Lipoprotein Oxidation and Lipoprotein-Induced Cytotoxicity

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The results of this study indicate that when human VLDL or LDL is prepared under conditions allowing oxidation, such oxidation renders the molecular complexes highly toxic to human skin fibroblasts growing in culture. The cytotoxicity can be predicted by assaying for the presence of thiobarbituric acid-reacting substances on the lipoprotein. However, malondialdehyde, which reacts with thiobarbituric acid and is known to be injurious to cells, was not cytotoxic in the same experimental system when dissolved in culture medium or covalently bound to non-toxic LDL. The toxic agent(s) on oxidized LDL is(are) located in a lipid-extractable moiety. Since lipid peroxides and oxidized sterols can occur in vivo under various pathological conditions, the cytotoxicity of these lipoprotein-associated substances observed in vitro may be related to certain manifestations of these conditions.


Several recent reports have demonstrated that lipoproteins are injurious or toxic to cells in vitro under certain conditions. Among these are the virtually simultaneous reports by Henriksen et al.1 showing injury to human umbilical vein endothelial cells by low density lipoprotein (LDL), and by Hessler et al.2 indicating cytotoxicity to both human umbilical endothelial cells and human arterial smooth muscle cells induced by LDL. Both groups2,3 reported inhibition of these effects by high density lipoprotein (HDL). The phenomenon was also mentioned by Alam et al.4 who observed death of human aortic smooth muscle cells at certain concentrations of LDL. That the phenomenon is not species-specific was indicated by the results of Tauber et al.,5 who confirmed the cytotoxicity of LDL, incubating bovine aortic endothelial cells with human LDL.

Under specific conditions, very low density lipoprotein (VLDL) has also been observed to be toxic to cultured cells. Chan and Pollard6,7 showed that VLDL fractions from rats in the late stage of pregnancy are toxic to tumorigenic and nontransformed cells. Gianturco et al.8 showed decreased viability of bovine aortic endothelial cells exposed to VLDL from hypertriglyceridemic humans, and Arbogast et al.9 found that porcine aortic endothelial cells died when exposed to VLDL from diabetic rat serum.

In addition to the above reports of lipoprotein-related cell injury and death, there are a number of studies describing the inhibition of proliferation of various stimulated leukocyte populations by lipoproteins, principally VLDL and LDL.10–14 Among these is the study by Schuh et al.12 who found that LDL was inhibitory in stimulated lymphocyte populations only when there was evidence of LDL "autoxidation."

The reports mentioned in the above paragraphs are evidence of a growing range of interests in the injurious effects of lipoproteins. Each of the above papers alludes to potential in vivo pathophysiological roles for these phenomena, since the lipoprotein concentrations involved are well within physiological limits.

Our preliminary data15 indicated that the cytotoxic phenomenon may be related to lipoprotein oxidation. The results of the present study indicate conclusively that oxidation renders LDL highly toxic to cultured skin fibroblasts in a concentration-dependent fashion. Our results also demonstrate that the toxic substance resides in the chloroform-methanol extractable fraction of the lipoprotein. Our measure of lipoprotein oxidation is in terms of the equivalents of
malondialdehyde (MDA) as measured by reactivity to thiobarbituric acid. Our results show that lipoprotein toxicity strongly correlates with the presence of thiobarbituric acid-reacting substances (TBARS). Although MDA has been shown to be injurious to mammalian cells in culture and to be carcinogenic in experimental animals, it appears from our results that MDA, itself, is not the agent responsible for the cytotoxic effects induced by oxidized LDL.

**Methods**

**Cell Preparation**

Human dermal fibroblasts were obtained from neonatal foreskin after circumcision. Epidermal layers were removed by careful dissection and portions of the underlying dermis were cut into small (1 mm) explants and placed in 25 cm² flasks (Costar, Cambridge, Massachusetts). Specimens were incubated at 37°C with Morgan’s M-199 medium (Microbiological Associates, Walkersville, Maryland) supplemented with 0.3 mg/liter glutamine, 10 mM HEPES buffer, and 15% fetal bovine serum (KC Biological, Lenexa, Kansas) in a humidified environment of 5% CO₂ in air. Culture medium was changed twice weekly. After 3 to 4 weeks, when sufficient peripheral growth had occurred, cells were subcultured into larger flasks (75 cm²) using 0.1% trypsin (Worthington Biochemical Corporation, Freehold, New Jersey) in Hank’s balanced salt solution (BSS) without Ca²⁺ and Mg²⁺ (GIBCO, Grand Island, New York). All experiments were performed with cells in passage 2 through 8. Cell densities of 1.70–2.4 × 10⁶ cells per Falcon culture dish (35 mm) were routinely used for experimentation. Many different dermal specimens were used in these studies.

