

Oxidized Cholesterol in the Diet Accelerates the Development of Atherosclerosis in LDL Receptor- and Apolipoprotein E-Deficient Mice

Ilona Stapanans, Xian-Mang Pan, Joseph H. Rapp, Carl Grunfeld, Kenneth R. Feingold

Abstract—The aim of the current study was to determine whether oxidized cholesterol in the diet accelerates atherosclerosis in low density lipoprotein receptor- (LDLR) and apolipoprotein E- (apo E) deficient mice. Mice were fed either a control diet or a diet containing oxidized cholesterol. For LDLR-deficient mice, the control diet consisted of regular mouse chow to which 1.0% cholesterol was added. The oxidized diet was identical to the control diet except that 5% of the added cholesterol was oxidized. In apo E-deficient mice, the control diet contained 0.15% cholesterol, whereas in the oxidized diet, 5% of the added cholesterol was oxidized. LDLR-deficient and apo E-deficient mice were fed the experimental diets for 7 and 4 months, respectively. In mice fed the oxidized-cholesterol diets, the levels of oxidized cholesterol in sera were increased. At the end of the experiment, aortas were removed and atherosclerosis was assessed. We found that in LDLR-deficient mice, feeding of an oxidized-cholesterol diet resulted in a 32% increase in fatty streak lesions ($15.93 \pm 1.59\%$ versus $21.00 \pm 1.38\%$, $P < 0.03$). Similarly, in apo E-deficient mice, feeding of an oxidized-cholesterol diet increased fatty streak lesions by 38% ($15.01 \pm 0.92\%$ versus $20.70 \pm 0.86\%$, $P < 0.001$). The results of the current study thus demonstrate that oxidized cholesterol in the diet accelerates fatty streak lesion formation in both LDLR- and apo E-deficient mice. (*Arterioscler Thromb Vasc Biol.* 2000;20:708-714.)

Key Words: oxidized cholesterol ■ atherosclerosis ■ oxidized-lipid diet ■ cardiovascular disease

There is compelling evidence that oxidized lipoproteins are atherogenic and play a key role in the pathogenesis of coronary heart disease.^{1,2} This hypothesis is supported by extensive in vitro and in vivo studies demonstrating that dietary antioxidants reduce lesion formation in experimental animal models.³⁻⁵ Oxidized lipoproteins have also been identified in atherosclerotic lesions in animals and humans.^{6,7} The origin of oxidized lipoproteins in vivo is not clear, since the site and mechanism whereby lipoproteins are oxidized have not been resolved. It has been suggested that the oxidation of lipoproteins may occur locally in the artery wall^{1,2} or that circulating oxidized lipoproteins are sequestered in atherosclerotic lesions.

Studies at our laboratory and others have shown that oxidized lipids in the diet may play a significant role in generating oxidized lipoproteins. In animals, oxidized fatty acids in the diet are absorbed, incorporated into serum lipoproteins, and transported to the liver,⁸⁻¹² where they are incorporated into VLDLs, which are secreted into the circulation.¹⁰ Moreover, consumption of diets containing oxidized fatty acids also results in increased levels of oxidized lipids in the postprandial chylomicrons of humans.^{13,14}

Similar to fatty acids, dietary cholesterol is also easily oxidized^{15,16} and is incorporated into serum lipoproteins in

both animals¹⁷⁻²⁰ and humans.²¹ Most importantly, we have recently shown that both oxidized fatty acids²² and oxidized cholesterol²³ in the diet accelerate fatty streak formation in cholesterol-fed rabbits. Thus, our observations suggest that oxidized fatty acids and cholesterol in the diet may constitute a risk factor for cardiovascular disease.

