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Invited Review

Potential Role of Oxidized Lipids and Lipoproteins in Antioxidant Defense

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The atherogenic oxidative modification of low-density lipoprotein is suggested to occur in the aortic intima. There is reasonable evidence to suggest that antioxidants might be beneficial in preventing or retarding the progression of atherosclerosis. Exercise, estrogens, and substitution of polyunsaturated fat for saturated fat are beneficial in the prevention of atherosclerosis. Yet, paradoxically, they are capable of inducing an oxidative stress. To reconcile with this paradox, we postulate that under certain conditions an oxidative stress might be beneficial by inducing antioxidant enzymes in arterial cells. However, those with genetic deficiency in antioxidant enzymes or those who poorly respond to oxidative stress or those with overwhelming plasma oxidative stress might need additional antioxidant protection.

Keywords: LDL, oxidative stress, exercise, estradiol, antioxidant

INTRODUCTION

Oxidation of low-density lipoprotein (LDL) was suggested to explain the formation of fatty streak lesions. When the hypothesis was proposed, the intent was to explain how macrophages could take up large quantities of LDL-derived cholesterol via mechanisms, independent of LDL receptor pathway.[1,2] The scavenger receptor concept was also originally proposed to describe alternate uptake mechanism(s) that would recognize modified apoprotein B_{100}.[3,4] The basic assumption in these studies was that the apoprotein B_{100} is intimately involved in the atherogenic process. During the past 15 years a number of studies have documented that the lipids of "oxidized LDL" could also contribute to atherogenesis.[2,5–11] More importantly, it appears that oxidized lipids themselves could contribute to enhanced uptake by macrophages via receptor(s), such as CD36, that

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do not appear to depend on the apoprotein modification.[12] Recently Steinberg and coworkers described that oxidized lipids covalently bound to apoprotein are recognized by macrophages.[13] In fact, there are more pro-atherogenic effects attributed to the presence of oxidized lipids as opposed to the oxidatively modified apoprotein.[11] These effects have been reviewed in a number of recent articles.[14–17]

OXIDATION HYPOTHESIS

In brief, the following evidence predominantly supports the oxidation hypothesis:

1. Oxidized LDL is potently atherogenic[1,2,18,19] and affect different types of cells in a profound manner eliciting multiple pro-atherogenic and pro-inflammatory responses. However, as pointed out later in this review, oxidized lipids also have anti-atherogenic effects on cells.

2. Components specific for oxidized LDL have been detected in atherosclerotic animals and humans.[20–22] The presence of receptors that interact with various components of oxidized LDL has also been detected in the atherosclerotic artery.[20,23–25] It still remains to be established whether these oxidized lipids are end products of cellular metabolism or are derived from extracellular oxidized LDL. For example, earlier studies by Mitchinson and coworkers have shown that macrophages that took up acetyl LDL (that did not have oxidized lipids) showed evidence of intracellular ceroid (oxidized lipid–protein complexes) accumulation suggesting that lipids were oxidized intracellularly.[26,27]

3. Epidemiological studies have established a correlation between low plasma antioxidant levels to the prevalence of CVD.[28–30] It should be pointed out that a higher presence of oxidation markers has not been correlated with increased CVD. However, a greater degree of correlation between low levels of β-carotene[31,32] (which has been shown to have little effect on the oxidation of LDL), the potential impact of diet (Mediterranean diet), consumption of increased amounts of red wine,[33,34] relaxed life style, and other factors have questioned the validity of these epidemiological studies.

4. Animal and human studies have suggested that oral supplementation of antioxidants may retard the progression of atherosclerosis.[35] However, very few human clinical trials are yet available and the conclusions from these studies have not been straightforward.[36–38] For example, the most recent HOPE trial has failed to establish a protective effect of vitamin E against CVD.[39] Studies by Steinberg and associates have provided a valuable insight that protection of oxidation of LDL in the plasma compartment might be of little consequence to the arterial progression of atherosclerosis.[40] When they treated animals with two structurally similar antioxidants, probucol and a close analog, only probucol had an effect on atherosclerosis. However, LDL isolated from both groups of animals was comparably protected from *in vitro* oxidation. The results were interpreted to suggest that antioxidant availability in the artery is far more important than the protection of LDL against oxidation in the plasma. Also, studies with probucol and mice models of atherosclerosis have cast doubts about the efficacy of antioxidants in this model.[41–44] However, the metabolism of synthetic and natural antioxidants in various animals might involve oxidative pathways and thus counteract the antioxidant effects.

