al (77) demonstrated a significant correlation between the extent of antioxidant protection to plasma LDL and the extent of inhibition of atherosclerosis. In addition, recent studies from our laboratory have pointed out that lipophilic antioxidants, such as vitamin E, which failed to provide the same degree of antioxidant protection as probucol, failed to protect against atherosclerosis, implying that a threshold level of antioxidant protection may be required to achieve protection against atherosclerosis. In other words, for any given degree of prooxidant stress, a threshold level of antioxidant protection must by achieved to confer a protective benefit against atherosclerosis. This concept becomes most important in discussing interventions in humans, where mild degrees of protection, such as that afforded by natural antioxidants, might be sufficient under minimal degrees of oxidant stress but might be ineffective in the face of severe stress, as may occur in hypercholesterolemia. 8. With regard to humans, many epidemiologic studies now suggest an inverse relationship between dietary intake and/or serum levels of antioxidants such as vitamin C, α-tocopherol, and β-carotene on the one hand, and coronary heart disease on the other (reviewed in 71). For example, Gey (32) noted in a cross-sectional study in European countries a strong inverse relationship between intake of vitamin C and vitamin E and rates of CAD. In the United States, several large cohort studies have demonstrated in both men and women that intake of vitamin E supplements is associated with a reduced risk for CAD. Those with the highest self-reported level of vitamin E intake (greater than 100 mg/day) had a 30–50% reduction in CAD events. In a 10-year follow-up of the First National Health and Nutrition Survey, which included a representative sample of over 10,000 US adults, vitamin C intake was inversely related to cardiovascular disease and overall mortality; however, the study was not controlled for vitamin E intake (25). Unfortunately, there are not yet any prospective interventional trials in humans designed to test the effectiveness of antioxidants in preventing coronary heart disease. In a widely publicized but preliminary presentation from the Harvard Physicians' Health Study, results were reported on a subset of 333 physicians with existing CAD who were prospectively followed for seven years while taking either β-carotene (50 mg) or placebo every other day. Those taking β-carotene had a 50% reduction in cardiovascular endpoints (31). Results from the large cohort of physicians in this study should be available in several years. In contrast, in a recent Finnish study in which middle-aged men who were smokers were given a similar dose of β-carotene to prevent lung cancer, no significant reduction

Table 2 Studies of antioxidants and atherosclerosis in animals

Antioxidant	Ref.	Animal	Lesion area	LDL oxidation
Probucol	12	WHHL rabbit	Decreased 50%	Decreased
Probucol	48	WHHL rabbit	Decreased 80%	Decreased
Probucol	17	Cholesterol-fed rabbit	Decreased 70%	Decreased
Probucol	81	Cholesterol-fed rabbit	No effect	ND[a]
Probucol	58	WHHL rabbit	Decreased 74%[b]	ND
			Decreased 54%[b]	ND
Probucol analogue	54	WHHL rabbit	Decreased 35%	Decreased
Probucol	77	Cholesterol-fed primate	Decreased 50%	Decreased
DPPD	79	Cholesterol-fed rabbit	Decreased 71%	Decreased
BHT	7	Cholesterol-fed rabbit	Decreased 68%	Decreased
Vit E	8	Cholesterol-fed rabbit	Decreased (in thoracic aorta only) 46%	ND

[a] ND, Not done.
[b] Probucol started after 8 months of lesion formation.

in cardiac endpoints was seen, and, in fact, the total mortality of this group was higher than those taking the placebo (85). Although this latter study was not designed to study the effect of antioxidants on cardiovascular disease, nevertheless it should give pause to anyone making widespread recommendations before appropriately designed interventional trials have been conducted.

DETERMINANTS OF SUSCEPTIBILITY OF LDL TO OXIDATION

To design effective therapeutic modalities to inhibit the modification of LDL, one has to understand the factors in vivo that influence the oxidation of LDL. Conceptually, these can be thought of as factors intrinsic or extrinsic to LDL, as outlined in Table 3. Susceptibility of an individual LDL particle to oxidation is clearly a primary determinant of the rate of overall LDL oxidation (26). What are those factors that we know contribute to the susceptibility of LDL to oxidation? Of chief importance seems to be the content of PUFA, the initial substrate for lipid peroxidation, as well as the content of endogenous antioxidant compounds. α-Tocopherol is probably the most important antioxidant found in LDL. Although β-carotene is the second most abundant compound classified as an "antioxidant" in LDL, it has not been possible to show that it functions as an antioxidant for LDL. Though ubiquinol-10 is a potent antioxidant, its quantitative importance appears questionable, as it is present in no more than one in ten LDL particles. The synthetic lipophilic antioxidant probu-

Table 3 Factors potentially affecting oxidation of LDL in vivo[a]

Factors intrinsic to LDL
Fatty acid composition (polyunsaturated fatty acid content in particular)
Content of antioxidants: natural (e.g. β-carotene, vitamin E, buiquinol-10); pharmacologic (e.g. probucol)
Phospholipase A_2 activity
Others; including size of particle, inherent properties of apoB-100, distribution of fatty acids (e.g. in surface phospholipids or in core triglycerides or cholesteryl esters), carbohydrate content, degree of nonenzymatic glycation
Factors extrinsic to LDL
Potential variation in cellular prooxidant activity (e.g. genetic variation in macrophage expression of 15-lipoxygenase activity or cellular superoxide anion secretion)
Concentration of plasma and extracellular fluid prooxidant components (e.g. trace metal concentrations)
Concentration of plasma and extracellular fluid antioxidant components (e.g. ascorbate, urate)
Concentrations of extracellular HDL and content of paraoxonase and PAF-acetylhydrolase
Concentrations of other factors influencing LDL oxidation (e.g. ceruloplasmin)
Factors influencing residence time of LDL in intima (e.g. factors that increase binding such as Lp(a); non-enzymatic glycosylation of LDL or matrix; differences in localized matrix proteins that bind LDL)

[a] apoB, Apolipoprotein B; PAF, platelet-activating factor; Lp(a), lipoprotein a.

col has proven to be the most effective agent in protecting LDL. Finally, a variety of other factors, such as the size of the particle, the content of carbohydrate, and the degree of nonenzymatic glycation, also appear to influence its susceptibility. Presumably, if one could develop effective and safe ways to retard the oxidation of the LDL particle itself, one could inhibit all the events that occur as a result of oxidation of LDL. For this reason, much effort has gone into developing effective strategies to achieve this aim.