In the rabbit, the majority of cholesterol in the circulation is carried in β -VLDL.²⁴ This animal model has been criticized in that it fails to mimic human lipoprotein metabolism and that the aortic lesions that develop with cholesterol feeding are not identical to those seen in humans. Recently, murine models, such as LDL receptor- (LDLR) deficient and apo E-deficient mice have been generated that develop extensive atherosclerosis and have been widely used as models in which to study the atherogenic process.²⁵ These animals mimic human lipoprotein disorders that are associated with an increased risk of coronary heart disease. In LDLR-deficient mice, which are similar to humans with familial hypercholesterolemia, the majority of cholesterol in the circulation accumulates in serum LDL particles, particularly after ingestion of a high-cholesterol diet.²⁶ In apo E-deficient mice, which are similar to humans with familial dysbetalipoproteinemia, the accelerated atherosclerosis is due to increased serum chylomicron and VLDL remnants. In this

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From the Department of Veterans Affairs Medical Center and the Departments of Surgery (I.S., X-M.P., J.H.R.) and Medicine (C.G., K.R.F.), University of California, San Francisco.

Correspondence to: Ilona Stapanans, PhD, Lipid Research Laboratory (151L), VA Medical Center, San Francisco, CA 94121. E-mail stapan@itsa.ucsf.edu

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model, aortic atherosclerosis resembles human lesions.²⁷ Therefore, we have now determined whether oxidized cholesterol in the diet accelerates atherosclerosis in LDLR- and apo E-deficient mice.

Methods

Animal Models

Homozygous LDLR-deficient and apo E-deficient mouse strains with a C57BL/6J background were generated from breeding pairs purchased from Jackson Laboratories (Bar Harbor, Me). Mice were maintained on a regular mouse chow diet in a temperature-controlled animal facility and were administered experimental diets at the age of 12 weeks. The project was approved by the Animal Care Committee at the San Francisco Veterans Affairs Medical Center.

Test Diets and Experimental Protocol

Mice in each strain were divided into 2 groups to receive 2 cholesterol-containing test diets: the control diet and the oxidized diet. One group of LDLR-deficient mice was fed the control diet of regular mouse chow to which 1.0% cholesterol had been added. The other group was fed the oxidized diet, which was identical to the control diet except that 5% to 10% of the added cholesterol consisted of cholesterol oxidation products (ie, the diet contained 1% cholesterol and 0.05% to 0.10% oxidized cholesterol). In the control diet group (n=11) there were 5 males and 6 females. In the oxidized diet group (n=11), there were 6 males and 5 females. Apo E-deficient mice received similar diets except that in the control diet, the cholesterol concentration was reduced to 0.15%. In the oxidized diet, 5% to 10% of the added cholesterol was oxidized (ie, the diet contained 0.15% cholesterol and 0.0075% to 0.015% oxidized cholesterol). In the control diet group (n=13) there were 7 males and 6 females. In the oxidized-diet group (n=17) there were 8 males and 9 females. The concentrations of cholesterol used were based on previous reports in the literature.²⁸

Oxidized cholesterol for the diets was prepared by heating. Cholesterol (10 g) was dissolved in ether in a 2-L round flask, and then ether was evaporated on a rotary evaporator, resulting in a thin film. The flask was placed in a 100°C oven and heated overnight (16 hours). The content of the flask was redissolved in ether, and the composition of cholesterol oxidation products was analyzed by gas-liquid chromatography (GLC).²⁹ Under these conditions, ≈90% of the cholesterol was not altered and the remainder (5% to 10%) was oxidized, yielding numerous oxidation products. When examined by GLC, ≈50% of the total altered cholesterol could be identified. Thus, 2.5% to 5% of heated cholesterol contained identifiable cholesterol oxidation products. Typically, the identifiable fraction of oxidized cholesterol had the following composition: 7% to 10% cholest-5-ene-3 β ,7 α -diol (7 α -hydroxycholesterol); 15% to 20% cholest-5-ene-3 β ,7 β -diol (7 β -hydroxycholesterol); 15% to 20% 5,6 β -epoxy-5 β -cholestan-3 β -ol (β -epoxycholesterol); 10% to 15% 5,6 α -epoxy-5 α -cholestan-3 β -ol (7 α -epoxycholesterol); 40% to 45% 3 β -hydroxycholest-5-ene-7-one (7-ketocholesterol); and a trace of cholest-5-ene-3 β ,25-diol (25 β -hydroxycholesterol). The control diet contained no detectable oxidation products. Cholesterol was added to diets in an ether solution, and the solvent was evaporated under an N₂ stream before feeding. Animals were given food and water ad libitum. LDLR-deficient and apo E-deficient mice were fed the experimental diets for 7 and 4 months, respectively.