5. Animal studies with genes for specific oxidative enzymes deleted (for example, 12/15-lipoxygenase that is presumably involved in the "seeding" of LDL with peroxides) have indicated potential oxidative stress in the development of the disease.[45] Specific inhibitors of these enzymes are also reported to inhibit atherosclerosis.[46,47] Contradictory evidence suggesting that an overexpression of 15-lipoxygenase affording a protection against atherosclerosis has also been reported.[45,48] Moreover, studies with myeloperoxidase (an enzyme that was touted to initiate the oxidation of LDL in the artery) deficient animals

appear to indicate enhanced atherosclerosis[49] suggesting that either oxidative processes are not major factors in the development of atherosclerotic lesions in these animals, or compensatory oxidative enzymes are activated or myeloperoxidase might actually serve to protect against atherosclerosis in some manner.

6. Oral supplementation of oxidized lipids such as oxidized cholesterol and oils appear to be atherogenic.[50–52] Heated oil was used as the source of oxidized lipids and no evidence was presented in these studies to suggest the extent of absorption of oxidized lipids or the observed effects were indeed due to the presence of oxidized lipids and not complicated by the presence of degradation products.

Thus, there is a great deal of confusion in literature regarding the oxidation hypothesis. Yet, a vast number of general public and notably cardiovascular physicians consume much higher than the required daily dose of antioxidant vitamins presumably with the convincing supportive evidence that they are at least safe to consume. The purpose of this review is to re-examine the oxidation hypothesis, particularly, as it pertains to the plasma compartment and to suggest that oxidation might have both beneficial and deleterious actions.

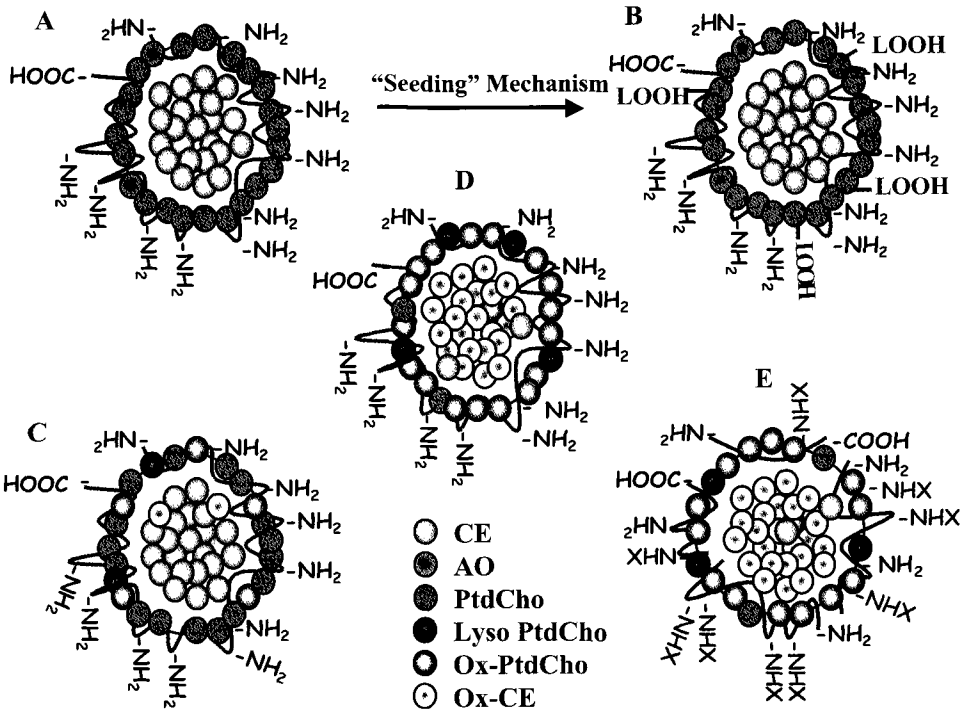
OXIDATION OF LDL IN THE INTIMA

Several lines of evidence suggest that oxidation of LDL might be more important in the subendothelial space as opposed to the oxidation that might occur in the plasma. In other words, the "atherogenic" oxidation of LDL might occur predominantly in the intima.