**Lipoprotein Preparation**

Lipoproteins were prepared from freshly drawn porcine blood. VLDL was prepared from a single ultracentrifugation at d = 1.006 from the serum of pigs that had fasted 12 hours. Hyperlipemia had been induced in these pigs by feeding them a diet supplemented with 1.5% cholesterol and 19.5% lard for 6 weeks. This diet has been shown to increase total serum cholesterol in pigs from a control level of about 110 mg/dl to 400 mg/dl by 4 weeks and to over 500 mg/dl by 12 weeks. Serum triglyceride levels, however, were not changed by the diet.

Preparations were dialyzed at 4°C for 48 hours against four changes of at least 50 volumes of saline (0.15 M NaCl), pH 8.0. Dialysis conditions were varied as indicated in the Results section. In the experiments for which dialysate pH was varied, the pH was adjusted (unbuffered) using 1.0 NaOH and was stable at the indicated level. Where EDTA was included, the concentration was 1.3 mM; where glutathione was included, the concentration was 0.65 mM. Where indicated, dialysis tubing was pretreated by boiling the tubing in 0.5 M EDTA solution for 30 minutes, decanting the solution, and repeating the boiling six times with distilled water.

After sterilization by filtration (0.45 μm Millipore membrane), cholesterol and triglyceride concentrations were determined by the Lipid and Lipoprotein Laboratories of the Cleveland Clinic Foundation (standardized and certified by the Center for Disease Control, Atlanta, Georgia). Protein concentrations were determined by the method of Lowry et al. Human lipoprotein preparations (LDL, HDL, and LPDS) were analyzed by agarose gel electrophoresis and immunoelectrophoresis (with antisera to human whole serum, albumin, alpha- and beta-lipoproteins). LPDS was found to be essentially lipid-free.

The method of Folch et al. was used for lipoprotein delipidation.

**Assay for Thiobarbituric Acid Reacting Substances**

Malondialdehyde content of lipoprotein samples was determined spectrophotometrically using a modification of the thiobarbituric assay described by Schuh et al. Specifically, 1.0 ml of 20% trichloroacetic acid (TCA) was added to 100 μl of a lipoprotein preparation of known cholesterol, protein, and triglyceride concentrations. Following precipitation, 1 ml of 1% thiobarbituric acid was added, and the mixture was incubated at 95°C for 45 minutes, cooled and subsequently centrifuged at 1000 × g for 20 minutes. The absorbance of the supernatant was determined immediately using a Gilford 250 spectrophotometer, at wavelength 532 nm. Freshly diluted malondialdehyde bis (dimethyl acetal), i.e., 1,1,3,3-tetramethoxypropane (TMP, Aldrich, Milwaukee, Wisconsin) was used as a standard. The absorbance was linear in the range of 0 to 10 nmol malondialdehyde.

The thiobarbituric acid test is one of the common methods used to detect the oxidation of lipids. A
characteristically colored adduct is formed by the reaction between malondialdehyde and thiobarbituric acid. The assay, however, is not specific for malondialdehyde since it may yield a positive reading in response to other short chain aldehydes produced by decomposition of lipid peroxides during the reaction. For this reason we have expressed the amount of thiobarbituric acid reacting substances in terms of malondialdehyde molar equivalents.

