Oxidatively Modified HDLs Are Potent Inhibitors of Cholesterol Biosynthesis in Human Skin Fibroblasts

Giancarlo Ghiselli, Laura Giorgini, Maurizio Gelati, and Roberto Musanti

Several biological properties of lipoproteins are modified by oxidative reactions. Modified lipoproteins are rapidly degraded by macrophages, and this is likely to be a major pathway for the formation of foam cells in the early phases of atherosclerosis. The effect of modification on other aspects of cholesterol homeostasis has, however, received lesser attention. In this study, the influence of copper ion— as well as rat aortic smooth muscle cell—oxidation-modified high density lipoprotein (HDL) on cholesterol biosynthesis in human skin fibroblasts has been investigated. Modified lipoproteins eluted at higher ionic strength than did control HDL on a Mono-Q 5/5 anion-exchange column. However, only copper ion—modified HDLs displayed greater electrophoretic mobility than did control lipoproteins on agarose gel electrophoresis. Both control and modified HDLs decreased cholesterol esterification in fibroblasts. On the other hand, whereas control HDLs were virtually ineffective in modulating cholesterol biosynthesis, modified HDLs had a significant suppressing effect. This was observed in normal as well as low density lipoprotein (LDL) receptor-defective fibroblasts, which are unresponsive to the LDL-mediated downregulation of cholesterol synthesis. These results are consistent with the concept that oxidative modification of HDLs drastically alters their effect on cholesterol homeostasis in fibroblasts. The data furthermore suggest the existence of a lipoprotein pathway for cholesterol biosynthesis regulation that is independent of the LDL receptor-mediated pathway. Downregulation of cholesterol biosynthesis would be a new function for oxidatively modified lipoproteins. (Arteriosclerosis and Thrombosis 1992;12:929–935)

KEY WORDS • high density lipoproteins • lipoprotein oxidation • human skin fibroblasts • cholesterol biosynthesis • low density lipoprotein receptor • reverse cholesterol transport • atherosclerosis

Interaction of oxidatively modified lipoproteins with macrophages has raised considerable interest and may be a key phenomenon in the conversion of resident monocytes/macrophages of the arterial wall into foam cells, the earliest histological evidence of atherosclerosis.1,2 Whereas much is known about the effect that oxidatively modified lipoproteins have on macrophage cholesterol homeostasis, considerably less information is available about the effects that these altered lipoproteins have on cells that express only the low density lipoprotein (LDL) receptor. In these cells, unlike macrophages, cholesterol metabolism is tightly coordinated through a process involving the specific uptake of LDL, leading to downregulation of both cholesterol biosynthesis and LDL receptor expression.3 In this way, further LDL uptake is decreased and intracellular accumulation of cholesterol is prevented. As much as 70% of the clearance of the circulating LDL is receptor mediated, and a large part of this process take place in the liver.4 Peripheral fluid is rather enriched with high density lipoproteins (HDLs).5,6 The functional role of HDL at this site is believed to concern reverse cholesterol transport of excess biosynthesized cholesterol by the peripheral tissues to the liver.7 Unlike LDLs, HDLs do not depress cellular cholesterol biosynthesis.8 In this communication, results are presented that show that oxidatively modified HDLs are potent suppressors of cellular cholesterol biosynthesis via a pathway that is LDL-receptor independent.

Methods

Lipoproteins

Blood from healthy volunteers was collected in tubes containing Na2EDTA (3 mM) as an anticoagulant. As a preservative, gentamicin sulfate (0.005%) and phenylmethylsulfonyl fluoride (1 mM) were added to the plasma, which was stored at 4°C and used for lipoprotein isolation within 36 hours.9 LDL (d=1.019–1.063 g/ml) and HDL (d=1.063–1.210 g/ml) were isolated by ultracentrifugation.10 All of the density solutions used to adjust the flotation density of the lipoproteins contained 1 mM Na2EDTA. After isolation, the lipoproteins were dialyzed overnight against 0.15 M potassium-sodium phosphate buffer, pH 7.4. After they were sterile filtered by passage through a Millex-HA 0.45-μm filter, HDL and LDL were stored at 4°C and used within 24 hours.