On the other side of the equation, the prooxidant conditions within the artery wall also play an equally important role in determining the overall rate of oxidation of LDL. Little is known about these factors. As noted in Table 3, presumably the prooxidant activity of the arterial wall cells must also be of major importance. Not only would this be determined by the cellular content of antioxidant compounds, by the activities of both prooxidant and antioxidant enzymes, environmental influences may be important as well. As examples, hypercholesterolemia (and lysophosphatidylcholine) appear to increase the ability of endothelial cells to release superoxide anion (60), and activation by immune complexes can also stimulate macrophages to release reactive oxygen species. In addition, the content in plasma and extracellular fluid of prooxidant compounds, such as ceruloplasmin and/or other complexes with trace metals, as well as the content of aqueous antioxidants, such as ascorbate, bilirubin,

urate, or even high density lipoproteins (HDL), also play important roles. The residence time of LDL in the intima is also of crucial importance, and anything that increases this half-life, as occurs in hypercholesterolemia, or in hyperglycemia and consequently in enhanced nonenzymatic glycation, would presumably increase the potential for prooxidant conditions. Our limited understanding of these factors in general make it conceptually more difficult to plan and test appropriate interventions. In addition, concerns about the safety of manipulating such basic factors in vivo suggest that more study is needed before interventions, such as alteration of cellular prooxidant activities, can be attempted. However, supplementation with water-soluble vitamin C may be an exception, as it is well-characterized and in moderate doses appears to be quite safe. Further studies with this agent are clearly needed, including intervention studies with animals.

In summary, although much research still needs to be done, for reasons of safety and practicality most efforts are now being aimed at protecting the LDL particle itself. These interventions have been reviewed elsewhere in detail (71), with a particular focus on the role of antioxidants. In the discussion below, we focus on those dietary modifications that could influence LDL oxidation.

THE ROLE OF DIETARY FAT IN LDL OXIDATION AND ATHEROSCLEROSIS

Effects on LDL Levels

Undoubtedly, one of the most important factors affecting the overall extent of LDL oxidation is the number of LDL particles present in the artery wall. Replacement of dietary saturated fatty acids with carbohydrates or with monounsaturated fatty acids (MUFA) or PUFA lowers total and LDL cholesterol levels. This reduction in LDL levels would likely decrease the amount of LDL that enters the artery wall and theoretically would directly reduce the amount of LDL available for oxidative modification. Replacing dietary saturated fatty acids with PUFA would achieve this hypocholesterolemic effect (35, 86). However, these diets lead to LDL particles enriched in PUFA, which should be more susceptible to lipid peroxidation and, in theory, more atherogenic. Enrichment of diets in n-3 PUFA may increase the unsaturated fatty acid ratio even further, perhaps making particles even more susceptible to oxidation. In addition, n-3 fatty acids can frequently raise LDL levels, even as they lower triglyceride levels. Again, by increasing the number of LDL particles available for oxidation, this might be counterproductive. On the other hand, replacement of dietary saturated fatty acids with MUFA has been shown to be as effective as replacement with PUFA in lowering plasma LDL levels (34, 55); it also has the advantage that it does not simultaneously lower HDL cholesterol levels

(34, 55). HDL has also been demonstrated to inhibit the oxidative modification of LDL in vitro (64). Thus, diets enriched in MUFA, rather than PUFA, might confer additional protection by generating LDL particles that are more resistant to oxidation while optimizing both LDL and HDL cholesterol levels.

Evidence of Effects of Dietary Fatty Acids on LDL Oxidation

Our group and others have demonstrated that diets enriched in linoleate (18:2), compared with diets enriched in oleate (18:1), lead to plasma LDL that are enriched in linoleate, and that such LDL are more susceptible to oxidation. Our laboratory first demonstrated this in New Zealand white rabbits, where linoleate-enriched diets led to LDL that was readily oxidized in comparison with oleate-enriched LDL (64). Subsequent studies have extended these findings to both normal and hypercholesterolemic human subjects (70, 73). In these studies, a diet enriched in linoleate led to plasma LDL that contained over 50% of the total fatty acids in the form of linoleic acid. Cholesteryl ester, triglyceride, and phospholipid fractions were all comparably enriched in PUFAs, demonstrating that the enrichment occurred uniformly throughout the LDL particle. HDL particles also demonstrated similar levels of enrichment with linoleic acid. Bonanome et al (9) reported similar results when grape seed oil (high in PUFA)–enriched solid food diets were compared with foods enriched in olive oil in a crossover study design. A slightly lower level of PUFA enrichment in LDL was obtained in this study compared with our previous studies; however, the rate of LDL oxidation during the PUFA diet phase was again increased compared with the MUFA-enriched diet. Abbey et al also compared linoleate-enriched diets with those enriched in MUFA (1). In this study, they compared a linoleate-enriched oil-based supplement with a similar supplement enriched in oleate, both of which provided less than 20% of the daily energy intake. This was sufficient, however, to achieve modest but significant differences in the 18:2 and 18:1 content of LDL particles from the two diet groups. LDL particles from the linoleate-supplemented group had a more rapid rate of oxidation and were more extensively oxidized, demonstrating that the amount of linoleate supplementation necessary to influence LDL oxidation may be less than initially was thought. These studies raise several interesting questions. In nearly all the studies described, the LDL content of 18:2 strongly correlated with either the rate of oxidation or the extent of oxidation, as demonstrated by data from our lab (Figure 1). However, it is important to note that in essentially all of these studies, as the content of LDL 18:2 increased, the amount of 18:1 decreased and the extent of oxidation also inversely correlated with the percentage of 18:1 in the LDL. Thus, given the reciprocal nature of the change in these two fatty acids, sorting out the independent effect of each individual fatty acid is difficult. For example, if an