1. Early studies by Van Berkel and associates[53] and also by Steinbrecher *et al.*[54] showed that when injected into the plasma, modified forms of LDL (including oxidized LDL) are more rapidly cleared by the liver as compared to LDL. Thus, an extensively oxidized LDL was cleared within minutes in contrast to "native LDL" that was cleared after several hours. These studies suggested that the liver might very rapidly clear oxidized LDL if it is generated in circulation thereby limiting its chance of penetration into the artery and initiating the disease process. This finding was viewed as evidence to suggest that "oxidized" LDL might not be present in plasma at sufficient concentrations to account for the entry into the arterial wall and to generate macrophage foam cells.

The concept of oxidized LDL has undergone numerous changes during the past 15 years. Oxidized LDL is not a single entity and as reviewed recently,[17] at least 4 different oxidized LDL species are theoretically possible (Scheme 1). These include, plasma LDL to which peroxides generated elsewhere in the body are associated, LDL with its own lipids oxidized to various extents, and a protein-modified LDL. These different forms of oxidized LDL are recognized by different receptors and not all these particles are as readily cleared from plasma as compared to heavily oxidized and modified LDL. Steinbrecher *et al.* demonstrated that the rate of clearance of oxidized LDL was indirectly proportional to the extent of oxidation.[54] Thus, minimally or moderately oxidized LDL or even extensively oxidized LDL that has not undergone protein modification may have a longer plasma half-life and these could not only interact with the endothelium and circulating cells including the leukocytes but also could more readily penetrate the intima.

Yet another problem with the interpretation of these studies is the lack of current understanding of the mechanism(s) that might be involved in the oxidation of LDL in the plasma, if it occurs. Copper-oxidized LDL was used in all the studies that determined the clearance of oxidized LDL. It is possible that LDL oxidized by other mechanisms that do not generate similar protein modifications as the copper-oxidized LDL might exist under physiological conditions with a longer half-life.



SCHEME 1 Oxidized LDL – different forms: A native LDL; B seeded LDL; C minimally oxidized LDL; D extensively oxidized LDL; E oxidatively modified LDL. (See Color plate I at the end of this issue.)

2. Another argument that was put forth to suggest that oxidation of LDL might not occur in the plasma is that the *in vitro* oxidation of LDL is reduced even by the presence of minuscule amount of serum.[1] However, several recent studies have described methods that would permit the oxidation of whole serum or plasma by copper.[55,56] It should be noted that in these studies, serum was diluted greatly before oxidation could be followed. The oxidation of total plasma by oxidants such as AAPH has been known for a long time.[55,57–60] It is unlikely that the human plasma will be exposed to hundreds of micromolar amounts of copper or to oxidants such as AAPH. On the other hand, myeloperoxidase (MPO)-mediated oxidation of lipoprotein is likely to occur in the plasma and as mentioned later could be of important consequence to atherosclerosis. Recently, MPO-mediated oxidation of plasma was described.[61]

3. A third line of evidence is presented to suggest that atherogenic oxidation might occur in the intima rather than in the plasma argues that other lipoproteins of plasma such as very low-density lipoprotein (VLDL) or high-density lipoprotein (HDL) also might compete for oxidation.[62–66] The logic behind such an argument is that only the oxidation of LDL is atherogenic and would affect the formation of foam cells and elicit pro-atherogenic changes in cells. However, there is no *a priori* reason to believe that oxidation of lipids in LDL is likely to generate atherogenic components (oxidized lipids) that are distinct from oxidized lipids associated with other lipoproteins. While the sheer mass of LDL as compared to other lipoproteins might make its

oxidation capable of generating quantitatively more atherogenic lipids, the oxidation of other lipoproteins should also render them atherogenic. There is evidence suggesting that oxidized HDL is less capable of inducing reverse cholesterol transport from cells and oxidized HDL and VLDL might promote lipid accumulation in macrophages.[67–69]

4. Particles resembling *in vitro* oxidized LDL have been isolated from atherosclerotic artery.[70] It should be clarified that the LDL isolated from the lesion has characteristics distinct from "modified LDL" fractions that have been isolated from circulation. These include apoprotein fragmentation, oxidation-specific protein alterations, evidence of antioxidant and polyunsaturated fatty acid (PUFA) depletion, and the generation of neoantigenic epitopes. No such "oxidized LDL" has been isolated from plasma, although several groups have claimed the demonstration of the presence of "oxidation-labile" or "mildly oxidized" fractions in plasma.