**Malondialdehyde Preparation**

The sodium salt of malondialdehyde was prepared by mild acid hydrolysis of TMP according to the method of Marnett et al. and Brown et al. Varied predetermined amounts of sodium malondialdehyde were dissolved in normal saline adjusted to pH 6.4 to yield 0–20 mM solutions. After sterile filtration (0.2 μm), 0.35 ml malondialdehyde solution was added for each milliliter of sterile LDL (approximately 500 mg/dl cholesterol) and incubated at 37°C for 6 hours. The MDA-LDL was dialyzed against four changes of normal saline and incubated at 37°C for 24 hours. The MDA-LDL was dialyzed against four changes of normal saline and incubated at 37°C for 6 hours. The MDA-LDL was dialyzed against four changes of normal saline and incubated at 37°C for 6 hours. The MDA-LDL was dialyzed against four changes of normal saline and incubated at 37°C for 6 hours. The MDA-LDL was dialyzed against four changes of normal saline and incubated at 37°C for 6 hours. The MDA-LDL was dialyzed against four changes of normal saline and incubated at 37°C for 6 hours.

**Preparation of Malondialdehyde-Treated LDL (MDA-LDL)**

LDL modified by treatment with malondialdehyde was prepared by minor modifications of the techniques used by Fogelman et al. and Brown et al. Varied predetermined amounts of sodium malondialdehyde were dissolved in normal saline adjusted to pH 6.4 to yield 0–20 mM solutions. After sterile filtration (0.2 μm), 0.35 ml malondialdehyde solution was added for each milliliter of sterile LDL (approximately 500 mg/dl cholesterol) and incubated at 37°C for 6 hours. The MDA-LDL was dialyzed against four changes of normal saline (with 0.5% EDTA at pH 8.8) for 48 hours at 4°C, filtered (0.45 μm, Millipore Corporation, Bedford, Massachusetts), and assayed for protein, cholesterol, and MDA before use in culture.

**In Vitro Incubations**

Fibroblasts were trypsinized and plated into 35 mm culture dishes 24 to 36 hours before the start of experimentation. After this period, all cultures were rinsed three times with Hank's BSS and placed in medium M-199 containing LPDS (2.5 mg protein/ml of medium in the experiments shown in Figures 1 and 2, and 4.0 mg/ml in all other experiments) and supplemented with the desired lipoprotein or isotonic saline (with or without EDTA) for up to 3 days.

In all trials, the medium composition by volume was 80% M-199, 10% LPDS, and 10% LDL or isotonic saline (with or without EDTA). LDL preparations routinely had cholesterol concentrations between 400 and 700 mg/dl and were diluted (with saline) before use so that the desired final concentration of each fraction could be attained in the culture medium with the 10% medium dilution. In experiments in which lipid extracts of lipoprotein were incubated with cultured cells, the nitrogen-dried extracts were redissolved in acetone/ethanol alcohol (1:1, vol/vol) and added to culture medium so as to make a final medium concentration of 2.5% solvent in both control and experimental dishes.

Population changes (growth or toxicity) were assessed qualitatively by daily inspection of the culture dishes using an inverted stage microscope, and quantified at termination by counting cells with a ZBICouler Counter (100 μm aperture), as previously described. Thus, our index of cytotoxicity is: the number of cells remaining at termination in the experimental groups expressed as a percentage of the cells remaining at termination in the control groups (± SEM).

In all experiments, manipulations were performed in triplicate. Statistical significance was calculated using the two-tailed t test for unpaired data.

**Results**

As seen previously with human smooth muscle and endothelial cells, fibroblasts exposed to LDL (100 to 250 μg cholesterol/ml of medium) underwent distinct morphological changes consistent with injury. The cells exhibited a disappearance of cellular processes or cytoplasmic extensions, assumption of a spherical shape and, subsequently, detachment from the bottoms of the culture dish. These changes could be observed by light microscopy as early as 24 to 30 hours after beginning the incubation with LDL. The injury was most extensive in the least dense areas of the culture. Following glutaraldehyde fixation and staining with Giemsa, the remaining attached cells in the dishes exposed to LDL exhibited nuclear pyknosis and retraction of cytoplasmic extensions. Attempts to recover detached cells by replating were unsuccessful, consistent with death preceding, or immediately following, cell detachment.