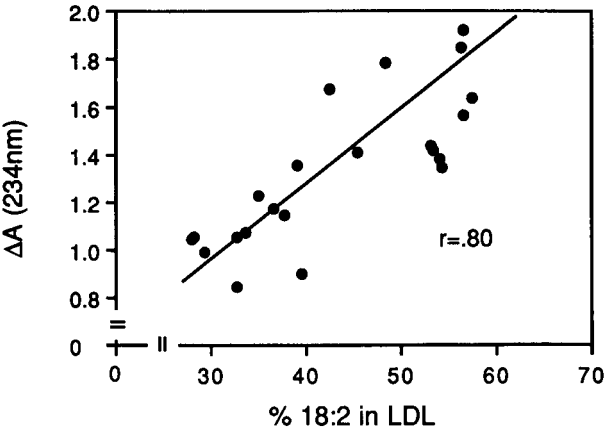

Figure 1 Correlation of conjugated diene formation with LDL content of linoleic acid. The absolute change in absorbance at 234 nm (representing conjugated diene formation) was correlated with linoleic acid content present in LDL after dietary supplementation in normal and hypercholesterolemic subjects from two separate studies (70, 73) ($r = 0.80$, $P < 0.05$). Absorbance was read after 16 h of LDL incubation in Ham's F-10 medium containing 5 mol of Cu^{2+} per liter at room temperature. [Reprinted with permission (70).]

oleate-enriched diet is used, is it the decrease in the content (of the readily oxidizable) 18:2 in LDL that decreases lipid peroxidation, or does the increasing 18:1 content somehow directly inhibit oxidation? Or do both fatty acids influence lipid oxidation independently? Additionally, comparisons of LDL susceptibility to oxidation were usually carried out only between MUFA or PUFA diet–supplemented groups, and comparisons with baseline susceptibility of LDL to oxidation were generally not done. Thus, although differences in extent of oxidation between dietary groups were consistent, it is not clear whether, when compared to a normal diet, a MUFA-enriched diet actually decreases LDL oxidation or whether, instead, a PUFA-enriched diet enhances oxidation. Fortunately, several recent observations may give some insight into these questions. Berry et al (6) compared a MUFA-enriched diet with a carbohydrate-enriched diet in a crossover study design involving 17 male yeshiva students. Compared with the carbohydrate diet group, freshly isolated LDL from the MUFA group contained less peroxides and was less susceptible to cell-mediated oxidation. Although LDL fatty acid composition was not measured, erythrocyte membranes were increased in 18:1 and decreased in 18:2 in the MUFA group. These results are consistent with the notion that a MUFA-enriched diet can reduce LDL oxidation. Aviram & Kassem (4) supplemented

ten subjects for two weeks with olive oil (50 g/day) and compared the susceptibility of their LDL to oxidation with that of LDL isolated at baseline. After both one and two weeks of the study diet, MUFA-enriched LDL showed a decreased susceptibility to oxidation. The investigators also demonstrated that, if added to LDL in vitro, oleic acid decreased LDL oxidation, whereas addition of linoleic or arachidonic acid increased LDL oxidation. These studies support the concept that an oleic acid–enriched diet reduces the susceptibility of LDL to oxidation, perhaps as a result both of intrinsic antioxidant properties of the diet and of the decrease in the content of 18:2 in LDL produced by such diets.

The influence of dietary fatty acids on LDL oxidation may be even more important when evaluating their effects on LDL subfractions. Individuals with increased amounts of small dense LDL, or the pattern B phenotype, may be at increased risk for CAD. This may be a direct result of the enhanced atherosclerotic properties of small dense LDL. For example, it has been previously demonstrated that small dense LDL is more readily oxidized than is larger, more buoyant LDL (20, 88). We recently found that vitamin E supplementation alone was less efficacious in inhibiting oxidation of dense LDL than it was for larger, more buoyant LDL. In contrast, the addition of MUFA-enriched diets to these same individuals generated a dense LDL particle that was quite resistant to oxidation (72). This suggests that 18:1 enrichment (and/or 18:2 depletion) of dense LDL may be a particularly useful therapeutic strategy.

The effect of diets enriched in long-chain polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), on LDL oxidation is less clear. Increasing the substrate for lipid oxidation (polyunsaturated fatty acids) by enriching LDL with EPA or DHA theoretically should enhance LDL oxidation. This has been supported by several studies of fish oil supplementation in humans. Harats et al (36) showed that MaxEpA (containing EPA and DHA) supplementation in smokers and nonsmokers led to increased susceptibility to oxidation of LDL. This prooxidant effect of fish oil was diminished if increased vitamin E was also added as a supplement. Harris et al (37) found that supplementation of the main components in fish oil (EPA and DHA ethyl esters) caused only a small increase in the content of long-chain PUFA but produced a significant increase in the extent of oxidation of a combined very low density lipoprotein (VLDL) plus LDL lipoprotein fraction. Several other studies showed small increases in lipoprotein oxidation or in plasma levels of lipid peroxides after fish oil supplementation (53, 57). In these latter studies, the extent of enrichment of plasma and LDL with EPA or DHA was small, as was the overall increase in total polyunsaturated fatty acids. Frequently, as the longer-chain PUFA increased in the plasma or LDL, there was a corresponding decrease in the percent of 18:2. Thus, the overall change in polyunsaturated content was small. This may explain why studies such as those by Frankel et al (29) and Brown & Wahle (10) showed no or only