OXIDATION OF LDL IN PLASMA

Despite controversies, the oxidation of LDL in the plasma has attracted considerable attention. If oxidation of LDL in plasma contributes to its atherogenicity, then its detection and prevention would be of immense value, not only in the diagnosis of cardiovascular diseases but also in its treatment.

Evidence for Oxidation of LDL in Plasma

There have been genuine concerns and questions about the potential of oxidized LDL to initiate proatherogenic events in the plasma compartment. Earlier studies by Avogaro and coworkers have documented that an electronegative subfraction is present even in the normal plasma and such LDL is internalized at a faster rate by macrophages as compared to normal plasma LDL.[71] This particle showed evidence of apoprotein aggregation and contained increased levels of malondialdehyde (MDA). Sevanian *et al.* provided definitive evidence to suggest that indeed such fractions contain large amounts of cholesterol oxidation products.[72,73] They also demonstrated that the supplementation of animals with cholesterol increase the amounts of oxidized cholesterol in this subfraction.[74]

The presence of oxidized fatty acid derivatives in plasma is known for a long time. Numerous studies have also documented the presence of MDA in plasma.[75,76] However, a definite connection between their presence and the atherogenic process had not been established. Studies by Christison and others showed that lipid peroxides are usually present in the normal cholesterol ester peroxides and are predominantly associated with the HDL and not LDL.[77] The presence of oxidized phospholipids is also recently suggested.[10,52,78] However, from these studies one cannot conclude whether these oxidized lipids were generated in the plasma or were derived from extra vascular tissues.

Recently oxidized LDL has been demonstrated in the peripheral blood.[79,80] An alternate possibility was raised that the oxidized LDL may be generated or released in the arterial blood[81,82] from arterial tissues. Salmon *et al.*[83] described a method for the detection of lipoproteins containing MDA-modified apolipoprotein B in the serum of patients with cardiovascular diseases. They coated ELISA plates with the antibodies to MDA–LDL and used peroxidase-labeled antibodies to LDL, which revealed only apolipoprotein B, the protein component of LDL. Similar approaches have been used by a number of workers to demonstrate the presence of oxidized LDL in the plasma.[81,84–87] In a study by Palinski *et al.*,[88] 13 monoclonal antibodies to various epitopes of oxidized LDL were cloned using the spleens of apoE-deficient mice (E0 antibodies). A sensitive double layered sandwich chemiluminescent immunoassay was established to determine if any of the E0 antibodies recognize the epitopes on the circulating LDL. The antibodies E06 (recognizes oxidized phospholipid epitope) and antibody

E014 (recognizes MDA–lysine like epitopes) clearly recognized three or four times the amount of epitope on the circulating LDL as did the other monoclonal antibodies or IgM controls. Thus, this study added to the findings of several other studies that some circulating LDL particles do contain selected oxidation specific epitopes, or that minimally modified forms of LDL may exist in the plasma.[81,84–87,89] The studies of Itabe *et al.*[81] reached the conclusion that the LDL oxidation occurs under some special clinical conditions and that the modified LDL is present in the circulating blood. The LDL oxidation level detected in the normal subjects was 0.52 ± 0.35 arbitrary units/5 μg protein of LDL.

In contrast to these studies, studies by Shimano *et al.* showed that there was no preformed oxidized lipoprotein in the plasma.[90] However, these investigators were able to isolate a subfraction of LDL that had an increased propensity to undergo further oxidation. This subfraction of LDL has been the topic of major importance as there is substantial evidence to indicate that plasma of subjects who are prone to coronary artery disease may contain LDL subfraction designated as subfraction B, which has the physical attributes of smaller size and heavier density.[91] This small, dense LDL subfraction has been shown to undergo oxidation at a faster rate as compared to normal LDL particle.[92] While there is no reason to expect that oxidized lipids from one particle would have a greater impact on atherosclerosis as compared to oxidized lipids on another particle, Reaven and colleagues argue that small dense LDL may enter the artery more readily as compared to larger LDL particles.[93,94]

Thus, three different speculations that can be made are, (a) the direct oxidation of LDL in the plasma compartment, (b) LDL is oxidized in the neighboring tissues such as the artery and then released into the blood stream by reverse transportation, and (c) the peroxides generated in the plasma compartment or in the neighboring tissues get associated with LDL and leads to its oxidation/modification.