The pattern of cytotoxicity to the fibroblasts induced by varying concentrations of LDL was similar to that observed previously for aortic smooth muscle cells. Figure 1 displays a collection of data pertaining to smooth muscle cultures replotted from a previous study plus two separate experiments using fibroblasts. As previously reported, the severity of the toxicity correlated with the proliferative activity of the cells. Figure 1 illustrates this; the indices of proliferative activity for the cells (the final cell counts in the control dishes divided by the starting cell counts) were 5.25 for the experiment showing more toxicity, and 2.05 for the experiment showing less toxicity.
**Effects of Dialysis Conditions**

Figure 2 displays the effects of altering the dialysate to favor conditions conducive to lipoprotein oxidation. Maintenance of sufficiently high concentration of EDTA throughout all of the preparative stages, including the addition of the LDL to the culture medium, prevented the cytotoxic effect as did the inclusion of the antioxidant, glutathione (GSH). By sparging the dialysate with oxygen, the effects of EDTA and glutathione combined were overcome and the LDL was rendered cytotoxic. The toxic LDL exhibited no difference in agarose gel electrophoretic mobility nor any change in cholesterol/protein ratio when compared to nontoxic LDL.

The presence of TBARS appeared to indicate a cytotoxic effect. Thiobarbituric acid assay revealed the absence of detectable TBARS in the LDL preparations shown in Figure 2 in which EDTA, GSH, or the combination without O₂ sparging was included. However, in the absence of EDTA ("saline only"), 4.2 nmol of MDA equivalents per mg LDL cholesterol were detected. In the absence of EDTA and the presence of O₂ sparging, 14 nmol/mg were found, and in the O₂-sparged case with added glutathione and EDTA, 5.0 nmol/mg were detected.

Cleare correlation between TBARS and cytotoxicity was revealed in experiments in which the dialysate pH was varied in the absence of antioxidants. Figure 3 shows that, when equivalent amounts of...
LDL were added to cultures, the concentrations of TBARS and the cytotoxicity increased with decreasing pH of the dialysate used during preparation. In other studies (data not shown) we found that pre-treatment of the dialysis bags had no effect on resulting toxicity of the LDL; i.e., in either treated or untreated bags, LDL was cytotoxic to the same degree if prepared in the absence of antioxidants and was not cytotoxic if antioxidants were present. The quality of the water in the dialysis had a marked effect for LDL prepared without EDTA. Use of distilled water (distributed to the laboratory from a stainless steel holding tank) resulted in LDL which was much more toxic (and had a higher level of TBARS) than did the use of glass distilled, deionized water (with resulting 3-day survivals of 7% and 39% of control cells remaining, respectively).

Cytotoxicity and Malondialdehyde

To further examine the correlation between thiobarbituric acid reactivity and the cytotoxic potential of LDL, we presented malondialdehyde to the cells in different forms. MDA was bound in varying molar ratios to the apolipoprotein of nontoxic LDL (i.e., LDL prepared using EDTA throughout). The resulting MDA concentrations were above and below that of a sample of the same batch of LDL prepared in the absence of antioxidants. In addition, various concentrations of MDA were added to lipoprotein-free culture media. In all the above, TBARS and cytotoxicity were measured (Table 1). Only the LDL that was allowed to oxidize by omission of EDTA or antioxidants from the dialysate was cytotoxic. The values appearing in Table 1 for the "cells remaining after 66 hours," which were between 73% and 82% of LPDS control dishes, are not indicative of toxicity due to the modified LDL. Experiments were performed (data not shown) that showed these were less than 100% due to undefined effects of EDTA entering the culture medium with the LDL preparation, but not present in the medium of the control dishes.

Oxidation and Toxicity of Selected Lipoprotein Fractions

LDL prepared without antioxidants (toxic) and LDL prepared with EDTA throughout (nontoxic) were delipidated and the recovered lipid extracts were added to the culture media as indicated in the Methods section. Table 2 shows that both the TBARS and the cytotoxic substance(s) resided in the lipid extract. HDL, which was dialyzed using the same procedure that rendered LDL cytotoxic, failed to exhibit detectable TBARS and failed to cause any detectable cytotoxicity when added to culture media at HDL cholesterol levels of 200 μg/ml. The HDL samples were tested for TBARS under the same conditions as LDL with respect to sample volume as well as lipoprotein cholesterol concentration. Figure 4 indicates that HDL inhibited or eliminated the cytotoxicity induced by toxic LDL in fibroblasts similarly to the inhibition reported for aortic smooth muscle and umbilical vein endothelial cells. Figure 4 also

Table 1. Concentration of Thiobarbituric Acid-Reacting Substance(s) (TBARS) and Fibroblasts Survival after Exposure to Various Sources of TBARS