transient increases in lipoprotein peroxidation after supplementation with fish oil. In the former study, no overall difference in PUFA was demonstrated, whereas in the latter study, no fatty acid measurements were reported. In a study by Nenseter et al (59), fish oil supplementation in the form of ethyl esters was compared with corn oil supplementation. Although the fish oil group did have LDL that was relatively more enriched in PUFA and had a higher unsaturation index, these differences were small, and the absolute amount of polyunsaturated fatty acids per milligram of LDL protein or per particle was greater in the corn oil group. LDL oxidation studies of two people from each dietary group did not reveal differences between the groups. However, finding differences in LDL peroxidation would be difficult when both dietary supplements increased the substrate for oxidation (polyunsaturated fatty acids) in LDL. The small sample size (two subjects per group) further decreased the probability of finding a difference between the two groups. An additional factor to consider when evaluating the effects of fish oil on LDL oxidation is the content of antioxidants provided in the fish oil supplements. Almost all fish oil supplements contain vitamin E to reduce the rate of oxidation during storage. The content and type of added antioxidants vary between preparations, and as they may also become incorporated into LDL, they may reduce the susceptibility of LDL to oxidation, possibly leading to an underestimation of the prooxidant effect of fish oil supplementation. Further studies of fish oil or their concentrated ethyl esters effects on LDL oxidation are needed.

Effects of Dietary Fatty Acids on Cellular Prooxidant Activity

The direct effect of dietary fatty acids on the susceptibility of LDL to oxidation is only one way that they may influence the atherosclerotic process. Dietary fatty acids can also have direct effects on cellular prooxidant activity. As noted earlier, the ability of artery wall cells, particularly monocytes and macrophages, to oxidize LDL may be particularly important. Monocytes and macrophage production of superoxide anion undoubtedly contributes to this process. Importantly, several investigators have demonstrated that dietary supplementation in humans with n-3 fatty acids led to increased monocyte membrane enrichment in DHA and EPA and reduced arachidonic acid. Such monocytes had decreased production of superoxide anion and of other reactive oxygen species (27, 78). Only one study has directly compared the effects of dietary supplementation with monounsaturated fatty acids (olive oil), n-6 fatty acids (corn oil), and n-3 fatty acids (DHA and EPA methyl esters) on cellular superoxide generation (78). The investigators noted a decrease in superoxide anion production from monocytes isolated from the n-3 fatty acid–supplemented group, whereas monocytes from all other diet groups showed no change or had increased superoxide anion levels. Interestingly, polymorphonuclear

leukocytes from the n-3 fatty acid–supplemented group had increased superoxide anion production, suggesting that the effect is cell specific. The mechanism by which n-3 fatty acids may decrease superoxide anion production is unknown, although it has been suggested that they may operate through reductions in arachidonic acid (AA) and active AA metabolites, such as leukotriene B4 (14, 28). Toward this end it should be noted that n-3 fatty supplementation invariably decreases AA content in LDL and cell membranes, perhaps reducing the subsequent formation of AA metabolites.

Effects of Dietary Fatty Acids on Cellular Resistance to Oxidative Stress

An additional consideration when evaluating the overall effect of dietary fatty acids on CAD is their effect on cellular response to oxidative stress. Exposure of cells to oxidative stress, such as extensively oxidized LDL, can lead to cell death (13). In the artery wall, this could lead to macrophage damage and rupture, with subsequent release of intracellular enzymes, oxidized LDL and its products, and other toxins. As described above, oxidative stress can also have more subtle effects, such as influencing gene expression or enzymatic activity. All these changes in cell activity and function caused by exposure to oxidized LDL may enhance the development of atherosclerosis. Cell susceptibility to oxidative stress appears to be influenced by membrane fatty acid composition. For example, enriching cells with monounsaturated fatty acids makes these cells less susceptible to oxidative damage (38, 39). In contrast, PUFA (18:3 and 20:3)–enriched cells were more susceptible to oxidative damage and death. Effects of other n-3 fatty acids, such as DHA and EPA, were not tested in this study. One explanation for the increased susceptibility of PUFA-enriched cells to oxidative damage is that once free radicals are formed, their propagation is accelerated by lipid peroxidation of their PUFA-enriched membranes. Alexander-North et al (3) have shown that, in response to iron-induced oxidant stress, free radical formation was enhanced in endothelial cells supplemented with PUFAs (both n-3 and n-6). Interestingly, this enhanced radical formation could be reversed by supplementing these same cells with MUFA (oleic acid). MUFA enrichment also had similar effects on monocyte-like cell lines, suggesting that inhibition of cell oxidation by MUFA enrichment may be applicable to other cell lines and tissues.

Summary of Effects of Dietary Modifications

Given the multitude of effects dietary fatty acids may have on lipoprotein oxidation, it would be difficult to predict the overall effect a given fatty acid would have on the development of atherosclerosis. However, studies of long-term supplementation with dietary fatty acids carried out with animals and

humans give us some insight into this question. The majority of animal studies have been done on rabbits or on a nonhuman primate, the African green monkey. Diets enriched in n-6 polyunsaturated fatty acids have consistently reduced atherosclerosis in the African monkey (92, 93). However, in these studies, plasma cholesterol levels decreased significantly as a result of the dietary manipulation and correlated with the extent of atherosclerosis. Thus, it appears that any proatherosclerotic effect n-6 diets may theoretically have by increasing LDL (or cellular) susceptibility to oxidation is overwhelmed by lipid lowering or other effects of this fatty acid. In general, similar findings are present when studying the effects of fish oil supplementation on atherosclerosis in animals. In studies where plasma cholesterol levels were reduced, atherosclerosis was also reduced (18, 62). However, in some studies, fish oil supplementation did not lower plasma cholesterol, and except for one study (94), atherosclerosis was not reduced in these cases and in some cases was in fact increased (46, 75, 87). Plasma thiobarbituric acid–reactive substances (TBARS) were increased when measured in these latter studies, suggesting that increased levels of lipid peroxidation were present in animals with increased lesion formation (46, 75, 87).