To induce modifications, HDLs were diluted to a concentration of 1–3 mg cholesterol/ml with PBS and incubated for 24 hours at 37°C in the presence of 50 μM

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Received February 19, 1992; revision accepted April 14, 1992.
CuSO₄. The copper sulfate oxidative challenge was terminated by chromatography of HDL through a PD-10 Sephadex column eluted with PBS. It was established earlier that removal of copper sulfate by this procedure soon after its addition to HDL effectively prevented HDL oxidation by further incubation. Cell-mediated lipoprotein modification was accomplished by incubating HDL (100 µg cholesterol/ml) with rat aortic smooth muscle cells in serum-free Ham’s F-12 medium. Dishes were placed in a humidified-air incubator with 5% CO₂ at 37°C for 24 hours. Copper ion- and cell-modified HDLs were reisolated by ultracentrifugation (d=1.063–1.210 g/ml), dialyzed against PBS, sterile filtered, and stored at 4°C. Modified lipoproteins were analyzed and added to the skin fibroblast cultures within 24 hours. HDLs that had been incubated at 37°C for 24 hours in dishes without cells were reisolated as described and are henceforth identified as “unmodified HDL.” The terms “native LDL” and “native HDL” refer to LDL and HDL preparations that had not been incubated but that were reisolated by ultracentrifugation.

Lipoprotein lipids were extracted from lyophilized lipoprotein preparations in the presence of 0.05% 2,6-di-t-butyl-4-methylphenol as an antioxidant, essentially as described by Kates, by using chloroform/methanol, 2:1 (vol/vol). The lipids were stored in the presence of the antioxidant at 4°C until used.

Lipoprotein Analysis

Lipoprotein protein content was determined according to Lowry et al by using a calibrated bovine serum albumin (BSA) standard from Bio-Rad. Cholesterol (total and unesterified), triglycerides, and choline-containing phospholipids were determined by automated enzymic assays13-15 on a Coulter CPA Autoanalyzer.

Thiobarbituric acid–reactive substances (TBARS) in lipoproteins as a measure of lipid peroxidation were quantified as described by Ohkawa et al by using malondialdehyde tetramethyl acetal-generated standard curve.

Agarose gel electrophoresis of native and modified lipoproteins was performed on Paragon Lipo-Gel strips from Beckman by using the Beckman electrophoresis apparatus at a constant voltage (100 V) for 30 minutes and following the manufacturer’s directions for electrophoretic conditions, gel staining, and destaining.

Fast protein liquid chromatography of lipoproteins was carried out on a Pharmacia apparatus fitted with a Mono-Q 5/5 (anionic exchange) column at a flow rate of 1 ml/min and a back pressure of 2 atm. Optical density of the eluate was recorded continuously at 280 nm. The starting elution buffer was 0.02 M tris(hydroxymethyl)aminomethane hydrochloride (Tris HCl), pH 7.4. Salt concentration was increased at 5 minutes to 0.2 M with the addition of sodium chloride and reached 0.6 M within 20 minutes by establishing a linear gradient of sodium chloride. Lipoproteins, previously dialyzed against 0.01 M Tris HCl, pH 7.4, were injected at a concentration of 1 mg protein/ml.

Cells

Normal human skin fibroblasts (N-HSFs) were grown from the foreskin biopsy of a 25-year-old healthy normolipidemic volunteer. The LDL receptor–defective fibroblasts (FH-HSFs) were purchased from the NIGMS Genetic Mutant Cell Repository and were from a patient with homozygous familial hypercholesterolemia. The cells were grown in a humidified incubator with 5% CO₂ in Dulbecco’s minimal essential medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum (FCS) and 0.01% streptomycin. For the experiments, cells between the eighth and 15th passage were plated onto 35-mm plastic dishes and used when they reached 90–95% confluence. Before the experiments, the cells were maintained for 24 hours in DMEM supplemented with 5% lipoprotein-deficient serum (LPDS).

Rat aortic smooth muscle cell cultures were grown in DMEM supplemented with 10% FCS and 0.01% streptomycin in humidified air with 5% CO₂. The cell cultures were used to modify HDL when the former reached the fifth to the eighth passage and 90–95% confluence.