<table>
<thead>
<tr>
<th>Experiment</th>
<th>TBARS source</th>
<th>TBARS† (nmol MDA/mg LDL chol)</th>
<th>TBARS† (nmol MDA/ml medium)</th>
<th>Cells remaining after 66 hrs‡ (% LPDS control)</th>
</tr>
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<tbody>
<tr>
<td>A*</td>
<td>LDL protected from oxidation</td>
<td>0.0</td>
<td>0.0</td>
<td>81.8 ± 2.1§</td>
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<td>Oxidized LDL</td>
<td>2.66</td>
<td>0.599</td>
<td>22.5 ± 1.6</td>
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<td>MDA-treated LDL (sham)</td>
<td>0.0</td>
<td>0.0</td>
<td>75.8 ± 4.8§§</td>
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<tr>
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<td>MDA-treated LDL</td>
<td>0.33</td>
<td>0.07</td>
<td>78.8 ± 2.3§§</td>
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<tr>
<td></td>
<td>MDA-treated LDL</td>
<td>0.94</td>
<td>0.21</td>
<td>78.5 ± 1.4§§</td>
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<tr>
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<td>MDA-treated LDL</td>
<td>2.03</td>
<td>0.46</td>
<td>74.7 ± 5.2§§</td>
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<tr>
<td></td>
<td>MDA-treated LDL</td>
<td>4.10</td>
<td>0.92</td>
<td>78.5 ± 6.1§§</td>
</tr>
<tr>
<td></td>
<td>MDA-treated LDL</td>
<td>10.30</td>
<td>2.32</td>
<td>73.4 ± 2.4§§</td>
</tr>
<tr>
<td>B</td>
<td>MDA in solution</td>
<td>—</td>
<td>0.1</td>
<td>112.3 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>MDA in solution</td>
<td>—</td>
<td>0.5</td>
<td>107.7 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>MDA in solution</td>
<td>—</td>
<td>1.0</td>
<td>109.1 ± 3.5§</td>
</tr>
<tr>
<td></td>
<td>MDA in solution</td>
<td>—</td>
<td>2.5</td>
<td>113.1 ± 3.1§</td>
</tr>
<tr>
<td></td>
<td>MDA in solution</td>
<td>—</td>
<td>5.0</td>
<td>109.3 ± 3.2§</td>
</tr>
<tr>
<td></td>
<td>MDA in solution</td>
<td>—</td>
<td>10.0</td>
<td>104.6 ± 7.5§</td>
</tr>
<tr>
<td></td>
<td>MDA in solution</td>
<td>—</td>
<td>45.0</td>
<td>108.1 ± 1.7§</td>
</tr>
</tbody>
</table>

*LDL concentration in Experiment A was 225 μg LDL-chol/ml medium.
†TBARS is the average of duplicates.
‡Relative cell survival is the average of triplicates ± SEM.
§Cell survival measurements of 73% to 82% in Experiment A were low due to the presence of EDTA that entered the culture medium with the LDL preparation in experimental groups but not controls. Matching EDTA content in the controls showed relative survivals of close to 100% in subsequent experiments (data not shown).
Table 2. Concentration of Thiobarbituric Acid-Reactive Substance(s) (TBARS) and Fibroblast Survival after Exposure to LDL or Extracted LDL Lipid

<table>
<thead>
<tr>
<th></th>
<th>LDL (w/o EDTA)*</th>
<th>Lipid extract</th>
<th>LDL (w/o EDTA)*</th>
<th>Lipid extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS† (nmol)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.46</td>
</tr>
<tr>
<td>MDA/mg LDL chol</td>
<td>0.0</td>
<td>0.0</td>
<td>0.48</td>
<td>0.46</td>
</tr>
<tr>
<td>TBARS† (nmol)</td>
<td>16.4±2.2</td>
<td>13.6±1.0</td>
<td>107.5±2.3</td>
<td>16.4±2.2</td>
</tr>
<tr>
<td>MDA/ml media</td>
<td>93.6±2.9</td>
<td>16.4±2.2</td>
<td>107.5±2.3</td>
<td>16.4±2.2</td>
</tr>
<tr>
<td>Cells remaining</td>
<td>66 hrs‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% LPDS control)</td>
<td>93.6±2.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*LDL concentration was 225 μg LDL-chol/ml medium. †TBARS values are averages of duplicates. ‡Cell survival data are averages of triplicates ± SEM. §LPDS concentration and lipid solvent vehicle concentration were identical in all experiments shown.

shows that nontoxic LDL was unable to inhibit the cytotoxicity of toxic LDL.