There are no human studies to date that compare the long-term effects on atherosclerosis of diets enriched in MUFA, PUFA, or fish oils. However, several population studies have suggested that diets enriched in MUFA (primarily oleic acid) will decrease CAD (47). Similar findings have been reported for populations that ingest diets high in n-3 fatty acids (22, 43, 50). Additionally, several intervention studies have demonstrated that lowering of plasma cholesterol by replacing diets rich in saturated acids with diets higher in PUFA modestly decreased atherosclerotic events (19, 44), although this is not a universal finding (74). None of these studies has demonstrated that dietary fatty acids influence CAD outcome independent of effects on changes in plasma cholesterol. However, a recent study did find that diets enriched in both alpha-linoleic acid (a precursor of n-3 long-chain fatty acids) and monounsaturated fatty acids reduced the number of coronary events and deaths in a male cohort of patients with preexisting CAD (21). In this study, serum lipid levels were similar in both the experiment and the control groups. Cardiac mortality was reduced by 76%, and the study was stopped early because of the clear improvement to those subjects following the Mediterranean-type diet. This study suggests that certain diets may have benefits independent of their lipid-lowering effects, but it does not provide further insight into the mechanism responsible for the decreased coronary events. The diet may have had antiatherogenic as well as antithrombogenic effects. One can also speculate that this combination of dietary fatty acids was particularly effective in decreasing cell and LDL susceptibility to oxidation, as well as in reducing cell-mediated oxidation of LDL. Clearly, additional long-term studies of this

combination of dietary fatty acids on atherosclerosis are needed, as are studies of the effect it has on cell prooxidant activity and LDL oxidation.

Atherosclerotic lesions develop slowly and may pass through many stages during their progression to complex plaques. Each stage of lesion progression may be influenced by a variety of artery wall events. It is not surprising, then, that our understanding of the atherosclerotic process is incomplete, and that our ability to effectively intervene is limited. The role that dietary fatty acids play in this process is also unclear. However, it is clear that diets that lower LDL cholesterol (primarily through replacement of saturated fatty acids) appear to reduce atherosclerotic events. Whether or not specific dietary fatty acids may influence LDL oxidation and cellular prooxidant activity in such a way as to inhibit atherosclerosis is not yet known. It seems prudent at this time, however, to recommend that if diets are greatly enriched in n-6 polyunsaturated fatty acids, consideration should be given to using antioxidant supplements, such as vitamin E, in addition. MUFA-enriched diets offer substantial advantages, as they deliver the same degree of cholesterol lowering and at the same time decrease the susceptibility to oxidation of LDL and possibly even cells. Fish oil supplementation, which lowers VLDL levels and triglycerides, may also have unique effects on thrombosis, blood pressure, and inflammation. In addition, fish oil may reduce cellular prooxidant activity and thus may reduce oxidation of LDL in vivo. Therefore, a particularly useful diet may be one in which saturated fatty acids are replaced primarily with MUFA along with small amounts of fish oil. Adequate antioxidants should also be included with the fish oil supplements or as part of the overall diet to prevent the possibility of enhanced lipid peroxidation.

Literature Cited

1. Abbey M, Belling GB, Noakes M, Hirata F, Nestel PJ. 1993. Oxidation of low density lipoproteins: intraindividual variability and the effect of dietary linoleate supplementation. *Am. J. Clin. Nutr.* 57:391–98
2. Acton SL, Scherer PE, Lodish HF, Krieger M. 1994. Expression cloning of SR-BI, a CD36-related class B scavenger receptor. *J. Biol. Chem.* 269:21003–9
3. Alexander-North LS, North JA, Kitonga KP, Buettner GR, Spector AA. 1994. Polyunsaturated fatty acids increase lipid radical formation induced by oxidant stress in endothelial cells. *J. Lipid. Res.* 35:1773–85
4. Aviram M, Kassem E. 1993. Dietary olive oil reduces low density lipoprotein uptake by macrophages and decreases the susceptibility of the lipoprotein to undergo lipid peroxidation. *Ann. Nutr. Metab.* 37:75–84
5. Benz DJ, Mol M, Ezaki M, Mori-Ito N, Zelan I, et al. 1995. Enhanced levels of lipoperoxides in low density lipoprotein incubated with murine fibroblast expressing high levels of human 15-lipoxygenase. *J. Biol. Chem.* 270(10): 5191–97