THE OXIDATION PARADOX

A number of risk factors, including oxidative stress, have been identified for coronary artery disease.[95] Conversely, a number of pharmacological agents, behavior modifications, and dietary modalities have been recommended to lower the risk of coronary artery disease (CAD). These include exercise or physical activity, substitution of polyunsaturated fat including fish oil for saturated fat, and hormone replacement therapy in women.[96–98] Numerous mechanisms have been suggested to explain the action of the beneficial effects of diet, exercise, or hormone therapy. This review is not too inadequate to discuss these mechanisms in detail. However, as pointed out in the subsequent paragraph, one common factor that stands out among these three is their connection to oxidative stress. All of them impose an oxidative stress and yet they are beneficial in preventing cardiovascular diseases.

(a) Exercise and Oxidative Stress

In recent years it has been recognized that even moderate levels of physical activity such as brisk walking can add to cardiovascular benefits.[99,100] Consequently, lack of physical or sedentary lifestyle has been recognized as a risk factor.[101–103] The precise mechanism by which exercise or regular physical activity may influence the progression of CAD is not known. One would expect that physical activity as a deterrent of cardiovascular disease would be compatible with the oxidation hypothesis.

The pro-oxidant nature of exercise has been recognized for a long time[104–110] (Table I). Maximal aerobic capacity has also been inversely related to LDL-cholesterol concentration.[111–114] Exercise appears to result in an initial activation of neutrophils (priming) which has been indicated by several means including degranulation which is accompanied by increased plasma level of granular enzymes.[115–117] Several studies have reported an exercise-induced increase in the

TABLE I Selected human studies of exercise and oxidative indices

Oxidative indices	Sample	Exercise type	References
MDA ↑	Human plasma	Cycling	[182]
	Human serum	Running	[183–186]
	Human urine	Running	[187]
	Human plasma	Running	[188–190]
	Human platelets	Cycling	[191]
CD ↑	Human muscle	Running	[187]
	Human plasma	Running	[189]
8-Hydroxy-deoxyguanosine ↑	Human urine	Running	[188,190]
Pentane ↑	Human expired air	Cycling	[192,193]
GSSG ↑, GSH ↓	Human plasma	Running	[194]
	Human plasma	Cycling	[106,195–197]
	Human erythrocytes	Running	[189,198]
Vitamin E ↓	Human serum	Running	[186]
	Human plasma	Running	[198,199]
	Human plasma	Cycling	[200,201]
SOD ↑	Human erythrocytes	Running	[190]
	Human platelets	Cycling	[191]
	Human plasma	Soccer	[202]
	Human plasma	Running	[203]
GPX ↑	Human platelets	Cycling	[191]
	Human erythrocytes	Aerobics	[204]
	Human plasma	Running	[205]
	Human plasma	Aerobics	[204]
Catalase ↑	Human platelets	Cycling	[191]
MPO ↑	Human plasma	Downhill running	[206]

↑ = Increase; ↓ = decrease; MDA = malondialdehyde; CD = conjugated diene; GSH = reduced glutathione; GSSH = oxidized glutathione; SOD = superoxide dismutase; GPX = glutathione peroxidase; MPO = myeloperoxidase.

plasma level of MPO, an enzyme implicated in the oxidation of LDL in the artery.[118,119] MPO is contained in large amounts within the neutrophilic granules. Upon stimulation, the granules release substantial amounts of MPO into the extracellular environment.