Cellular Lipid Quantification and Cholesteryl Ester Formation Assay

The cell monolayers were washed twice with DMEM and supplemented with 2 ml DMEM containing 0.2% BSA (DMEM-BSA). Lipoproteins were added to the cells at a calculated concentration that was based on their cholesterol content. After 18 hours, [¹⁴C]Oleate (1 µCi/ml) as a complex with BSA17 was added. Incubation was continued for an additional 6 hours and terminated by washing the cells three times with 2 ml DMEM-BSA and then twice with 2 ml DMEM while keeping the dishes on ice. The lipids were extracted in situ by pouring 2 ml hexane/isopropanol 3:2 (vol/vol), over the cells. The dishes were kept at room temperature for 30 minutes, and after removal of the solvent, the procedure was repeated. The combined extracts were evaporated under nitrogen and the lipids redissolved in hexane. Aliquots of the extract were taken for cholesterol and cholesteryl ester determination by the fluorometric procedure of Gamble et al. Another aliquot was applied onto a silica-gel 60 F254 plastic sheet from Merck and chromatographed by using heptane/ethyl ether/acetic acid, 90:30:1 (vol/vol), as the developing system. Phospholipid, cholesterol, triglyceride, and cholesteryl ester bands were positively identified by comparison with egg phosphatidylcholine, recrystallized cholesterol, tripalmitin, and cholesteryl oleate standards. The lipid bands were enhanced with 8% phosphomolybdic acid, cut out, and finally counted in a Packard Tricarb 1900 CA beta counter with automatic correction for quenching. The radioactivity recovered in the cholesteryl ester band was regarded as a direct measure of the cellular cholesteryl ester synthetic activity. The remaining adherent cells were dissolved in 0.1 M NaOH overnight at room temperature. Cellular proteins were determined according to Lowry et al by using a calibrated BSA standard.

Cellular Lipid Biosynthesis

The cells were washed twice with DMEM and then received DMEM-BSA as for the previous experiments. The lipoproteins were supplemented to the cells based on cholesterol content of the previous 2 hours before addition of [¹⁴C]Oleate (2 µCi/ml). The lipopro-
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FIGURE 1. Fast protein liquid chromatography of native (panel A), unmodified (panel B), rat aortic smooth muscle cell-modified (panel C), and copper ion-modified (panel D) human high density lipoprotein (HDL). Plasma HDLs (d=1.063–1.210 g/ml) were isolated by ultracentrifugation and incubated with copper sulfate or rat aortic smooth muscle cells as described in “Methods.” Unmodified HDLs were prepared by incubating native HDL at 37°C for 24 hours in a humidified incubator. Lipoproteins were resolated by ultracentrifugation, dialyzed against 0.02 M tris(hydroxymethyl)aminomethane (Tris HCl), pH 7.4, and applied onto a Mono-Q 5/5 anion-exchange column at a concentration of 1 mg protein/mL. The starting elution buffer was 0.02 M Tris HCl, pH 7.4. Salt concentration was increased at 5 minutes to 0.2 M by addition of sodium chloride and reached 0.6 M within 20 minutes by establishing a linear gradient of sodium chloride. Eluate optical density (OD) was recorded continuously at 280 nm.

TBARS content of native and unmodified HDL was 4.2±1.2 and 4.3±1.3 nmol/mg protein (n=3), respectively. Incubation with copper ions or rat aortic smooth muscle cells enhanced the TBARS content of HDL to 56.3±8.9 or 18.5±3.9 nmol/mg (n=3), respectively. Control, copper ion-modified, and smooth muscle cell-modified HDLs were further analyzed by fast protein liquid chromatography using a Mono-Q 5/5 anion-exchange column (Figure 1). The elution of oxidized HDL was delayed compared with that of both native and unmodified HDL, indicating that modified HDL had gained a more negative charge. This was also evidenced by the migration.

Assay of Cellular HMG-CoA Reductase Activity

Cells grown in 100-mm dishes were washed with DMEM before receiving DMEM-BSA. After addition of lipoprotein, the incubation was continued for 8 hours. Cellular 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity was assayed and determined as described by Goldstein et al.17 Briefly, the cells were harvested by scraping from the dishes and were lysed at room temperature by addition of 0.1 ml 50 mM phosphate solution containing 0.25% 10 Brij 96, 5 mM dithiothreitol, 5 mM Na2EDTA, and 0.2 M KCl, pH 7.4. The resulting detergent-solubilized suspension was centrifuged for 1 minute at 12,000 rpm in a Beckman microfuge, and an aliquot of the supernatant was assayed for HMG-CoA reductase activity by using [14C]HMG-CoA. The formed, labeled mevalonate was lactonized by incubation at 37°C for 30 minutes, adsorbed over disodium sulfate, and extracted with diethyl ether. The mevalonolactone was separated by thin-layer chromatography, and the band corresponding to the product was cut out and counted.