6. Berry EM, Eisenberg S, Friedlander Y, Harats D, Kaufmann NA, et al. 1992. Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins—the Jerusalem Nutrition Study. II. Monounsaturated fatty acids vs. carbohydrates. *Am. J. Clin. Nutr.* 56:394–403
7. Björkhem I, Henriksson-Freyschuss A, Breuer O, Diczfalusy U, et al. 1991. The antioxidant butylated hydroxytoluene protects against atherosclerosis. *Arterioscler. Thromb.* 11:15–22
8. Bocan TMA, Mueller SB, Brown EQ, Uhlendorf PD, Mazur MJ, Newton RS. 1992. Antiatherosclerotic effects of antioxidants are lesion-specific when evaluated in hypercholesterolemic New Zealand White rabbits. *Exp. Molec. Pathol.* 57:70–83
9. Bonanome A, Pagnan A, Biffanti S, Opportuno A, Sorgato F, et al. 1992. Effect of dietary monounsaturated and polyunsaturated fatty acids on the susceptibility of plasma low density lipoprotein to oxidative modification. *Arterioscler. Thromb.* 12:529–33
10. Brown JE, Wahle KWJ. 1990. Effect of fish-oil and vitamin E supplementation on lipid peroxidation and whole-blood aggregation in man. *Clin. Chim. Acta* 193:147–56
11. Bucala R, Makita Z, Kochinsky T, Cerami A, Vlassara H. 1993. Lipid advanced glycosylation: pathway for lipid oxidation *in vivo*. *Proc. Natl. Acad. Sci. USA* 90:6434–38
12. Carew TE, Schwenke DC, Steinberg D. 1987. Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: Evidence that antioxidants *in vivo* can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks slowing the progression of atherosclerosis in the WHHL rabbit. *Proc. Natl. Acad. Sci. USA* 84:7725–29
13. Cathcart MK, Morel DW, Chisolm GM III. 1985. Monocytes and neutrophils oxidized low density lipoproteins making it cytotoxic. *J. Leukoc. Biol.* 38:341–50
14. Corey SJ, Rosoff PM. 1991. Unsaturated fatty acids and lipoxygenase products regulate phagocytic NADPH oxidase activity by a nondetergent mechanism. *J. Lab. Clin. Med.* 118:343–51
15. Cushing SD, Berliner JA, Valente AJ, Territo MC, Navab M, et al. 1990. Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells. *Proc. Natl. Acad. Sci. USA* 87(13):5134–38
16. Daugherty A, Dunn JL, Rateri DL, Heinecke JW. 1994. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *J. Clin. Invest.* 94:437–44
17. Daugherty A, Zweifel BS, Schonfeld G. 1989. Probucol attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. *Br. J. Pharmacol.* 98: 612–18
18. Davis HR, Bridenstine RT, Vesselinovitch D, Wissler RW. 1987. Fish oil inhibits development of atherosclerosis swine model. *Arteriosclerosis* 7:441–49
19. Dayton S, Pearce ML, Hashimoto S, Dixon, WJ, Tomiwasu U. 1969. A controlled clinical trial of a diet high in unsaturated fat in preventing complications of atherosclerosis. *Circulation* 40 (Suppl. 2):II1–II63
20. de Graaf J, Hak-Lemmers HLM, Hectors MPC, Demacker PNM, Hendriks JCM, Stalenoef AFH. 1991. Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. *Arterioscler. Thromb.* 11:298–306
21. de Lorgeril M, Renaud S, Mamelle N, Salen P, Martin J-L, et al. 1994. Mediterranean alpha-linoleic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* 343:1454–59
22. Dolecek TA. 1992. Epidemiological evidence of relationships between dietary polyunsaturated fatty acids and mortality in the multiple risk factor intervention trial. *Proc. Soc. Exp. Biol. Med.* 200:177–82
23. Ehrenwald E, Chisolm GM, Fox PL. 1994. Intact human ceruloplasmin oxidatively modifies low density lipoprotein. *J. Clin. Invest.* 93:1493–501
24. Endemann G, Stanton LW, Madden KS, Bryant CM, White RT, Protter AA. 1993. CD36 is a receptor for oxidized low density lipoprotein. *J. Biol. Chem.* 268:11811–16
25. Enstrom JE, Kanim LE, Klein MA. 1992. Vitamin C intake and mortality among a sample of the United States population. *Epidemiology* 3(3):194–202
26. Esterbauer H, Gebicki J, Puhl H, Jürgens G. 1992. The role of lipid peroxides and antioxidants in oxidative modification of LDL. *Free Rad. Biol. Med.* 13:341–92
27. Fisher M, Levine PH, Weiner B, Johnson MH, Doyle EM, et al. 1990. Dietary n-3 fatty acid supplementation reduces superoxide production and chemiluminescence in a monocyte-enriched preparation of leukocytes. *Am. J. Clin. Nutr.* 51:804–8

28. Fisher M, Upchurch KS, Levine PH, Johnson MH, Vaudreuil CH, et al. 1986. Effects of dietary fish oil supplementation on polymorphonuclear leukocyte inflammatory potential. *Inflammation* 10: 387–92
29. Frankel EN, Parks EJ, Xu R, Schneeman BO, Davis PA, German JB. 1994. Effect of n-3 fatty acid-rich fish oil supplementation on the oxidation of low density lipoprotein. *Lipids* 29:233–36
30. Galis ZS, Muszynski M, Sukhova GK, Simon-Morrissey E, Unemori EN, et al. 1994. Cytokine-stimulated human vascular smooth muscle cells synthesize a complement of enzymes required for extracellular matrix digestion. *Circ. Res.* 75:181–89
31. Gaziano JM, Manson JE, Branch LG, LaMotte F, Colditz GA, et al. 1992. Dietary beta carotene and decreased cardiovascular mortality in an elderly cohort. *J. Am. Coll. Cardiol.* 19(3):377A (Abstr.)
32. Gey FK. 1990. Lipids, lipoproteins and antioxidants in cardiovascular dysfunction. *Biochem. Soc. Trans.* 18:1041–45
33. Goldstein JL, Ho YK, Basu SK, Brown MS. 1979. Binding site on macrophage that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc. Natl. Acad. Sci. USA* 76:333–37
34. Grundy SM. 1986. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. *N. Engl. J. Med.* 314:745–48
35. Grundy SM, Denke MA. 1990. Dietary influences on serum lipids and lipoproteins. *J. Lipid Res.* 31:1149–72
36. Harats D, Dabach Y, Hollander G, Ben-Naim M, Schwartz R, et al. 1991. Fish oil ingestion in smokers and nonsmokers enhances peroxidation of plasma lipoproteins. *Atherosclerosis* 90:127–39
37. Harris WS, Windsor SL, Caspermeyer JJ. 1993. Modification of lipid-related atherosclerosis risk factors by ω3 fatty acid ethyl esters in hypertriglyceridemic patients. *J. Nutr. Biochem.* 4:706–12
38. Hart CM, Tolson JK, Block ER. 1991. Supplemental fatty acids alter lipid peroxidation and oxidant injury in endothelial cells. *Am. J. Physiol.* 260(4): L481–88
39. Hart CM, Tolson JK, Block ER. 1992. Quantitative fatty acid analyses in cultured porcine pulmonary artery endothelial cells: the combined effects of fatty acid supplementation and oxidant exposure. *J. Cell. Physiol.* 153:76–87
40. Heinecke JW, Rosen H, Chait, A. 1984. Iron and copper promote modification of low density lipoprotein by human arterial smooth muscle cells in culture. *J. Clin. Invest.* 74(5):1890–94
41. Heinecke JW, Rosen H, Suzuki LA, Chait A. 1987. The role of sulfur-containing amino acids in superoxide production and modification of low density lipoprotein by arterial smooth muscle cells. *J. Biol. Chem.* 262(21):10098–103
42. Hessler JR, Robertson AL Jr, Chisolm GM, III. 1979. LDL-induced cytotoxicity and its inhibition by HDL in human vascular smooth muscle and endothelial cells in culture. *Atherosclerosis* 32:213–29
43. Hirai A, Terano T, Tamura Y, Yoshida S. 1989. Eicosapentaenoic acid and adult diseases in Japan: epidemiologic and clinical aspects. *J. Int. Med.* 225 (Suppl. 1):69–75
44. Hjermann I, Velve BK, Holme I, Leren P. 1981. Effect of diet and smoking intervention on the incidence of coronary heart disease: report from the Oslo Study Group of a randomized trial in healthy men. *Lancet* 2:1303–10
45. Kawamura M, Heinecke JW, Chait A. 1994. Pathophysiological concentrations of glucose promote oxidative modification of low density lipoprotein by a superoxide-dependent pathway. *J. Clin. Invest.* 94:771–78
46. Kazdova L, Vrana A, Urbanova D, Vidlakova M, Novakova V, Matejckova M. 1993. Effect of dietary fish oil on the development and regression of atherosclerosis in rabbits. *Ann. NY Acad. Sci.* 683:376–77
47. Keys A, Menotti A, Karvonen MJ, Aravanis C, Blackburn H, et al. 1986. The diet and 15-year death rate in the seven countries study. *Am. J. Epidemiol.* 124:903–15
48. Kita T, Nagano Y, Yokode M, Ishii K, Kume N, et al. 1987. Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. *Proc. Natl. Acad. Sci. USA* 84:5928–31
49. Krieger M, Acton S, Ashkenas J, Pearson A, Penman M, Resnick D. 1993. Molecular flypaper, host defense, and atherosclerosis. Structure, binding properties, and functions of macrophage scavenger receptors. *J. Biol. Chem.* 268: 4569–72
50. Kromhout D, Bosschieter EB, de Lezenne Coulander C. 1985. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N. Engl. J. Med.* 312: 1205–9