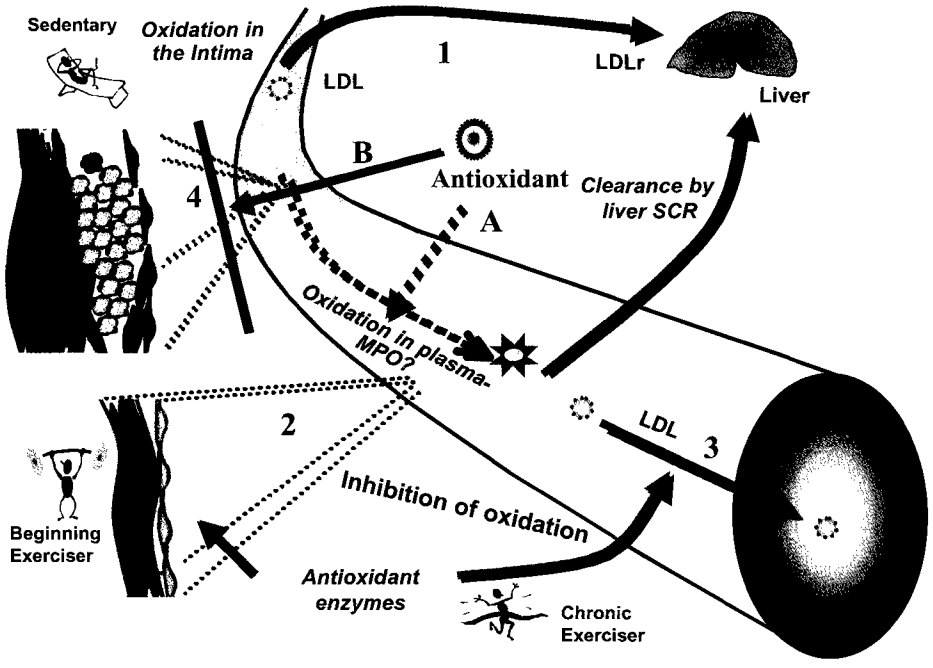
How do we reconcile with the fact that exercise is an oxidative stress and yet is a deterrent of cardiovascular disease if oxidative stress plays an important role in the etiology of the disease? There are at least two solutions to this paradox: (1) the overwhelming cardiovascular benefit of exercise might override the potential negative effects, and (2) the oxidative stress itself could be a part of the beneficial package.

This paradox was addressed recently by Shern-Brewer *et al.*,[120,121] who showed that beginning exercisers suffered an oxidative stress and as a consequence the isolated LDL was more readily oxidized *in vitro* as compared to LDL isolated from sedentary subjects. In contrast, LDL isolated from chronic exercisers was less readily oxidized as compared to sedentary subjects. One interesting observation in this study was that women regardless of the status of physical activity appeared to be well protected against oxidative damage. These seemingly contradictory results can be explained if the oxidative stress induced by exercise would promote antioxidant defense in the artery. In other words, sustained oxidative stress in the plasma might induce antioxidant defense in the arterial cells by inducing antioxidant enzymes. The overall result would be a protection of LDL against intimal oxidation in chronic exercisers despite the potential of the ongoing physical activity to induce an

oxidative stress. In other words the antioxidant enzymes associated with the arterial cells might block the seeding mechanisms of LDL so that the isolated LDL undergoes oxidation much less readily as compared to LDL isolated from beginning exercisers (Scheme 2).

Other studies in the literature, including ours,[120–122] have looked at the effects of acute exercise and have reported an enhanced susceptibility of isolated LDL to oxidation. Sanchez-Quesada *et al.*[122] had six well-trained runners run continuously for 4 h. After the exercise bout, LDL susceptibility to oxidation, measured as conjugated dienes formation, was increased significantly and the lag time *decreased*. In contrast, as would be expected from our study, Vasankari *et al.*[123] observed eight trained male runners who participated in a 31 km run and 22 male runners who participated in a marathon. After these exercise bouts, no changes were seen in LDL diene conjugation. Beard *et al.*[124] investigated 25 participants in the Pritikin Longevity Center 3-week training study. The training protocol consisted of exercise classes 5 days a week with 45 min of aerobic exercise on a treadmill or exercise. At the end of the 3-week training period, LDL was isolated from subjects and oxidized using a copper system. There was a significant *increase* in lag time ($p < 0.05$) after the training period as compared to before the training period.

The ability of younger women to resist exercise-induced oxidative changes presented an interesting possibility. In these women, estrogen might protect against oxidative injury by inducing antioxidant enzymes such as nitric oxide synthase (NOS) in the artery.[125] However, these studies do not exclude the direct effect of estrogen as an antioxidant.



SCHEME 2 Oxidative stress and LDL oxidation in plasma: 1. Native LDL is cleared predominantly by liver *via* the classical LDL receptor. 2. When there is an oxidative stress (as in beginning exercisers) LDL may be oxidized by MPO. The oxidation of LDL might result in the induction of antioxidant enzymes in the artery and its enhanced clearance *via* the liver scavenger receptor(s). 3. The increased antioxidant defense would prevent subsequent oxidative stress as seen in chronic exerciser. 4. When there is inadequate antioxidant defense or when there is overwhelming oxidative stress, further oxidation of LDL in the intima would result in atherosclerotic changes. (See Color plate II at the end of this issue.)