Results

TBARS content of native and unmodified HDL was 4.2±1.2 and 4.3±1.3 nmol/mg protein (n=3), respectively. Incubation with copper ions or rat aortic smooth muscle cells enhanced the TBARS content of HDL to 56.3±8.9 or 18.5±3.9 nmol/mg (n=3), respectively. Control, copper ion-modified, and smooth muscle cell-modified HDLs were further analyzed by fast protein liquid chromatography using a Mono-Q 5/5 anion-exchange column (Figure 1). The elution of oxidized HDL was delayed compared with that of both native and unmodified HDL, indicating that modified HDL had gained a more negative charge. The changes brought about by incubation of HDL with 50 μM CuSO4 appeared more extensive than those produced by smooth muscle cells. This was also evidenced by the migration.
to the pre-α position of copper ion-modified but not cell-modified HDL, which, on the contrary, retained the α-mobility of native HDL (data not shown). Compared with native and unmodified HDL, which had virtually the same chemical composition (Table 1), incubation with smooth muscle cells and copper ions decreased the HDL content of cholesteryl esters by 8.2% and 22.0%, of triglycerides by 6.1% and 24.2%, and of phospholipids by 6.7% and 21.1%, respectively, whereas such incubation increased the content of protein by 6.0% and 17.5% and of cholesterol by 8.1% and 19.0%, respectively.

Preliminary experiments showed that modified lipoproteins were not toxic to the fibroblasts when the former were added to the cultures up to a concentration of 250 μg cholesterol/ml medium. Specifically, modified lipoproteins did not affect cell adherence to the plates, and cell morphology at the light-microscopic level was normal. In addition, cell protein recovery from the plates was constant, independent of the amount of lipoprotein added. Incubation of native, copper ion-modified, and cell-modified HDL over a concentration range of 10-50 μg cholesterol/ml medium led to a noticeable reduction of intracellular cholesteryl esters. Results are presented in Table 2. Modified HDLs were as effective as native HDL. Native HDL, copper ion-modified, and cell-modified HDLs also significantly reduced [14C]oleate incorporation into cholesteryl esters (Table 3). Ten micrograms per milliliter of any of the lipoproteins caused, on average, a 48% reduction in cholesterol esterification. Higher lipoprotein concentrations decreased slightly further the cholesterol esterification rate. By comparison, 50 μg/ml native LDL increased twofold the [14C]oleate incorporation into cholesteryl esters. Incorporation of [14C]oleate into phospholipids and triglycerides was not significantly affected by native and modified HDL at any of the concentrations tested. This suggests that the lipoproteins acted specifically on the cholesterol esterification reaction.

The effect of control and modified HDLs on cellular cholesterol biosynthesis was investigated by using [14C]acetate as the precursor. In these studies, LDL receptor-deficient fibroblasts (FH-HSFs) were also used. Results are illustrated in Figure 2. Whereas native and unmodified HDLs had virtually no effect on the [14C]acetate incorporation into cholesterol in both N-HSF and FH-HSF cells, copper ion- and cell-modified HDLs dramatically decreased acetate incorporation. The effect was dose dependent. A dose as low as 3 μg/ml copper ion-modified HDL decreased [14C]acetate incorporation by 22% in N-HSFs and by 24.5% in FH-HSFs. Higher concentrations of copper ion-modified HDL were increasingly active. In N-HSFs, the effect of 30 μg/ml copper ion-modified HDL was comparable to that achieved by 100 μg/ml native LDL. In FH-HSFs, which are insensitive to the cholesterol biosynthesis downregulation of LDL, copper ion-modified HDLs were as effective as they were in N-HSFs. The effect of cell-modified HDL was similar to that of copper ion-modified HDL, although higher lipoprotein concentrations were necessary to achieve comparable effects. Chloroform/methanol lipid extracts of both copper ion- and cell-modified HDLs were effective as they were in N-HSFs. The effect of cell-modified HDL was similar to that of copper ion-modified HDL, although higher lipoprotein concentrations were necessary to achieve comparable effects. Chloroform/methanol lipid extracts of both copper ion- and cell-modified HDLs were as effective as they were in N-HSFs. The effect of cell-modified HDL was similar to that of copper ion-modified HDL, although higher lipoprotein concentrations were necessary to achieve comparable effects.