51. Kume N, Cybulsky MI, Gimbrone MA. 1992. Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. *J. Clin. Invest.* 90:1138–44
52. Liao F, Andalibi A, deBeer FC, Fogelman AM, Lusis AJ. 1993. Genetic control of inflammatory gene induction and NF-κB-like transcription factor activation in response to an atherogenic diet in mice. *J. Clin. Invest.* 91(6):2572–79
53. Lussier-Cacan S, Dubreuil-Quidoz S, Roederer G, Leboeuf N, Boulet L, et al. 1993. Influence of probucol on enhanced LDL oxidation after fish oil treatment of hypertriglyceridemic patients. *Arterioscler. Thromb.* 13:1790–97
54. Mao SJT, Yates MT, Parker RA, Chi EM, Jackson RL. 1991. Attenuation of atherosclerosis in a modified strain of hypercholesterolemic Watanabe rabbits using a probucol analog (MDL 29,311) that does not lower serum cholesterol. *Arterioscler. Thromb.* 11:1266–75
55. Mattson FH, Grundy SM. 1985. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *J. Lipid Res.* 26:194–202
56. McMurray HF, Parthasarathy S, Steinberg D. 1993. Oxidatively modified low density lipoprotein is a chemoattractant for human T lymphocytes. *J. Clin. Invest.* 92:1004–8
57. Meydani M, Natiello F, Goldin B, Free N, Woods M, et al. 1991. Effect of long-term fish oil supplementation on vitamin E status and lipid peroxidation in women. *J. Nutr.* 121:484–91
58. Nagano Y, Nakamura T, Matsuzawa Y, Cho M, Ueda Y, Kita, T. 1992. Probucol and atherosclerosis in the Watanabe heritable hyperlipidemic rabbit—long-term antiatherogenic effect and effects on established plaques. *Atherosclerosis* 92:131–40
59. Nenseter MS, Rustan AC, Lund-Katz S, Søyland E, Mælandsmo G, et al. 1992. Effect of dietary supplementation with n-3 polyunsaturated fatty acids on physical properties and metabolism of low density lipoprotein in humans. *Arterioscler. Thromb.* 12:369–79
60. Ohara Y, Peterson TE, Zheng B, Kuo JF, Harrison DG. 1994. Lysophosphatidylcholine increases vascular superoxide anion production via protein kinase C activation. *Arterioscler. Thromb.* 14: 1007–13
61. Ottnad E, Parthasarathy S, Sambrano GR, Ramprasad MP, Quehenberger O, et al. 1994. A macrophage receptor for oxidized low density lipoprotein distinct from the receptor for acetyl low density lipoprotein: partial purification and role in recognition of oxidatively damaged cells. *Proc. Natl. Acad. Sci. USA* 92(5): 1391–95
62. Parks JS, Rudel LL. 1990. Effect of fish oil on atherosclerosis and lipoprotein metabolism. *Atherosclerosis* 84:83–94
63. Parthasarathy S. 1987. Oxidation of low-density lipoprotein by thiol compounds leads to its recognition by the acetyl LDL receptor. *Biochim. Biophys. Acta* 917(2):1328
64. Parthasarathy S, Barnett J, Fong L. 1990. High-density lipoprotein inhibits the oxidative modfication of low-density lipoprotein. *Biochim. Biophys. Acta* 1044:275–83
65. Parthasarathy S, Khoo JC, Miller E, Barnett J, Witztum JL, Steinberg D. 1990. Low density lipoprotein enriched in oleic acid is protected against oxidative modification: implications for dietary prevention of atherosclerosis. *Proc. Natl. Acad. Sci. USA* 87:3894–98
66. Parthasarathy S, Printz DJ, Boyd D, Joy L, Steinberg D. 1986. Macrophage oxidation of low density lipoprotein generates a modified form recognized by the scavenger receptor. *Arteriosclerosis* 6:505–10
67. Quinn MT, Parthasarathy S, Steinberg D. 1988. Lysophosphatidylcholine: a chemotactic factor for human monocytes and its potential role in atherogenesis. *Proc. Natl. Acad. Sci. USA* 85:2805–9
68. Rajavashisth TB, Andalibi A, Territo MC, Berliner JA, Navab M, et al. 1990. Induction of endothelial cell expression of granulocyte and macrophage colony-stimulating factors by modified low-density lipoproteins. *Nature* 344:254–57
69. Rankin SM, Parthasarathy S, Steinberg D. 1991. Evidence for a dominant role of lipoxygenase(s) in the oxidation of LDL by mouse peritoneal macrophages. *J. Lipid Res.* 32:449–56
70. Reaven P, Parthasarathy S, Grasse BJ, Miller E, Steinberg D, Witztum JL. 1993. Effects of oleate-rich and linoleate-rich diets on the susceptibility of low density lipoprotein to oxidative modification in hypercholesterolemic subjects. *J. Clin. Invest.* 91:668–76
71. Reaven P, Witztum JL. 1995. The role of oxidation of LDL in atherogenesis. *Endocrinologist* 5:44–54
72. Reaven PD, Grasse BJ, Tribble DL.