Because of reports of MDA release by aggregating platelets and reports of cytotoxicity induced by VLDL from hypertriglyceridemic patients, LDL from normolipemic porcine serum and LDL and VLDL from hyperlipemic porcine serum were separately tested for TBARS and cytotoxicity to human fibroblasts. None was found to be positive for TBARS or cytotoxic unless allowed to oxidize in preparation. If allowed to oxidize by dialysis without EDTA, all were positive for TBARS and all were cytotoxic.

Our study shows that the presence of TBARS in LDL, an indicator of oxidation, may be used as a predictor of LDL-induced toxicity to cultured human fibroblasts. However, while detectable TBARS is a predictor of cytotoxic potential, our experiments reveal that malondialdehyde delivered to the cell cultures in solution or covalently bound to the apoprotein moiety of LDL does not cause cytotoxicity in the same experimental model in which oxidized LDL is markedly cytotoxic. This indicates that, despite the documented potential for cell damage that MDA represents, it is probably not the substance responsible for cell death in our studies.

The present study also shows that VLDL, similarly allowed to oxidize, is also a potent cytotoxic agent. Other reports of specific instances of VLDL-induced cytotoxicity differ because EDTA was used for lipoprotein preparation. However, even in these cases, the cytotoxicity may be due to lipoprotein-borne lipid peroxides existing in vivo. Such speculation is indirectly supported by the report of Arbogast et al. showing that VLDL from diabetic rat serum is cytotoxic. While these researchers did not report lipid peroxide levels, others have observed elevated serum TBARS in rats with streptozocin-induced diabetes mellitus and elevated lipid peroxides in the lipoprotein fractions of diabetic humans. This elevated lipid peroxide level may be related to decreased levels of copper- and zinc-containing superoxide dismutase in certain tissues of streptozocin-induced diabetic rats. Insulin treatment in vivo restored or increased superoxide dismutase levels in the diabetic rats in the studies by Loven et al. and in vivo insulin treatment of the diabetic rats used in the study by Arbogast et al. rendered the VLDL of the rats nontoxic.

These speculations suggest the potential importance of lipoprotein-induced cellular damage in vivo.
in situations such as diabetes in which elevated lipid peroxides have been identified. Damage to cells by peroxidation of cellular lipids has been an important area of study for many years. Recently interest has been rekindled as lipid peroxidation has been shown to have far-reaching physiological effects. Lipid peroxides are increased in rabbit plasma and aorta in experimental hypercholesterolemia, in plasma of vitamin E-deficient animals, in the lung after oxygen toxicity, and in certain drug toxicities, in aging, and in human atherosclerotic arteries. MDA from platelets is enhanced both in hyperlipemic humans and in patients with known cerebrovascular disorders. That lipid peroxides residing on lipoproteins are toxic in vitro may be indicative of a role for such substances in the tissue damage accompanying the above situations.

HDL, however, was not toxic to the fibroblasts after exposure to oxidative conditions during preparation when added to the cultures in HDL-cholesterol concentrations equivalent to the LDL cholesterol levels used. As in previous reports using vascular cells, HDL was found to prevent LDL-induced cytotoxicity when added with the lower of the toxic concentrations of LDL (Figure 4 A) and partially inhibitory to the higher concentrations of toxic LDL (Figure 4 B). Nontoxic LDL, when added at similar cholesterol ratios, did not alleviate the effects of toxic LDL.

The mechanism by which the oxidized lipid fraction of LDL or VLDL might damage cultured cells and the identity of the toxic agent remain subjects of speculation. The possibilities include oxidized sterols, which have been found in the lipoprotein fractions of experimental animals and have been shown to be toxic to aortic smooth muscle cell cultures, and oxidized fatty acids, which can act as calcium ionophores and thus, presumably, interfere with certain normal cellular functions.

Acknowledgments

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