### Table 1. Percent Chemical Composition of Native, Unmodified, and Oxidatively Modified HDL

<table>
<thead>
<tr>
<th>Component</th>
<th>N-HDL</th>
<th>U-HDL</th>
<th>Cu-HDL</th>
<th>SMC-HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>3.1±0.1*</td>
<td>3.0±0.1</td>
<td>3.8±0.1*</td>
<td>3.4±0.1*</td>
</tr>
<tr>
<td>Cholesteryl esters</td>
<td>15.0±0.3</td>
<td>14.9±0.2</td>
<td>11.7±0.3*</td>
<td>13.8±0.1*</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>26.8±0.7</td>
<td>27.2±0.6</td>
<td>21.2±0.5*</td>
<td>25.0±0.6*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>5.2±0.1</td>
<td>5.2±0.1</td>
<td>3.9±0.1*</td>
<td>4.9±0.2</td>
</tr>
<tr>
<td>Protein</td>
<td>49.9±1.1</td>
<td>49.7±1.3</td>
<td>59.4±1.6*</td>
<td>52.9±1.2*</td>
</tr>
</tbody>
</table>

N-HDL, native high density lipoprotein; U-HDL, unmodified HDL; Cu-HDL, copper ion-modified HDL; SMC-HDL, rat aortic smooth muscle cell-modified HDL (see text for details). Mean±SD from three lipoprotein preparations.

*p<0.05 vs. N-HDL by t test.

### Table 2. Cholesterol and Cholesteryl Ester Content of Normal Human Skin Fibroblasts After Incubation With Native and Modified HDL

<table>
<thead>
<tr>
<th>Component</th>
<th>N-HDL</th>
<th>Cu-HDL</th>
<th>SMC-HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>25.2±3.2</td>
<td>22.8±2.4</td>
<td>21.0±3.5</td>
</tr>
<tr>
<td>Cholesteryl esters</td>
<td>12.4±2.3</td>
<td>9.6±1.2</td>
<td>6.4±1.2*</td>
</tr>
</tbody>
</table>

N-HDL, native high density lipoprotein; Cu-HDL, copper ion-modified HDL; SMC-HDL, rat aortic smooth muscle cell-modified HDL (see text for details). Mean±SD from three experiments in micrograms per milligram of cell protein.

*p<0.05 vs. "None" group by t test.

### Discussion

Oxidative modification has important consequences for the interaction of lipoproteins with cells. Oxidatively modified LDLs interfere with several cell functions, alter organ responses to pharmacological stimuli, and
have received considerably less attention. The effect on lipidogenesis is an example. In the present study, we found that unlike native circulating HDL, oxidized HDLs potently inhibited cholesterol biosynthesis in fibroblasts. This effect was also evident in cells unresponsive to the LDL-mediated suppression of cholesterol biosynthesis. This suggests that the cholesterol biosynthesis downregulation mediated by oxidized HDL is independent of a functional LDL receptor pathway. Previously, we have shown that oxidatively modified HDLs inhibit cholesterol biosynthesis in an established line of macrophages,23 supporting the concept that oxidatively modified HDLs are efficacious in different cell types.

The effect of copper ion-oxidized HDL on the fibroblast's cholesterol biosynthesis was mimicked, albeit less intensively, by HDLs that had been modified by incubation with rat aortic smooth muscle cells. Although the cell-mediated alteration of HDL could not be detected by examining the electrophoretic mobility of these lipoproteins on agarose gels, both the enhancement of the TBARS content and the delay in elution from the Mono-Q 5/5 column support the idea that HDL had undergone oxidative modification. A crucial event in both cell- and metal-induced modification of LDL is peroxidation of the lipoprotein lipids,24–27 with the formation of fatty acid endoperoxides28 and oxysterols.29 The effect of oxysterols on cholesterol homeostasis is particularly well recognized.29 Some oxidized products of cholesterol completely mimic the effect elicited by the receptor-mediated uptake of LDL, i.e., they cause cholesterol biosynthesis suppression at the mevalonate formation site, activate cholesterol esterification, and decrease LDL receptor expression.3 Other oxysterols have different effects on these processes. For example, it has been shown that oxysterols that are formed during copper ion-mediated oxidation of LDL are responsible for the suppression of cholesterol esterification that is observed in macrophages exposed to oxidized lipoproteins.31 Akin to LDL, oxidation of HDL has perhaps engendered the formation of bioactive lipid peroxides. In fact, the lipid extracts of both cell- and copper ion-oxidized HDL suppressed cholesterol biosynthesis like that produced by the intact oxidized lipoproteins.