1993. Effects of linoleate-rich and oleate-rich diets in combination with α-tocopherol on the susceptibility of low-density lipoproteins (LDL) and LDL subfractions to oxidative modification in humans. *Arterioscler. Thromb.* 14:557–66

73. Reaven PD, Parthasarathy S, Grasse BJ, Miller E, Almazan F, et al. 1991. Feasibility of using an oleate-rich diet to reduce the susceptibility of low density lipoprotein to oxidative modification in humans. *Am. J. Clin. Nutr.* 54:701–6
74. Research Committee to the Medical Research Council. 1965. Low-fat diet in myocardial infarction—a controlled trial. *Lancet* 2:501–4
75. Rogers KA, Adelstein R. 1990. MaxEPA fish oil enhances cholesterol-induced intimal foam cell formation in rabbits. *Am. J. Pathol.* 137:945–51
76. Ross R. 1993. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 362(6423):801–9
77. Sasahara M, Raines EW, Carew TE, Steinberg D, Wahl PW, et al. 1994. Inhibition of hypercholesterolemia-induced atherosclerosis in *Macaca nemestrina* by probucol. I. Intimal lesion area correlates inversely with resistance of lipoproteins to oxidation. *J. Clin. Invest.* 94:155–64
78. Sirtori CR, Gatti E, Tremoli E, Galli C, Gianfranceschi G, et al. 1992. Olive oil, corn oil, and n-3 fatty acids differently affect lipids, lipoproteins, platelets, and superoxide formation in type II hypercholesterolemia. *Am. J. Clin. Nutr.* 56: 113–22
79. Sparrow CP, Doebber TW, Olszewski J, Wu MS, Ventre J, et al. 1992. Low density lipoprotein is protected from oxidation and the progression of atherosclerosis is slowed in cholesterol-fed rabbits by the antioxidant N,N′-diphenyl-phenylenediamine. *J. Clin. Invest.* 89:1885–91
80. Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willet WC. 1993. Vitamin E consumption and the risk of coronary disease in women. *N. Engl. J. Med.* 328(20): 1444–49
81. Stein Y, Stein O, Delplanque B, Fesmire JD, Lee DM, Alaupovic P. 1989. Lack of effect of probucol on atheroma formation in cholesterol-fed rabbits kept at comparable cholesterol levels. *Atherosclerosis* 75:145–55
82. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. 1989. Beyond cholesterol: modifications of low density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* 320: 915–24
83. Steinberg D, Witztum JL. 1990. Lipoproteins and atherogenesis: current concepts. *JAMA* 264(23):3047–52
84. Steinbrecher UP, Parthasarathy S, Leake DS, Witztum JL, Steinberg D. 1984. Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. *Proc. Natl. Acad. Sci. USA* 83:3883–87
85. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. 1994. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N. Engl. J. Med.* 333:1029–35
86. The National Diet-Heart Study. 1968. *American Heart Association Monograph No. 18.* New York: Am. Heart Assoc.
87. Thiery J, Seidel D. 1987. Fish oil feeding results in an enhancement of cholesterol-induced atherosclerosis in rabbits. *Atherosclerosis* 63:53–56
88. Tribble DL, Holl LG, Wood PD, Krauss RM. 1992. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. *Atherosclerosis* 93: 189–99
89. Witztum JL. 1993. Role of oxidized low density lipoprotein in atherogenesis. *Br. Heart J.* 69:512–18
90. Witztum JL. 1994. The oxidation hypothesis of atherosclerosis. *Lancet* 344: 793–95
91. Witztum JL, Steinberg D. 1991. Role of oxidized LDL in atherogenesis. *J. Clin. Invest.* 88:1785–92
92. Wolfe MS, Parks JS, Morgan TM, Rudel LL. 1993. Childhood consumption of dietary polyunsaturated fat lowers risk for coronary artery atherosclerosis in African Green monkeys. *Arterioscler. Thromb.* 13:863–75
93. Wolfe MS, Sawyer JK, Morgan TM, Bullock BC, Rudel LL. 1994. Dietary polyunsaturated fat decreases coronary artery atherosclerosis in a pediatric-aged population of African Green monkeys. *Arterioscler. Thromb.* 14:587–97
94. Zhu B, Smith DL, Sievers RE, Isenberg WM, Parmley WW. 1988. Inhibition of atherosclerosis by fish oil in cholesterol-fed rabbits. *J. Am. Coll. Cardiol.* 12: 1073–78