Oxidized Cholesterol Determination in Serum

Oxidized cholesterol levels in the sera of mice were determined by GLC as described previously by Hughes et al.²⁹ Oxidized cholesterol levels in serum lipoprotein fractions were determined in a Hewlett-Packard model 5890 GLC utilizing a DB-1 bonded-phase capillary column (30 m \times 0.25-mm inner diameter, J & W Scientific). A Hewlett-Packard 3600 integrator series II was used for data analysis. Lipoproteins were extracted as described by Folch et al.³⁰ and the lipid-containing fraction was analyzed for cholesterol and oxidized cholesterol (7 β -hydroxycholesterol, 7 α -hydroxycholesterol, 25 α -hydroxycholesterol, 7-ketocholesterol, α -epoxycholesterol, and β -epoxycholesterol). Standards for oxidized cholesterol were determined

by GLC were obtained from Steraloids, Inc. To correct for preparative losses during the extraction procedure and sample injection, a 5 α -cholestane standard was added to the serum sample. To minimize the destruction of any oxidized cholesterol products, the saponification in this procedure was performed at room temperature for 12 hours as described by Addis et al.³¹ For the determination of oxidized cholesterol levels in the serum, a separate set of male LDLR-deficient and female apo E-deficient mice on both diets was used.

Determination of Serum Lipid Peroxide Levels

Lipid peroxide levels in the serum were determined by measuring conjugated dienes as described by us previously.^{13,14} Serum samples were extracted, and conjugated dienes were measured by second-derivative UV spectroscopy. It should be noted that this method does not yield an absolute measurement of lipid peroxides but rather a comparison of lipid oxidation between groups. Serum lipid peroxide levels were determined in male LDLR-deficient and female apo E-deficient mice.

Fatty Streak Lesion Quantification

Lesion areas in mouse aortas were quantified as described by Tangirala et al.²⁸ The stained fatty streak lesions in the enlarged photographs were traced on a digital tablet (Kurta IS/ADB, Inmac Inc), and the areas of the lesions were measured using MacDraft software on a Macintosh computer.

Analytical Methods

Total serum cholesterol (kit No. 352-20, Sigma Chemical Co) and triglycerides (kit No. 339-20, Sigma) were determined by enzymatic assays. HDL cholesterol was determined after removing apo B-containing particles by the magnesium/dextran sulfate precipitation method as described by the manufacturer (Sigma).

Statistical Analysis

All data are presented as mean \pm SEM. Student's *t* test was used to test for significance. Differences of *P*<0.05 between diet groups were considered significant. Calculations were performed with the Biostatistics program by Stanton Glanz (New York, NY; McGraw-Hill, Inc; 1988) for the Macintosh.

Results

Serum Lipid Levels and Body Weights

At the end of the experiments in LDLR- and apo E-deficient mice, body weights were similar in animals fed the cholesterol or the oxidized diet (Table 1). In LDLR-deficient mice, total serum cholesterol levels were significantly lower (*P*=0.049) in animals fed the oxidized diet. There was no difference in serum cholesterol levels when male animals were compared with female animals (19.59 \pm 4.43 versus 18.70 \pm 2.61 mmol/L, *P*=0.861 for control diet; 13.67 \pm 0.80 versus 15.80 \pm 3.03 mmol/L, *P*=0.478 for oxidized diet). In apo E-deficient mice, the concentration of oxidized cholesterol in the diet was lower, and no reduction in serum cholesterol levels was observed in animals fed the oxidized diet. There was no difference in serum cholesterol levels between male and female animals (17.42 \pm 1.77 versus 20.39 \pm 0.49 mmol/L, *P*=0.161 for control diet; 17.78 \pm 0.84 versus 18.88 \pm 4.60 mmol/L, *P*=0.827 for oxidized diet). Serum HDL levels were not significantly altered by ingestion of the oxidized-cholesterol diet in either LDLR- or apo E-deficient mice. In LDLR-deficient mice, serum triglyceride levels were higher in mice fed the oxidized diet, but this difference was not statistically significant (*P*=0.084).