(b) Estrogen and Oxidative Stress

It has been noted for a long time that women of child bearing age are protected from coronary artery diseases as compared to men of the same age.[126] However, once women reach the menopausal age they appear to be equally if not more susceptible to the development of atherosclerosis.[127,128] Based on these findings it was suggested that estrogen might protect against coronary artery disease in premenopausal women. Considering that estrogen (estradiol) is a phenol, in recent years there have been attempts to link its potential antioxidant properties with the oxidation hypothesis.[129–133] In other words, there have been suggestions that the beneficial effects of estradiol could be related to its antioxidant effects. Accordingly, a number of studies have shown that estradiol is an antioxidant, at least in *in vitro* studies.[134–141] However, its antioxidant affect could be established only under conditions that are unphysiological, using micromolar concentrations. Normal concentration of estradiol in the plasma of younger women ranges between 50 and 200 pg per ml, a concentration well below 1 nM. Therefore, it is very unlikely that under physiological conditions estradiol would have an antioxidant effect. More importantly, there is no logical reason to expect that estradiol, present in nanomolar concentration would have a greater antioxidant effect than vitamin E which is present in micromolar concentrations in LDL.

It was pointed out recently that LCAT-reacted estradiol in which the 17-β-position is esterified to a fatty acid could inhibit the oxidation of LDL at a more physiological concentration.[142] If this is true, the esterified estradiol would be the most potent antioxidant ever known to man, acting at nanomolar concentrations to inhibit the oxidation of LDL. However, there has been no demonstration that such a compound exists *in vivo* or acts as an antioxidant to suppress the oxidation of LDL. It is difficult to comprehend the formation of esterified estradiol when massive amounts of free cholesterol or other potential substrates for LCAT are available in the plasma. One has to imagine a K_m of less than 1 nM of estradiol for LCAT reaction.

The antioxidant property of estradiol was put to test recently by Santanam *et al.* who showed that LDL isolated from estradiol-rich younger and estradiol-poor older women were oxidized at similar rates *in vitro*.[143] They also demonstrated that LDL isolated from women during various stages of their menstrual cycle (with different levels of plasma estradiol levels) again were oxidized at similar rates *in vitro* by copper demonstrating that physiologically relevant estradiol concentration had no effect on the rate of oxidation of LDL. The results of Santanam *et al.*[143] argues against the possibility that esterified estradiol is a physiological antioxidant unless the concentration of these derivatives were the same in older and younger women or in younger women during various time periods of their menstrual cycle (despite vast differences in plasma estradiol values).

Women with low estrogen levels are often infertile and when they undergo ovarian hyperstimulation for *in vitro* fertilization the estrogen levels often raises several thousand pg per ml. When we tested LDL samples isolated from such women to undergo oxidation *in vitro*, such LDL showed reduced susceptibility to undergo oxidation by copper as compared to LDL isolated before hyperstimulation.[143] This could be interpreted as an antioxidant effect. Surprizingly, high levels of free MPO protein was present in the plasma of such women and accordingly the isolated LDL was more readily oxidized by peroxidases as compared to LDL isolated before hyperstimulation. It has been shown for a long time that estradiol can release MPO from neutrophils.[144] Administration of animals with estrogens lead to an increase in leukocyte recruitment in steroidogenic tissues and under these conditions there is an increase in the levels of MPO, eosinophil peroxidase and uterine peroxidase, enzymes that are involved in oxidation reactions.[145–150] More importantly, estrogen

induced lipid peroxidation is well documented in literature.[151–154] Is it possible that the beneficial effects of estradiol could be related to its pro-oxidant effects? An increase in plasma MPO in women who have high estradiol levels might lead to the oxidation of LDL in the plasma. As mentioned earlier, such oxidized LDL is expected to be cleared rapidly from circulation by the liver.

Interestingly, exercise also has been noted to increase neutrophil degranulation[155,156] and the release of MPO protein.[120] We have documented an increase in plasma MPO in exercisers as compared to sedentary subjects.[120] How this increase relates to the lowering of plasma LDL can only be speculated. If MPO is involved in the oxidation of LDL in the plasma, then oxidative clearance of LDL by liver could, at least in theory, explain the lowering of cholesterol. Far fetched, as it may seem, low plasma cholesterol has been observed in subjects with infectious diseases who have increased neutrophil activity and increased plasma MPO levels.[157–159] In addition to increasing antioxidant defense in the artery, such oxidation might also deliver oxidized sterols to the liver and limit *de novo* cholesterol synthesis.