In this study, native and oxidized HDLs decreased [14C]oleate incorporation into cholesterol of human skin fibroblasts from a normal individual (panel A) and a familial hypercholesterolemia homozygous subject (panel B). Fibroblasts were grown in Dulbecco's minimal essential medium with 10% fetal calf serum. For the experiments, cells between the eighth and 15th passage were plated onto 35-mm plastic dishes and used when they reached 90–95% confluence. Growth medium was replaced with Dulbecco's minimal essential medium containing 5% lipoprotein-deficient serum, and 24 hours later, cell cultures were prepared for lipoprotein experiments as described in "Methods." N-HDL, native high density lipoprotein; U-HDL, unmodified HDL; N-LDL, native low density lipoprotein; Cu-HDL, copper ion-modified HDL; LCU-HDL, lipids extracted from copper ion-modified HDL; SMC-HDL, rat aortic smooth muscle cell-modified HDL; LSMC-HDL, lipids extracted from rat aortic smooth muscle cell-modified HDL. Added lipoproteins had been reisolated by ultracentrifugation and were added in amounts based on total cholesterol content. Lipid extracts were prepared as described in text and were also added in amounts based on cholesterol content. Points are the mean of two experiments, each with duplicate samples.

cytotoxic, and the increase of the cholesterol content of macrophages20–22 (also see the reviews in References 1 and 2). Other aspects of the effect that oxidatively modified lipoproteins have on cellular cholesterol homeostasis

Table 3. [14C]Oleate Incorporation (as Percent of Control) Into Lipids of Normal Human Skin Fibroblasts Elicited by Native LDL and HDL and Modified HDL

<table>
<thead>
<tr>
<th>Component</th>
<th>Lipoprotein added (μg/ml)*</th>
<th>N-HDL</th>
<th>Cu-HDL</th>
<th>SMC-HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>50</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Cholesteryl esters</td>
<td>100.0</td>
<td>204.1</td>
<td>48.1</td>
<td>39.6</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>100.0</td>
<td>120.3</td>
<td>100.2</td>
<td>107.1</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>100.0</td>
<td>94.2</td>
<td>105.0</td>
<td>103.7</td>
</tr>
</tbody>
</table>

N-LDL, native low density lipoprotein; N-HDL, native high density lipoprotein; Cu-HDL, copper ion-modified HDL; SMC-HDL, rat aortic smooth muscle cell-modified HDL (see text for details). Average values from two experiments, each with duplicate samples.

*As lipoprotein cholesterol.
Cholesteryl ester formation has not been significantly affected by oxidation, cannot be presently established. It is noteworthy that incubation with modified HDL also decreased fibroblast cholesteryl ester content. This finding suggests that the ability of native HDL to promote cellular cholesterol efflux22 had been maintained by modified HDL. Unlike apolipoprotein B in LDL,33 apolipoprotein A-I in HDL does not fragment not be critically affected by oxidation,24 and its lipid-organizing properties may not be critically affected by oxidation.

The effect of modified lipoproteins appeared to be the same (on the basis of lipoprotein cholesterol added) as that of LDLS, which suppress cholesterol biosynthesis in N-HSFs via the LDL receptor pathway.3 Clearly, the oxidative modification of HDL is changing an otherwise inactive lipoprotein species into a potent cholesterol biosynthesis suppressor. The functional significance of this finding can only be speculative at present. The ability of rat aortic smooth muscle cells to modify HDL raises the possibility that, as is thought for LDL,3 oxidation of HDL might also occur in vivo. Given the large amount of circulating antioxidants, however, there is considerable skepticism as to whether lipoprotein oxidation would be a significant process in the bloodstream. More likely, oxidation is confined to sites where lipoprotein turnover is slowed by diffusional barriers and where HDL may be affected by the resident cell population. At these sites, formed, oxidized HDLs may act as autacoids to modulate cholesterol biosynthesis. Perhaps while native HDLs are active in the reverse cholesterol transport mechanism,3 oxidized HDLs may prevent the upregulation of cholesterol biosynthesis caused by cholesterol efflux from the cell.23 In this sense, oxidized HDL would back up the LDL-mediated downregulation of cholesterol biosynthesis. This backup mechanism may be of particular significance in patients with a defective LDL receptor mechanism. Clearly, further studies will be necessary to corroborate these hypotheses. Akin to the situation with cell-modified HDL, we found that oxidatively modified LDL also can decrease cholesterol biosynthesis in normal as well as LDL receptor–deficient cells (G. Ghiselli et al, unpublished observations). Downregulation of cholesterol biosynthesis may therefore be a common feature of oxidized lipoproteins and in this sense would be a new, previously poorly recognized property of oxidized lipoproteins.

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doi: 10.1161/01.ATV.12.8.929

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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