TABLE 1. Comparison of Serum Cholesterol, HDL Cholesterol, and Triglyceride Levels and Body Weights in LDLR- and Apo E-Deficient Mice

Variable	LDLR-Deficient Mice		Apo E Receptor-Deficient Mice	
	Control Diet (n=11)	Oxidized Diet (n=11)	Control Diet (n=13)	Oxidized Diet (n=17)
Cholesterol, mmol/L	19.4±2.12	14.44±1.35*	18.62±0.93	18.82±1.22
HDL cholesterol, mmol/L	1.22±0.18	1.66±0.14	0.25±0.02	0.20±0.01
Triglycerides, mmol/L	1.07±0.11	1.69±0.29	0.93±0.06	1.29±0.23
Body weight, g	24.6±0.98	25.00±1.53	30.20±1.59	29.60±2.32

Results are expressed as mean±SEM.

* $P=0.049$.

Oxidized Cholesterol in Serum

When oxidized cholesterol levels were measured in LDLR-deficient mice, no cholesterol oxidation products could be detected by our method in the sera of mice fed the control diet that contained no detectable oxidized cholesterol (Figure 1). In contrast, in LDLR-deficient mice fed a diet containing oxidized cholesterol, measurable levels of 7-ketocholesterol (0.85 ± 0.30 $\mu\text{g}/\text{mg}$ cholesterol) and α -epoxycholesterol (1.17 ± 0.19 $\mu\text{g}/\text{mg}$ cholesterol) were detected. Oxidized cholesterol detected in the sera of LDLR-deficient mice fed an oxidized-cholesterol diet is likely to be derived from dietary sources.

In apo E-deficient mice, oxidized cholesterol was detected in the sera of both diet groups (Figure 2). 7-Ketocholesterol (1.13 ± 0.29 $\mu\text{g}/\text{mg}$ cholesterol), 7 β -hydroxycholesterol (4.40 ± 0.92 $\mu\text{g}/\text{mg}$ cholesterol), β -epoxycholesterol (15.28 ± 1.00 $\mu\text{g}/\text{mg}$ cholesterol), and 7 α -hydroxycholesterol (1.49 ± 0.68 $\mu\text{g}/\text{mg}$ cholesterol) were present in the sera of mice fed the control diet that contained no detectable oxidized cholesterol (Figure 2). Mice that were fed the oxidized-cholesterol diet had a 4-fold increase in serum concentrations of 7-ketocholesterol (5.5 ± 0.56 $\mu\text{g}/\text{mg}$ cholesterol, $P<0.001$) and a 100% increase in 7 β -hydroxycholesterol (9.95 ± 0.81 $\mu\text{g}/\text{mg}$ cholesterol, $P<0.001$), the 2 main oxidized-cholesterol components identified in the diet. Moreover, mice fed the oxidized diet had α -epoxycholesterol in their sera (1.97 ± 0.22 $\mu\text{g}/\text{mg}$ cholesterol),

which was undetectable in mice fed the control diet. No increase was observed in 7 α -hydroxycholesterol and β -epoxycholesterol levels in the sera of apo E-deficient mice after feeding of the oxidized-cholesterol diet.

Determination of Serum Lipid Peroxide Levels

To determine whether a diet containing oxidized cholesterol is associated with increased levels of fatty acid-derived lipid peroxides, we next measured serum conjugated dienes in mice fed the control and oxidized-cholesterol diets. Very low levels of conjugated dienes were detected in LDLR-deficient mice fed the control diet, and no increase was observed when mice were fed a diet containing oxidized cholesterol (1.76 ± 0.19 versus 1.58 ± 0.32 nmol/mg serum triglyceride, Figure 3). In apo E-deficient mice fed the control diet, the serum levels of endogenous conjugated dienes were higher than in the sera of LDLR-deficient mice (1.76 ± 0.19 versus 4.88 ± 0.47 nmol/mg serum triglyceride). Moreover, there was a 70% increase in serum conjugated dienes in apo E-deficient mice fed the oxidized-cholesterol diet when compared with the control diet group (7.87 ± 0.24 versus 4.88 ± 0.47 nmol/mg serum triglyceride, $P=0.005$; Figure 3). Thus, in LDLR-deficient mice, the levels of lipid peroxides in serum were low and were not affected by the dietary intake of