(c) PUFA and Oxidative Stress

PUFA are vulnerable to peroxidation.[160] Plethora of studies have documented the increased presence of lipid peroxidation products and depletion of antioxidants in subjects who consume predominantly PUFA-enriched diet. This is more so in the case of subjects who consume higher amounts of fish oil-derived PUFA.[161] *In vitro* studies have documented the vulnerability of lipoproteins enriched in PUFA to oxidation as compared to lipoproteins enriched in monounsaturated fatty acids (MUFA).[162] Similarly cells that are enriched in PUFA appear to release more reactive oxygen species and appear to be affected more by oxidative stress.[163] Based on these findings, one would expect MUFA, which lowers cholesterol as much as PUFA[164,165] would be more beneficial in preventing atherosclerosis. Yet, a low fat diet, with predominant intake of PUFA is recommended by the AHA for lowering plasma cholesterol. Rudel and associates have convincingly established that feeding of PUFA to cholesterol-fed monkeys decreases the formation of atherosclerotic lesions as compared to animals fed MUFA or saturated fatty acids.[166] On the contrary, MUFA appears not only to be ineffective but seem to increase the severity of lesions as observed in mutant mouse models.[167]

There might be several mechanism(s) by which PUFA might protect against CAD. There is overwhelming evidence to suggest that increased consumption of PUFA impose an oxidative stress and even in the monkeys in which PUFA retarded the progression of the lesions, there was evidence of oxidative stress.[168,169]

SUMMARY

In summary, it can be concluded that physical activity, estrogens, and the consumption of PUFA could be an oxidative stress. If so, either such a stress is beneficial or the overwhelming benefits derived from these override the deleterious effects of oxidative stress. How can the oxidative stress be beneficial? Long time ago, the induction of prostacyclin synthesis by oxidized LDL was reported[170,171] but went unnoticed. A number of recent studies have documented the induction of antioxidant enzymes by oxidants. Superoxide dismutase (SOD),[172,173] heme oxygenase,[174–176] NOS[177] and catalase,[178] have been shown to be induced by H_2O_2 and lipid peroxides. In addition, a number of potentially anti-atherogenic effects of oxidized lipids could be viewed as anti-atherogenic[17] (Table II). For example, the induction of VCAM-1, an adhesion molecule that might be involved in the recruitment of sub-endothelial monocytes, by oxidants has been well established.[179,180] On the other hand, the soluble form of VCAM-1 that is released into the plasma, has been recognized as an angiogenic factor and thus could help to promote neovascularization.

TABLE II Anti-atherogenic effects of oxidized lipids

Oxidized lipid	Gene induced	Anti-atherogenic effect
Hydroperoxy linoleic acid (13-HPODE), 13-HODE, oxidized LDL	VCAM-1[8]	s-VCAM-1, the soluble form of VCAM-1 is an angiogenic factor that would promote neo-vascularization[207]
13-HPODE	NOS[177]	Potent vasodilator and antioxidant[208]
13-HPODE	Heme oxygenase[176]	Generates antioxidant products such as bilirubin, biliveridin, and carbon monoxide[209]
Lyso PtdCho		Causes cholesterol efflux from cells[210] Generated in vast quantities by LCAT and might promote *de novo* HDL synthesis by liver[78]
Cholesterol oxidation products		Inhibit cholesterol synthesis[211] Could potentially inhibit smooth muscle cell growth?
Unknown components of oxidized LDL	Mn-SOD[173]	Quench superoxide radicals, which may be involved in the oxidation of LDL
Unknown components of oxidized LDL		Increased glutathione synthesis[212]
13-HPODE, 13-HODE	Catalase	Antioxidant enzyme[213,214]

Similarly, the oxysterol of oxidized LDL could help to reduce cholesterol synthesis.[181]

From the above discussion, one can imagine a scenario in which low and sustained levels of oxidative stress in the extracellular milieu and in plasma might help to maintain adequate antioxidant enzyme presence in the arterial cells (Scheme 2). When there is overwhelming oxidative stress or when the antioxidant enzyme genes fail to respond, there might be a need for external supplementation of antioxidants. Aging population and those with established disease states in which the oxidative stress is exacerbated as in diabetes or chronic hypertension or those with physical inactivity might thus depend on external antioxidant supplementation. Again, the antioxidant should be available where it is needed and at concentrations sufficient enough to overwhelm the oxidative stress. More importantly, the antioxidant itself should not become an oxidative problem by imposing an oxidative metabolic threat.

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