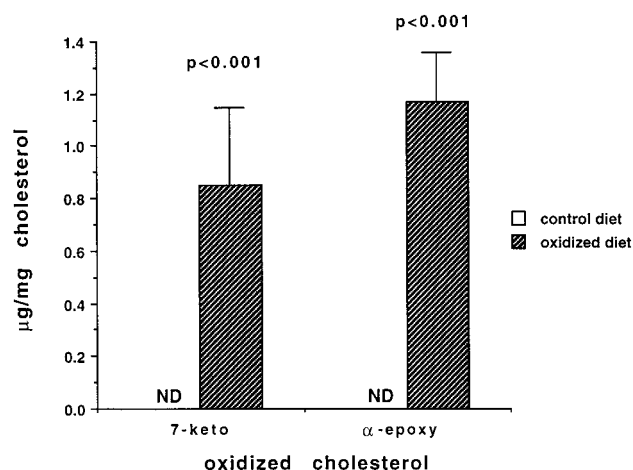


Figure 1. Oxidized cholesterol levels in the sera of LDLR-deficient male mice. Mice were fed (for 7 months) either a control diet containing 1.0% cholesterol (n=6) or an oxidized-cholesterol diet containing 1.0% cholesterol and in which 5% of the added cholesterol was oxidized (n=6). 7-Keto indicates 7-ketocholesterol; α -epoxy, α -epoxycholesterol; ND, not detected.

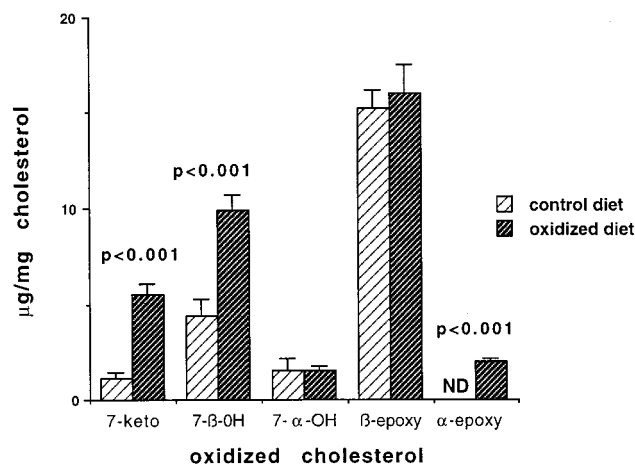


Figure 2. Oxidized cholesterol levels in the sera of apo E-deficient female mice. Mice were fed (for 4 months) either a control diet containing 0.15% cholesterol (n=6) or an oxidized-cholesterol diet containing 0.15% cholesterol and in which 5% of the added cholesterol was oxidized (n=6). 7-Keto indicates 7-ketocholesterol; 7-β-OH, 7 β -hydroxycholesterol; 7-α-OH, 7 α -hydroxycholesterol; β-epoxy, β-epoxycholesterol; α-epoxy, α-epoxycholesterol; ND, not detected.

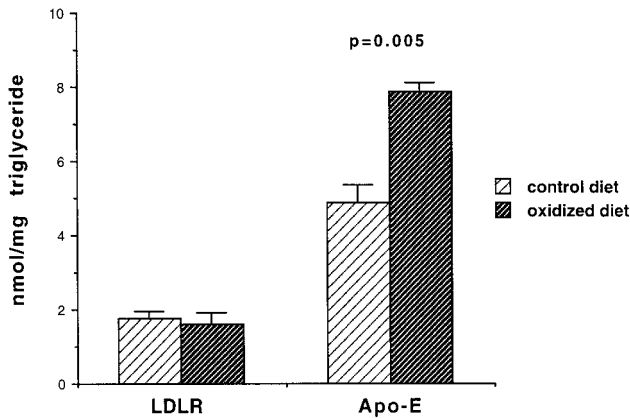


Figure 3. Lipid oxidation in the sera of LDLR- and apo E-deficient mice measured as conjugated dienes. Mice were fed either the control or oxidized diet. $n=4$ for each group.

oxidized cholesterol. On the other hand, in apo E-deficient mice, dietary oxidized cholesterol significantly increased the levels of conjugated dienes, indicating an increase in serum lipid peroxides.

Effect of Oxidized Dietary Cholesterol on Fatty Streak Lesion Formation

At the end of the experiment, aortas from LDLR- and apo E-deficient mice were removed and the percent fatty streak lesions determined. In LDLR-deficient mice (Figure 4), despite their lower serum cholesterol concentrations, feeding of an oxidized-cholesterol diet resulted in a 32% increase in fatty streak lesions ($15.93 \pm 1.59\%$ versus $21.00 \pm 1.38\%$, $P < 0.03$). Similarly, in apo E-deficient mice, feeding of an oxidized-cholesterol diet resulted in a 38% increase in fatty streak lesions ($15.01 \pm 0.92\%$ versus $20.70 \pm 0.86\%$, $P < 0.001$). These results demonstrate that oxidized cholesterol in the diet increases fatty streak lesions in both LDLR- and apo E-deficient mice.

Previous studies by other investigators have shown that sex influences the extent of fatty streak lesions in these murine models of atherosclerosis.²⁸ Therefore, we analyzed our data separately for male and female animals. As shown in Table 2, in LDLR-deficient mice, feeding of an oxidized-cholesterol diet increased fatty streak lesions by 63% in males, whereas

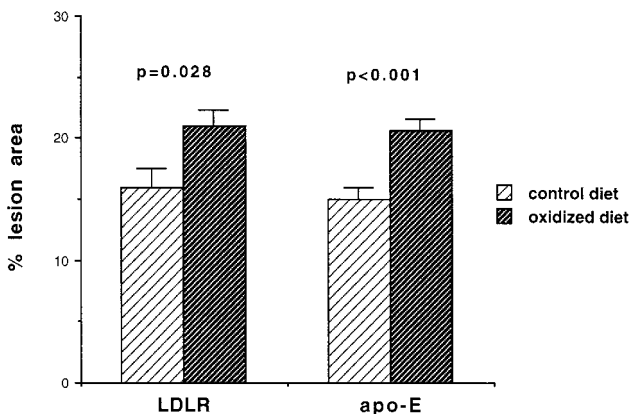


Figure 4. Areas of fatty streak lesions in aortas of LDLR- and apo E-deficient mice fed control and oxidized diets. In LDLR-deficient mice, there were 11 animals in the control and the oxidized groups. In apo E-deficient mice, there were 13 animals in the control group and 17 animals in the oxidized group.

in female mice the extent of fatty streak lesion formation was not significantly altered by feeding of the oxidized-cholesterol diet. Thus, male LDLR-deficient mice appear to be more susceptible to the atherogenic effects of dietary oxidized cholesterol. In contrast, in apo E-deficient mice, feeding of an oxidized-cholesterol diet increased fatty streak lesions in both males and females by 45% and 25%, respectively. Moreover, differences in lesion areas were observed between male and female mice when fed the control diet (Table 2); however, these differences did not reach statistical significance. In LDLR-deficient mice, the percent lesion areas for male and female mice were $13.16 \pm 1.15\%$ and $18.20 \pm 2.47\%$, respectively ($P = 0.119$). For apo E-deficient mice, the percent lesion areas were $13.49 \pm 0.60\%$ and $16.78 \pm 1.60\%$ for males and females, respectively ($P = 0.065$).

Discussion

It is well established that the typical diet in Western countries contains high concentrations of cholesterol oxidation products.^{31–39} When exposed to heat, air, light, and oxidizing agents, cholesterol undergoes spontaneous oxidation, forming products that can sometimes be identified and quantified by GLC.^{16,29} Food processing, especially heat treatment and drying, induces cholesterol oxidation in foods including dairy products, eggs, meat, and fish.^{31–39} Oxidized cholesterol is also present in bakery products, since the major ingredients, eggs and butter, contain large amounts of oxidized cholesterol. Thus, many foods in the Western diet contain cholesterol oxidation products.

Studies have demonstrated that in rabbits, dietary oxidized cholesterol is absorbed by the small intestine and becomes incorporated into serum chylomicrons and VLDL and LDL fractions.¹⁷ Our laboratory has shown that in cholesterol-fed rabbits, oxidized cholesterol in the diet is absorbed by the small intestine and is transported via serum lipoproteins to the liver.²³ There is also evidence that dietary oxidized cholesterol is absorbed and recovered in serum VLDL and lymph chylomicrons of rats.^{19,20} Moreover, in humans, a meal containing oxidized cholesterol results in increased levels of oxidized cholesterol in chylomicrons and in total plasma.²¹ Thus, different studies by several investigators have demonstrated that dietary oxidized cholesterol results in increased levels of oxidized lipids in the circulation.

In the current study, LDLR- and apo E-deficient mice were fed control and oxidized-cholesterol diets, and serum cholesterol and oxidized-cholesterol levels were determined. In LDLR-deficient mice, mice fed the oxidized-cholesterol diets had significantly lower levels of serum cholesterol when compared with cholesterol-fed animals (Table 1). The mechanism for this reduction in serum cholesterol levels in mice fed oxidized cholesterol is not clear, but it may have resulted from impaired cholesterol absorption due to the presence of oxidized cholesterol in the diet, as previously shown in rats by Osada et al.²⁰ Those authors demonstrated that in the presence of oxidized cholesterol, the absorption of other fats, including cholesterol, is decreased.

Increased serum levels of oxidized cholesterol after ingestion of oxidized cholesterol-containing diets were detected in both LDLR- and apo E-deficient mice (Figures 1 and 2). In these mice, dietary oxidized cholesterol is absorbed and enters the circulation, thus contributing to the overall oxida-

TABLE 2. Comparison of Aortic Lesion Areas in Males and Females of the LDLR- and Apo E-Deficient Mouse Strains

	LDLR-Deficient Mice		Apo E Receptor-Deficient Mice	
	Control Diet	Oxidized Diet	Control Diet	Oxidized Diet
Male	13.16±1.15 (5)	21.45±1.43 (6)*	13.49±0.6 (7)	19.52±1.30 (8)*
Female	18.20±2.47 (6)	19.34±2.51 (5)	16.78±1.6 (6)	21.74±1.07 (9)†

Results are expressed as mean±SEM. Numbers in parentheses indicate number of animals in each group.

* $P=0.002$, † $P=0.02$.

tive stress in these mice. Moreover, in apo E-deficient mice, oxidized cholesterol was present in their sera even when they were fed the control diet that did not contain any detectable cholesterol oxidation products. The explanation for the presence of cholesterol oxidation products in apo E-deficient mice fed the diet containing no detectable oxidized cholesterol and not in LDLR-deficient mice is not clear. However, one can speculate that oxidized cholesterol is observed in animal models in which the clearance of diet-derived chylomicron remnants is impaired, as occurs in cholesterol-fed rabbits²⁴ and apo E-deficient mice.²⁷ This impairment in remnant clearance might allow for the accumulation of oxidized cholesterol that is present in the diet at very low levels, although in vivo generation cannot be ruled out.

The relative amounts of the various species of oxidized cholesterol present in the diet were not reflected in the sera of mice fed the oxidized-cholesterol diet. This difference between diet and serum is probably due to differences in the extent of absorption and clearance from the circulation of each individual oxidized-cholesterol species.^{20,40} The extent to which each of the oxidized-cholesterol species found in the diet contributes to the observed increase in fatty streak lesion formation in mouse aortas remains to be determined. Moreover, in apo E-deficient mice, ingestion of the oxidized-cholesterol diet resulted in increased levels of oxidized fatty acids in serum. The mechanism by which the ingestion of oxidized cholesterol results in an increase in serum lipid peroxides is not clear at the present time, but one can speculate that the increase in oxidized cholesterol in these lipoproteins serves as a nidus to increase the susceptibility to endogenous oxidation. At present, it is not known exactly how a nidus of oxidized cholesterol leads to increased susceptibility to oxidation. Several mechanisms are possible, including the following: (1) oxidized cholesterol accelerates the use of antioxidants such as vitamin E, thereby increasing the ability of other pro-oxidants to oxidize fatty acids or lipoproteins; (2) oxidized cholesterol, when present in lipoproteins, may alter the lipoprotein structure, so that fatty acids are more exposed and therefore more prone to oxidation; (3) oxidized cholesterol may inhibit the action of enzymes such as paraoxonase that protect lipoproteins from being oxidized.^{41,42}

Most importantly, our results demonstrate that oxidized cholesterol in the diet increases fatty streak lesions in aortas of both LDLR- and apo E-deficient mice (Figure 4). In LDLR-deficient mice fed diets that contained oxidized cholesterol, fatty streak lesions increased from 15.93% to 21.00% (32% increase). It should be noted that in LDLR-deficient mice, the 32% lesion increase is probably underestimated

because of the lower serum cholesterol levels observed in these mice when fed the oxidized diet. In apo E-deficient mice, the lesion area increased from 15.01% to 20.70% (38% increase).

These results confirm and extend our observations in cholesterol-fed rabbits, wherein we demonstrated that the addition of oxidized cholesterol to the diet increased atherosclerosis by 100%.²³ Moreover, the pathogenesis of atherosclerosis is different in these animal models. Apo E-deficient mice represent a model for impaired chylomicron remnant and IDL clearance,²⁷ whereas LDLR-deficient mice represent a model for impaired LDL and IDL clearance.²⁶ In the cholesterol-fed rabbit, the pathogenesis of atherosclerosis is due to increased formation of β -VLDL.²⁴ Thus, we have shown that oxidized cholesterol in the diet promotes fatty streak lesion formation in several different animal models of atherosclerosis.

Our observations demonstrating the atherogenicity of dietary oxidized cholesterol are supported by studies from other investigators. Jacobson et al⁴³ observed a 5-fold increase in coronary atherosclerosis in White Carneau pigeons after feeding oxidized cholesterol in amounts that were comparable to the average US dietary intake. Recently, Vine et al¹⁸ demonstrated that rabbits fed oxidized cholesterol had markedly increased aortic cholesterol deposition, suggesting an increase in aortic fatty streak lesion development. On the other hand, Higley et al⁴⁴ reported that oxidized cholesterol has a protective effect on cholesterol-induced atherosclerosis in rabbits. However, in that study, oxidized cholesterol concentrations in the diet were very high (120 to 240 mg/d), and such high concentrations of oxidized cholesterol have been shown to impair the absorption of both cholesterol and oxidized cholesterol from the diet,¹⁹ thus making it difficult to interpret the results.

Atherosclerosis is a complex process, and there are several mechanisms by which oxidized cholesterol in lipoproteins could accelerate atherosclerosis.^{1,2,45} Oxidized cholesterol is cytotoxic to arterial wall cells, including endothelial cells in vitro.^{46–49} Moreover, several in vivo studies have demonstrated that oxidized cholesterol causes endothelial cell injury in animals.^{50–56} Oxidized cholesterol-containing lipoproteins could also accelerate lipid uptake by macrophages, induce monocyte recruitment and adhesion to the endothelium, and alter cell migration and growth.^{1,2,45} The incorporation of dietary oxidized cholesterol into serum lipoproteins could also accelerate atherosclerosis by increasing the susceptibility of serum lipoproteins to further oxidation in vivo, as described by Vine et al¹⁸ and supported by our finding of increased serum lipid peroxidation in apo E-deficient mice.

Regardless of the precise mechanism by which oxidized cholesterol leads to atherosclerosis, our studies and those of others demonstrate that the addition of oxidized cholesterol to the diet increases atherosclerosis in multiple animal models.

In summary, the current study demonstrates that oxidized cholesterol in the diet accelerates fatty streak lesion formation in both LDLR- and apo E-deficient mice, 2 different animal models of atherosclerosis. Given our observation in rabbits and the results of others, the data suggest that dietary oxidized cholesterol is atherogenic and may constitute a risk factor for atherosclerotic disease. Reducing the consumption of cholesterol oxidation products could reduce atherosclerosis.

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Ilona Staprans, Xian-Mang Pan, Joseph H. Rapp, Carl Grunfeld and Kenneth R. Feingold

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